Parasites of Feral Cats and Native Fauna from Western Australia: The Application of Molecular Techniques for the Study of Parasitic Infections in Australian Wildlife

Peter John ADAMS

B. Sc. (Hons)

This thesis is presented for the degree of Doctor of Philosophy of Murdoch University, 2003.

I declare that this thesis is my own account of my research and contains as its main content work, which has not previously been submitted for a degree at any tertiary education institution.

…….

Peter John ADAMS

Abstract

A survey of gastro-intestinal parasites was conducted on faecal samples collected from 379 feral cats and 851 native fauna from 16 locations throughout Western Australia. The prevalence of each parasite species detected varied depending upon the sampling location. Common helminth parasites detected in feral cats included *Ancylostoma* spp. (29.8%), *Oncicola pomatostomi* (25.6%), *Spirometra erinaceieuropaei* (14%), *Taenia taeniaeformis* (4.7%), *Physaloptera praeputialis* (3.7%) and *Toxocara cati* (2.6%). The most common protozoan parasites detected in feral cats were *Isospora rivolta* (16.9%) and *I. felis* (4.5%). The native mammals were predominately infected with unidentified nematodes of the order Strongylida (59.1%), with members of the orders Rhabditida, Spirurida and Oxyurida also common. Oxyuroid nematodes were most common in the rodents (47.9%) and western grey kangaroos (27.8%). Several species of *Eimeria* were detected in the marsupials whilst unidentified species of *Entamoeba* and coccidia were common in most of the native fauna.

Primers anchored in the first and second internal transcribed spacers (ITS1 and ITS2) of the ribosomal DNA (rDNA) were used to develop a polymerase chain reaction-linked restriction fragment length polymorphism (PCR-RFLP) technique to differentiate the species of *Ancylostoma* detected in feral cats. Amplification of the ITS^{+} region (ITS1, ITS2 and 5.8S gene) followed by digestion with the endonuclease *Rsa*I produced characteristic patterns for *A. tubaeforme*, *A. ceylanicum* and *A. caninum*, which were detected in 26.6%, 4.7% and 0% of feral cats respectively.

Giardia was detected in a cat, dingo, quenda and two native rodents. Sequence analysis at the small subunit rDNA gene (SSU-rDNA) identified the cat and dingo as harbouring

G. duodenalis infections belonging to the genetic assemblages A and D respectively. Subsequent analysis of the SSU-rDNA and elongation factor 1 alpha (*ef1α*) identified a novel species of *Giardia* occurring in the quenda. Attempts to genetically characterise the *Giardia* in the two native rodents were unsuccessful.

Serological detection of *Toxoplasma gondii* was compared to a one tube hemi-nested PCR protocol to evaluate its sensitivity. PCR was comparable to serology in detecting *T. gondii* infections, although PCR was a much more definitive and robust technique than serology for large numbers of samples. Amplification of *T. gondii* DNA detected infections in 4.9% of feral cats and 6.5% of native mammals. The distribution of *T. gondii* does not appear to be restricted by environmental factors, which implies that vertical transmission is important for the persistence of *T. gondii* infections in Western Australia.

These results demonstrate that cats carry a wide range of parasitic organisms, many of which may influence the survival and reproduction of native mammals. As such, the large-scale conservation and reintroduction of native fauna in Western Australia must not disregard the potential influence parasites can have on these populations.

Table of Contents

Publications and Presentations

Scientific Papers

Adams, P. J., Monis, P. T., Burrows, N. D., Elliot, A. D. and Thompson, R. C. A. (2003) Cyst morphology and sequence analysis of the small subunit rDNA and *ef1α* identifies a novel *Giardia* genotype in a quenda (*Isoodon obesulus*) from Western Australia. *Infection, Genetics and Evolution*. (Submitted).

Adams, P.J. and Thompson, R.C.A. (2002) Characterization of a novel genotype of *Giardia* from a Quenda (*Isoodon obesulus*) from Western Australia. In *Giardia: The Cosmopolitan Parasite*. Olson, B.E., Olson, M.E., and Wallis, P.M. (eds.) Wallingford: CABI Publishing, pp. 287-291.

Adams, P.J. and Chavand, O.L.A. (2002) Assessment of feral cat numbers and parasite burdens in the Walpole district: A Report to the Department of Conservation and Land Management. In Perth: Murdoch University, pp. 9.

O'Handley, R.M., Olson, M.E., Fraser, D., **Adams, P.J.** and Thompson, R.C.A. (2000) Prevalence and genotypic characterisation of *Giardia* in dairy calves from Western Australia and Western Canada. *Veterinary Parasitology*. **90**: 193-200.

Algar, D., Angus, G.J., **Adams, P.J.** and Chavand, O.L.A. (2000) Assessment of feral cat numbers and control options in the bait exclusion area on Mt. Keith: A Report to WMC Resources. In Perth: Department of Conservation and Land Management, pp. 9.

Conference Abstracts (Oral Presentations)

Adams, P. J., Burrows, N. D. and Thompson, R. C. A. (2001) Detection and prevalence of *Toxoplasma gondii* in feral cats and native fauna from Western Australia. The VIIIth International Coccidiosis Conference and Annual General Meeting of the Australiasian Society for Parasitology, Cairns, Queensland, Australia.

Adams, P. J., Burrows, N. D. and Thompson, R. C. A. (2001) Parasites of feral cats and native fauna from Western Australia. The 47th Scientific amd Annual General Meeting of the Australian Mammal Society, Brisbane, Queensland, Australia.

Adams, P. J., Burrows, N. D. and Thompson, R. C. A. (2000) Characterisation of a novel genotype of *Giardia* found in a southern brown bandicoot (quenda) (*Isoodon obesulus*) in the south west of Western Australia. *Giardia* in the Rockies Conference, Canmore, Alberta, Canada. (Inaugural Postgraduate Student Award).

Conference Abstracts (Poster Presentation)

Adams, P. J. (2000) Prevalence and distribution of gastro-intestinal parasites in feral cats and native fauna throughout Western Australia. Annual Australian Veterinary Association Conference; Symposium on Veterinary Conservation Biology, Perth, Australia.

List of Tables

List of Figures

Acknowledgements

Firstly, I would like to thank my principal supervisor Professor R. C. Andrew Thompson for his support and encouragement throughout this project. Thanks also go to my co-supervisor Dr. Neil Burrows for his encouragement and advice, and without whose support this project would not have come to fruition.

I am extremely grateful to Aileen Elliot and Russ Hobbs for availing me with their parasitological expertise. For their tireless efforts in regards to processing samples, parasite identification and answering of questions I am deeply indebted. I also owe a great debt to Dr. Jennifer Rodger for her assistance in proofreading and formatting of this thesis.

I am extremely grateful to Dr. David "Marvin" Algar and John Angus from the Department of Conservation and Land Management, Woodvale, for their assistance in the collection of feral cat samples for this project. Thanks go also to Adrian Wayne and the Manjimup Crew; Ian "Wheels" Wheeler, Graham "Tub" Liddlelow, Bruce Ward, John Rooney and Colin Ward for their undying enthusiasm and assistance in the collection of native fauna samples for this project. Thanks also go to Dr. Colleen Sims, Mark Britza and Gary Desmond from Shark Bay for their contribution and assistance.

I would like to make mention of my office buddies whose combined insanity helped keep everything in perspective; Rebecca Traub, Jill Meinema, Joyce Eade and Cheeks. Special thanks to Olivier "The French Fighter Pilot" Chavand, whose friendship and support throughout this project has made it bearable.

Thanks also go to Dr. Paul Monis for his help with the phylogenetic analysis of Giardia*, Rebecca Traub for designing the species-specific* Ancylostoma *primers and Frances Brigg for her assistance with sequencing.*

Most importantly, I would like to thank my family, Ron, Delma, Matthew and Melinda for their support and sufferance over the years. My eternal gratitude goes to Abbie Fall for her support and love over the last four years, which has helped keep me positive and focussed on the job at hand.

Last but not least, to the Leave It Alone Tuggers indoor cricket team, thanks for nothing.

Angry

Chapter 1 – General Introduction

1.1 INTRODUCED ANIMALS IN AUSTRALIA

Humans have the innate ability to alter their surrounding habitat through a variety of ways, not least of which includes the introduction of non-native organisms to new environments. This anthropogenic form of invasion, also known as "biological pollution", is increasing global biogeographical homogeneity, resulting in a global loss of biodiversity (Daszak *et al.*, 2000). In Australia this process of homogenisation is thought to have first begun when dingoes (*Canis familiaris dingo*) were imported from South East Asia approximately 4000 years ago (Low, 1999; Breckwoldt, 1988).

Since European colonisation of the Australian continent a veritable flood of exotic organisms has been introduced to Australia and many of our offshore islands. Each of these introduced organisms present their own unique suite of problems for the Australian environment, and have managed to establish a feral population in some part or parts of Australia (Freeland, 1994). However, only one introduced animal is to be found throughout the entire continent of Australia including Tasmania and most of our off shore islands. That animal is the cat (*Felis catus*).

1.2 THE CAT

There are three categories assigned to describe the structure of the cat population in Australia:

Domestic - refers to pet or house cats that are housed, fed and generally cared for by human "owners". During periods of daytime or nocturnal freedom however, these pets, though well fed, still engage in predatory capture, play and killing behaviours.

Stray - refers to street, alley, farm or semi dependent cats that may or may not receive some food directly from humans, however they do so indirectly by scavenging scraps from rubbish bins, dump sites or from slaughter remains on farms. No attempt is made to house these animals yet they may inhabit man made structures such as farm buildings, factories, wharves or abandoned vehicles.

Feral - refers to true feral, bush or wild cats that have no reliance whatsoever on human contact for their survival and obtain all their food and shelter requirements from the natural environment. They may however, have indirect contact with human activity through the predation or scavenging of livestock and refuse.

These three categories are not well defined nor easily identifiable and individuals may vary their status due to factors such as the availability of food, group pressure and the migration of humans (Izawa and Doi, 1993; Izawa *et al.*, 1982). Visual differentiation between the categories cannot be easily accomplished. In general, true feral cats in good condition show greater muscle development around the head, neck and shoulders, giving the animal a more robust appearance. However, this is a generalisation and exceptions to this rule are common.

1.2.1 The Feline Advantage

Cats differ from canids in that they have the added advantage of an arboreal dimension, with retractable claws aiding their ability to climb as well as run, jump, dig and stalk. Unlike canids, cats also have a well developed tapetum lucidum in their eyes which gives cats excellent night vision (May and Norton, 1996; Jones, 1988). A unique aspect of the cat is that they do not require water where live prey is readily available (Newsome, 1991; Jones, 1988). Adequate moisture is provided by blood and other bodily fluids of prey species caught, with the cat able to exist in almost total isolation of water, with perhaps the exception of females suckling young and individuals in poor condition (Cross, 1990). Cats also appear to be much more selective in their choice of prey species than canids and are generally not scavengers unless adverse environmental conditions dictate such behaviour (Read and Bowen, 2001; Jones and Coman, 1981; Bayly, 1978).

1.2.2 Feline Social Structure

The feral cat has been most commonly described as a solitary species, with non-overlapping female ranges included within the larger ranges of one or more males (Newsome, 1991; Brothers *et al.*, 1985; Jones and Coman, 1982). This exclusively solitary social system is true to most felids and is attributed to their stealth and ambush hunting strategy (Izawa and Doi, 1993). However, the lion (*Panthera leo*), cheetah (*Acinonyx jubatus*) and the domestic cat are the only members of the Felidae that exhibit group formation (Denny *et al.*, 2002; Izawa and Doi, 1993).

Worldwide, cat densities vary from less than one to more than 2000 cats km^{-2} and appear to be dependant on food supply (Liberg and Sandell, 1994). However, a rich

food supply does not necessarily decrease the size of a cat's home range (Yamane *et al.*, 1994; Dards, 1978). Whilst these home ranges are maintained, they often overlap with those of other cats and are generally not defended, rather the sharing of an area is temporal with different cats utilising the same area at different times (Dards, 1983; Izawa *et al.*, 1982; Laundre, 1977; Leyhausen, 1965).

Studies of groups of cats exploiting resource-rich sites (e.g. rubbish tips) have suggested that rather than being *ad hoc* collections of animals, they are structured and functional populations of group-living cats (Denny *et al.*, 2002; Mirmovitch, 1995; Yamane *et al.*, 1994; Dards, 1983). Rubbish tips are a ubiquitous feature throughout much of rural Australia and are associated with most towns, mining sites, tourist resorts and homesteads, and are known to support high densities of cats (Denny *et al.*, 2002; Wilson *et al.*, 1994).

1.2.3 The Feral Cat Diet

The feral cat's diet is as wide and varied as the habitats it has colonised. Feral cats are known to prey upon 186 species of native bird, 64 species of native mammal (in addition to introduced mammals), 87 species of reptiles, 10 species of amphibians and numerous invertebrates (Barratt, 1997; Paltridge *et al.*, 1997; Martin *et al.*, 1996; Paton, 1993; Jones and Coman, 1981; Bayly, 1978). Along with the introduced red fox, feral cats are at least partially responsible for the extinction and decline of many species of Australian wildlife (Dickman, 1996a; Gibson *et al.*, 1994; Morton, 1990; Burbidge and McKenzie, 1989). Ground nesting birds on islands and small to medium sized mammals have been particularly vulnerable to these exotic predators (Burbidge and McKenzie, 1989; Brothers, 1984; Taylor, 1979).

Recent studies on the impact of feral cats on native fauna suggest that they may play a far more destructive role than has previously been assumed and indeed appear to be a considerably greater menace to native fauna than the fox (Risbey *et al.*, 2000; Risbey *et al.*, 1999; Christensen and Burrows, 1994; Gibson *et al.*, 1994). Studies on feral cats and foxes in arid environments show many similarities in their respective diets (Read and Bowen, 2001; Catling, 1988), however the fox is typically an opportunist relying predominantly on rabbits and carrion (Risbey *et al.*, 1999; Bayly, 1978; Croft and Hone, 1978; Coman, 1973a). In contrast, the feral cat appears to be a selective feeder that can readily adapt to different prey items (Read and Bowen, 2001; Risbey *et al.*, 1999; Paltridge *et al.*, 1997; Martin *et al.*, 1996; Catling, 1988; Triggs *et al.*, 1984; Bayly, 1978, 1976). The ability of the cat to effectively switch prey species depending on their relative abundance allows it to avoid population declines in unfavourable seasons, unlike the fox (Read and Bowen, 2001).

1.3 DECLINE OF NATIVE SPECIES IN AUSTRALIA

Australian native species have accounted for 50% of all the mammal species that have become extinct worldwide (Short and Smith, 1994). The majority of these extinctions and declines have come from two taxonomic groups, the marsupials and the rodents (Smith and Quin, 1996; Short and Smith, 1994), however not all species in these two taxonomic groups appear to be as equally vulnerable to decline or extinction. The most affected species fall into the weight range of 35 g to 5500 g; denoted as being the "critical weight range" by Burbidge and McKenzie (1989). The two areas of Australia most affected by these extinctions are the southern arid zone and the wheat belt of

Western Australia (Short and Smith, 1994). In fact, Australian arid zone mammals have suffered extinctions and contractions of range to a far greater degree than any other vertebrate group in Australia (Morton, 1990).

A potential explanation may have its basis in the long biogeographical isolation of the Australian land mass and the resultant evolution of its diverse and different fauna, unusually vulnerable to whatever the cause(s) of extinction may be (Freeland, 1994). Australia's native fauna has been subjected to a series of significant ecological disturbances over the past 200 plus years of European settlement including: hunting; habitat clearing and fragmentation; grazing by introduced livestock; altered fire regimes; as well as increased predation from introduced foxes and cats (Smith and Quin, 1996; Short and Smith, 1994; Morton, 1990; Lunney and Leary, 1988). Each of these disturbances has had varying levels of impact on the native fauna, however more recently disease has been considered as a potential factor in the decline and extinction of the Australian native fauna.

1.4 PARASITES AND DISEASE

1.4.1 Pathogens and Food Webs

There are three potentially density dependent factors regulating populations and food webs: nutrient limitation; predators; and parasites (Freeland, 1994). Of these three factors it is the parasites that have the greatest capacity for stabilising natural communities (Freeland, 1994; Freeland and Boulton, 1992). Indeed, pathogens have been the dominant selective forces in human populations for the last 10,000 years and are still the main cause of the dramatic differences of survivorship curves in both

developed and developing countries today (May, 1988; Bradley, 1974). Because of their profound influence on individual fitness, parasites are a major evolutionary force and an important factor in the maintenance of biodiversity (Gulland, 1995; May, 1988; Scott, 1988). The consequences of parasites may well be as important at the immediate level of the individual as at the level of the population (Lyles and Dobson, 1993), whilst the effects of a parasite on one species may also have an impact on other species via direct or indirect interactions (Cunningham, 1996).

This complexity of interaction led to the notion that the stability of nature was in some way related to the species-richness of communities; the more complex the system, the greater the stability (Freeland, 1994). We now know that the reverse of this is true and that the simpler the system the more likely it is to be able to persist without loss of species (Freeland, 1994). However, complex communities such as the species-rich rainforests and coral reefs do exist and appear to exhibit a relatively high level of stability. If these systems are to be preserved for posterity, it is important that our understanding of their population dynamics does not disregard the involvement of parasites as a potential regulatory force.

The large number of parasite species in natural food webs and the existence of parasitic life cycles that link host species of different trophic levels indicates their importance as agents of community structure and stability (Daszak *et al.*, 2000; Freeland, 1994). Indeed, Freeland and Boulton (1992) working with experimentally constructed food webs showed that the addition of parasites to these systems resulted in higher levels of stability, indicating a greater capacity for them to recover from perturbation. It has also been demonstrated that the removal of parasites from natural systems may generate

instability (loss of species), and increase the potential for alien parasites to disrupt communities where they do not have co-evolved relationships with their hosts (Freeland and Boulton, 1992).

1.4.2 Impact of Parasites on Populations

The impact of parasites on the survival, reproduction or dispersal of host individuals will depend upon the virulence of the parasite, the infective dose and the resistance of the host to infection (Gulland, 1995; Anderson and May, 1978). These parameters can be modified by a number of factors such as malnutrition, overcrowding, stress and multiple parasitism that complicate the dynamics of the host-parasite interaction (Gulland, 1995). Parasites may also indirectly affect the survival of the host by increasing their susceptibility to predation or by reducing their competitive fitness (Webster, 2001; Berdoy *et al.*, 1995; Scott, 1988).

Parasites that influence survival, reproduction, or dispersal of the host may also influence the genetic structure of the local host population (Scott, 1988). Conservation biologists hypothesize that endangered species are especially vulnerable to infectious disease due to their low numbers leading to reduced genetic diversity and a reduced ability of the host to respond to pathogens in an evolutionary sense (Lyles and Dobson, 1993; O'Brien and Evermann, 1988). A lack of genetic variability in a population significantly improves the odds of an infectious disease causing devastating effects, because when it overcomes one individual defence system it more likely than not will overcome the others in a genetically uniform population (O'Brien and Evermann, 1988; Ralls *et al.*, 1979). This is exemplified by the high susceptibility and subsequent mortality rates of cheetahs to feline infectious peritonitis, due to their lack of genetic

variability resulting from a severe population bottleneck in the cheetah's recent evolutionary history (O'Brien *et al.*, 1985; O'Brien *et al.*, 1983).

1.4.3 Impact of Introduced Pathogens

Transmission of pathogens by an introduced species to susceptible natives can have a more profound impact on the native species than any direct impact of the introduced species itself (Freeland, 1994). Well known examples of the devastating impact introduced disease can have on native fauna include the decimation of the native lowland bird fauna of Hawaii by avian malaria (van Riper and van Riper, 1986), the impact of air-sac mite on Australia's Gouldian finch (Tidemann *et al.*, 1992) and the virus mediated relationship between native red squirrel and introduced grey squirrel in the United Kingdom (Vizoso, 1968). Recently, the first definitively proven example of extinction by infection was reported and involved the extirpation of the captive remnant population of the Polynesian tree snail (*Partula turgida*) by a microsporidian parasite (Cunningham and Daszak, 1998).

Host population size has a profound effect on the dynamics of a pathogen as every parasite requires a minimum density of hosts whereby it can maintain itself, known as the threshold population size (H_T) (Lyles and Dobson, 1993; Dobson and May, 1986; Bartlett, 1960). If the host population exceeds H_T, the parasite is able to maintain itself within the population (Dobson and May, 1986). This is exemplified by the emergence of brucellosis in bison in the Yellowstone National Park, which occurs whenever the herd size exceeds approximately 200 animals (Dobson and Meagher, 1996).

For any pathogen, prevalence falls to zero in susceptible host populations that are smaller than H_T, unless they are maintained by another source (Lyles and Dobson, 1993). Ironically, the presence of a threshold for establishment suggests that endangered species are relatively protected from virulent pathogens as their population sizes may be too small to continuously support an infection (Lyles and Dobson, 1993). However, this perceived level of protection actually increases the susceptibility of endangered species to catastrophic disease outbreaks, as small populations of species are at a greater risk from non-specific pathogens than host specific pathogens.

Thus, the movement of pathogens to new locations poses a serious threat to global biodiversity due to the potential for the catastrophic depopulation of new and naïve host populations (Daszak *et al.*, 2000). When introduced diseases become enzootic, initial declines in host populations may be followed by chronic population depression, and if the threshold host density for disease transmission is lowered, local extinctions may also occur (Daszak *et al.*, 2000).

1.4.4 Emergence of Disease

Human environmental modification is suggested as the primary driving force for the emergence of human, domestic animal and wildlife infectious diseases (Daszak *et al.*, 2001; Schrag and Wiener, 1995). In particular, the continued expansion of human populations brings us into contact with a large pool of known and unknown zoonotic pathogens (Mahy and Brown, 2000). The threat of emerging infectious disease outbreaks in wildlife populations leads to complicated (and costly) conservation issues, whilst threatening biodiversity, human health and wellbeing in a complex, inter-related manner. Loss of biodiversity itself has economic ramifications (Costanza *et al.*, 1997),

and its conservation is of increasing economic interest and ethical concern (Myers *et al.*, 2000).

The key event in the emergence of most infectious diseases is a change in host–parasite ecology resulting from changes in human demography, behaviour or social structure (Dobson and Carper, 1996; Garnett and Holmes, 1996). These changes allow an increase in transmission between individual hosts, new host populations or species and selection pressure, leading to the dominance of pathogen strains adapted to these new conditions (Daszak *et al.*, 2001). Wildlife populations have long been considered a link in the chain of pathogen emergence, by forming the reservoirs from which zoonotic pathogens may emerge (Daszak *et al.*, 2001). However, wildlife populations are seldom the guilty party in the event of a disease outbreak, though more often than not they bear the brunt of its effects.

Emerging infectious wildlife diseases have been responsible for mass mortalities as well as local (population) extinctions and global (species) extinctions (Daszak and Cunningham, 1999; Cunningham and Daszak, 1998). This direct loss of biodiversity due to infectious disease may lead to further impacts on ecosystems via 'knock-on' effects. For example, the introduction of rinderpest into Africa in the late 19th century resulted in massive changes in grazing pressure and a perturbation of succession in the savannah flora that persists to date (Dobson and Crawley, 1994). Such knock-on effects may lead to the extinction of species further up the food chain that remain uninfected by the pathogen. Hence, apart from the immediate direct and indirect effects on individual animal species, the introduction of disease may also have broad, long-term, and unforeseeable effects on ecosystems (Cunningham, 1996).

A number of high-impact wildlife diseases have also emerged due to 'spill-over' of pathogens from domestic animals into wildlife populations. Because domestic animals often outnumber the wildlife hosts of shared pathogens, they act as maintenance hosts, enabling the pathogen to avoid the threshold density effect and drive the smaller population of wild animals to virtual extinction (Cleaveland and Dye, 1995). Generally it is only when these pathogens threaten to 'spill-back' into domestic animals that these issues may become politically charged as conservationist and commercial interests clash (Dobson and Meagher, 1996). However, a more common factor driving the emergence of wildlife disease is the anthropogenic movement of pathogens into new geographic locations – a phenomenon termed 'pathogen pollution' (Daszak *et al.*, 2000; Cunningham, 1996).

1.4.5 Pathogen Pollution and Apparent Competition

Pathogen pollution is rooted in the unprecedented globalisation of agriculture, commerce, human travel and the transport of domestic animals and their products (Daszak *et al.*, 2001). Human landscape changes that remove portions of host populations, alter host migration patterns or increase host density are also likely to increase the risk of pathogen emergence (Dobson and May, 1986). Pathogens may also be spread or amplified following the introduction of uninfected hosts into new geographic areas (Daszak *et al.*, 2001). Pathogen introductions have a particularly high impact when naïve host populations are involved and introduced pathogens may contribute to the competitive success of the invading carrier hosts by the process of 'apparent competition' (Hudson and Greenman, 1998).

Apparent competition is mediated via a shared pathogen, where one species can be a superior competitor simply by harbouring and transmitting this pathogen to a more vulnerable species (Hudson and Greenman, 1998; Bonsall and Hassell, 1997; Holt and Lawton, 1994). This is exemplified by the helminth parasite *Parelaphostrongylus tenuis* and its suggested role as the agent that allows white-tailed deer to prevent moose and caribou from establishing in larger areas of the eastern United States (Schmitz and Nudds, 1994; Anderson, 1972). Competition is widely accepted as a major force influencing biodiversity, so it stands to reason that apparent competition could play a significant role in shaping community structures (Hudson and Greenman, 1998).

1.4.6 Parasites in Small Populations

Endangered species generally have small populations and as such are at a much greater risk of being adversely affected by the loss of a given percentage of the population, or even individuals, from disease (Cunningham, 1996). Diseases and parasites pose particularly severe problems in captive or managed populations in which animals are held at high densities (McCallum and Dobson, 1995). As animals are forced to exist at these higher densities, virtually every aspect of the life and health of the animals will be affected (Scott, 1988). Stress associated with captivity may also increase the pathological effect of common infectious agents and further debilitate animals already compromised by their containment (Viggers *et al.*, 1993). Animals held in captivity are also at an increased risk of exposure to infection from foreign parasites.

The susceptibility of small populations to pathogens may be enhanced by the loss of endemic diseases once the population size falls below the critical levels required for the maintenance of such diseases (Cunningham, 1996). These populations risk becoming

immunologically naïve as most individuals within a small population are never exposed, resulting in low levels of acquired immunity within these populations (Cunningham, 1996; Viggers *et al.*, 1993). Without this level of exposure, these populations are at an increased risk of being adversely affected by epidemics of what were previously endemic diseases, as well as new and emerging diseases (McCallum and Dobson, 1995).

Additionally, due to their small population sizes, endangered species will tend to acquire virulent infections only after exposure to infected hosts of another more common and widespread species (McCallum and Dobson, 1995). These more common and widespread species are defined as reservoir and/or maintenance hosts, and are capable of independently maintaining the pathogen as well as acting as a source of infection to other species. The presence of a maintenance host enables a pathogen to avoid the threshold density effect and can drive the smaller population of susceptible animals to virtual extinction (Cleaveland and Dye, 1995). Thus, when an epidemic does occur it tends to infect a large proportion of the susceptible population and mortality levels may be high (McCallum and Dobson, 1995). A prime example of this phenomenon was the rinderpest epizootic in East Africa during the 1890's which decimated 95% of the wildebeest and cape buffalo populations though was relatively benign to its domestic cattle hosts (McCallum and Dobson, 1995; Dobson and Crawley, 1994).

Likewise, introduced or translocated animals may be susceptible to parasites that are relatively non-pathogenic in their primary host(s), whilst animals released into previously unoccupied areas may be exposed to new parasites and vectors of disease

(Cunningham, 1996; Viggers *et al.*, 1993). The translocation of animals to new or existing habitats can also import new parasites with them that may primarily affect other hosts (Viggers *et al.*, 1993), this risk is increased if the animals have been held in captivity prior to release (Cunningham, 1996). In these ways, parasites are not only capable of nullifying the potential benefits of captive breeding programs, but they can also have an overall negative effect on wildlife conservation where translocations have been carried out (Cunningham, 1996; Viggers *et al.*, 1993; May, 1988; Scott, 1988).

1.4.7 Conservation and Disease

Extinction is a natural process, but the current rate of loss of genetic variability of populations and of species is ongoing and far above background rates, representing an irreversible global change (Vitousek *et al.*, 1997). The Earth's most vulnerable terrestrial species can be identified within 25 "biodiversity hotspots" that individually occupy no more than 2% of the ice-free land surface (Myers *et al.*, 2000). As such, introduced species are a serious threat to global biodiversity, second only to habitat loss (Vitousek *et al.*, 1997; Vitousek *et al.*, 1996), whilst the introduction of their pathogens to new areas is of worldwide concern given the potential for pathogens to reduce host abundance (Hudson and Greenman, 1998; Bonsall and Hassell, 1997; Lyles and Dobson, 1993).

Along with the increasing pace of habitat destruction, there has been an increase in captive breeding and translocation programs for endangered species (Abbott, 2000; Fischer and Lindenmayer, 2000; Short and Turner, 2000). Although the primary objectives of these programs revolve around the requirement to maintain local and global genetic diversity within the species of interest, little regard has been given to the

role of their pathogens (Cunningham, 1996; Lyles and Dobson, 1993; Viggers *et al.*, 1993; Scott, 1988). However, as we set about attempting to conserve the last vestiges of our remaining natural habitats and the species within them, parasites will play an increasingly important role in our management of species. Abnormally high population densities that may arise when species are crowded into improperly managed wildlife reserves can facilitate the establishment of pathogens which may result in the reduced survival and reproduction of host species (Scott, 1988; Dobson and May, 1986).

Many attempts are made at keeping animals free from infection, especially in zoos and captive breeding facilities. In many ways this is a sensible strategy for it should enable animals to survive and reproduce at rates close to maximum which would have to be good for the conservation of the species. However, protecting animals from disease may do more to weaken a population and increase its susceptibility to infection than it does to improving survivorship (Cunningham, 1996; Lyles and Dobson, 1993; Scott, 1988). For example, a study on the lungworm-pneumonia complex in bighorn sheep in western Canada found that the "fittest" bighorn sheep population was also the one with the highest level of lungworm infection (Uhazy *et al.*, 1973).

Establishment of disease-free wild populations is unnatural and overall not a feasible ideal. To attempt to eliminate parasites and the role they play in host population ecology and evolution, for the "good" of captive, reintroduced and wild populations would be short sighted indeed to say the least. Infectious disease is a normal, constant and continuing feature in the lives of most animals and plants. Parasites have the ability to regulate the growth of host populations even in the complete absence of other influences such as predation or intraspecific competition (May and Anderson, 1978).

Pathogens can and do exert important effects on host population dynamics both as endemic and epidemic infections (Gulland, 1995).

There is little point in conserving animal populations unless those animals are kept in conditions that promote their health and continued wellbeing (Scott, 1988). Recognition of the potential impact of disease in wild animal populations is the first step in prevention. Even non-pathogenic parasites may become important when animal populations are malnourished or stressed, and the combined effects may predispose animals to other population pressures. It must be remembered however, that a disease-free wild population is unnatural and policies of keeping managed populations parasite free should mostly be replaced with maintaining moderate levels of infection for the long-term benefit of those populations (Lyles and Dobson, 1993).

1.4.8 The Role of Disease in Australian Extinctions

The argument regarding declines in range and abundance of populations of native mammals in Australia due to disease has mainly been based on evidence considered to be anecdotal (How *et al.*, 1987; Archer, 1984; White, 1952). Burbidge & McKenzie (1989) argue that if disease was a primary cause of extinction or decline in mammal species it is unlikely that it would have selectively affected the "critical weight range" species, which also come from a number of unrelated groups. However, Freeland (1994) argues that declined species of dasyurid are larger than those that have not declined, bandicoot and macropod species that have declined are smaller than other members of their taxa, and that there is no difference between the mean body sizes of rodents that have and have not declined. This apparent lack of a single critical size class indicates that it might be a combination of body size and taxonomic affinity that is

associated with the probability of species extinction or decline. Freeland (1994) also suggests that the mammal species suffering the greatest declines (and extinction) following European colonisation of Australia are those most likely to acquire parasites from introduced animals.

However, any hypothesis sufficient to explain the high rate of extinction of Australian mammals must provide a causal mechanism as well as account for the unusual vulnerability of Australian mammals. Freeland (1994) proposes that the high rate of extinction of Australian mammals reflects the instability of ecosystems that have a paucity of coevolved, host-parasite relationships resulting from an unusually severe extinction of Australia's Pleistocene predators and their parasites. Freeland (1994) also suggests that the recent imposition of alien parasites on the mammalian fauna, the extinction of Australian parasites that colonise introduced hosts and a paucity of parasites among introduced mammals have further perturbed the instability of Australia's ecosystems.

1.4.9 Australia's Historical Ecosystem Restructuring

The Australian continent, along with all the other continents except Africa, suffered major losses of mammals, large birds and large terrestrial reptiles during and following the Pleistocene era (Horton, 1984). Reasons for these extinctions include hunting pressure from invasive humans and a combination of climate change and human use of fire in the modification of environments (Owen-Smith, 1987; Guthrie, 1984). In North and South America the net result of the Pleistocene extinctions was the development of a relatively rich fauna of predatory mammals whilst Australia appears to have retained only the thylacine (*Thylacinus cyanocephalus*), the relatively small Tasmanian devil

(*Sarcophilus harrisi*) and the even smaller tiger quoll (*Dasyurus maculatus*) (Freeland, 1994). This relatively poor survival of large mammals may have been due to Australia's limited topographic diversity, resulting in fewer refugia during periods of dramatic climate change (Freeland, 1994).

The loss of Australia's large predators and many herbivores together with their parasites during the Pleistocene may have resulted in species communities with lower levels of stability (Freeland, 1994). Although the Australian fauna continues to maintain parasites with complex life cycles involving reptiles, birds and small mammals as ultimate hosts, the Tasmanian devil is perhaps the only "large" mammalian predator to host such parasites. The thylacine is known to have had parasites, but these have presumably become extinct along with the animal itself (Sprent, 1970; Ransom, 1907). Ironically, introduced pathogens have been convincingly portrayed as the cause for the thylacine's extinction (Guiler, 1961), however this hypothesis is yet to be proven and definitive proof might be difficult to obtain.

1.4.10 Introduction of Pathogens to Australia

Inevitably, the introduction of animals to the Australian environment has nonetheless resulted in the introduction of new parasites that have colonised the native fauna. The introduced dog, cat and fox support a total of nine helminth species whose intermediate or paratenic stages now infest the Australian fauna (Coman *et al.*, 1981a; Coman, 1972b, a). Examples of introduced parasites colonising Australia's native animals include the cestode *Echinococcus granulosus*, common in kangaroos and other macropods of south eastern Australia (Grainger and Jenkins, 1996; Lymbery *et al.*, 1990; Howkins, 1966b), as well as the protozoan *Toxoplasma gondii*, which is known to

occur in numerous marsupial species (Canfield *et al.*, 1990; Johnson *et al.*, 1989; Johnson *et al.*, 1988). These parasites, like many introduced parasites, have intermediate life history stages that are relatively non host-specific, increasing the potential for their transmission to both wild and domestic populations of animals (Pozio, 2000; Baldock *et al.*, 1985; Howkins, 1966b, a).

Whilst the introduction of potential hosts into new geographic locations without the co-introduction of their pathogens can result in disease emergence (Daszak *et al.*, 2000), the parasites of Australia's kangaroos and wallabies do not appear to present any such problem for introduced herbivorous mammals (Freeland, 1994). Likewise, the majority of our native frog parasites have failed to colonise the introduced cane toad (*Bufo marinus*) (Delvinquier and Freeland, 1988; Freeland *et al.*, 1986), and there appears to be only one helminth parasite of Australian dasyurids (*Cyathospirura dasyuridis*) that has successfully colonised the feral cat and fox (Coman *et al.*, 1981a; Coman, 1972b, a). Freeland (1994) theorised that it was the unusual phylogenetic composition of the Australian fauna combined with its loss of parasites and predators during the Pleistocene, that has resulted in introduced animals having no or little in the way of a "parasitological" barrier to overcome prior to successful invasion.

Unfortunately the reverse is not true and the incorporation of the Australian native fauna into the life cycles of introduced parasites is a common occurrence. This may be considered by some as constituting the beginnings of evolutionary re-establishment of density dependent controls on the stability of Australian ecosystems, however the transfer of parasites from introduced to native species cannot be regarded as invariably benign or positive (Daszak *et al.*, 2001; Freeland, 1994). The destabilisation of food

webs resulting from the introduction of alien parasites may result in extinctions or an increased probability of extinctions during periods of environmental perturbation (Freeland, 1994).

The more subtle and sinister threat pathogens pose to Australia's biodiversity is only now gaining recognition and understanding as a serious global threat (Pimm and Raven, 2000; Cassis, 1998; Vitousek *et al.*, 1997; Vitousek *et al.*, 1996). However, our incredible lack of knowledge of the Australia fauna and the ecosystems in which they dwell is a major stumbling block for the progression of any research into this relatively new field, which is further complicated by the large number of feral pests and their parasites in Australia. As such, the study of these effects requires sensitive techniques for the detection and identification of pathogens in both the native and introduced fauna.

1.5 PARASITE DETECTION

1.5.1 The Importance of Taxonomy

Knowledge of taxonomic relationships and the phylogeny of parasite species and their variants provides a better understanding of infections and is crucial for the development of vaccines and new diagnostic methods (McManus and Bowles, 1996). Unfortunately, traditional approaches have often been of limited use for parasite taxonomy or identification, due in some cases to a lack of morphological variation in parasites either because of constraints caused by specialised body-plans or because species are too closely related (Monis, 1999). Despite the increasing use of electron microscopy, the range of morphological features is limited in many parasitic organisms and morphologically indistinguishable sibling species are common (McManus and Bowles,

1996). A prime example of this is the protozoan parasite *Neospora caninum*, which was only identified as being distinct from *Toxoplasma gondii* in 1988 (Dubey *et al.*, 1988), and whose "correct" taxonomy is still a contentious issue today (Dubey *et al.*, 2002). For the effective diagnosis, treatment and control of parasitic diseases, it is essential that parasite isolates can be accurately and reliably identified (McManus and Bowles, 1996).

1.5.2 Molecular Detection of Parasitic Infections

The differentiation and detection of parasites as well as the diagnosis of parasitic infections can be achieved by a variety of methods, however molecular techniques are becoming increasingly more important as a dearth of biological data on the mode of reproduction, ploidy, host range and/or life cycle of parasite species limits progress (Zarlenga and Higgins, 2001; Monis, 1999). Direct testing for the presence of parasites through molecular assays can avoid many of the ambiguities associated with indirect detection methods and help improve our understanding of parasite epidemiology (Zarlenga and Higgins, 2001). Molecular markers provide valuable tools for the study of disease transmission and diagnosis which require simple, reproducible and practical methods for accurate identification (Monis and Andrews, 1998; McManus and Bowles, 1996). The nature of the polymerase chain reaction (PCR) makes it an ideal tool for parasite identification, especially in a diagnostic context as it is technically simple to use, rapid, sensitive and specific (Monis and Andrews, 1998).

PCR has provided the basis for the development of a new generation of tools that are increasingly being used for diagnosis and epidemiology in both veterinary and human parasitology (Morgan and Thompson, 1998). PCR-based diagnostic assays are able to detect multiple genotypes of infectious agents directly in clinical or environmental

samples without the need to produce large quantities of the agent by *in vitro* or *in vivo* laboratory amplification (Monis and Andrews, 1998; Morgan and Thompson, 1998). Direct sequence analysis of the amplified DNA can also be used for the characterisation of distinct species, subspecies and/or strain groups of parasite, or to infer their phylogenetic relationships and thus identify routes of disease transmission (Monis and Andrews, 1998; McManus and Bowles, 1996).

The relatively stable nature of DNA, particularly in comparison to enzymes, allows the collection and storage of material for molecular techniques to be less stringent than that required for many other identification methods (McManus and Bowles, 1996). The ability to rapidly select, isolate, amplify and sequence parasite DNA from small amounts of tissue applies itself to the screening of crude material, which is important for the detection of infectious agents that are refractory to culture and hence, unlikely to be detected by traditional methods (Monis and Andrews, 1998; McManus and Bowles, 1996). Additionally, for the purposes of identification and phylogenetic study, it is not necessary that genetic characters are directly related to functional or other phenotypic differences between forms, it is sufficient that they consistently act as markers of particular known epidemiological types (McManus and Bowles, 1996).

1.5.3 Molecular Systematics and Parasitology

The control and prevention of many pathogenic infections depends upon knowledge of how the aetiological agents survive and are transmitted in different environments (Morgan and Thompson, 1998). Molecular epidemiology of pathogenic organisms allows the distinction of closely related infectious agents and the documentation of the mechanisms by which they are transmitted between hosts, and how these mechanisms

may affect their dispersal both within and between populations of hosts (Monis and Andrews, 1998; Thompson *et al.*, 1998). Thus, molecular techniques provide predictive and quantifiable tools not previously available, allowing precise determinations to be made about aetiological agents, their characteristics and source of infection (Thompson *et al.*, 1998). However, DNA characterisation methods still need to be combined with traditional techniques and matched by appropriate methods of analysis and interpretation to achieve the greatest benefit (Thompson *et al.*, 1998).

The phylogenetic benefit of using DNA based approaches is that they focus on genes rather than gene products and so avoid problems of life cycle stage variation, environmentally and host-induced modification, and post translational modification (McManus and Bowles, 1996). Except in extremely rare instances, the content and integrity of parasite DNA is essentially invariant throughout its many life-cycle stages and does not succumb to short term environmental stress factors, thus molecular based identification methods are generally not limited to any particular developmental stage (Zarlenga and Higgins, 2001). Additionally, the level of variability detected can be extremely sensitive and depends on the technique used as well as the region of DNA examined, since even "silent" nucleotide positions can be compared (Thompson *et al.*, 1998; McManus and Bowles, 1996).

However, there is a tendency to refer to groups characterised by molecular methods as "strains" or "variants" – terms that are not taxonomically valid (Hyde, 1990). At present, there is no universally accepted criterion to allow the identification of species from such data due to the lack of appropriate models for defining populations and species of parasitic protozoans (Monis, 1999). This is an issue that needs to be resolved

as not only have molecular methods in many cases enhanced the sensitivity and specificity of the detection process, but they have also reduced much of the subjectivity inherent in interpreting morphological and biological data (Zarlenga and Higgins, 2001).

1.6 INTRODUCED PARASITES OF INTEREST

Introduced animals to Australia not only bring with them the added pressures of competition, habitat alteration and predation they also bring new parasites. Invariably, animals introduced by humans to new environments bring only a minority of their natural parasites, however this does not make them any less potent in their effect upon the native fauna. Detailed surveys of introduced pathogens are not available for all groups of native vertebrates and records are particularly lacking for reptiles and amphibians, however sharing of both external and internal parasites between cats and the Australian native fauna appears to occur most in the marsupials (Dickman, 1996b).

Whilst 38 helminth species, 10 protozoan species, two flagellates and one blood-borne parasite have been reported from the cat in Australia (Prescott, 1984), only two helminths, *Cyathospirura dasyuridis* and *Anoplotaenia dasyuri*, have been acquired from the Australian fauna (Coman *et al.*, 1981a). Conversely, introduced dogs, cats and foxes support at least nine helminth species and an unknown number of protozoan parasites whose intermediate life history stages are known to infest the Australian fauna (Coman *et al.*, 1981a; Coman *et al.*, 1981b; Coman, 1972b, a). Particular interest has been given to the investigation of *Ancylostoma*, *Giardia* and *Toxoplasma gondii*

infections in the current study due to their cosmopolitan distribution and potential impact on host species.

1.6.1 Hookworms

Hookworms are parasitic helminths belonging to the order Strongylida (family Ancylostomatidae), and are typically harboured in the small intestine, where they attach to the mucosa and feed on blood (Monti *et al.*, 1998). The most outstanding symptom of hookworm infestation is anaemia, with a burden of 1000 adult worms able to draw almost a cup of blood from their host each day (Hotez and Pritchard, 1995). As such, hookworm disease remains one of the most important and widespread helminth infections world-wide with roughly one fifth of the world's human population infected (Monti *et al.*, 1998; Hotez and Pritchard, 1995). Despite their medical and economic significance world wide, hookworms are one of the least well represented parasite groups in the Australian fauna (Beveridge, 2002). This poor representation is probably a reflection of the fact that they are primarily parasites of eutherian groups which are either absent from the Australian fauna or which have only recently arrived in Australia (Beveridge, 2002).

The majority of the hookworm species in Australia have been introduced by humans and the importation of domestic animals within the last 200 years (Beveridge, 2002). The only known native species of hookworm in Australia occur in seals and sea-lions (*Uncinaria hamiltoni*), and in the small intestine of the Australian water rat (*U. hydromyidis*) (Beveridge, 1980). The isolation of the Australian continent is the principal reason for the relative paucity of ancylostomatoid species present (Beveridge,

2002; Beveridge and Spratt, 1996), however feline hookworm infections are particularly common in the northern regions of Australia (Prescott, 1984).

1.6.2 Giardia

Species of the flagellated protozoan parasite *Giardia*, inhabit the intestinal tracts of virtually all classes of vertebrates (Thompson, 2000), however *Giardia duodenalis* (syn. *G. intestinalis*; *G. lamblia*) is the only recognised species found in most mammals including dogs, cats, cattle, pigs, sheep and horses (O'Handley *et al.*, 2000; Thompson *et al.*, 2000b; Thompson *et al.*, 1998; Thompson *et al.*, 1993b). The close association between humans and domestic animals, particularly pets, has been suspected as a means whereby zoonotic giardiasis occurs (Healy, 1990).

While it has been shown that *Giardia* of human origin can infect animals and vice versa (Bettiol *et al.*, 1997; Majewska, 1994), many epidemiological details still require clarification. Molecular characterisation of *Giardia* recovered from domestic and wild animals is helping to improve our understanding of the aetiological agents that are responsible for causing giardiasis in mammals as well as the dynamics of both endemic and zoonotic transmission.

1.6.3 Toxoplasma gondii

Toxoplasma gondii is a ubiquitous, obligate intracellular coccidian parasite that is capable of infecting an unusually wide range of hosts (Dubey *et al.*, 1998; Dubey and Beattie, 1988). *T. gondii* is of both veterinary and medical importance worldwide due to its implication in abortion and congenital disease in its intermediate hosts. *T. gondii* infects virtually all species of warm-blooded animals including humans, though only

cats and other members of the family Felidae are the definitive hosts (Dubey and Lappin, 1998). In Australia the only definitive host is the domestic or feral cat (with the exception of those felids in zoos).

T. gondii tissue cysts have a high affinity for neural and muscular tissues, being located predominantly in the central nervous system, the eye as well as skeletal and cardiac muscles and infected animals may exhibit pathology including obscured vision, difficulty in walking and calcification of the heart (Tenter *et al.*, 2000; Dubey and Beattie, 1988). Australian marsupials have been reported in the wild as being blinded, stumbling and dying from toxoplasmosis (Patton *et al.*, 1986; Ashton, 1979). Deaths from toxoplasmosis may constitute a major form of mortality for some native mammals, though *T. gondii* has also been implicated in increasing the susceptibility of the intermediate host to predation (Berdoy *et al.*, 2000; Webster, 1994a; Webster *et al.*, 1994).

The epidemiology of *T. gondii* infections in the Australian native fauna is poorly understood. The cat is believed to be essential for the maintenance of *T. gondii* in endemic areas and is theorised to be the main source of infection for wildlife (Obendorf *et al.*, 1996; Hartley and Munday, 1974). Given that the cat has successfully colonised the entire continent of Australia, and that Australia possesses approximately 90% of the world's marsupial species (Strahan, 1983), toxoplasmosis can be considered one of the most potentially threatening diseases facing Australia's marsupial fauna and a very real threat to global biodiversity.

1.7 OBJECTIVES

The potential exists for parasites carried by introduced animals, such as the cat, to be responsible for the extinction of native species throughout Australia. Indeed most mammal species that have become extinct following European colonisation of Australia appear to have been those most likely to acquire parasites from introduced animals (Freeland, 1994). In some rare cases introduced parasites may have the potential to ultimately stabilise Australian ecosystems, though in the vast majority of cases the native mammals' physiological susceptibility to introduced parasites is such that rather than aiding the stabilisation of these newly created food webs, the introduced parasite(s) further perturb an already destabilised situation. Although feral cats may contribute to the dissemination or maintenance of some pathogens in populations of native fauna, impacts are difficult to discern and to date have been little studied. Therefore the aims of this study were to:

1) Identify the common protozoan and helminth parasites occurring in the gastro-intestinal tract of both feral cats and selected native mammals throughout Western Australia;
2) Develop molecular tools to identify between species of hookworm occurring in feral cats from Western Australia;
3) Identify the occurrence of any uncommon parasites occurring in either feral cats and/or native mammals, and determine the potential risk they may pose to conservation and reintroduction programs;
4) Determine, by the use of molecular detection methods, the prevalence of *T. gondii* infections in feral cats throughout Western Australia and the reservoir potential of both feral cat and native fauna populations in the spread and persistence of this parasite in the environment.

Chapter 2 – Epidemiological Survey of Gastro-intestinal Parasites of Feral Cats and Native Fauna in Western Australia

2.1 INTRODUCTION

Investigations into species interaction and community structure have historically been dominated by studies involving competitive interactions between predators and their prey. In recent years the role of pathogens in host populations and the structure of biological communities has become much more of a focus of attention, not only from an ecological perspective (Hudson *et al.*, 1998; Hudson and Greenman, 1998; Grenfell and Harwood, 1997; Schrag and Wiener, 1995; Holt and Lawton, 1994), but also from a conservational one as well (Cleaveland *et al.*, 1999; Viggers *et al.*, 1993; May, 1988; Scott, 1988). Whilst the objectives of animal conservation programs need not be preoccupied with parasites, at the same time they must not disregard them either, as it is widely recognised that parasites have a considerable impact on host population dynamics resulting in their regulation and stabilisation (Anderson and May, 1979; May and Anderson, 1979; Anderson and May, 1978; May and Anderson, 1978). Indeed, an increasing number of studies have shown that parasites also have the potential to structure species assemblages as well as entire ecosystems (Tompkins and Begon, 1999; Hudson *et al.*, 1998; Bonsall and Hassell, 1997; May, 1988; Scott, 1988).

Sound, long-term disease management approaches for the conservation and management of most species requires a level of knowledge of the dynamic relationships

between animals and their parasites that is seldom achieved or available. Therefore, an understanding of the endemic as well as the introduced parasites that occur within host populations should be a high priority if they are to be cared for or managed properly. With this in mind, the aims of this research were to:

1) Conduct an epidemiological survey of the gastro-intestinal parasites occurring in both feral cats and selected native mammal species throughout different geographical and climatic regions of Western Australia;
2) Investigate the potential of parasite transfer between feral cats and the native fauna and identify any possible threats.

2.2 MATERIALS AND METHODS

2.2.1 Sampling Locations

2.2.1.1 Feral Cats

A total of 379 faecal samples and 23 intestinal tracts were obtained from feral cats over a two and a half year period from 1999 to 2001. These animals were collected from eleven different geographical regions throughout Western Australia, including the southwest, coastal northwest and arid interior of the state as well as the Montebello and Cocos Islands (Figure 2.1). The specimens were collected in conjunction with ongoing feral cat control programs around Western Australia, initiated by the Department of Conservation and Land Management (DCLM). Cats were caught using Victor 'Soft Catch'No. 2 leg hold traps (Algar *et al.*, 1999; Meek *et al.*, 1995), and euthanased humanely in accordance with the guidelines set out by the Murdoch University Ethics Committee (permit # 820R/00). Samples were collected from each cat and preserved and stored for later screening. Twentythree feral cat stomachs and intestines were also collected for examination, primarily from Shark Bay. In all collection areas care was taken to ensure that only feral animals were collected, with the exception of stray cats collected from metropolitan and rural tips, as well as from the Cocos Islands.

2.2.1.2 Native Fauna

A total of 851 native mammals were captured during the same two and a half year trapping period as the feral cats for comparative parasitological examination. Native fauna samples were collected from many of the same sample regions as the feral cats were, however it was not always possible to combine a comprehensive trapping

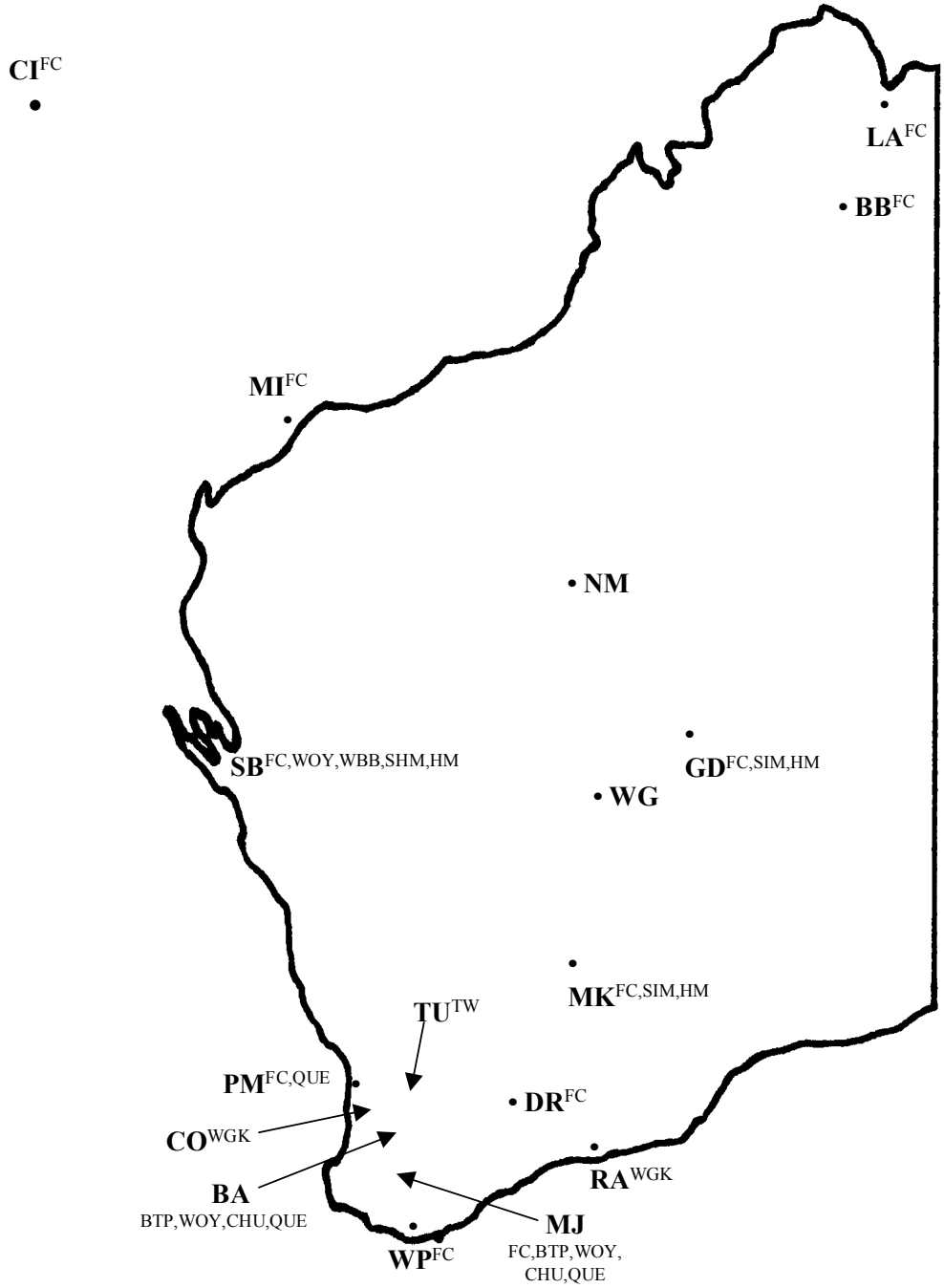

Figure 2.1 Location of sampling sites within Western Australia showing mammal species collected from each site. Key to sampling site abbreviations: BA = Batalling; BB = Bungle Bungles; CI = Cocos Islands; CO = Collie; DR = Dragon Rocks; GD = Gibson Desert; LA = Lake Argyle; MI = Montebello Islands; MJ = Manjimup; MK = Mount Keith; NW = Newman; PM = Perth Metropolitan Region; RA = Ravensthorpe; SB = Shark Bay; WG = Wongawol Station; WP = Walpole. Key to species abbreviations: BTP = Brushtail Possum (*Trichosurus vulpecula*); FC = Cat (*Felis catus*); CHU = Chudich (*Dasyurus geoffroii*); HM = House Mouse (*Mus musculus*); QUE = Quenda (*Isoodon obesulus*); SHM = Spinifex Hopping Mouse (*Notomys alexis*); SIM = Sandy Inland Mouse (*Pseudomys hermannsbergensis*); TW = Tammar Wallaby (*Macropus eugenii*); WBB = Western Barred Bandicoot (*Perameles bougainville*); WGK = Western Grey Kangaroo (*Macropus fuliginosus*); WOY = Woylie (*Bettongia penicillata*).

program for both cats and native mammals. As such, both native mammal and feral cat data are not available for all of the sampling sites (Figure 2.1). Native animals were live-trapped using wire cage or Elliot traps as well as pit fall traps. Trapping of native animals was performed in conjunction with the DCLM's ongoing fauna monitoring programs throughout the state. Trapping sessions were typically run over three successive nights, with the predominant species caught being brushtail possums (*Trichosurus vulpecula*), woylies (*Bettongia penicillata*), quenda (*Isoodon obesulus*) and chudich (*Dasyurus geoffroii*) from the southwest region, and various species of native mice as well as introduced house mice (*Mus musculus*) from the northern regions. Due to the monitoring nature of the DCLM trapping programs and the endangered status of the majority of the fauna being trapped, catch and release protocols only allowed the collection of faeces. Western barred bandicoot (*Perameles bougainville*) samples were sourced from the captive breeding colony run by the DCLM as part of Project Eden at Shark Bay. Samples from kangaroos (*Macropus fuliginosus* and *M. rufus*) were collected with the assistance of professional shooters operating in the south western and central interior regions of Western Australia.

2.2.2 Faecal Samples

Faecal samples of both feral cats and native fauna were preserved in three different solutions: 10% Formalin, for the identification of eggs of helminth parasites; 2% potassium dichromate, for the identification of cyst stages of protozoan parasites; and 20% dimethyl sulphoxide (DMSO) in saturated salt solution, for future molecular based screening.

Parasite eggs and other stages were isolated from preserved faecal samples via three methods. *Cryptosporidium* oocysts were detected via light microscopy by staining with 5% malachite green stain and visually scanning microscope slides at ×10 and ×40 magnifications. Other protozoan and helminth parasites were detected using both Fecalyzer (Evsco Pharmaceuticals, NJ, USA) and zinc sulphate flotation (Bartlett *et al.*, 1978) methods to concentrate eggs and cysts and viewed via light microscopy at ×10 and ×40 magnification. Identification of parasite species was performed based on egg and cyst morphology for the well documented species (Soulsby, 1982). Eggs for wildlife hosts could only be identified to family or order level in most cases.

2.2.3 Tissue Samples

As mentioned in section 2.2.1.2, the collection of tissue samples from native mammals was limited due to ethical considerations regarding the threatened status of many of the species sampled, with the majority of tissue samples collected being from feral cats. Tissue samples collected primarily consisted of brain, muscle, liver, spleen and whole blood. Tissue samples were preserved in 20% DMSO in saturated salt solution to facilitate the extraction of DNA for the application of molecular testing. A limited number of samples involved the collection of serum, lymph nodes, spinal cord and tongue, however the collection of these, as well as the standard tissue samples was predominantly concerned with the detection of the parasite *Toxoplasma gondii*, which will be further discussed in Chapter 5.

2.2.4 Gastro-Intestinal Tracts

Cat stomachs and intestines were opened and the contents examined for the presence of visible parasites. Suspected tumours/cysts were cut out and preserved in 10% formalin

for later dissection and examination under a dissecting microscope. Intestinal tracts were removed and perforated along their length prior to placing in 10% formalin to facilitate the "fixing" of the intestinal contents. Preserved intestines were externally washed in distilled water and allowed to soak for up to 12 hours to remove excess formalin prior to examination. Intestines were cut open along their length and the intestinal walls as well as any material present within the intestines was examined closely using a dissecting microscope for the presence of small or immature parasites. All observed parasites were removed and collected for identification.

2.3 RESULTS

2.3.1 Prevalence of Intestinal Parasites in Feral Cats

Parasites were detected in faecal samples in 76.8% of the 379 cats examined. No particular species of parasite was present across all eleven sampling regions. Thirteen helminth species and five protozoan genera were identified as occurring in feral cats throughout Western Australia. *Ancylostoma* spp. and *Oncicola pomatostomi* were the most common helminth parasites occurring among all cats, whilst *Isospora rivolta* and *I. felis* were the most commonly occurring protozoan parasites detected (Table 2.1). Twentythree gastro-intestinal tracts were collected from cats, predominantly from Shark Bay, to facilitate and confirm the identification of similar helminth parasites such as taeniid cestodes.

2.3.1.1 Age Distribution of Feral Cats

Exact determination of the age of cats caught was not performed, however body measurements (weight and size) were used to classify cats as either: kittens; juveniles; sub-adults; or adults. Cats were predominantly either juvenile/sub-adult or adult cats, with kittens only rarely being caught.

2.3.1.2 Helminth Parasites

Ancylostoma spp. were the most common parasites occurring in feral cats even though they were present in only five of the eleven different regions sampled, being most prevalent in cats from the Cocos Islands (89.5%) and Walpole (80%). Three species of *Ancylostoma* are known to occur in cats in Australia: *A. tubaeforme*; *A. ceylanicum*; and *A. caninum*. No *Uncinaria stenocephala* infections were observed in any cats. Other nematodes encountered were *Toxocara cati*, *Aelurostrongylus abstrusus*, *Cyathospirura*

dasyuridis, *Cylicospirura felineus*, *Gnathostoma spinigerum* and *Physaloptera* spp. (Table 2.1).

T. cati was present at moderate levels in the south west regions (10%-15%), with three cats from the Shark Bay region also infected. All three infected cats were collected from Dirk Hartog Island, whilst none of the cats from the Peron Peninsula or Faure Island were infected (Figure 2.2). The cat lungworm *A. abstrusus* was found in two stray cats from the Perth metropolitan region. Two nodules in the stomach wall of a feral cat from Walpole contained a total of 12 specimens of both *C. dasyuridis* and *C. felineus* worms, with each nodule containing representatives of both species. Additionally, two cats from Lake Argyle were diagnosed with infections of *G. spinigerum*.

The occurrence of a *Physaloptera* species in cats from the Mount Keith and sub tropical northern regions as well as the Cocos Islands was presumed to be *P. praeputialis*. Eggs identified as an *Abbreviata* sp. which were found in the faeces of one cat from the Gibson Desert, are presumed to be *A. hastaspicula*.

Taeniid eggs were present in feral cats from Walpole, Mount Keith and Shark Bay at moderate levels (10%, 11.3% and 7.7% respectively). *Taenia taeniaeformis* is the most common *Taenia* species recorded in cats in Australia. However, differentiation between species of *Taenia* or *Echinococcus granulosus* cannot be achieved based solely on egg morphology, although the examination of gastro-intestinal tracts collected from feral cats detected only *T. taeniaeformis*. Whilst no specimens of *T. ovis*, *T hydatigena*, *T. pisiformis* or *E. granulosus* were detected, their absence from feral cats in this study

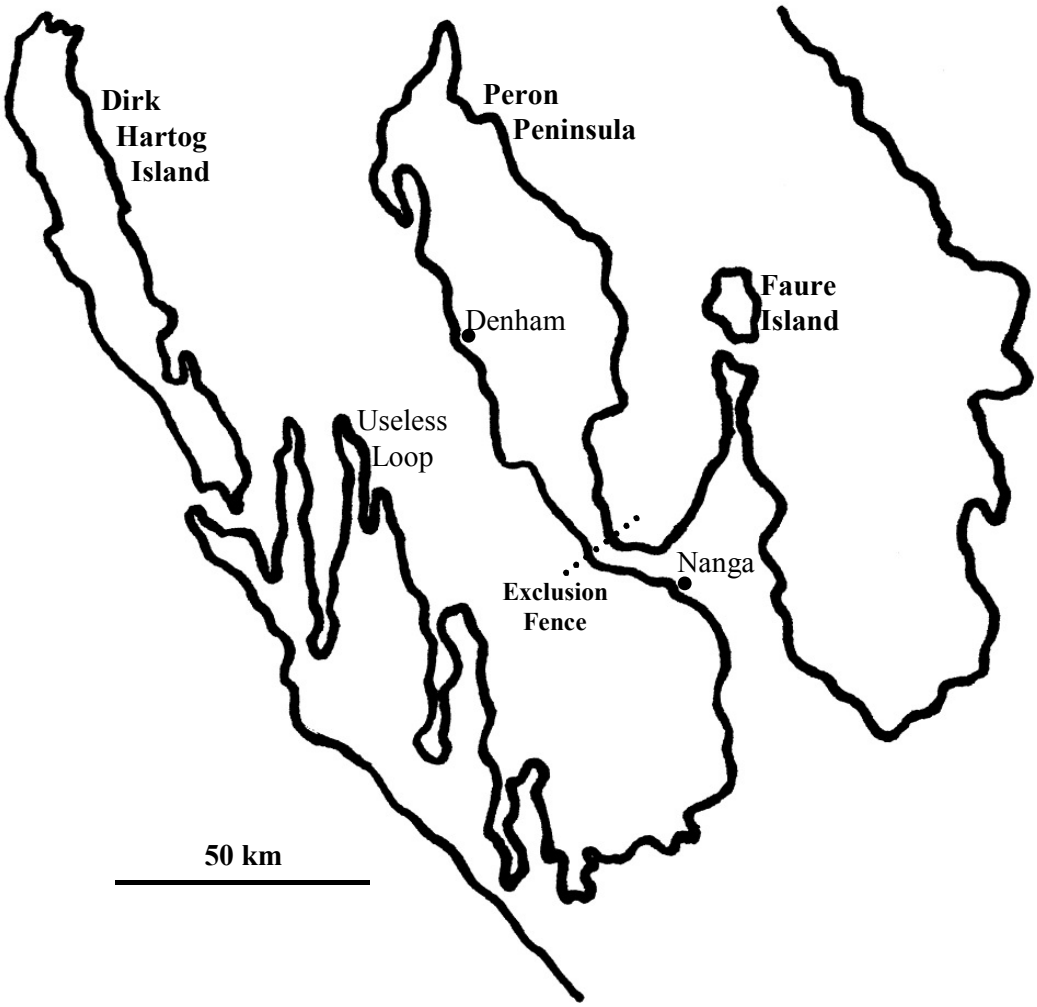

Figure 2.2 Map of Shark Bay region indicating location of Dirk Hartog Island and Faure Island relative to the Peron Peninsula.

cannot be guaranteed. Only one case of *Dipylidium caninum* was found in a cat from Shark Bay. *Spirometra erinaceieuropaei* was present in cats from Lake Argyle (79.4%), the Bungle Bungles (75%), Perth (40%), Walpole (30%), Manjimup (13.3%), Mount Keith (9.1%) and Shark Bay (1.5%). *S. erinaceieuropaei* infections from the Shark Bay region only occurred in three cats from Faure Island (Figure 2.2).

An acanthocephalan worm was highly prevalent in feral cats from the more arid regions, particularly Mount Keith, the Gibson Desert and Shark Bay. Acanthocephalan parasites

occurring in feral cat populations, commonly referred to as *Oncicola* sp., have since been identified as *Oncicola pomatostomi* (O'Callaghan and Beveridge, 1996; Schmidt, 1983).

2.3.1.3 Protozoan Parasites

Protozoan oocysts were found in all sampling regions with the exception of the Cocos Islands and the Bungle Bungles (Table 2.1). *I. felis* and *I. rivolta* were the most common protozoan parasites found in feral cats, with *Sarcocystis* spp. occurring at moderate to low prevalence only in the more arid regions as well as at Lake Argyle. *T. gondii* oocysts were not found in any of the 379 feral cat faecal samples examined. An unidentified *Entamoeba* sp. was found in only three cats from Shark Bay, whilst *Giardia* was found in one cat from Dragon Rocks Nature Reserve situated in the southeast wheat belt of the state. *Cryptosporidium* was detected in three cats from Mount Keith, one cat from Manjimup and one cat from Shark Bay. *Cryptosporidium* was believed to have been detected in three cats from Mount Keith, one cat from Manjimup and one cat from Shark Bay, however due to the degraded nature of the samples identification could not be confirmed. Further attempts at isolation and identification of all five samples proved unsuccessful.

2.3.2 Prevalence of Intestinal Parasites in Native Fauna

A total of 851 faecal samples from native (and some introduced) mammals were examined for intestinal parasites, comprising: 249 *Trichosurus vulpecula*; 238 *Bettongia penicillata*; 77 *Isoodon obesulus*; 65 *Dasyurus geoffroii*; 42 *Notomys alexis*; 36 *Macropus fuliginosus*; 35 *Macropus eugenii*; 35 *Perameles bougainville*; 18 *Pseudomys hermannsbergensis*; 13 *Mus musculus*; 8 *Pseudomys nanus*; 8 *Lagostrophus fasciatus*; 6

Table 2.1 Regional and overall parasite occurrence and prevalence in feral cats throughout Western Australia based on the examination of 379 faecal samples and 23 gastro-intestinal tracts.

Parasite	Regional Prevalence[a,b]											Total Prevalence
	PM[c] (20)	WP (20)	MJ (15)	DR (6)	MK (44)	SB (194)	GD (20)	LA (34)	BB (4)	MI (3)	CI (19)	
Toxocara cati	15.0%	10.0%	13.3%	0.0	0.0	1.5%	0.0	0.0	0.0	0.0	0.0	2.6%
Ancylostoma spp.	0.0	80.0%	0.0	0.0	13.6%	34.5%	0.0	26.5%	0.0	0.0	89.5%	29.8%
Aelurostrongylus abstrusus	10.0%	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.53%
Cyathospirura dasyuridis	0.0	5.0%	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.25%
Cylicospirura felineus	0.0	5.0%	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.25%
Gnathostoma spinigerum	0.0	0.0	0.0	0.0	0.0	0.0	0.0	5.9%	0.0	0.0	0.0	0.53%
Physaloptera sp.	0.0	0.0	0.0	0.0	15.9%	0.0	0.0	8.8%	75.0%	0.0	5.3%	3.7%
Abbreviata sp.	0.0	0.0	0.0	0.0	0.0	0.0	5.0%	0.0	0.0	0.0	0.0	0.25%
Dipylidium caninum	0.0	0.0	0.0	0.0	0.0	0.5%	0.0	0.0	0.0	0.0	0.0	0.25%
Taenia taeniaeformis	0.0	10.0%	0.0	0.0	11.3%	7.7%	0.0	0.0	0.0	0.0	0.0	4.7%
Spirometra erinaceieuropaei	40.0%	30.0%	13.3%	0.0	9.1%	1.5%	0.0	79.4%	75.0%	0.0	0.0	14.0%
Oncicola pomatostomi	0.0	0.0	0.0	16.6%	43.2%	33.5%	40.0%	0.0	75.0%	33.3%	0.0	25.6%
Isospora felis	5.0%	10.0%	6.6%	0.0	11.3%	3.6%	10.0%	2.9%	0.0	0.0	0.0	4.5%
Isospora rivolta	5.0%	10.0%	0.0	33.3%	18.2%	20.1%	20.0%	23.5%	0.0	33.3%	0.0	16.9%
Sarcocystis spp.	0.0	0.0	0.0	0.0	11.3%	0.5%	5.0%	2.9%	0.0	0.0	0.0	2.1%
Entamoeba sp.	0.0	0.0	0.0	0.0	0.0	1.5%	0.0	0.0	0.0	0.0	0.0	0.79%
Giardia	0.0	0.0	0.0	16.6%	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.25%
Cryptosporidium	0.0	0.0	6.6%	0.0	6.8%	0.5%	0.0	0.0	0.0	0.0	0.0	1.3%
Unidentified Spiruroid	0.0	0.0	0.0	0.0	4.5%	2.0%	20.0%	23.5%	100.0%	33.3%	5.3%	6.3%
Eimeria spp.	0.0	5.0%	0.0	0.0	18.2%	2.0%	30.0%	23.5%	0.0	0.0	0.0	7.1%

[a]Numbers in parentheses denote sample size.
[b]PM = Perth Metropolitan region, WP =Walpole, MJ = Manjimup, DR = Dragon Rocks Nature Reserve, MK = Mount Keith, SB = Shark Bay, GD = Gibson Desert Nature Reserve, LA = Lake Argyle, BB = Bungle Bungles, MI = Montebello Islands, CI = Cocos Islands.
[c]Includes cats from Leonora Tip (n=3), Harvey Tip (n=2), Canning Dam (n=2) and Rottnest Island (n=3).

Bettongia lesueur; 6 *Leporillus conditor*; 5 *Rattus tunneyi*; 5 *Canis lupus dingo*; 3 *Pseudomys deliculatus*; 1 *Macropus rufus*; and 1 *Ningaui ridei*. The majority of animals trapped appeared to be in good health with no visible signs of disease detected. A cross section of all age groups was sampled, though no attempt was made to group the animals relative to their age due to the low numbers of some species sampled. Of the 851 native (and introduced) animals sampled, 71.9% were infected with one or more parasite species. To aid in the analysis and comparison of parasitological results with published studies, only the ten most commonly sampled host species (comprising 807 individuals) will be discussed.

2.3.2.1 Helminth Parasites

The marsupial helminth fauna was dominated by nematodes of the order Strongylida, which were present in 59.1% of all animals examined, with nematodes of the order Rhabditida, Spirurida and Oxyurida also common (Table 2.2). *D. geoffroii* were predominantly parasitised by strongyle nematodes (89.2%), with only a single animal being infected with a Spiruroid nematode whilst two others were found to have an oxyuroid nematode present in their faeces. *T. vulpecula* were found to have a lower helminth infection rate than most of the other host species with less than half of them(46.6%) harbouring strongyle nematodes and 9.6% harbouring unidentified nematodes. Similarly, the parasite fauna of *M. eugenii*, *M. fuliginosus* and *B. penicillata* were dominated by strongylid nematodes, however only *B. penicillata* were infected by *Strongyloides* sp., spiruroids and unidentified nematodes, whilst 27.8% of the *M. fuliginosus* sampled were infected with oxyuroid nematodes. The bandicoots appeared to be parasitised by the widest range of nematode parasites of all the host species sampled, and strongyle nematodes were again the most common group of

Table 2.2 Prevalence of common helminth parasite groups in selected mammal species from Western Australia based on the examination of faecal samples.

	Nematodes						**Oxyuroid Nematodes**			
	Strongyle eggs	*Strongyloides* sp.	Spiruroid	*Trichuris* sp.	*Linstowinema* sp.	Unidentified Nematode	Unidentified Oxyuroid	Unidentified *Syphacia* sp.	*Syphacia obvelata*	Trematode
CHU* (n = 65)	89.2%	0.0	1.5%	0.0	0.0	1.5%	3.1%	0.0	0.0	3.1%
QUE (n = 77)	68.8%	3.9%	3.9%	11.7%	37.7%	5.2%	0.0	0.0	0.0	0.0
WBB (n = 35)	60.0%	0.0	25.7%	22.9%	11.4%	11.4%	2.9%	0.0	0.0	0.0
WOY (n = 238)	76.5%	5.0%	2.5%	0.0	0.0	10.1%	0.0	0.0	0.0	0.0
BTP (n = 249)	46.6%	0.0	0.0	0.0	0.0	9.6%	0.0	0.0	0.0	0.0
TW (n = 35)	82.9%	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
WGK (n = 36)	88.9%	0.0	0.0	0.0	0.0	0.0	27.8%	0.0	0.0	0.0
SHM (n = 42)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	40.5%	0.0	0.0
SIM (n = 18)	0.0	0.0	5.6%	0.0	0.0	5.6%	0.0	44.4%	0.0	0.0
HM (n = 13)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	46.2%	30.8%	0.0

*CHU: Chudich (*Dasyurus geoffroii*), QUE: Quenda (*Isoodon obesulus*), WBB: Western Barred Bandicoot (*Perameles bougainville*), BTP: Brushtail Possum (*Trichosurus vulpecula*), WOY: Woylie (*Bettongia penicillata*), TW: Tammar Wallaby (*Macropus eugenii*), WGK: Western Grey Kangaroo (*Macropus fuliginosus*), SHM: Spinifex Hopping Mouse (*Notomys alexis*), SIM: Sandy Inland Mouse (*Pseudomys hermannsbergensis*), HM: House Mouse (*Mus musculus*).

parasites detected in these species. A single *P. bougainville* was found to be harbouring oxyuroid nematodes though none were detected in any of the *I. obesulus*. Both *I. obesulus* and *P. bougainville* were the only native fauna sampled to be parasitised by *Trichuris* and *Linstowinema* species. An unidentified species of *Syphacia* was the most commonly occurring nematode in each of the three murid species sampled, whilst four *M. musculus* were also harbouring infections of *Syphacia obvelata*. An unidentified spiruroid nematode was detected in a single *P. hermannsbergensis* whilst a second was found to be harbouring an unidentified nematode. Trematode eggs were detected in two

D. geoffroii collected from Manjimup, but were not detected in any other host species sampled. Cestode parasites were not detected in any of the faecal samples collected.

2.3.2.2 Protozoan Parasites

Protozoan infections in the native fauna sampled consisted primarily of unidentified coccidia, *Eimeria* and *Entamoeba* species (Table 2.3). Unidentified coccidia were detected in all host species sampled except for *D. geoffroii* and *M. musculus*. The occurrence of unidentified coccidia was significantly higher in *M. eugenii* (74.3%) than in *I. obesulus*, which had the second highest prevalence (27.9%) whilst their occurrence in all other host species was relatively low, with none detected in *D. geoffroii* or *M. musculus*. *Entamoeba* infections were most common in *N. alexis* (14.3%) and *T. vulpecula* (7.2%), with additional infections detected in only one *D. geoffroii*, two *I. obesulus* and one *P. bougainville*. *Eimeria* species were detected in *M. fuliginosus*, *B. penicillata*, *T. vulpecula* and both species of bandicoot sampled. Whilst unidentified species of *Eimeria* were detected in each of these host species, *B. penicillata* also harboured infections of *E. gaimardi*, *E. potoroi* and *E. aepyrmni*. *Giardia* was detected in only one *I. obesulus* collected from Manjimup. Additional cases of *Giardia* were detected in one pale field rat (*Rattus tunneyi*), one western chestnut mouse (*Pseudomys nanus*) and one dingo (*Canis lupus dingo*) which were all collected from Lake Argyle (results not shown).

Table 2.3 Prevalence of common protozoan parasite groups in selected mammal species from Western Australia based on the examination of faecal samples.

	Protozoa						
	Unidentified *Entamoeba*	Unidentified *Eimeria*	*Eimeria gaimardi*	*Eimeria potoroi*	*Eimeria aepyrmni*	*Giardia*	Unidentified Cocidia
CHU* (n = 65)	1.5%	0.0	0.0	0.0	0.0	0.0	0.0
QUE (n = 77)	2.7%	4.5%	0.0	0.0	0.0	0.9%	27.9%
WBB (n = 35)	2.9%	2.9%	0.0	0.0	0.0	0.0	14.3%
WOY (n = 238)	0.0	4.6%	2.5%	1.3%	0.4%	0.0	0.8%
BTP (n = 249)	7.2%	2.4%	0.0	0.0	0.0	0.0	2.4%
TW (n = 35)	0.0	0.0	0.0	0.0	0.0	0.0	74.3%
WGK (n = 36)	0.0	16.7%	0.0	0.0	0.0	0.0	5.6%
SHM (n = 42)	14.3%	0.0	0.0	0.0	0.0	0.0	4.8%
SIM (n = 18)	0.0	0.0	0.0	0.0	0.0	0.0	11.1%
HM (n = 13)	0.0	0.0	0.0	0.0	0.0	0.0	0.0

*CHU: Chudich (*Dasyurus geoffroii*), QUE: Quenda (*Isoodon obesulus*), WBB: Western Barred Bandicoot (*Perameles bougainville*), BTP: Brushtail Possum (*Trichosurus vulpecula*), WOY: Woylie (*Bettongia penicillata*), TW: Tammar Wallaby (*Macropus eugenii*), WGK: Western Grey Kangaroo (*Macropus fuliginosus*), SHM: Spinifex Hopping Mouse (*Notomys alexis*), SIM: Sandy Inland Mouse (*Pseudomys hermannsbergensis*), HM: House Mouse (*Mus musculus*).

2.4 DISCUSSION

2.4.1 Parasites of Feral Cats

The general consensus regarding feral cats is that they are largely solitary in their habits and population densities are low (Jones and Coman, 1982). Therefore, the probability of transmitting infections between feral cats would be reduced, which has been used to describe the fact that many parasite infections occur at lower rates in feral cats than in domestic cats (Coman *et al.*, 1981a). However, the prevalence and occurrence of parasite infections of feral cats found in this study provides evidence that parasite burdens of feral cats from different environments throughout Western Australia are not necessarily lower than in domestic cats.

The range of helminth and protozoan species found in feral cats was generally similar to that reported in earlier surveys of both feral and domestic cats in Australia (Milstein and Goldsmid, 1997; O'Callaghan and Beveridge, 1996; O'Callaghan *et al.*, 1984; Wilson-Hanson and Prescott, 1982; Coman *et al.*, 1981a; Coman *et al.*, 1981b; Gregory and Munday, 1976; Ryan, 1976a; Kelly and Ng, 1975; Coman, 1972b). However, prevalence figures for particular parasite species differed greatly from many of these earlier surveys. Regional variation in the prevalence of some parasite species is explicable in terms of dietary and environmental differences between cats and sampling areas. The feral cat is an opportunistic predator and the composition of its diet typically reflects the relative availability of different prey species (Read and Bowen, 2001; Risbey *et al.*, 1999; Catling, 1988; Jones and Coman, 1981; Coman and Brunner, 1972).

2.4.2 Nematode Parasites of Feral Cats

2.4.2.1 *Toxocara cati*

Toxocara cati is a large roundworm found in the small intestine of the cat and other wild Felidae, with heavy infections most commonly reported from catteries with poor hygiene conditions (Prescott, 1984). *T. cati* has a cosmopolitan distribution and occurs in 35-85% of young adult cats and about 60% of kittens (Bowman *et al.*, 2002; Prescott, 1984). Rabbits and small rodents may act as facultative intermediate or paratenic hosts with the larvae becoming distributed in somatic tissues, whilst earthworms, cockroaches, chickens and other animals may act as transport hosts (Prescott, 1984; Coman *et al.*, 1981a). Additionally, Swerczek *et al.* (1971) showed that transmammary passage of *T. cati* is common in the cat.

Ryan (1976a) theorised that *T. cati* was better adapted to the cooler climates of Victoria than the drier regions of New South Wales. Likewise, Coman (1972a) and Coman *et al.* (1981a) found *T. cati* to be more prevalent in the cooler sampling areas (85.1%, 86.4% and 50%, 53% respectively) as opposed to the drier sampling area (0% and 6% respectively) in their surveys of feral cats from Victoria. Similarly, Gregory and Munday (1976) detected *T. cati* in 86% and 90.5% of feral cats from Tasmania whilst only 1% of feral cats from the Northern Territory were infected (O'Callaghan and Beveridge, 1996), providing further evidence of the effect of climate on the distribution and prevalence of *T. cati* as hypothesised by Ryan (1976a).

The occurrence of *T. cati* in this study was predominantly limited to the three most southern sampling regions (Perth metropolitan area 15%, Walpole 10%, Manjimup 13.3%), although three cats from Shark Bay were also infected. All three cats from the

Shark Bay region infected with *T. cati* were collected from Dirk Hartog Island (Figure 2.2), which provided a localised prevalence of 42.9%. The absence of *T. cati* in cats from the Peron Peninsula and Faure Island indicates that this parasite became established on Dirk Hartog Island after the initial colonisation of this region by cats. The likelihood of this occurring is extremely high given the pastoral history of the island.

At the time of sampling Dirk Hartog Island was an active pastoral lease and supported a population of feral cats sharing a limited number of focal resources (mainly food and shelter). These conditions are conducive to the formation of structured populations of cats centred around food-rich areas such as livestock watering points, grain storage areas and refuse dumps (Denny *et al.*, 2002; Izawa and Doi, 1993; Dards, 1983; Izawa *et al.*, 1982). These populations of group-living cats are considered to be true social groups that are dominated by related females, which provide communal care and defence of kittens (Denny *et al.*, 2002; Liberg and Sandell, 1994). Therefore, given that transmammary transmission of *T. cati* commonly occurs in cats (Swerczek *et al.*, 1971), the communal nursing of kittens by related females in a social group may be responsible for maintaining a relatively high prevalence of *T. cati* in an otherwise unfavourable environment.

2.4.2.2 *Ancylostoma*

Ancylostoma tubaeforme is the common hookworm of cats and is frequently found in domestic cats from Brisbane (Prescott, 1984; Wilson-Hanson and Prescott, 1982) and Sydney (Kelly and Ng, 1975). The occurrence of *A. tubaeforme* in cats appears to be more prevalent in the warmer northern parts of Australia (O'Callaghan and Beveridge,

1996; Meloni *et al.*, 1993; Thompson *et al.*, 1993a; Wilson-Hanson and Prescott, 1982). Several studies have failed to detect *A. tubaeforme* in cats in the southern states or in the southwest of Western Australia (O'Callaghan *et al.*, 1984; Shaw *et al.*, 1983; Gregory and Munday, 1976; Ryan, 1976a; Coman, 1972b), however the present study detected *Ancylostoma* infections in feral cats from Walpole, Shark Bay, Lake Argyle and Mount Keith.

Both feral and domestic cats are known to harbour *A. caninum* and *U. stenocephala* infections in Australia (Bowman *et al.*, 2002; Setasuban and Waddell, 1973), however both these species are relatively uncommon parasites of cats, being much more common in dogs (Johnston and Gasser, 1993; O'Callaghan *et al.*, 1984; Blake and Overend, 1982; Kelly and Ng, 1975; Gardiner and Fraser, 1960; Pullar, 1946). Cats are also known to harbour *A. braziliense* and *A. ceylanicum*, two closely related hookworms that are commonly responsible for infecting humans (Schad and Banwell, 1990), though unlike *A. braziliense*, *A. ceylanicum* is able to mature in humans (Schad, 1991). There are no published reports of *A. braziliense* occurring in Australia, though *A. ceylanicum* appears to be common among cats in north Queensland (Stewart, 1994). The distribution, occurrence and characterisation of hookworm infections in feral cats from Western Australia is discussed in further detail in Chapter 3.

2.4.2.3 *Aelurostrongylus abstrusus*

The cat lungworm *Aelurostrongylus abstrusus* has been reported as occurring in cats from all states of Australia (Prescott, 1984). True intermediate hosts for *A. abstrusus* include slugs and snails, though rodents and birds that ingest these infected molluscs can serve as paratenic hosts (Bowman *et al.*, 2002; Prescott, 1984). Therefore, cats

most probably become infected through the ingestion of infected rodents and small birds. A study of gastro-intestinal parasites in 726 urban and 30 rural cats from Perth found the occurrence of *A. abstrusus* as "occasional", though no further data were provided (Shaw *et al.*, 1983). Only two cats from the Perth metropolitan region were infected with *A. abstrusus* in the present study, though the lack of infections from other regions could be a reflection of the timing of the sampling regimes, the majority of which took place when *A. abstrusus* infections are apparently at their lowest level in Australia (between August and February) (Wilson-Hanson and Prescott, 1982). Intermediate stages and hosts of *A. abstrusus* generally require a moist conditions which may influence the seasonal and regional variation in prevalence of this parasite observed in previous studies (Coman *et al.*, 1981a; Gregory and Munday, 1976; Kelly and Ng, 1975). Thus, the absence of *A. abstrusus* infections in feral cats from the arid and northern regions of Western Australia cannot be confirmed without a sampling regime that would detect any such variation.

2.4.2.4 *Cyathospirura dasyuridis* and *Cylicospirura felineus*

The spiruroid nematodes *Cyathospirura dasyuridis* and *Cylicospirura felineus* have been reported as occurring in feral cats from New South Wales (Ryan, 1976a), Victoria (Coman *et al.*, 1981a; Coman, 1972b), Tasmania (Gregory and Munday, 1976), the Northern Territory (O'Callaghan and Beveridge, 1996) and South Australia (O'Callaghan *et al.*, 1984). *C. dasyuridis* has also been reported as occurring in foxes in New South Wales (Ryan, 1976b) and Victoria (Coman, 1973b) as well as dingoes and feral dogs in Victoria (Coman, 1972a). Very little is known in regard to the life cycle of these two parasites, though arthropods are suspected of being intermediate hosts whilst lizards may act as paratenic hosts (Bowman *et al.*, 2002). Neither *C. dasyuridis* nor *C.*

felineus have been previously reported in feral cats from Western Australia (Thompson *et al.*, 1993a; Shaw *et al.*, 1983).

C. dasyuridis is a native Australian parasite which occurs in *Dasyurus viverrinus* (eastern quoll) and *D. maculatus* (tiger quoll) (Mawson, 1968), and is one of the few native parasites to have successfully colonised the introduced cat, fox, dingo and dog. The adult worms of *C. dasyuridis* commonly occur in the lumen of the stomach whilst *C. felineus* encysts in the stomach wall, though both are commonly associated with nodular lesions in the stomach (Beveridge *et al.*, 1978). The occurrence of these two species co-existing within the one stomach nodule has previously been reported in feral cats (Gregory and Munday, 1976) and appears to be common. Whilst *C. felineus* occurs in both feral and domestic cats (Pavlov and Howell, 1977), *C. dasyuridis* has only been reported from feral cats (O'Callaghan and Beveridge, 1996; O'Callaghan *et al.*, 1984; Coman *et al.*, 1981a; Gregory and Munday, 1976; Ryan, 1976a; Coman, 1972b).

The localisation of *C. dasyuridis* and *C. felineus* in tumour-like lesions or nodules in the stomach wall means that their prevalence cannot be accurately determined without proper examination of the stomach, particularly as the shedding of eggs is not constant. As previously mentioned, the regular removal of cat stomachs for a separate dietary analysis greatly reduced the sensitivity of detecting parasites located in the stomach in the current study. Consequently, it is probable that the prevalence and distribution of these two parasites in feral cats throughout Western Australia is greater than reported here.

2.4.2.5 *Gnathostoma spinigerum*

The parasite *Gnathostoma spinigerum* is considered rare in Australia with reports of it occurring in only two feral cats from New South Wales (Coman *et al.*, 1981a; Beveridge *et al.*, 1978) and several cases reported from domestic cats in Queensland (Wilson-Hanson and Prescott, 1982; Trueman and Ferris, 1977; Olds, 1952; Heydon, 1929). The occurrence of *G. spinigerum* infections in only two feral cats from the Lake Argyle region in the present study suggests that this parasite is equally as rare in Western Australia. *G. spinigerum* encysts in the stomach wall of the definitive host which includes cats and dogs as well as several other wild carnivores (Prescott, 1984). The life cycle of this parasite involves two intermediate hosts: the first is a cyclopoid copepod of the genus *Cyclops*, *Eucyclops*, *Mesocyclops* or *Thermocyclops*; whilst the second intermediate host can include a wide variety of animals (e.g. fish, frogs, reptiles, birds and rodents) (Bowman *et al.*, 2002). Paratenic hosts play an important role in the persistence of *G. spinigerum* and the advanced third-stage larvae can be transferred by feeding from one host to a second host (Bowman *et al.*, 2002). When a cat ingests an infected host the cycle is completed and the worm penetrates the stomach wall where it becomes encapsulated and reaches maturity in three to seven months (Miyazaki, 1960; Prommas and Daengsvang, 1937).

The pathogenesis of *G. spinigerum* is not well defined, though *Gnathostoma* was once considered to be the most pathogenic parasite to occur in cats (Chandler, 1925). However, the spontaneous cure of both natural and experimental infections of *G. spinigerum* in cats has since been reported (Daengsvang *et al.*, 1969). Likewise, both fatal and non-fatal cases of *G. spinigerum* infections have been reported in cats from north Queensland (Trueman and Ferris, 1977; Olds, 1952). The pathological effect of

G. spinigerum on the two infected cats from Lake Argyle was not determined as no clinical examination was performed on either cat.

The global distribution of *G. spinigerum* suggests that it is a tropical/sub-tropical parasite and the environmental conditions at Lake Argyle would facilitate the completion of the parasite's life cycle (Trueman and Ferris, 1977). However, as infected cats do not constantly shed eggs, the accurate diagnosis of *G. spinigerum* infections requires the examination of the stomach wall for adult worms and their associated cysts. Therefore, as with *C. dasyuridis* and *C. felineus*, the removal of cat stomachs for a separate dietary analysis during this study may have resulted in an underestimation of *G. spinigerum* infections in feral cats from Lake Argyle and other sampling regions within Western Australia.

2.4.2.6 *Physaloptera* and *Abbreviata*

Nematodes of the genus *Physaloptera* are commonly found in goannas (*Varanus* spp.) throughout Australia (Pichelin *et al.*, 1999), and the occurrence of *Physaloptera* species in feral cats has generally been attributed to their predation of reptiles (Strong and Low, 1983; Ryan, 1976a). Indeed, Ryan (1976a) detected an unidentified *Physaloptera* sp. in the intestines of three feral cats from New South Wales and attributed its occurrence to the predation of *V. gouldii*, as one of the cats contained a *V. gouldii* that was heavily infected with *Physaloptera*. However, *P. praeputialis* is a common parasite of cats in most parts of the world (Bowman *et al.*, 2002), and a number of cases of *P. praeputialis* have been found in feral cats from the Northern Territory (O'Callaghan and Beveridge, 1996; Barton and McEwan, 1993), suggesting it is a common parasite of feral cats in central Australia.

Arthropods are known intermediate hosts for *P. praeputialis*, though cats are considered to most likely become infected through the ingestion of paratenic hosts such as lizards (Bowman *et al.*, 2002). Studies on the dietary composition of feral cats from arid regions of Australia have shown that both reptiles and invertebrates are frequently predated by feral cats, though neither are considered a to be a major prey item (Read and Bowen, 2001; Paltridge *et al.*, 1997; Martin *et al.*, 1996). Therefore, both intermediate and paratenic hosts may be important sources for the transmission of *P. praeputialis* to feral cats in arid regions.

Although not identified to species level in this study, the *Physaloptera* species detected in feral cats from Mount Keith, Lake Argyle, the Bungle Bungles and the Cocos Islands is believed to be *P. praeputialis*. The prevalence of *P. praeputialis* in the present study is much lower than the 40.4% prevalence recorded by O'Callaghan and Beveridge (1996) in feral cats from the Northern Territory. Likewise, the lack of any *P. praeputialis* infections from the Gibson Desert contradicts Barton and McEwan (1993), who considered it to be a common parasite of feral cats in central Australia. However, due to storage conditions and the length of time some faecal samples spent in preservative, not all samples collected in the present study allowed accurate identification of parasite eggs. Indeed, some of the unidentified spiruroid eggs that were found in cats from all of the central and northern sampling areas (Table 2.1), could well be *P. praeputialis* or even *Abbreviata* species that were not identified as such.

Species of nematode in the genus *Abbreviata* are also common parasites of *V. gouldii* and occur throughout Australia, although *A. hastaspicula* occurs most commonly in

Western Australia (Pichelin *et al.*, 1999; Jones, 1983). *A. hastaspicula* has previously been found in a single feral cat and a dog from an Aboriginal settlement in the Northern Territory (Barton and McEwan, 1993). In these two cases, despite recovering *A. hastaspicula* worms attached firmly to the mucosa of the stomach of both animals, the infections were considered to most likely be adventitious. However, the study by O'Callaghan and Beveridge (1996) showed that *A. hastaspicula* occurred in 4.3% of feral cats sampled from the Northern Territory and is apparently able to develop in the stomach of cats. Therefore, the occurrence of an unidentified *Abbreviata* species in a feral cat from the Gibson Desert may be *A. hastaspicula*.

2.4.2.7 *Ollulanus tricuspis*

Ollulanus tricuspis is an extremely small nematode (0.7-1.0 mm long) that occurs in the stomach of the cat, dog and fox (Bowman *et al.*, 2002; Prescott, 1984). There are no known intermediate or paratenic hosts for this nematode and transmission from cat to cat is believed to be direct through the consumption of vomitus (Bowman *et al.*, 2002). Gregory and Munday (1976) found *O. tricuspis* in 11.4% and 4.8% of feral cats from the Tasmania Midlands and King Island respectively, whilst Shaw *et al.* (1983), Wilson-Hanson and Prescott (1982) and Kelly and Ng (1975) did not detect any cases in their surveys of cats in Perth, Brisbane or Sydney respectively. *O. tricuspis* was not detected in any cats in this survey due to the removal of cat stomachs for separate dietary analysis and the small size of the adult worms, although a single case of *O. tricuspis* has previously been detected in a dog presented to the Murdoch University Veterinary Hospital in 1993 (Hobbs, pers. comm.).

2.4.3 Cestode Parasites of Feral Cats

2.4.3.1 *Dipylidium caninum*

Dipylidium caninum is the most common tapeworm of cats, and also occurs dogs dingoes and foxes in Australia (Ryan, 1976b; Coman, 1973b, 1972a). The transmission potential of *D. caninum* is a function of the density of the flea intermediate host (Bowman *et al.*, 2002). Indeed, Wilson-Hanson and Prescott (1982) observed a seasonal prevalence of *D. caninum* infections in cats from Brisbane, which they attributed to the fluctuation of the flea population. Ryan (1976a) detected a greater prevalence of *D. caninum* in feral cats from New South Wales (11.6%) than Coman *et al.* (1981a) and Coman (1972a) detected in feral cats from Victoria (2.0% and 2.4% respectively), whilst Shaw *et al.* (1983) found a higher prevalence of *D. caninum* in domestic cats (33.7%) than rural cats (11.5%) in their survey in Perth.

The detection of only a single case of *D. caninum* in a feral cat from Shark Bay is most likely an underestimation, due to the reduced sensitivity of the faecal flotation method used in the current study for detecting eggs shed in gravid segments such as for *D. caninum*. Kelly and Ng (1975) detected *D. caninum* in 39.4% of cats in Sydney, though only 0.25% of those infected showed eggs in their faeces. Likewise, Shaw *et al.* (1983) did not detect eggs of *D. caninum* in any of the cat faeces they examined using a flotation technique. This lack of sensitivity could easily be responsible for the detection of only one case of *D. caninum* infection out of 379 faecal samples examined.

2.4.3.2 *Anoplotaenia dasyuri*

The native cestode *Anoplotaenia dasyuri* is typically a parasite of the Tasmanian Devil (*Sarcophilus harrisi*) and the Tiger Quoll (*Dasyurus maculatus*), though it has been

found to infect feral cats and dogs (Gregory *et al.*, 1975). The occurrence of immature *A. dasyuri* in six cats from the Tasmanian Midlands by Gregory and Munday (1976) were considered to be accidental parasites resulting from the use of wallaby meat as bait. *A. dasyuri* was not detected in any of the cats in this survey which is not surprising given its natural hosts are restricted to Tasmania and the eastern coast of Australia.

2.4.3.3 Taeniids

Morphological examination of taeniid eggs does not permit the identification of species of *Taenia* and *Echinococcus* due to the eggs being largely indistinguishable. However, cats are not usually considered to be definitive hosts for *T. hydatigena*, *T. pisiformis* or *T. serialis*, instead these parasites are more commonly found in feral dogs and foxes, and to a lesser extent domestic dogs (Ryan, 1976b; Kelly and Ng, 1975; Coman, 1973b, 1972a). A single feral cat from New South Wales has been recorded as being infected with *T. serialis* (Ryan, 1976a), whilst *T. pisiformis* has been found in one domestic kitten from Brisbane (Wilson-Hanson and Prescott, 1982). *T. ovis* was found in a single cat from South Australia (O'Callaghan *et al.*, 1984), though the feral or domestic status of this cat was not mentioned. Although feral cats predate rabbits, *T. hydatigena*, *T. pisiformis* and *T. serialis* have not been reported in cats despite numerous surveys throughout Australia (Milstein and Goldsmid, 1997; O'Callaghan and Beveridge, 1996; Shaw *et al.*, 1983; Coman *et al.*, 1981a; Gregory and Munday, 1976; Coman, 1972b).

Similarly, the hydatid tapeworm *Echinococcus granulosus* has not been found to occur in feral cats in Australia (Milstein and Goldsmid, 1997; O'Callaghan and Beveridge, 1996; Shaw *et al.*, 1983; Coman *et al.*, 1981a; Gregory and Munday, 1976; Ryan, 1976a; Coman, 1972b). The definitive hosts for *E. granulosus* in Australia are the dog,

dingo and red fox, with two cycles that may interact; a dog-sheep cycle and a macropod-dingo cycle (Thompson, 2001). However, as with *T. pisiformis* and *T. serialis*, the feral cat appears to be an unsuitable host for *E. granulosus*.

The examination of a number of gastro-intestinal tracts collected from feral cats from Shark Bay identified the presence of adult *T. taeniaeformis* worms though no other taeniid parasites were detected. Therefore, based on this result and the corroboration of previous surveys of feral cat parasites (Milstein and Goldsmid, 1997; O'Callaghan and Beveridge, 1996; Shaw *et al.*, 1983; Coman *et al.*, 1981a; Gregory and Munday, 1976; Coman, 1972b), it was presumed that the presence of taeniid eggs in the faeces of feral cats indicated a *T. taeniaeformis* infection.

The prevalence of *T. taeniaeformis* was much lower in this survey than recorded in previous surveys of feral cats from Victoria, New South Wales and the Northern Territory (O'Callaghan and Beveridge, 1996; Coman *et al.*, 1981a; Ryan, 1976a; Coman, 1972b). This may be a reflection of the decreased sensitivity of faecal examination in detecting *T. taeniaeformis* infections, as Kelly and Ng (1975) found only 10.7% of cats infected with *T. taeniaeformis* in Sydney showed eggs in their faeces. Despite the decreased sensitivity, *T. taeniaeformis* was detected in cats from Walpole, Mount Keith and Shark Bay at similar levels (10%, 11.3% and 7.7% respectively). These rates are significantly higher than those recorded for feral cats by Gregory and Munday (1976) from the Tasmanian Midlands (2.3%), though much lower than they found on King Island (42.9%).

The cystic stage of *T. taeniaeformis* develops in the liver of the intermediate hosts which are most commonly rodents (mainly rats and mice) (Bowman *et al.*, 2002; Prescott, 1984). The prevalence of this cestode parasite in feral cat populations has been shown to correlate with the importance of mice and small rodents in their diet (Gregory and Munday, 1976). Likewise, rodents were a common prey item of cats collected from Walpole, Mount Keith and Shark Bay where *T. taeniaeformis* was also detected.

2.4.3.4 *Spirometra erinaceieuropaei*

Spirometra erinaceieuropaei, like *G. spinigerum*, requires at least two intermediate hosts to complete its life cycle, the first being a fresh-water crustacean of the genus *Mesocyclops* or *Eucyclops*, whilst the second may include frogs, snakes or mammals rather than fish (Bowman *et al.*, 2002; Sandars, 1953; Bearup, 1948). However, unlike *G. spinigerum*, *S. erinaceieuropaei* does not require a tropical/sub-tropical climate and is equally widespread in the temperate southern states of Australia as it is in the northern regions, particularly around wetlands (Beveridge *et al.*, 1998; Gordon *et al.*, 1954; Bearup, 1948). *S. erinaceieuropaei* occurs in cats throughout Australia, generally with a higher prevalence in adult and stray/feral cats that reflects the increased success and reliance upon hunting in these cats (Shaw *et al.*, 1983; Wilson-Hanson and Prescott, 1982).

S. erinaceieuropaei infections were detected in cats from all sample regions in the present study except Dragon Rocks, the Gibson Desert, the Montebello Islands and the Cocos Islands. The occurrence of *S. erinaceieuropaei* in cats from the southern, coastal and northern sampling regions of Western Australia is expected as the environmental

conditions in these areas are conducive to completion of the parasite's life cycle (Bowman *et al.*, 2002; Prescott, 1984; Coman *et al.*, 1981a). The occurrence of *S. erinaceieuropaei* in cats from the Perth Metropolitan area (40%) and Manjimup (13.3%) indicates that these cats, which were predominantly sourced from tips, were actively predating intermediate hosts as well as scavenging. Additionally, the incidence of *S. erinaceieuropaei* in feral cats from Walpole (30%) emphasises the availability of intermediate hosts as well as the diverse nature of the feral cat diet in this region. Gregory and Munday (1976) found a strong correlation between *S. erinaceieuropaei* infections and the presence of frogs and lizards in the stomachs of feral cats from Tasmania.

The prevalence of *S. erinaceieuropaei* in cats from Lake Argyle (79.4%) and the Bungle Bungles (75%) is much higher than the 14.7% reported in cats from Aboriginal communities in the northwest of Western Australia by Thompson *et al.* (1993b) and Meloni *et al.* (1993). However, Thompson *et al.* (1993b) observed that although these cats exhibited hunting and scavenging behaviour, they were still treated as pets and spent much of their time inside houses. This decreased reliance upon hunting would explain the lower levels of *S. erinaceieuropaei* observed in these cats compared to the feral cats from Lake Argyle and the Bungle Bungles which have much higher levels of exposure.

The occurrence of *S erinaceieuropaei* (albeit at low prevalence) in feral cats from Mount Keith and Shark Bay is of interest as both these areas experience an arid climate where the availability of surface water is limited. However, both the Mount Keith and Shark Bay regions have a pastoral history, with mining also occurring at Mount Keith.

Features such as livestock watering points and waste water discharge from mine processes as well as human dwellings present in these areas clearly provide conditions that allow the persistence of intermediate hosts required by *S. erinaceieuropaei* within these arid landscapes.

However, the only cats infected with *S. erinaceieuropaei* from Shark Bay were collected from Faure Island (Figure 2.2), even though similar environmental conditions conducive to the persistence of this parasite would be expected to occur on both Dirk Hartog Island and the Peron Peninsula given their similar pastoral history. The anthropogenic movement of cats onto Faure Island could explain this, as the introduction of a single infected cat would be sufficient to establish an infection of *S. erinaceieuropaei* on the island. Much like the occurrence of *T. cati* in cats from Dirk Hartog Island (section 2.4.2.1), the isolated nature of the Faure Island cats indicates that *S. erinaceieuropaei* became established in these cats via anthropogenic means some time after the initial colonisation of this region by cats.

2.4.4 Acanthocephalan Parasites of Feral Cats

2.4.4.1 *Oncicola pomatostomi*

Acanthocephalan parasites occurring in feral cats throughout Australia have previously been referred to as *Oncicola* sp. (Coman *et al.*, 1981a; Ryan, 1976a; Coman, 1972b). However, Schmidt (1983) identified the species as *Oncicola pomatostomi* based on both juvenile and adult worms collected from feral cats. Both the dingo and the feral cat act as definitive hosts (Schmidt, 1983), though Coman (1972b) failed to detect *O. pomatostomi* in any of the 204 dingoes and feral dogs he surveyed in Victoria. The intermediate host (presumed to be an arthropod) of *O. pomatostomi* is not known,

though cats are considered to become infected through the ingestion of birds which act as paratenic hosts (Bowman *et al.*, 2002; Schmidt, 1983).

Foxes do not appear to harbour *O. pomatostomi* (Ryan, 1976b; Coman, 1973b), this may be due to the fact that birds are a more important food item of feral cats than of foxes, particularly in arid regions (Read and Bowen, 2001; Risbey *et al.*, 1999; Bayly, 1978). As such, *O. pomatostomi* infections are most commonly detected in cats from the arid regions of Australia (O'Callaghan and Beveridge, 1996; Coman *et al.*, 1981a; Ryan, 1976a), although the parasite has been found in feral cats from Kangaroo Island in South Australia (O'Callaghan *et al.*, 1984), as well as wetter regions of New South Wales (Ryan, 1976a). *O. pomatostomi* infections were prominent in feral cats collected from the arid regions in the present study and absent in those from the wetter southwest regions.

Whilst birds were a rare occurrence in the stomachs of feral cats from the arid sampling areas in the present study, grasshoppers, insects and other arthropods were frequently encountered. It may be that the frequent occurrence of arthropods as well as birds in the diet of feral cats from arid regions accounts for the increased prevalence of *O. pomatostomi* in cats from these areas and its absence from the wetter climates, at least in Western Australia.

2.4.5 Protozoan Parasites of Feral Cats

Estimates of the total number of protozoan species present in the Australian vertebrate fauna range from 1306 to 3194 species (Adlard and O'Donoghue, 1998). So far only 488 species have been identified, with 255 of these being recorded from the 35% of the

Australian mammal fauna that has been investigated (Adlard and O'Donoghue, 1998). This dearth of information on Australian protozoa is due not only to a lack of investigation, but also to a lack of distinguishing morphological characteristics and the availability of appropriate tools for studying them (Monis, 1999; McManus and Bowles, 1996). As such, surveys of the protozoan parasites of feral cats in Australia are few.

2.4.5.1 *Isospora felis* and *I. rivolta*

The coccidian parasites *Isospora felis* and *I. rivolta* were the most common protozoan parasites detected in feral cats for this study. At least one of these two parasite species was present in cats from all except two sample regions throughout Western Australia: the Bungle Bungles and the Cocos Islands. Whilst the absence of *I. felis* and *I. rivolta* in cats from the Bungle Bungles may be due to the small sample size (n = 4), the lack of any protozoan parasites from the Cocos Islands cannot be explained. The introduction of cats to this island environment may have resulted in a protozoan-free population, though this is unlikely.

Like most coccidian parasites, the life cycle of *I. felis* and *I. rivolta* is typically direct, although rodents have been shown to act as paratenic hosts for both these species (Dubey and Frenkel, 1972b; Frenkel and Dubey, 1972). However, it is unclear whether cats are infected more commonly by oocysts or by the ingestion of paratenic hosts (Bowman *et al.*, 2002). The prevalence of coccidian parasites in cats appears to be at least partially age dependent with kittens typically having a higher infection rate than adults (Prescott, 1984; Collins *et al.*, 1983; Dubey, 1973), though a study of 1,294 cats in 1978 found no relation between age and prevalence of coccidia (Visco *et al.*, 1978).

Collins *et al.* (1983) found only four of 71 cats examined from Sydney to be infected with enteric protozoa, whist Coman *et al.* (1981b) detected *I. rivolta* in 3% and *I. felis* in 4% of feral cats from Victoria and western New South Wales. Likewise, Gregory and Munday (1976) found *I. rivolta* in 5.4% and *I. felis* in 12.7% of feral cats from Tasmania. This is not unusual as *I. felis* is reported to occur more frequently in cats than *I. rivolta* (Prescott, 1984), although the overall prevalence of *I. rivolta* (16.9%) was higher than *I. felis* (4.5%) in the present study. This difference in prevalence of *I. rivolta* and *I. felis* in feral cats from Western Australia may be due to a climatic influence, as *I. rivolta* was most prevalent in cats collected from drier regions.

2.4.5.2 *Sarcocystis*

The life cycle of *Sarcocystis* is indirect and requires two hosts, a herbivorous and/or omnivorous intermediate host and a predatory or scavenging definitive host (Buxton, 1998; Savini *et al.*, 1992a). *Sarcocystis* causes disease in the intermediate host and appears to have little or no pathogenicity in the definitive host carnivores (Prescott, 1984). Whilst cats are known to harbour two species of *Sarcocystis* infectious to sheep (Savini *et al.*, 1993), dogs, dingoes and red foxes are more often responsible for the transmission of *Sarcocystis* to both sheep and cattle (Savini *et al.*, 1993, 1992b).

Studies on *Sarcocystis* infections in Australia have predominantly been concerned with those species infecting sheep and cattle due to their potential economic impact, however *Sarcocystis* spp. have been shown to occur in numerous species of Australian wildlife (Munday and Mason, 1980; Munday *et al.*, 1980; Munday *et al.*, 1979; Munday *et al.*, 1978). Despite these investigations, the cat has only been identified as a definitive host for *Sarcocystis* in rabbits (Munday *et al.*, 1980).

A survey of protozoan parasites in feral cats from Victoria and western New South Wales failed to detect *Sarcocystis* in any of the 300 cats examined (Coman *et al.*, 1981b). Additionally, a survey by Gregory and Munday (1976) of feral cats from Tasmania found *Sarcocystis* in only one of 55 cats examined and tentatively attributed the presence of this infection to the possible scavenging of dead sheep or offal. The occurrence of *Sarcocystis* in feral cats from Western Australia was similarly uncommon with an overall prevalence of only 2.1%, with a single case of *Sarcocystis* detected from Shark Bay, the Gibson Desert and Lake Argyle, whilst five cats from Mount Keith were infected.

Savini *et al.* (1992a) reported a low prevalence (9%) of *Sarcocystis* infections in cattle from arid regions of Western Australia, and concluded that aridity coupled with low densities of both definitive and intermediate hosts are the factors most likely to limit the prevalence of *Sarcocystis* in an area. However, the results from the present study seem to show a preference for *Sarcocystis* infections in cats from dryer areas, which may reflect a seasonal or opportunistic infection source for these areas.

2.4.5.3 *Toxoplasma gondii*

None of the 379 cats examined in this study were found to be shedding *Toxoplasma gondii* oocysts in their faeces. Cats collected for this study were predominantly adults and as older cats are less likely to harbour active infections of *T. gondii* in the gut, the fact that this parasite was not detected in faeces is not surprising. Therefore, further investigation into the occurrence of *T. gondii* in feral cats was performed using more sensitive detection methods, which is presented in greater detail in Chapter 5.

2.4.5.4 *Giardia*

Species of the flagellated protozoan *Giardia* inhabit the intestinal tracts of virtually all classes of vertebrates, however *G. duodenalis* is the only recognised species to occur in most mammals (O'Handley *et al.*, 2000; Thompson *et al.*, 2000b; Thompson *et al.*, 1998; Thompson *et al.*, 1993b). A single *Giardia* infection was detected in a cat from the Dragon Rocks Nature Reserve. Molecular analysis of the purified oocysts revealed the isolate to be *G. duodenalis* of the genotype belonging to the zoonotic genetic Assemblage A, which is most commonly found in humans. This finding is further discussed in Chapter 4.

2.4.5.5 *Cryptosporidium*

Cryptosporidium was found in a total of four cats, however the oocysts were in poor condition and speciation was not possible. Further attempts to purify and characterise the parasite using molecular procedures proved unsuccessful. The poor quality of the oocysts may have been due to the preservative they were stored in or an effect of passing through the feline digestive tract if the oocysts originated from an infection source other than the cat. Given the wide host range and zoonotic potential of this parasite (Nizeyi *et al.*, 1999; Graczyk *et al.*, 1998; Olson *et al.*, 1997b), it is impossible to determine if the *Cryptosporidium* detected in these cats were due to active infections or passive transfer without applying the proper diagnostic techniques.

2.4.5.6 *Eimeria*

Cats and other carnivorous vertebrates are not known to harbour any infections of *Eimeria* spp. The presence of *Eimeria* in cats from several different sampling regions,

often at high levels, is considered to originate from ingested prey infected with *Eimeria*. This is supported by the damaged condition of most *Eimeria* oocysts detected, which indicated that they were merely being passed through the cat intestine and did not represent an active infection. These artefact infections are believed to be a result of cat depredation of lizards and rodents with prevalence levels generally indicating the importance of these animals in the diet of feral cats.

2.4.6 Parasites of Native Fauna

Australian marsupials are parasitised by a broad range of helminth parasites which constitute 24% to 50% of the helminth families known from mammals (Beveridge and Spratt, 1996). The Trematoda is represented by nine families, the Cestoda by five families, the Acanthocephala are represented by two genera, whilst the Nematoda are by far the most numerous helminths with 20 families (Beveridge and Spratt, 1996). These major groups of parasites are similar to those that you would expect to find in eutherian mammals occupying similar ecological niches on other continents (Beveridge and Spratt, 1996). Thus, it appears that the convergent evolution of marsupials and their analogous eutherian mammals on other continents and the overall similarity between them, extends to the types of helminth parasites they harbour (Beveridge and Spratt, 1996). Whilst Australian marsupials harbour a relatively cosmopolitan range of helminth families, their species of helminths are highly endemic due to the prolonged period of isolation during which they evolved (Beveridge and Spratt, 1996).

Due to the destructive techniques of collecting and identifying internal parasites, most investigations have focussed on the study of helminths in the more common native species (Spratt *et al.*, 1990). Some comprehensive reviews of marsupial diseases have

previously been published (Munday, 1988; Arundel *et al.*, 1977), though these focus primarily on disease and their symptoms rather than parasites. More recently, O'Donoghue and Adlard (2000) compiled a catalogue of recorded protozoan parasites occurring in the Australian vertebrate fauna. However, systematic surveys of native species are rare and many studies still rely upon opportunistic sampling methods such as the collection of road-kill or professional shooters (Oakwood and Spratt, 2000; Beveridge *et al.*, 1992; Barker *et al.*, 1989; Arundel *et al.*, 1979; Beveridge and Arundel, 1979).

The present study is in no way intended to be a comprehensive survey of the parasites present in Western Australia's wildlife. Instead, it is a preliminary investigation into the basic composition of the parasite fauna of selected native mammal species based primarily on the examination of faecal samples collected from different regions of Western Australia.

2.4.7 Native Fauna Parasite Communities

Dominance of helminth communities by the strongyloid nematodes is common in marsupials (Beveridge and Spratt, 1996; Spratt, 1987), and was observed for all species of marsupials examined in the present study (Table 2.2). It appears that this general dominance of strongyle nematodes in helminth communities occurs not only in macropods where it has been most commonly reported (Beveridge *et al.*, 1992; Beveridge and Arundel, 1979), but also in the dasyurids, peramelids, phalangerids, and potoroidids as well (Spratt, 1987).

The protozoan parasite communities of Australian marsupials are less well known than the helminth communities, presumably due to the lack of appropriate diagnostic (molecular) tools which means that traditionally complex biological parameters such as host range, growth characteristics or serology are required for their identification and speciation (Monis, 1999). Many species of Australian marsupials have no protozoan parasites recorded for them.

2.4.7.1 *Dasyurus geoffroii*

The dasyurids typically harbour the widest range of helminth families of any group of marsupials including helminths from the three parasite phyla: Platyhelminthes; Acanthocephala; and Nemathelminthes (Beveridge and Spratt, 1996). Within the dasyurid marsupials, the families of cestode parasites and their life cycle patterns parallel those found in comparable eutherian mammals. However, the two cyclophyllidean genera *Anoplotaenia* and *Dasyurotaenia* that occur in the larger carnivorous dasyurids are enigmas (Beveridge and Jones, 2002). Although both genera were originally placed in the family Taeniidae, they have since been removed and left as genera of indeterminate affinities within the Cyclophyllidea (Jones and Bray, 1994; Khalil *et al.*, 1994; Rausch, 1994, 1985).

Interestingly, *D. geoffroii* had the highest prevalence of strongyles (89.2%) of all the marsupials examined for this study despite previous studies reporting higher prevalences in macropodids. Beveridge and Spratt (1996) reported a frequency of occurrence of strongyle nematodes in members of the Dasyuridae as approximately 50% whilst members of the Macropodidae typically experienced frequencies of 60% or more. However, research on the helminth fauna of dasyurids is far from

comprehensive, and the majority has been based on those species occurring in the northern, eastern and southern regions of Australia (i.e. *D. hallucatus*, *D. maculatus* and *D. viverrinus*) (Oakwood and Spratt, 2000; Spratt *et al.*, 1990). Only three species of nematode have been recorded for *D. geoffroii* (*Mackerrastrongylus mawsonae*, *Sprattellus waringi* and *Woolleya cathiae*) (Spratt *et al.*, 1990), all belonging to the order Strongylida. Similarly, the recorded helminth fauna of other dasyurids (e.g. *D. albopunctatus*, *D. hallucatus* and *D. maculatus*) is also dominated by nematodes, many of which are strongyles (Oakwood and Spratt, 2000; Spratt *et al.*, 1990).

One possible explanation for the high prevalence of strongyle nematode eggs detected in the faeces of *D. geoffroii* in the present study may be that they are passing strongyle nematode eggs originating from ingested prey species. The presence of oxyuroid eggs in two *D. geoffroii* samples adds support to this hypothesis, as this nematode group normally parasitises herbivorous and folivorous marsupials (Spratt *et al.*, 1990) as well as reptiles (Pichelin *et al.*, 1999). Similarly, Oakwood and Spratt (2000) deemed that the occurrence of an oxyuroid nematode in the stomach of a *D. hallucatus* in the Northern Territory originated from the ingestion of invertebrate or vertebrate prey, and as such was not an active infection. The occurrence of artefact parasites was also observed in feral cats, with the presence of unidentified spiruroid and *Eimeria* species in numerous faecal samples (section 2.4.5.6). However, without proper speciation of the helminths occurring in both dasyurids and other marsupials, the status of these parasites as active or artefact infections cannot be confirmed nor disproved, though the majority of these strongyle eggs would be expected to belong to the Trichostrongyloidea, which commonly occur in dasyurids.

Two *D. geoffroii* were the only marsupials sampled found to be infected with a trematode parasite. This low prevalence appears somewhat uncommon, as there are several genera of trematode reported to occur in dasyurids, many of which are undescribed (Spratt *et al.*, 1990). However, Oakwood and Spratt (2000) detected a single echinostomatid trematode in only one of 62 *D. hallucatus* autopsied for internal parasites in the Northern Territory. This low frequency of occurrence of trematode parasites may be common in dasyurids. Additionally, Oakwood and Spratt (2000) found only a single species of cestode (*S. erinaceieuropaei*) occurring inter-muscularly and in the peritoneal cavity of three *D. hallucatus*. The nature and location of these infections means that they would not have been detected in *D. geoffroii* in the present study.

Protozoan parasites recorded for species of the genus *Dasyurus* consist of unidentified *Sarcocystis* sp., *Toxoplasma* and *Hepatozoon dasyuri*, though there are no protozoan parasites specifically recorded for *D. geoffroii* (O'Donoghue and Adlard, 2000). The majority of coccidia reported as occurring in the Dasyuridae have not been described, though *Entamoeba* has not previously been recorded for any species of *Dasyurus* (O'Donoghue and Adlard, 2000). However, the limited information available for dasyurids does not necessarily mean that the detection of an unidentified *Entamoeba* species in a single *D. geoffroii* is an uncommon occurrence. This was the only protozoan parasite detected from *D. geoffroii* in the present study. Similarly, Oakwood and Spratt (2000) detected only a single protozoan parasite (unidentified *Sarcocystis* sp.) in their survey of *D. hallucatus*. As such, it appears that members of the genus *Dasyurus* may harbour a restricted fauna of protozoan parasites.

2.4.7.2 *Isoodon obesulus* and *Perameles bougainville*

The diversity and range of helminth parasite groups represented in the peramelids resembles that of dasyurids in many ways. The major groups of nematodes in these marsupials consist of the superfamilies Trichostrongyloidea, Metastrongyloidea and superfamilies of the order Spirurida (Beveridge and Spratt, 1996). Additionally, a range of trematode parasites have been recorded whilst cestodes consist of the families Hymenolepididae, Dilepididae and Linstowiidae (Beveridge and Jones, 2002), all of which generally have insects as intermediate hosts (Beveridge and Spratt, 1996). However, no trematode or cestode parasites were detected in *I. obesulus* or *P. bougainville* for this study, which was most likely due to the use of a flotation technique for the examination of faecal samples.

Strongyle nematodes were detected in the faeces of *I. obesulus* and *P. bougainville* at similar prevalences (68.8% and 60.0% respectively) despite the *P. bougainville* being sourced from a captive breeding colony. Trichostrongyloid nematodes constitute the most diverse helminth superfamily in the bandicoots comprising three families; Mackerrastrongylidae; Dromaeostrongylidae and Herpetostrongylidae (Beveridge and Spratt, 1996). The peramelids are typically dominated by the family Mackerrastrongylidae which is represented by the three genera: *Asymmetracantha*; *Mackerrastrongylus*; and *Tetrabothriostrongylus* (Beveridge and Spratt, 1996; Spratt *et al.*, 1990). Each of these genera has been recorded from *I. obesulus* but not from *P. bougainville*, however they have been recorded in other species of *Perameles* (Spratt *et al.*, 1990). Additionally, the representative genera of the Dromaeostrongylidae and Herpetostrongylidae families have also been recorded from *I. obesulus* (Spratt *et al.*, 1990).

Three *I. obesulus* from Manjimup were found to have *Strongyloides*-like eggs or L1 larvae though none were detected in any of the *P. bougainville*. A single species, *Parastrongyloides australis*, has been recorded as occurring in *I. obesulus* (Mawson, 1960). Parasites of the family Strongyloididae typically have direct life cycles and involve a free-living stage where infection of the host occurs via ingestion or skin penetration of L3 larvae. The sourcing of *P. bougainville* from a captive breeding colony may explain the absence of these parasites, as the management of this *P. bougainville* population included periodic anthelminthic treatment which could have broken the life cycle and contributed to the elimination of these parasites from these animals. However, the prevalence of these parasites in the wild *I. obesulus* populations sampled was still low (3.9%).

The occurrence of spiruroid nematodes in only three of the 77 wild *I. obesulus* (3.9%) appears to be significantly lower than its occurrence in nine of the 35 captive *P. bougainville* (25.7%). These parasites typically have an indirect life cycle involving arthropods as intermediate hosts and spiruroid larvae have previously been reported to occur in *I. obesulus*, though none have been identified to species level (Spratt *et al.*, 1990). The higher prevalence of spiruroid nematodes in *P. bougainville* as opposed to *I. obesulus* in the present study could be related to their captivity. The constant presence of a readily accessible food source would be expected to attract numerous insects and enhance the transmission of spiruroid nematodes among *P. bougainville*, resulting in the higher prevalence observed.

Similarly, the difference in prevalence of *Trichuris* between *I. obesulus* (11.7%) and *P. bougainville* (22.9%) may also be due to the influence of captivity. Whilst *Trichuris* species have direct life cycles, periodic anthelminthic treatment would most likely not be intensive enough to control infections. Therefore, the captive bandicoots would be continuously exposed to infective stages, which would be exacerbated by the movement of both animals and soil/bedding. Although the species of *Trichuris* in the bandicoots was not determined, *T. peramelis* is the only species of this cosmopolitan genus described from marsupials (Beveridge and Spratt, 1996), and has been reported in several species of the bandicoot genera *Isoodon* and *Perameles*.

The parasite genus *Linstowinema* is represented by four species and is endemic in Australian marsupials (Beveridge and Spratt, 1996). This nematode genus is commonly found in peramelids (Beveridge and Spratt, 1996), and was present in 37.7% of *I. obesulus* and 11.4% of *P. bougainville* sampled. Only one species of *Linstowinema* (*L. cinctum*) has previously been recorded for *P. bougainville*, though in the present study all four infected *P. bougainville* were identified as harbouring *L. inglisi*. Five of the 29 infected *I. obesulus* were harbouring *L. inglisi,* whilst the remaining infections were an unidentified species of *Linstowinema*. Two species (*L. inglisi* and *L. meridionalis*) as well as a third unidentified species of *Linstowinema* have previously been identified in *I. obesulus* (Spratt *et al.*, 1990).

The detection of oxyuroid nematodes in a single *P. bougainville* is unusual as these nematodes typically occur in herbivorous and/or folivorous diprotodonts (Beveridge and Spratt, 1996), whilst several species of lizard are also parasitised by oxyuroid nematodes (Pichelin *et al.*, 1999). The occurrence of this parasite in only a single *P.*

bougainville suggests that this is an accidental infection, most likely resulting from the ingestion of soil contaminated with oxyuroid eggs or an infected lizard attracted to the readily available food in the breeding pens.

Both *I. obesulus* and *P. bougainville* had the widest range of protozoan parasites of the host species sampled in the present study. Unidentified species of *Entamoeba* and *Eimeria* species were present in both *I. obesulus* and *P. bougainville* at similar prevalences. Whilst these two parasite genera have previously been recorded in *I. obesulus*, there are no records for *P. bougainville* and neither of these protozoan parasites have been recorded for other members of the genus *Perameles* (O'Donoghue and Adlard, 2000). *Giardia* was detected in only a single *I. obesulus* from Manjimup, though Bettiol *et al.* (1997) found *Giardia* in 62% of bandicoots examined from Tasmania. *Giardia* was also detected in a dingo (*Canis lupus dingo*), a pale field rat (*Rattus tunneyi*) and a western chestnut mouse (*Pseudomys nanus*), all collected from Lake Argyle (results not shown). These results, along with that of the *Giardia* found in the *I. obesulus*, are further discussed in Chapter 4.

2.4.7.3 *Bettongia penicillata*

Knowledge of the helminth fauna of the potoroid marsupials is relatively sparse as many of them are endangered and few have been examined extensively for parasites (Beveridge and Spratt, 1996). The helminth fauna of potoroids appears to be quite limited, with small numbers of species of trichostrongyloid, strongyloid and oxyuroid nematodes and a few cestodes having been reported (Beveridge and Spratt, 1996), whilst the recorded helminth fauna for *B. penicillata* consists of a single cestode, an

acanthocephalan, and six nematodes (only three described to species) (Spratt *et al.*, 1990).

The occurrence of Rhabditida nematodes in *B. penicillata* has not been previously reported, though such a low occurrence of these parasites as observed in the present study (5.0%) may have contributed to their previous lack of detection. Additionally, strongyle nematodes identified as belonging to the genus *Potorostrongylus* were detected in two *B. penicillata* from Manjimup. A single species of this genus, *Potorostrongylus finlaysoni*, is the sole member of the Strongyloidea known to occur in the genera *Potorous* and *Bettongia* (Beveridge and Spratt, 1996; Spratt *et al.*, 1990), though a second species, *P. aepyprymnus*, has been reported from the rufous bettong (*Aepyprymnus rufescens*) (Beveridge *et al.*, 1992; Mawson, 1974).

The shift of the centre of fermentative digestion in the Macropodidae from the large bowel to the stomach allowed the subsequent radiation of strongyloid nematodes in these hosts (Beveridge and Spratt, 1996). However, in potoroids the primary site of fermentative digestion is in the caecum, as their saccular forestomach functions primarily as a storage organ (Beveridge and Spratt, 1996; Frappel and Rose, 1986; Hume, 1982). Therefore, the limited production of volatile fatty acids in the stomach of potoroids has resulted in a limited fauna of strongyloid nematodes. The absence of these stomach-inhabiting strongyloid nematodes in potoroids is considered to be the reason for their observed lack of helminth diversity, as these nematodes commonly dominate the parasite communities in most macropodid hosts (Beveridge *et al.*, 1992).

Records of protozoan parasites of bettongs are very limited (O'Donoghue and Adlard, 2000), and there are no records of protozoan parasites from *B. penicillata* though the species found in the present study appear to correlate with those previously found in other members of the Potoroidae family. *Eimeria* is a commonly reported protozoan in potoroids and was detected in *B. penicillata* from both Batalling and Manjimup, with three species identified: *E. gaimardi*; *E. potoroi*; and *E. aepyrmni*. Each of these species has previously been reported as occurring exclusively in *B. gaimardi*, *Potorous tridactylus* and *A. rufescens* respectively (Barker *et al.*, 1988). Despite this, all three of these species were detected in *B. penicillata* in the present study.

The unidentified *Eimeria* species as well as the unidentified coccidia detected in *B. penicillata* suggests that there may be additional species yet to be identified and further investigation is required to elucidate the extent of the protozoan fauna in members of the Potoroidae. *Eimeria* was not detected in any of the *B. penicillata* sampled from Shark Bay, though this may have been due to a combination of the small sample size (n = 7) and the origin of the *B. penicillata* population, which was reintroduced to Shark Bay only a year prior to sampling.

2.4.7.4 *Trichosurus vulpecula*

Beveridge and Spratt (1996) proposed that the evolution of herbivory in Australian marsupials could have initially led to a decrease in parasite diversity. This is illustrated by the detection of only one group of helminths (strongyle nematodes) in *T. vulpecula* in the present study. In addition, the prevalence of these helminths is markedly lower than for all the other species of marsupial examined. It has been proposed that this lack of helminth diversity may most likely be due to their arboreal nature (Beveridge and

Spratt, 1996). The shift from a terrestrial to an arboreal existence would have resulted in reducing their access to infective parasite stages in soil thus leading to a decrease in parasite diversity (Beveridge and Spratt, 1996; Beveridge *et al.*, 1992). Hence, the observed lack of diversity of major helminth groups in *T. vulpecula* in the present study.

As with other marsupials the helminth fauna of *T. vulpecula* is dominated by species of nematodes, with only two platyhelminths being known to occur, the common liver fluke (*Fasciola hepatica*) and the cestode *Bertiella trichosuri* (Beveridge and Spratt, 1996). The trematode *F. hepatica* was introduced to Australia with domestic livestock and is relatively common in *T. vulpecula* in the eastern states, however it does not occur in Western Australia. The cestode *B. trichosuri* is a common parasite of *T. vulpecula* (Beveridge, 1985), though was not detected in the present study, possibly due to the reduced sensitivity of the flotation technique used to screen faecal samples.

Of the fifteen nematode species known to occur in *T. vulpecula*, eight are trichostrongyloid nematodes most of which are accidental acquisitions from grazing ruminants (Beveridge and Spratt, 1996), and appear to occur at a low prevalence and intensity of infection (O'Callaghan and Moore, 1986). Whilst speciation of strongyle parasites was not performed, the occurrence of accidental species originating from introduced ruminants would not be unexpected given the proximity of grazing livestock to many of the areas where *T. vulpecula* were sampled in this study. *T. vulpecula* are also known to harbour the oxyuroid nematode *Adelonema trichosuri* (Beveridge and Spratt, 1996; Spratt *et al.*, 1990), though again none were detected in the present study.

Recorded protozoan infections of *T. vulpecula* include parasites of the genera *Eimeria*, *Sarcocystis*, *Toxoplasma* and *Entamoeba* (O'Donoghue and Adlard, 2000), though the presence of *Sarcocystis* and *Toxoplasma* can generally only be detected by the examination of tissue samples, which were not collected in the present study. Both *Eimeria* and *Entamoeba* were detected in *T. vulpecula* at relatively low levels (2.4% and 7.2% respectively), however none of the *Eimeria* could be identified to species. The protozoan fauna of *T. vulpecula* from Western Australia appears to correlate with the recorded protozoan parasites of *T. vulpecula*, though this itself appears to be somewhat lacking. Excluding the introduced trichostrongylid nematodes and the common liver fluke, *T. vulpecula* harbour a restricted range of parasites (Beveridge and Spratt, 1996), and this appears to be even more so for *T. vulpecula* in Western Australia.

2.4.7.5 *Macropus fuliginosus* and *M. eugenii*

Despite the initial indication that the Macropodidae harbour a very limited diversity of parasites (Table 2.2), it must be remembered that the classification of strongyle eggs represents all parasites of the order Strongylida, with the exception of the superfamily Metastrongyloidea. Therefore, classifying parasites only to their order has concealed the existence of a potentially large diversity of helminth fauna in both *M. fuliginosus* and *M. eugenii* from Western Australia. Indeed, the Macropodidae are known to harbour the most diverse range of parasites within the Australian marsupial radiation (Beveridge and Spratt, 1996), and this diversity has been attributed to their development of a complex sacculated forestomach (Hume, 1982).

The evolution of a saccular forestomach in the Macropodidae shifted the centre of fermentative digestion from the large bowel to the stomach allowing the subsequent

radiation of numerous strongyloid nematode genera that contribute significantly to the diversity of the helminth fauna of kangaroos and wallabies (Beveridge and Spratt, 1996; Beveridge and Arundel, 1979). Typically, the Macropodidae have a helminth fauna characteristic of grazing eutherians, with members of the Trichostrongyloidea and Metastrongyloidea present, though members of the Strongyloidea are the most common nematodes (Beveridge and Spratt, 1996; Beveridge and Arundel, 1979). Therefore, the helminth communities of kangaroos and wallabies are relatively uniform, composed of numerous species with no one species or group of species being dominant (Beveridge *et al.*, 1992).

A single genus of oxyuroid nematode, *Macropoxyuris*, occurs in the caecum and colon of kangaroos with worm burdens sometimes in excess of 500,000 (Beveridge and Arundel, 1979). Two species of *Macropoxyuris* have been described though several more remain undescribed (Spratt *et al.*, 1990). Beveridge and Arundel (1979) found that species of *Macropoxyuris* usually occurred in kangaroos in mixed infections. Therefore, the oxyuroid eggs detected in 27.8% of *M. fuliginosus* sampled in the present study most likely belong to the genus *Macropoxyuris*. An unidentified species of *Macropoxyuris* has been recorded as occurring in *M. eugenii* (Spratt *et al.*, 1990), though oxyuroid eggs were not detected in any of the *M. eugenii* examined in the present study. Likewise, a two year study of the seasonal and geographical variation of *M. eugenii* nematodes on Kangaroo Island also failed to detect oxyuroid nematodes (Smales and Mawson, 1978).

The occurrence of unidentified coccidia in 74.3% of *M. eugenii* and 5.6% of *M. fuliginosus* sampled may represent a wide variety of protozoan species that have

previously been reported from these hosts (O'Donoghue and Adlard, 2000). The most commonly occurring protozoans reported from both *M. eugenii* and *M. fuliginosus* belong to the genus *Eimeria* (O'Donoghue and Adlard, 2000). Unidentified species of *Eimeria* were detected in 16.7% of *M. fuliginosus*, however many of the unidentified coccidia detected in *M. eugenii* are believed to be *Eimeria* species though they were not identified.

The transmission of direct life cycle parasites, such as *Eimeria* and many other protozoan parasites may be enhanced in small and highly fragmented host populations (Viggers *et al.*, 1993; Scott, 1988). Therefore, the higher prevalence of protozoan parasites in *M. eugenii* compared to *M. fuliginosus*, may be a reflection of the fragmented and localised distribution of *M. eugenii* in Western Australia compared to the broad distribution of *M. fuliginosus* (Menkhorst and Knight, 2001; Smales and Mawson, 1978).

2.4.7.6 *Notomys alexis*, *Pseudomys hermannsbergensis* and *Mus musculus*

In this study, the predominant eutherian mammals examined consisted of spinifex hopping mice (*Notomys alexis*), sandy inland mice (*Pseudomys hermannsbergensis*) and introduced house mice (*Mus musculus*). Due to the general difficulty in finding and trapping native rodents as opposed to the more common introduced species, very little is known of their helminth fauna (Warner, 1998). In contrast to the marsupials, the Muridae in this study appeared to harbour a sparse range of helminth parasites, with the most obvious difference being the absence of nematodes belonging to the order

Strongylida. However, all three murid species sampled had a high prevalence of oxyuroid nematodes belonging to the genus *Syphacia*.

Nematodes of the genus *Syphacia* were the most common parasites detected in all three rodent species examined and although the majority of these were unidentified, *Syphacia obvelata* was detected in 30.8% of *M. musculus*. Adult *Syphacia* worms were recovered from each of the murid species examined in the present study, however *S. obvelata* was only detected in *M. musculus*, whilst an unidentified *Syphacia* species was detected in *N. alexis*, *P. hermannsbergensis* and *M. musculus*. The detection of *S. obvelata* in only *M. musculus* correlates with the previous finding that separate helminth communities exist between native rodents and *M. musculus* (Spratt, 1987). However, the occurrence of an unidentified *Syphacia* species at similar prevalence levels in both the native rodents and *M. musculus* indicates that each of these host species is exposed to similar levels of infective stages of these nematodes.

Oxyuroid nematodes have a typically direct life cycle, hence their affinity for herbivorous mammals. However, *S. obvelata* has been shown to be transmitted primarily by direct contact between host individuals (Grice and Prociv, 1993), largely negating any environmental or external constraints on its transmission. This requirement for physical contact between hosts would explain the occurrence of *S. obvelata* in the *M. musculus* and its absence from either species native mice. However, the similar prevalence levels of the unidentified species of *Syphacia* in both the native mice and *M. musculus* suggest that it does not require direct contact between individuals for its transmission.

Protozoan parasites were not detected in any of the *M. musculus* examined in the present study. Indeed, the occurrence of protozoan parasites in all three murid species examined was very low with unidentified coccidia detected in two *N. alexis* and two *P. hermannsbergensis*, and unidentified *Entamoeba* species in six *N. alexis*. There are no protozoan parasites recorded for *P. hermannsbergensis* and *Giardia duodenalis* is the only protozoan recorded for *N. alexis* (O'Donoghue and Adlard, 2000). Information on the prevalence and origin of the *Giardia* infection in this host indicates that it was an isolated case (Ey *et al.*, 1993). *Giardia* was not detected in any of the *N. alexis* examined from Shark Bay, though it was detected in one pale field rat (*Rattus tunneyi*), one western chestnut mouse (*Pseudomys nanus*) and one dingo (*Canis lupus dingo*) all from Lake Argyle, and will be further discussed in Chapter 4.

The absence of protozoan parasites in *M. musculus* in the present study may be a reflection of the sample size (n = 13), however a similar sample size (n = 18) succeeded in detecting coccidia in *P. hermannsbergensis*. Therefore, the apparent absence of protozoan parasites in *M. musculus* appears to support the hypothesised lack of interaction between them and native mice based on their dietary requirements (Norton, 1987; Cockburn, 1980; Watts and Braithwaite, 1978). However, the occurrence of an unidentified *Syphacia* species in both *M. musculus* and native mice contradicts this hypothesis. Further investigation of the parasite fauna of introduced and native mice species is clearly required to gain a better understanding of the parasite communities present in both native and introduced rodents in Western Australia.

2.4.8 Parasite Transfer Between Feral Cats and Native Fauna

Initial observations indicate that the transfer of parasite between cats and the native wildlife appears to be negligible. A similar conclusion was reached by Dickman (1996b) who reviewed the published literature on the coincidence of non-specific feline parasites in the Australian vertebrate fauna. However, only 25% of known marsupial host species have been examined for parasites (Beveridge and Spratt, 1996), whilst only 35% of all Australian mammals have been examined for protozoan parasites (Adlard and O'Donoghue, 1998).

Despite the lack of any observed sharing and/or interaction with feline pathogens to native fauna in the present study, the occurrence of certain parasites within feral cat populations indicates the involvement of the native fauna. Obvious examples of this include those parasites with complex life cycles such as *S. erinaceieuropaei* and *G. spinigerum* as well as *T. taeniaeformis* and *O. pomatostomi*, which readily involve native species as intermediate or paratenic hosts. The occurrence of generally non-specific parasites, such as *T. gondii* (see Chapter 5) and *Sarcocystis*, in feral cat populations further increases the potential impact cats have on the native fauna.

Further evidence of the cross-transmission of parasite between feral cats and the native fauna is provided by the nematode *C. dasyuridis*, a native parasite of dasyurids (Mawson, 1968). The survey of helminth parasites of feral cats on King Island by Gregory and Munday (1976) detected 9.5% of cats infected with *C. dasyuridis*, despite the last recorded sighting of a dasyurid on the island being in 1922. Despite the presumed extinction of dasyurids from King Island approximately 50 years earlier (Gregory and Munday, 1976), *C. dasyuridis* managed to persist in the feral cat

population, demonstrating the ability of some parasites to acquire new hosts to their advantage.

The importance of this finding is the potential for foreign parasites to demonstrate similar abilities in the Australian fauna. This has already been documented in *T. vulpecula*, where a significant portion of their helminth communities include species they have acquired from introduced ruminants (Beveridge and Spratt, 1996). This is of particular concern for conservation efforts, as this "apparent competition" may continue to impact upon native species in areas where introduced species have been removed.

The effects at population level of introduced disease in the Australian fauna are not adequately known for any species, though the transmission of pathogens to susceptible native wildlife by an invading species has been shown to have a more profound effect than any direct impact of the invader itself. The well known examples of avian malaria in Hawaii (van Riper and van Riper, 1986), and rinderpest in East Africa (McCallum and Dobson, 1995) serve to show us the magnitude of the effects that introduced parasites can exert on wild animal populations. Whilst such severe impacts have not yet been scientifically demonstrated as occurring in Australia, the present study shows that cats, along with other introduced carnivores, carry a wide range of parasitic organisms, some of which may have the potential to have a dramatic impact on not only native fauna survival and reproduction, but also on community structure.

Chapter 3 – Development of a PCR-RFLP for the Identification of *Ancylostoma* Species: Occurrence and Distribution in Feral Cats Throughout Western Australia

3.1 INTRODUCTION

3.1.1 Hookworm Infections

Hookworms are parasitic helminths that belong to the order Strongylida (family Ancylostomatidae). They are dioecious and have a direct life-history pattern (Crompton, 2000), typically being harboured in the host's small intestine where they attach to the mucosa and feed on blood (Monti *et al.*, 1998). Hookworms feed by ingesting plugs of intestinal villi and adjoining tissue into their mouth opening, where small tooth-like lancets abraid the mucosal plug and enzymes are released which cause villus capillary loops to burst, which along with the cell contents and tissue fluids, provides the parasite's food (Schad, 1991). Due to the secretion of an anticoagulant whilst feeding, the former attachment sites continue to bleed after the hookworms have reattached elsewhere (Behnke, 1991; Hotez and Cerami, 1983).

As a consequence of their blood-sucking activity, hookworms can affect physical and cognitive development in children (Stephenson *et al.*, 2000). Anaemia is the classical manifestation of hookworm disease and is a result of iron deficiency, which can vary from slight to severe (Crompton, 2000; Migasena and Gilles, 1991). Up to 25% of individuals infected with hookworm suffer from anaemia and protein malnutrition,

which can also lead to extreme lethargy and weakness (Hotez and Pritchard, 1995). Hookworm infection can also occasionally be fatal, especially in infants (Hotez and Pritchard, 1995).

After mating in the small intestine, each individual female hookworm can release between 5000 and 25,000 eggs per day that are passed out of the small intestine with the faeces (Crompton, 2000). The soil and local environment frequented by infected hosts becomes contaminated with free-living first and second stage larvae which prefer well aerated soil that is shielded from direct sunlight for optimum growth (Crompton, 2000; Hotez and Pritchard, 1995). The survival of hookworm larvae is favoured in damp, sandy or friable soil, with decaying vegetation, and a temperature range of 24ºC - 32ºC (Bundy and Keymer, 1991). In these conditions the transition from egg to third stage infective larvae can be completed within a week (Prescott, 1984), though the prevailing climate greatly affects the rate of development of the free-living stages and the survival of the infective larvae (Crompton, 2000). Temperatures above 37ºC inhibit the embryonation of hookworm eggs whilst exposure to temperatures below 12ºC reduces egg hatching rates and causes disruption to the normal development of the free-living larvae (Nwosu, 1978; Croll, 1972).

The infective L3 larvae can migrate upwards in the soil as much as three metres to make contact with a host (Hotez and Pritchard, 1995; Bundy and Keymer, 1991). The most common infection routes are via ingestion and cutaneous penetration, though some hookworm species can also infect animals prenatally via mother's milk and the ingestion of paratenic hosts (Hendrix *et al.*, 1996). Typically the barely visible third stage hookworm larvae burrow into the skin of the host animal's legs or feet and begin

an extensive migration route through the body, eventually establishing themselves in the small intestine (Hendrix *et al.*, 1996; Hotez and Pritchard, 1995; Croll *et al.*, 1975). Oral ingestion of third stage larvae provides a much more direct route to the small intestine, where they become sexually mature and begin to mate and feed (Hotez and Pritchard, 1995). Females generally begin releasing eggs within about two months after entering the body as larvae (Hotez and Pritchard, 1995).

3.1.2 Hookworm Occurrence in Australia

The hookworm fauna of Australian mammals is limited with all but two of the species present having been introduced (Beveridge, 2002). These introduced hookworm species are of considerable medical and veterinary importance due to their occurrence in humans and livestock (Beveridge, 2002), however the Australian fauna's susceptibility to them is unknown. Until the distribution and prevalence of these parasites is known, their potential impact on the native wildlife cannot be fully recognised.

The poor representation of hookworms in the Australian fauna is probably a reflection of the fact that they are primarily parasites of eutherian groups which are either absent or which have only recently reached the Australian continent (Beveridge, 2002). Native or endemic species of hookworms in Australia are restricted to two species *Uncinaria hamiltoni* and *U. hydromyidis*, which occur in pinnipeds and the Australian water rat respectively (Beveridge, 2002). The hookworm fauna of Australian mammals is therefore limited in terms of the number of species, though the majority of the ancylostomatoid fauna in Australia is represented by introduced species of man and domestic animals (Beveridge, 2002).

Currently, eleven species of hookworms have been recorded in Australia, with the principal genera being *Ancylostoma*, *Bunostomum*, *Necator* and *Uncinaria* (Beveridge, 2002). The only ancylostomatoids which have reached Australia prior to the arrival of man and his domesticated animals appear to be species present in marine, or at least aquatic, mammals (Beveridge, 2002). Thus the isolation of the Australian continent over a long period of time is the principal reason for the relative paucity of ancylostomatoid species present (Beveridge, 2002; Beveridge and Spratt, 1996).

The human hookworm species, *Ancylostoma duodenale* and *Necator americanus*, were once common in Australia, particularly in the north eastern regions (Beveridge, 2002). However, the Australian Hookworm Campaign run from 1919 to 1924 was highly successful in controlling infections and both species have become rare, though *A. duodenale* does remain a significant problem in some isolated Aboriginal communities (Beveridge, 2002). The more common species of hookworm in Australia include *A. tubaeforme*, *A. caninum*, *A. ceylanicum* and *U. stenocephala*, which generally occur in dogs and cats though may also infect humans to differing degrees (Schad and Banwell, 1990). *A. braziliense* has been detected in both dogs and cats in north Queensland (Stewart, 1994; Heydon and Bearup, 1963), though its distribution and host range in Australia is not known.

Whilst *A. tubaeforme* has been reported in cats from Brisbane (19%, 81%) (Prescott, 1984; Wilson-Hanson and Prescott, 1982) and Sydney (35%) (Kelly and Ng, 1975), it has only been found in feral cats from the Northern Territory (12.8%) (O'Callaghan and Beveridge, 1996) and the north west of Western Australia (20%) (Meloni *et al.*, 1993; Thompson *et al.*, 1993a). Multiple studies have failed to detect *A. tubaeforme* in feral

and/or domestic cats from the southern regions of Australia (O'Callaghan *et al.*, 1984; Shaw *et al.*, 1983; Gregory and Munday, 1976; Ryan, 1976a; Coman, 1972b).

As its name suggests, *A. caninum* is the commonly recognised hookworm of dogs, occurring at high prevalences in northern regions of Australia (Dunsmore and Shaw, 1990; Kelly and Ng, 1975). It has also been found in dingoes in Queensland (Dunsmore and Shaw, 1990; Seddon, 1967) and foxes in New South Wales (Ryan, 1976b). In Australia there appears to be an association between the prevalence of *A. caninum* and latitude, with higher prevalences occurring in the warmer northern regions (Dunsmore and Shaw, 1990). Studies of *A. caninum* in Townsville have shown that it is not specific to dogs, but is also capable of systematically infecting both cats and humans (Prociv and Croese, 1996; Stewart, 1994), and has been identified as a common cause of eosinophilic enteritis in humans in Queensland (Beveridge, 2002; Prociv and Croese, 1996).

A. ceylanicum is able to mature in humans, but throughout most of its range it is primarily a parasite of dogs and cats, occurring in humans only rarely, except in New Guinea (Schad, 1991). *A. ceylanicum* has been shown to be much more infective orally than percutaneously in human volunteers, which helps explain its rarity in humans in most of its pantropical range (Yoshida *et al.*, 1971). *A. ceylanicum* and *A. braziliense* were not considered separate species until it was demonstrated that *A. braziliense* does not have the capacity to develop to maturity in humans, while *A. ceylanicum* does (Yoshida, 1971). Hence, *A. braziliense* is most commonly associated with cases of cutaneous larva migrans in humans (Behnke, 1991). In Australia, *A. braziliense* has been reported in dogs in north Queensland (Setasuban and Waddell, 1973; Seddon,

1967) with a single occurrence in Sydney (Kelly and Ng, 1975). Its distribution has also been stated as extending to the Northern Territory and the north west of Western Australia (Kelly, 1977), though supporting references were not provided.

U. stenocephala is a common parasite of dogs in the southern regions of Australia, being reported from New South Wales (Kelly and Ng, 1975), Victoria (Johnston and Gasser, 1993; Blake and Overend, 1982; Pullar, 1946), South Australia (O'Callaghan *et al.*, 1984), Tasmania (Gregory and Munday, 1976) and the south west of Western Australia (Gardiner and Fraser, 1960), though not in Queensland (Beveridge, 2002). It is a less common parasite of cats, having only been detected in 3% of feral cats from New South Wales (Ryan, 1976a) and 2.3% of feral cats from Tasmania (Gregory and Munday, 1976).

3.1.3 Identifying Hookworm Species

Accurate identification of hookworm species and the specific diagnosis of the infections they cause have important implications for studying fundamental aspects relating to anthelmintic efficacy, population biology and epidemiology as well as for effective control (Monti *et al.*, 1998). The most common means of diagnosing the presence of hookworm infections is the examination of faecal samples for the occurrence of hookworm eggs (Crompton, 2000; Monti *et al.*, 1998). However, accurate identification of *Ancylostoma* species cannot be achieved solely on the basis of egg morphology (Crompton, 2000). Species identification can be performed on adult worms based on the morphology of their buccal apparatus (Crompton, 2000; Monti *et al.*, 1998), though adult worms can generally only be obtained following highly intrusive anthelmintic expulsion chemotherapy or surgical techniques (Crompton, 2000). Culturing larval

stages from eggs can also allow identification of hookworm species (Pawlowski *et al.*, 1991; Mueller *et al.*, 1989), however 'larval culture' is tedious, time consuming (1 week) and requires skilled personnel for accurate differentiation (Monti *et al.*, 1998).

Detailed morphological examinations of adult worms of *Ancylostoma* from both dogs and cats have also revealed variation in morphology (morphotypes) within species (Stewart, 1994). The taxonomic status of such morphotypes, which may represent either morphologically similar but genetically distinct species (i.e. cryptic species), subspecies or population variants that are capable of infecting different host species, may cause confusion (Gasser *et al.*, 1996). The existence of such cryptic species with only slight or no morphological differences has been previously demonstrated in other bursate nematodes (Beveridge *et al.*, 1993; Chilton *et al.*, 1993, 1992), as well as more recently in *A. caninum* and *A. duodenale* (Hu *et al.*, 2002). Therefore, as our understanding of particular parasite taxonomy improves, techniques other than morphological examination are becoming necessary for the accurate identification of species and subspecies. Molecular and biochemical techniques have been shown to be useful tools for the identification of parasite species (Monti *et al.*, 1998; Stevenson *et al.*, 1995; Bowles and McManus, 1993; Chilton *et al.*, 1993), especially for those species where identification is complicated by a lack of morphologically distinct characters (Monis, 1999; Morgan and Thompson, 1998).

Direct PCR assays have been developed for the species-specific detection of DNA from numerous species of parasitic worms and their eggs (Epe *et al.*, 1997; Newton *et al.*, 1997; Romstad *et al.*, 1997; von Samson-Himmelstjerna *et al.*, 1997), and are based on the use of primers that amplify species-specific sequences within the internal

transcribed spacers (ITS) of the ribosomal DNA (rDNA) (Monti *et al.*, 1998). One exceptionally useful advantage of these techniques is the potential to quickly and accurately differentiate between parasite species based on DNA obtained from eggs shed in the faeces of infected hosts (Chilton and Gasser, 1999; Monti *et al.*, 1998; Gasser *et al.*, 1993). The highly sensitive nature of PCR has the added advantage of being able to differentiate closely related nematode species, based on the DNA from a single egg (Campbell *et al.*, 1995; Stevenson *et al.*, 1995; Gasser *et al.*, 1993), reducing the time required to identify species from 10 to 14 days for larval culturing to less than one day (Campbell *et al.*, 1995).

Polymerase chain reaction-linked restriction fragment length polymorphism (PCR-RFLP) analysis has shown that different developmental stages of *Ancylostoma* hookworms can be identified to species level using the variation present in their rDNA (Gasser *et al.*, 1998; Gasser *et al.*, 1996). This non-invasive method of identifying hookworm infections also negates the requirement to examine adult worms, the collection of which may involve highly invasive methods (Prociv and Croese, 1996). Thus, molecular techniques such as species specific PCR and PCR-RFLP have important implications for studying the systematics of hookworms and the epidemiology/population biology of hookworm infections. As such, the aim of this study was to develop a PCR-RFLP method for the effective identification of *Ancylostoma* species from eggs collected in the faeces of feral cats from Western Australia, utilizing the sequence variation present in the ITS regions of the rDNA.

3.2 MATERIALS AND METHODS

3.2.1 Screening for Hookworm Positive Samples

Faecal samples were collected from 379 feral cats collected from eleven sampling locations throughout Western Australia (Figure 2.1). Samples were screened for the presence of hookworm and other helminth and protozoan parasites as previously described in section 2.2.2. The collection of feral cat samples from Shark Bay and Mount Keith occurred across three distinct time periods over a three year period: August 1998, November 1999-January 2000, and November 2000 for cats from Shark Bay; and November 1998, April 1999 and September 2000 for cats from Mount Keith. All other feral cat collection sites were sampled once. Significance of differences between hookworm prevalence in cats from Shark Bay and Mount Keith over their three sampling periods was determined by chi-square analysis using the computer program Statview 4.0 (Abacus Concepts Inc., 1992).

3.2.2 Purification and DNA Extraction

Of the 119 faecal samples identified as being hookworm positive via light microscopy, 56 had sufficient material preserved in 20% dimethyl sulphoxide (DMSO) in a saturated salt solution for the isolation of hookworm eggs. Eggs were separated from the faecal pellet via sucrose gradient purification (Meinema, unpublished). The presence of hookworm eggs following sucrose gradient purification was confirmed via light microscopy before undergoing DNA extraction.

DNA was extracted from purified hookworm eggs using glassmilk (Biorad) as per manufacturer's instructions. Hookworm DNA was resuspended in 50 µl of TE buffer (1 mM Tris-HCl; 0.1 mM EDTA; pH 7.2) prior to amplification of the ITS region.

3.2.3 Designing Hookworm Primers

Species specific hookworm primers were designed with the computer program Amplify 1.2 (Engels, 1993) to amplify the rDNA region comprising the first (ITS-1) and second (ITS-2) internal transcribed spacers plus the 5.8S gene (known as the ITS^+ region) using the published sequence information from Genbank for *A. tubaeforme* (Y19182, AJ001592, Z70741), *A. ceylanicum* (Y19183, AJ001593, Z70740) and *A. caninum* (Y19181, AJ001591, Z70739). There was no sequence information available for *A. braziliense*, therefore a specific primer could not be designed. Primers consisted of a degenerate forward primer (RTHF1), which amplified all four species and was anchored in the ITS1 portion of the rDNA, and four species specific reverse primers: RTATR1 (specific for *A. tubaeforme*); RTAYR1 (specific for *A. ceylanicum*); and RTACR1 (specific for *A. caninum*). These primers were anchored in the ITS2 region of the rDNA (Table 3.1).

Table 3.1 Primers designed for the amplification of specific *Ancylostoma* species.

Primer	Sequence	Species
RTHF1	5'-CGTGCTAGTCTTCACGACTTTG-3'	*Ancylostoma* spp.
RTAYR1	5'-CTGCTGAAAAGTCCTCAAGTCC-3'	*A. ceylanicum*
RTATR1	5'-CTAGAACGGGAATCGCTAAAAGC-3'	*A. tubaeforme*
RTACR1	5'-CTAGAACGGGAATCGCTAAATGC-3'	*A. caninum*

3.2.4 Amplification of Hookworm ITS^+ rDNA

PCR amplification was performed in 25 µl volumes with the final mix containing 5-50 ng of hookworm DNA, 12.5 pMol of each primer, 200µM each of dATP, dCTP, dGTP, dTTP, 67 mM Tris-HCl (pH 8.8), 16.6 mM $(NH_4)_2SO_4$, 0.45% Triton X-100, 0.2 mg/ml gelatin, 4 mM $MgCl_2$ and 0.5 units Tth^+ polymerase (Biotech International, Perth,

Australia). PCR mix was made up fresh for each reaction and were amplified in a Perkin Elmer 2400 thermocycler machine (Perkin Elmer Cetus) under the following conditions: preheated to 94ºC for 2 min; followed by 35 cycles of 94ºC (denaturation) for 30 s; 64ºC (annealing) for 30 s; 72ºC (extension) for 30 s; followed by a final extension step at 72ºC for 7 min.

3.2.5 Sequencing of Hookworm rDNA

Templates were purified using the freeze/squeeze method. In brief, PCR bands were cut from the gel using sterile techniques and placed into separate eppendorf tubes and frozen overnight at -20ºC or for 15 min at -80ºC. Whilst still frozen the gel slices containing the PCR band were squeezed between sterile parafilm to separate liquid from the agarose. On average 50-100 µl of liquid was recovered from each band (dependent on original size). The DNA was precipitated using salt and EtOH and resuspended in 20 µl of sterile dH_2O. Sequencing reactions were performed using an ABI Prism™ Dye Terminator Cycle Sequencing Core kit (Applied Biosystems, Foster City, California) according to the manufacturer's instructions. Reactions were electrophoresed through an ABI 373 automatic sequencer and sequencing profiles analysed using SeqEd v1.0.3 (Applied Biosystems).

3.2.6 Agarose Gel Electrophoresis

Each hookworm sample was checked post PCR for sufficient amplification of DNA by running 5 µl of each PCR on an agarose gel. Agarose gel electrophoresis was carried out using 1% gels in TAE buffer (40 mM Tris-HCl; 20 mM acetate; 2mM EDTA; pH 7.9). Electrophoresis was performed using horizontal gels, in electrophoretic cells (Biorad). Ethidium bromide was included in the gel at a final concentration of 0.15

µg/ml. After electrophoresis, DNA was visualised under UV-illumination. Amplified hookworm DNA was compared to a 100 bp DNA ladder (New England Biolabs).

3.2.7 RFLP Analysis

RFLP analysis of amplified hookworm DNA was performed using the restriction enzyme *Rsa*I (New England Biolabs), which cleaves the recognition sequence 5'…GT↓AC…3'. Selection of the restriction enzyme *Rsa*I was based on the differential digestion patterns generated with DNA Strider 1.2 (Marck, 1988) for the ITS^+ sequence data for *A. caninum*, *A. ceylanicum* and *A. tubaeforme*. Restriction digests contained 10 mM Bis Tris Propane-HCl, 10 mM $MgCl_2$, 1 mM dithiothreitol (pH 7.0), 2.5 units of *Rsa*I (New England Biolabs) and 15 µl of PCR product in a final reaction volume of 20µl. No inhibitory effect of the PCR buffer on digestion with *Rsa*I was detected. PCR products were digested for 3 h at 37ºC and the resultant restriction patterns were visualised on 1% agarose gels as above (section 3.2.6). Size of fragments was estimated by comparison with a 100 bp DNA ladder (New England Biolabs). Every RFLP gel contained uncut, control hookworm PCR product for comparison with the resulting digestion patterns.

3.3 RESULTS

3.3.1 Hookworm Occurrence in Feral Cats

Hookworm eggs were present in 119 of the 379 faecal samples screened via microscopy, and occurred in cats from Walpole, Shark Bay, Lake Argyle, Mount Keith and the Cocos Islands (Table 3.2).

Table 3.2 Hookworm prevalence in feral cats throughout Western Australia.

Sample Location	Prevalence (%)	Sample Size
Perth*	0.0	20
Manjimup	0.0	15
Walpole	80.0	20
Dragon Rocks	0.0	6
Mount Keith	13.6	44
Gibson Desert	0.0	20
Shark Bay	36.6	194
Montebello	0.0	3
Lake Argyle	26.5	34
Bungle Bungles	0.0	4
Cocos Islands	89.5	19
Total	31.4	379

* Includes Leonora Tip (n=3), Harvey Tip (n=2), Canning Dam (n=2) and Rottnest Island (n=3)

The prevalence of hookworm infections in feral cats from Shark Bay varied markedly across the three distinct sampling periods of August 1998, November 1999-January 2000 and November 2000 (Table 3.3). Chi-square analysis revealed significant differences in hookworm prevalence between cats from August 1998 and those sampled in November 1999-January 2000 and November 2000 ($P < 0.0001$), though the difference in hookworm prevalence between cats collected in November 1999-January 2000 and November 2000 was less pronounced ($P < 0.05$). The feral cat population size estimated by officers from the Department of Conservation and Land Management (DCLM) based on cat tracks and trapping success as part of their cat eradication program at Shark Bay during this time, revealed a marked decrease in the feral cat

population between the years 1998 to 1999, followed by a rising trend, which may indicate that the population was beginning to recover following its apparent collapse (Figure 3.1).

Table 3.3 Prevalence of hookworm infection in feral cats collected from Shark Bay from 1998 to 2000.

Sample Time	Sample Size	Prevalence
Aug 1998	60	68.3%
Nov 1999-Jan 2000	68	7.4%
Nov 2000	55	23.6%

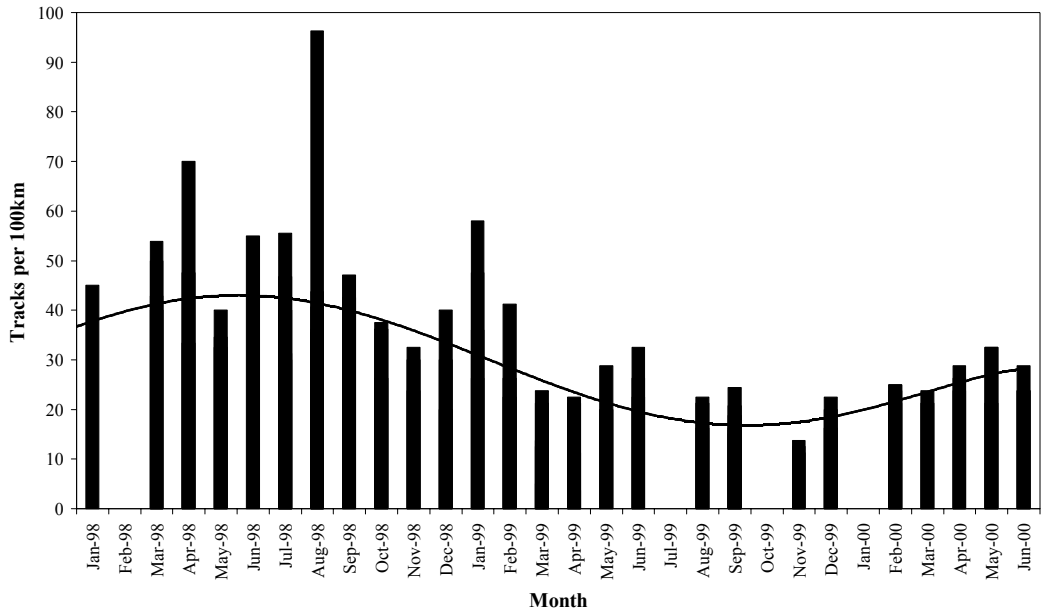

Figure 3.1 Number of feral cats per 100 km transect at Shark Bay from February 1998 to June 2000 based on track counts and trapping success. Graph modified and used with permission (Algar and Angus, 2000).

Sampling of feral cats from Mount Keith in November 1998, April 1999 and September 2000 showed some variation in hookworm prevalence (Table 3.4). However, this apparent variation was not significant as determined by chi-square analysis and was most likely due to the small sample sizes obtained.

Table 3.4 Prevalence of hookworm infection in feral cats collected from Mount Keith from 1998 to 2000.

Sample Time	Sample Size	Prevalence
Nov 1998	25	8.0%
Apr 1999	6	33.3%
Sept 2000	13	15.4%

3.3.2 Identification of Ancylostoma *by Species-Specific PCR*

Specific amplification of *A. ceylanicum* DNA produced a band of approximately 650 bp in length, whilst both *A. caninum* and *A. tubaeforme* produced bands of approximately 550 bp in size (Figure 3.2). Cross-reaction did not occur when attempting to amplify either *A. caninum* or *A. tubaeforme* DNA using the *A. ceylanicum* specific reverse primer (RTAYR1). Likewise, no cross-reaction occurred when attempting to amplify *A. ceylanicum* DNA using either the *A. caninum* (RTACR1) or *A. tubaeforme* (RTATR1) specific reverse primers. However, cross-reaction occurred between the *A. caninum* (RTACR1) and *A. tubaeforme* (RTATR1) primers, producing a fragment of approximately 550 bp in size when amplifying either *A. caninum* or *A. tubaeforme* DNA (Figure 3.2). As sequence information and/or control DNA was not available, cross-reaction with *A. braziliense* DNA could neither be confirmed nor discounted.

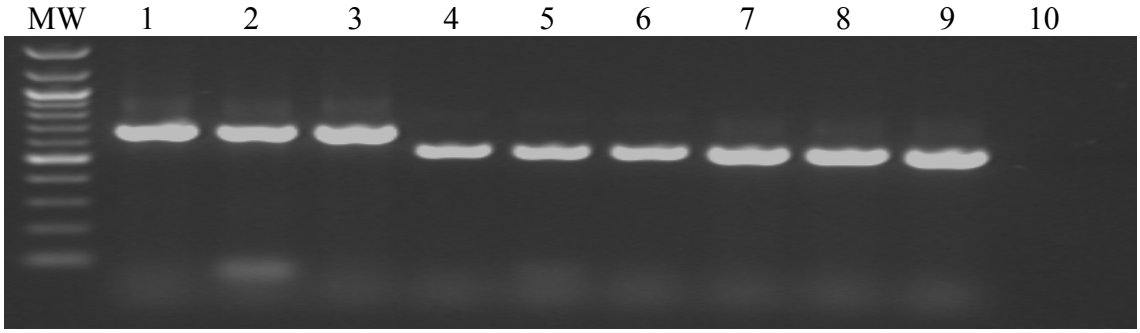

Figure 3.2 PCR amplification of the ITS^{+} region of *A. ceylanicum*, *A. tubaeforme* and *A. caninum*. Lanes: MW, 100 bp ladder; 1-3, *Ancylostoma ceylanicum*; 4-6, *A. tubaeforme*; 7-9, *A. caninum*; 10, negative control.

3.3.3 Identification of Ancylostoma *Species by PCR-RFLP*

Patterns produced by the digestion of the ITS^{+} region with *Rsa*I allowed the differentiation of *A. caninum*, *A. ceylanicum* and *A. tubaeforme* from each other (Figure 3.3). Amplification of *A. caninum* and *A. tubaeforme* DNA with each other's "specific" reverse primer (RTATR1 or RTACR1 respectively) did not affect the digestion pattern of either hookworm species.

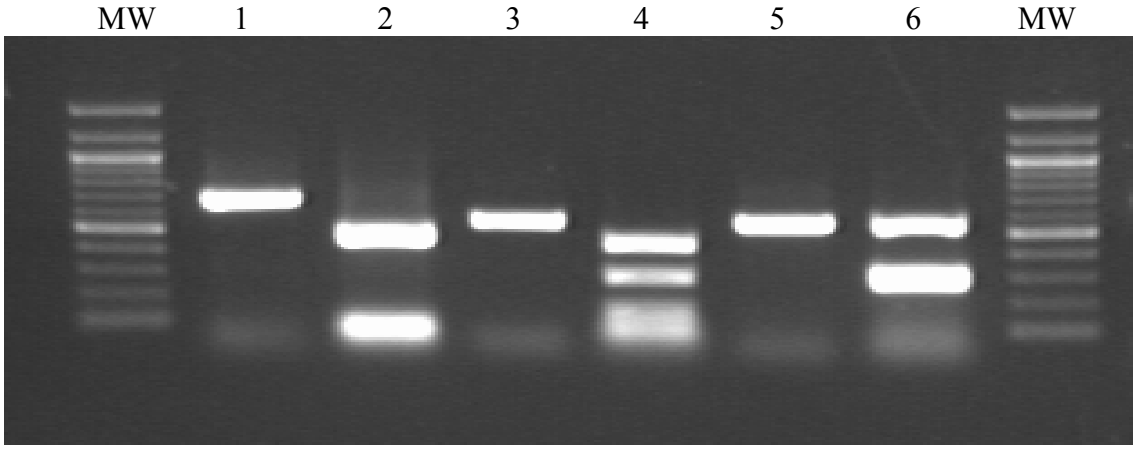

Figure 3.3 PCR-RFLP analysis of the ITS^{+} region of *A. ceylanicum*, *A. tubaeforme* and *A. caninum* using *Rsa*I. Lanes: MW, 100 bp ladder; 1, *Ancylostoma ceylanicum* uncut; 2, *A. ceylanicum* cut; 3, *A. tubaeforme* uncut; 4, *A. tubaeforme* cut; 5, *A. caninum* uncut; 6, *A. caninum* cut.

Two hookworm species were identified as occurring in feral cats from Western Australia: *A. tubaeforme* and *A. ceylanicum*. The most common hookworm species identified was *A. tubaeforme*, which occurred in cats from four of the five different sampling locations where hookworm was detected whilst *A. ceylanicum* occurred in cats from the Cocos Islands and a single case from Lake Argyle (Table 3.5). The Cocos Islands, Mount Keith, Shark Bay and Walpole had only a singular hookworm species infecting feral cats, whilst both *A. tubaeforme* and *A. ceylanicum* occurred in the feral cat population from Lake Argyle (Table 3.5). Mixed hookworm infections were not detected in any of the feral cats sampled. *A. caninum* and *A. braziliense* were not

detected in any of the cats from this study. Due to the lack of *A. braziliense* control DNA, the absence of this hookworm species in feral cats was confirmed using sequence analysis of ITS^{+} PCR products in comparison with the published sequence data of other *Ancylostoma* species.

Table 3.5 PCR-RFLP identification of *Ancylostoma* species in 56 feral cats collected from Western Australia.

Sample Location	Hookworm Species		
	A. tubaeforme	*A. ceylanicum*	*A. caninum*
Walpole	10	0	0
Mount Keith	4	0	0
Shark Bay	20	0	0
Lake Argyle	5	1	0
Cocos Islands	0	16	0
Total	39	17	0

3.4 DISCUSSION

3.4.1 Identification of Ancylostoma *spp. by PCR-RFLP*

The species-specific PCR was capable of differentiating *A. ceylanicum* from *A. caninum* and *A. tubaeforme* due to a 100 bp size difference in the fragments produced, though was unable to differentiate between *A. caninum* and *A. tubaeforme* (Figure 3.2). Cross-reaction between the species-specific *Ancylostoma* primers and *A. braziliense* could not be determined due to the lack of any *A. braziliense* control DNA. The inability to obtain any adult *A. braziliense* worms serves to highlight the rarity of this species in Australia, though does not exclude its presence. As such, sequence analysis was used in conjunction with the PCR-RFLP to confirm the species identity of hookworm eggs collected from feral cats throughout Western Australia.

This study has demonstrated the ability of PCR-RFLP to accurately identify the three hookworm species *A. caninum*, *A. ceylanicum* and *A. tubaeforme* from eggs collected in faeces, despite cross-reaction between the reverse primers RTACR1 (*A. caninum*) and RTATR1 (*A. tubaeforme*) (Figure 3.3). This result demonstrates the suitability of the ITS^+ region for the differentiation of hookworm species. Although the present study has focussed primarily on the application of the ITS^+ PCR-RFLP to the identification of hookworms from cat, previous studies have also shown the ITS to be equally effective at differentiating between human hookworm species as well (Monti *et al.*, 1998; Hawdon, 1996).

The advantage of the hookworm PCR-RFLP technique is that it allows for the accurate identification of hookworm species, irrespective of developmental stage (Chilton and Gasser, 1999). This has important implications for studying the epidemiology and

population biology of hookworms and for accurate diagnosis of the infections they cause. The nature of this method negates the need for invasive sampling methods and allows species identification from as little as a single egg. The PCR-RFLP provides a useful alternative to less specific and more time consuming identification methods such as serology and copro-culture (Chilton and Gasser, 1999; Prociv and Croese, 1996), and lends itself to processing large numbers of samples. Genetic characterisation of hookworm infections also provides the potential for investigating the occurrence and taxonomic status of "morphotypes" within populations and subpopulations (Hu *et al.*, 2002).

3.4.2 Hookworm Occurrence in Feral Cats

The overall prevalence of hookworm infections in feral cats collected throughout Western Australia was 31.4% and did not appear to be limited to any particular climatic region, occurring in cats collected from Walpole (80%), Mount Keith (13.6%), Shark Bay (36.6%), Lake Argyle (26.5%) and the Cocos Islands (89.5%) (Table 3.2). PCR-RFLP identified the presence of two species of ancylostomatoid hookworm occurring in feral cats throughout Western Australia, *A. tubaeforme* and *A. ceylanicum*. The most common species was *A. tubaeforme*, whilst *A. ceylanicum* was only detected in cats from the Cocos Islands as well as a single cat from Lake Argyle (Table 3.5).

According to the literature, four species of *Ancylostoma* (*A. braziliense*, *A. caninum*, *A. ceylanicum* and *A. tubaeforme*) occur in cats in Australia, throughout a range of climatic regions, though appear to be primarily limited to coastal regions of the northern half of Australia (Beveridge, 2002; Prescott, 1984). Given that only 56 of the 119 cats infected with hookworm were identified to species by PCR-RFLP in the present study, a higher

prevalence of *A. ceylanicum*, or the occurrence of *A. caninum* and/or *A. braziliense* in feral cats in Western Australia cannot be ruled out.

Beveridge (2002) recently reviewed the distribution and occurrence of hookworm species in Australia, and *A. tubaeforme* has not previously been observed in cats from the southern states or the southwest of Western Australia before, despite numerous surveys of both feral and domestic cats (Milstein and Goldsmid, 1997; O'Callaghan *et al.*, 1984; Shaw *et al.*, 1983; Gregory and Munday, 1976; Ryan, 1976a; Coman, 1972b). O'Callaghan and Beveridge (1996) found *A. tubaeforme* to be widely distributed in feral cats from the Northern Territory whilst Meloni *et al.* (1993) and Thompson *et al.* (1993b) reported *A. tubaeforme* in cats from Aboriginal communities in the far north of Western Australia.

The occurrence of *A. tubaeforme* in 80% of the feral cats collected from Walpole contradicts previous studies which report an absence of this species in the southern states or the southwest of Western Australia (O'Callaghan *et al.*, 1984; Shaw *et al.*, 1983; Gregory and Munday, 1976; Ryan, 1976a; Coman, 1972b). Additionally, the absence of *U. stenocephala* from cats in the southwest of Western Australia is not unexpected as *U. stenocephala* is commonly found in foxes, dingoes and feral dogs (Ryan, 1976b; Coman, 1973b, 1972a), though it is seldom found infecting cats (Gregory and Munday, 1976; Ryan, 1976a).

The reports by Meloni *et al.* (1993) and Thompson *et al.* (1993b) detected *A. tubaeforme* in 18.2% and 20.6% of cats examined from Aboriginal communities in the far north of Western Australia respectively. These findings are similar to the 26.5%

hookworm prevalence found in the present study, however neither of the previous studies detected *A. ceylanicum*. Whilst *A. tubaeforme* is not considered to be a zoonotic risk (Schad and Banwell, 1990), *A. ceylanicum* is known to be capable of infecting humans and is the only zoonotic hookworm known to consistently cause human gut infections (Prociv and Croese, 1996; Chowdhury and Schad, 1972). In New Guinea, human infection with *A. ceylanicum* is common and occurs at abundances comparable to that of the human hookworms *A. duodenale* and *N. americanus* (Schad, 1991; Schad and Banwell, 1990). Whilst *A. ceylanicum* has been shown to be much more infective orally than percutaneously in human volunteers (Yoshida *et al.*, 1971), its occurrence in feral cats is still of major zoonotic importance.

Nowhere is this more crucial in Western Australia than the Cocos Islands, where 89.5% of cats examined were harbouring *A. ceylanicum* infections. This high prevalence of *A. ceylanicum* in cats, coupled with centralised human and cat populations on the islands, represents a serious zoonotic threat to public health. With such a high prevalence of *A. ceylanicum* infection in the cat population and a climate that is conducive to the growth and development of hookworm larvae, it is suspected that hookworm infection, or at the very least cutaneous larva migrans, present a significant public health threat to the local inhabitants of the Cocos Islands.

This same zoonotic potential exists at Lake Argyle, where *A. ceylanicum* was detected in the feral cat population, even though *A. tubaeforme* was more prevalent. The higher *A. tubaeforme* infection rate may be due to this species out competing *A. ceylanicum* in the environment or the host, though no significant difference in the development of these two species has been observed under varying temperature and osmotic stress

conditions (Matthews, 1985). However, paratenic hosts have been implicated in the life cycle of *A. tubaeforme* (Norris, 1971), which may play an important role in the persistence of this hookworm species within Western Australia.

A. tubaeforme is much more infective in cats when inoculated via ingestion (Norris, 1971), which suggests the involvement of paratenic hosts in its transmission within the environment. Paratenic and transport hosts of *A. tubaeforme* include predominantly rodents and arthropods respectively (Prescott, 1984; Norris, 1971), presumably due to their role in the diets of cats, their foraging habits and life histories which increase their potential exposure to infective stages in the soil. However, our understanding of the epidemiology of *A. tubaeforme* is limited (Prociv and Croese, 1996), and the involvement of paratenic hosts in the transmission of feline hookworm in Australia requires further investigation.

This study has demonstrated that the occurrence of hookworm in feral cats throughout Western Australia is much more widespread than was previously thought. The detection of *A. tubaeforme* in feral cats from both arid and semi-arid environments (Mount Keith and Shark Bay respectively) is of particular interest as O'Callaghan and Beveridge have also identified *A. tubaeforme* and a single case of *A. caninum* in feral cats from central Australia. Therefore, previous studies that have limited the occurrence of this species to the warmer northern regions of Australia may have failed to appreciate the role of paratenic hosts or the existence of suitable microenvironments within otherwise inhospitable regions.

3.4.3 Hookworm and Microenvironments in Western Australia

The occurrence of hookworm infections in cats from Shark Bay and Mount Keith was unexpected as these areas experience an arid climate with extremes of temperature and un-seasonal rainfall. Temperature has the greatest effect on the free-living hookworm larvae, with temperatures between 20ºC and 30ºC being most favoured by *A. tubaeforme* (Nwosu, 1978). These temperatures are optimal for development under "adequate moisture or humidity" conditions (Prociv and Croese, 1996; Schad and Banwell, 1990; Nwosu, 1978). Matthews (1985) and Nwosu (1978) investigated the effects of osmotic stress on the development of *A. tubaeforme* eggs, however they failed to clearly define what constitutes "adequate" moisture or humidity. A general consensus appears to indicate that tropical or sub-tropical environments as well as areas that are regularly watered such as grass or garden soil are sufficiently moist to facilitate hookworm development (Prociv and Croese, 1996; Hotez and Pritchard, 1995; Schad and Banwell, 1990).

Shark Bay and Mount Keith do not experience tropical or sub-tropical climates and temperatures can range from below 0ºC to over 40ºC within a 24 h period. Likewise, the un-seasonal rainfall for these areas also leads to extended periods of dry heat. The occurrence of *A. tubaeforme* in feral cats from these two regions suggests the existence of microenvironments within the landscape where *A. tubaeforme* can complete its life cycle. The presence of these microenvironments is believed to be associated with human modification of the environment. Both the Shark Bay and Mount Keith sampling regions have a pastoral history with many of the livestock water reservoirs still in working order. Additionally, Mount Keith is an active mining lease with an on-site mine village complete with sewage treatment plant and tailings dam. These sources

of moisture in an otherwise predominantly dry landscape would not only facilitate the development of infective stages of *A. tubaeforme*, they would also act as "sinks" for the local wildlife. The presence of these water points in the landscape would increase the potential for hookworm transmission in cats through direct contact with contaminated soil as well as via predation of paratenic hosts in the immediate vicinity.

This anthropogenic influence on the distribution of *A. tubaeforme* in cats throughout Western Australia is supported by the absence of hookworm in cats from the Gibson Desert (Table 3.2). The climatic conditions of this region are similar to those of Mount Keith, however it is extremely remote and the landscape has experienced very little in the way of grazing or mining. Similarly, this hypothesis can be applied to other areas such as the Bungle Bungles and the Montebello Islands where hookworm infections were also absent in feral cats from these areas. However, the small sample size collected from these areas does not allow a definite conclusion to be drawn. Nevertheless, modification of the environment to provide permanent water sources for livestock or other purposes has facilitated the spread of hookworm into regions of Western Australia where it otherwise would not occur.

3.4.4 Hookworm Fluctuation in Feral Cats from Shark Bay

The hookworm prevalence in feral cats from Shark Bay fluctuated dramatically over the three years that sampling took place (Table 3.3). However, there was no significant change in hookworm prevalence for cats collected from Mount Keith during the same time period, indicating that the variation in prevalence at Shark Bay was due to a localised influence (Table 3.4). Estimates of the feral cat population size at Shark Bay were collated from trapping success and track counts from January 1998 through to

June 2000 by DCLM staff undertaking an eradication program on the enclosed Peron Peninsula, Shark Bay (Figure 2.2). Plotting the number of cats observed shows that the feral cat population on the peninsula suffered a steady decline in numbers from mid 1998 till the end of 1999, when it began to recover (Figure 3.1). Likewise, the hookworm prevalence in feral cats during this period dropped from 68.3% in August 1998 to 7.4% at the end of 1999.

Host population size has a profound effect on the dynamics of a pathogen and every parasite requires a minimum density of hosts whereby it can maintain itself, known as the threshold population size (H_T) (Lyles and Dobson, 1993; Dobson and May, 1986; Bartlett, 1960). So long as the host population exceeds the threshold density, the parasite is able to maintain itself (Dobson and May, 1986). As the feral cat population declined in size on the Peron Peninsula, the hookworm prevalence also experienced a significant decline (Table 3.3), suggesting the cat population was approaching H_T.

The high hookworm prevalence observed in feral cats in 1998 (68.3%) indicates a high level of interaction between individuals, presumably due to resource sharing and an overlapping of home ranges (Denny *et al.*, 2002; Izawa and Doi, 1993; Izawa *et al.*, 1982). Reduction of the cat population (by approximately half) due to the intensive trapping regime throughout 1998/1999 would have reduced the need for resource sharing and interaction within the feral cat population due to the resultant lower densities. This decreased interaction is evident in the significantly ($P < 0.0001$) lower hookworm prevalence in cats collected at the end of 1999 (7.4%).

Izawa and Doi (1993) reported that the level of interaction and resource sharing in populations of cats varies with the density of cats and the availability of resources. Therefore, the low density of cats at Shark Bay in 1999 was reflected in the low prevalence of hookworm infections due to the reduced interaction between individual animals. However, as population size decreases in an enclosed system such as the Peron Peninsula, so too does the efficiency of trapping as a control measure (Algar, pers. comm.). A reduction in the trapping intensity at Shark Bay after 1999 permitted the feral cat population to recover (indicated by trendline in Figure 3.1), resulting in an increase in hookworm prevalence observed in cats collected in 2000 (23.6%), as their interaction increased.

3.4.5 Potential Impact of Hookworm Infections

The potential impact of hookworm infections on native and introduced species in Western Australia is not obvious, although the presence of *A. ceylanicum* in feral cats from both Lake Argyle and the Cocos Islands clearly presents a potential zoonotic risk to humans. Whilst the transmission potential of *A. ceylanicum* at Lake Argyle is unknown, it would appear to be extremely high at least in the Cocos Islands. Hookworm parasitism may be common in cat populations, however clinical signs are generally not observed and the occurrence of skin lesions in cats is less severe than in humans (Prescott, 1984). The most outstanding feature of hookworm infection is anaemia which can lead to inappetence and ill-thrift, and can occasionally be fatal, particularly in young animals (Hotez and Pritchard, 1995; Prescott, 1984).

Chapter 4 – Detection of *Giardia* in Introduced and Native Wildlife: Molecular Characterisation of a Novel Species of *Giardia* from a Quenda (*Isoodon obesulus*)

4.1 INTRODUCTION

4.1.1 Giardia – *Impact and Importance*

The flagellated protozoan *Giardia* was first observed by Van Leeuwenhoek in 1681 and more fully described by Lambl in 1859 (Ortega and Adam, 1997). Initially it was thought to be commensal in humans, but it is now clearly recognised as a common cause of diarrhoea and malabsorption and is the most commonly recognised pathogenic intestinal parasite in developed countries as well as in developing areas where hygiene and nutritional standards are difficult to maintain (Ortega and Adam, 1997; McRoberts *et al.*, 1996). *Giardia* infects millions of people throughout the world in both epidemic and sporadic forms and is transmitted through ingestion of contaminated water and food or by direct faecal-oral transmission (Ortega and Adam, 1997). Species of *Giardia* inhabit the intestinal tracts of virtually all classes of vertebrates (Thompson, 2000), however *Giardia duodenalis* (syn. *Giardia intestinalis*; *Giardia lamblia*) is the only recognised species found in most mammals (O'Handley *et al.*, 2000; Thompson *et al.*, 2000b; Thompson *et al.*, 1998; Thompson *et al.*, 1993b).

The life cycle of *Giardia* is simple and direct, consisting of two developmental forms, the trophozoite and the cyst. The cyst is the infectious form and is relatively inert and

environmentally resistant (Ortega and Adam, 1997). After ingestion, excystation occurs in the duodenum as a result of exposure to the acidic gastric pH and the pancreatic enzymes chymotrypsin and trypsin, producing two trophozoites from each cyst (Hill, 1993; Feely *et al.*, 1991). The trophozoites replicate in the crypts of the duodenum and upper jejunum and reproduce asexually by binary fission (Ortega and Adam, 1997).

The prepatent period of giardiasis and the duration of infection are not related to the size of the initial inoculum (Islam, 1990). The incubation period for people with symptomatic infection is 1-2 weeks but can vary from 1 to 75 days (Wolfe, 1990), though in the majority of cases the infection remains asymptomatic. Malabsorption in cases of giardiasis has been well documented and may be responsible for the substantial weight loss that can occur following infection (Meloni *et al.*, 1993; Roberts *et al.*, 1988).

The diagnosis of giardiasis is most commonly established by the identification of cysts or, less frequently, trophozoites in faecal specimens. The passage of cysts is somewhat sporadic, and for some cases with chronic diarrhoea and malabsorption, the results of faecal sample examinations can be repeatedly negative despite ongoing suspicion of giardiasis (Ortega and Adam, 1997).

4.1.2 Nomenclature of Giardia

Five morphologically distinct species of *Giardia* have been recognised; *G. duodenalis* (syn. *G. intestinalis*, *G. lamblia*) (infects a wide range of wild and domestic mammals including humans), *G. agilis* (amphibians), *G. muris* (rodents) and *G. psittaci* and *G. ardeae* (birds) (Thompson *et al.*, 2000a). This taxonomy is largely based on the shape

of the trophozoite, the size of the ventral adhesive disc relative to the cell length and the shape of the median bodies (Monis *et al.*, 1999). The phylogenetic affinities of *Giardia* have been a matter of controversy for a number of years, however there is now broad consensus of its primitive origins (Simpson *et al.*, 2002). *Giardia* has thus become a key organism in attempts to understand the evolution of eukaryotic cells given it has a very simple intracellular organization (Marti *et al.*, 2003a; Marti *et al.*, 2003b).

Attempts at speciation of *Giardia* isolates based on host specificity have been discarded due to the high level of ambiguity between host specific and zoonotic infections. As such, *G. duodenalis* is currently split into eight major assemblages commonly infecting a wide range of wild and domestic mammals and humans. These genotypes were initially segregated based on host species specificity, however further research has shown many of them to be capable of cross transmission (Thompson *et al.*, 2000a; Thompson *et al.*, 2000b). The four other species; *G. muris*, *G. agilis*, *G. ardeae* and *G. psittaci* appear to maintain a reasonable level of host specific infectivity. A sixth species, *G. microti*, has been described on the basis of cyst morphology and small subunit ribosomal RNA (SSU-rDNA) sequence analysis (van Keulen *et al.*, 1998).

4.1.3 Giardia *Phylogenetics*

The SSU-rDNA is a well conserved group of genes widely recognised as a means of detecting genetic diversity among species of organisms (Appels and Honeycutt, 1986). The different regions of the ribosomal RNA provide varying levels of phylogenetically useful information. The coding regions (18S, 5.8S and 28S) are highly conserved whilst the internal and external transcribed spacers are moderately conserved (Hillis and Davies, 1986). It is widely accepted that analysis of the rRNA region can be used to

differentiate between strains or species of organisms (McManus and Bowles, 1996). Of particular interest is the 5' end of the 18S gene, which has been used extensively for genotyping and identifying isolates of *Giardia*. Indeed, sequence analysis of the SSU-rDNA has supported the distinctiveness between the genotypes of *G. duodenalis*, as well as that of *G. ardeae* and *G. muris* as proposed based on their morphological characteristics (Thompson *et al.*, 2000a; Monis *et al.*, 1999; Hopkins *et al.*, 1997).

The use of SSU-rDNA analysis has been previously used to describe the occurrence of a distinct *Giardia* species found in voles and muskrats (van Keulen *et al.*, 1998). Based on morphology and analysis of the SSU-rDNA gene, van Keulen *et al.* (1998) concluded that the *Giardia* they isolated was a new species that they called *G. microti*. Additionally, the use of the Glutamate Dehydrogenase (*gdh*), Triose Phosphate Isomerase (*tpi*) and Elongation Factor 1 Alpha (*ef1α*) genes has been widely accepted as a reliable means for the characterisation of both *Giardia* species and genotypes (McIntyre *et al.*, 2000; Hopkins *et al.*, 1999; Monis, 1999; Monis *et al.*, 1999; Monis and Andrews, 1998; Monis *et al.*, 1998; Hopkins *et al.*, 1997; McRoberts *et al.*, 1996).

These loci are useful tools for the characterisation of novel *Giardia* isolates as well as phylogenetic studies. Both the SSU-rDNA gene and the *ef1α* gene have previously been used to segregate the *G. duodenalis* genotypes as well as the species *G. ardeae*, *G. muris* and *G. microti* for phylogenetic and epidemiological studies (Monis *et al.*, 1999). Here we investigate the occurrence of *Giardia* in both introduced and native wildlife throughout Western Australia, and characterise a novel isolate of *Giardia* found in a southern brown bandicoot (quenda) (*Isoodon obesulus*) in the south west of Western Australia using both SSU-rDNA and *ef1α* gene sequence analysis.

4.2 METHODS

4.2.1 Detection, Purification and Measurement of Cysts

Faecal samples were collected from 851 native mammals and 379 feral cats throughout Western Australia. The 851 native mammals comprised: 249 *Trichosurus vulpecula*; 238 *Bettongia penicillata*; 77 *Isoodon obesulus*; 65 *Dasyurus geoffroyi*; 42 *Notomys alexis*; 36 *Macropus fuliginosus*; 35 *Macropus eugenii*; 35 *Perameles bougainville*; 18 *Pseudomys hermannsbergensis*; 12 *Mus musculus*; 8 *Pseudomys nanus*; 8 *Lagostrophus fasciatus*; 6 *Bettongia lesueur*; 6 *Leporillus conditor*; 5 *Rattus tunneyi*; 5 *Canis lupus dingo*; 3 *Pseudomys deliculatus*; 1 *Macropus rufus*; 1 *Macropus robustus*; and 1 *Ningaui ridei*.

Faecal samples were examined by direct stool microscopy stained with 5% Malachite Green stain and by both Sodium Nitrate and Zinc Sulphate flotation (Bartlett *et al.*, 1978). Samples positive for *Giardia* cysts were subsequently purified via saturated salt and glucose gradient purification (Meinema unpublished). Purified quenda (*Isoodon obesulus*) *Giardia* cysts were measured using the Optimas Image Analysis Package version 5.2 at ×1,000 magnification. The area of analysis was set to enclose each individual cyst in order to minimise computational time. Threshold was optimised by eye to differentiate the cyst background and once the optimal threshold was reached, the software was set to recognise the cyst as an area object. All measurements (length, width, area and circularity) were transferred to a Microsoft Excel spreadsheet for analysis.

4.2.2 DNA Extraction and PCR Amplification

DNA was extracted from purified and concentrated *Giardia* cysts using glassmilk (Biorad) as per manufacturer's instructions. Preliminary genetic characterisation of the extracted *Giardia* DNA was performed by amplifying a 292 bp region of the 5' end of the small subunit ribosomal RNA (SSU-rRNA) gene (Hopkins *et al.*, 1997), using the conditions set out below (section 4.2.2.1). Characterisation of the quenda *Giardia* isolate was performed using molecular techniques studying the entire SSU-rRNA gene as well as the Elongation Factor 1 alpha (*ef1α*) gene.

4.2.2.1 SSU-rDNA PCR

Amplification of the SSU-rDNA was performed using conditions previously set out by Hopkins *et al.* (1997), and the primers RH11 (5'-CATCCGGTCGATCCTGCC-3') and RH4 (5'-AGTCGAACCCTGATTCTCCGCCAGG-3') to generate a 292 bp fragment for genotyping *G. duodenalis* genotypes and primers RH11 and RM3' (5'-CAGGTTCACCTACGGATACC-3') for amplifying the entire 1.4 kb SSU-rRNA gene. In short, PCR's contained 1-50 ng of extracted *Giardia* DNA; 1x Taq DNA polymerase reaction buffer consisting of 67 mM Tris-HCl, 16.6 mM $(NH_4)_2SO_4$, 0.45% Triton X-100 and 0.2 mg/ml gelatin at pH 8.8 (Biotech International Ltd., Perth, Western Australia); 2 mM $MgCl_2$, 200 µM of each deoxyribonucleotide triphosphate, 5% DMSO, 12.5 pMol of both the forward and reverse primers, 0.5 units of *Taq* polymerase (Biotech International Ltd., Perth, Western Australia) and 0.5 units of *Taq* Extender™ PCR Additive (Stratagene, Integrated Sciences, Sydney). Ultra pure water was used to make up the final volume of each reaction to 25 µl. PCR's were performed in 0.2 ml thin walled PCR tubes on a Perkin Elmer GeneAmp PCR System 2400 thermocycler. Reaction conditions consisted of a pre-incubation at 96ºC for 2 min and an initial 3

cycles of 96ºC for 20 s, 45ºC for 30 s and 72ºC for 2 min, followed by 32 cycles of 96ºC for 20 s, 55ºC for 30 s and 72ºC for 1 min with a final 72ºC extension for 7 min. Due to the size of the SSU-rDNA gene (1433 bp), amplified products were cloned prior to sequencing (see section 4.2.4 below).

4.2.2.2 *ef1α* PCR

Amplification of the *ef1α* gene was performed as previously described by Monis (1999) and generated a band of approximately 750 bp. In brief, each PCR contained 1-50 ng of extracted *Giardia* DNA; 1x Taq DNA polymerase reaction buffer consisting of 67 mM Tris-HCl, 16.6 mM $(NH_4)_2SO_4$, 0.45% Triton X-100 and 0.2 mg/ml gelatin at pH 8.8 (Biotech International Ltd., Perth, Western Australia); 4 mM $MgCl_2$; 200 µM of each deoxyribonucleotide triphosphate; 5% Dimethyl Sulphoxide (DMSO); 12.5 pMol of both the forward (GLongF 5'-GCTCSTTCAAGTACGCGTGG-3') and reverse (EF1AR 5'-AGCTCYTCGTGRTGCATYTC-3') primers; 0.5 units of *Taq* polymerase (Biotech International Ltd., Perth, Western Australia); and 0.5 units of *Taq* Extender™ PCR Additive (Stratagene, Integrated Sciences, Sydney). Ultra pure water was used to make up the final volume of each reaction to 25 µl. PCR's were performed in 0.2 ml thin walled PCR tubes on a Perkin Elmer GeneAmp PCR System 2400 thermocycler. Reaction conditions consisted of a pre-incubation at 94ºC for 2 min followed by 35 cycles of 94ºC for 30 s, 55ºC for 30 s and 72ºC for 1 min, with a final 72ºC extension for 7 min. Sequencing of the *ef1α* gene was performed directly from the amplified product without cloning as the gene (750 bp in length) could be sequenced in a single step.

4.2.2.3 Agarose Gel Electrophoresis

Agarose gel electrophoresis was carried out using 1% gels in TAE (40 mM Tris-HCl; 20 mM acetate; 2 mM EDTA; pH 7.9). Electrophoresis was performed using horizontal gels in electrophoretic cells (BioRad). Ethidium bromide was included in the gel at a final concentration of 0.15 µg/ml. After electrophoresis, DNA was visualised under UV-illumination.

4.2.3 Cloning

Multiple SSU-rDNA gene products were amplified in isolation from each other under the conditions described above (section 4.2.2.1). These PCR fragments, originating from unique PCR master mixes, were excised from their gels using sterile techniques and purified using spin columns (Qiagen, Hilden, Germany) as per manufacturer's instructions. Purified PCR products were cloned into TOP 10 F' competent cells using the TOPO™ Cloning Kit (Invitrogen, Carlsbad, California) as per manufacturer's instructions. Transformed cells were plated onto LB (Bacto-tryptone, 10 g/L; Bacto-yeast extract, 5 g/L; NaCl, 10 g/L; pH 7.0) plates containing 50 µg/ml of ampicillin. Colonies were grown up overnight (16 h) at 37ºC.

4.2.4 Automatic Sequencing

Sequencing was performed as previously described (section 3.2.5), with the exception that PCR templates were purified using spin columns (Qiagen, Hilden, Germany) and three independent sequences for both the SSU-rDNA and *ef1α* were obtained in both the forward and reverse direction prior to analysis.

4.2.5 Sequence Comparison and Phylogenetic Analysis

Sequence information for the SSU-rDNA and *ef1α* loci was obtained from Genbank for comparison with the sequence data from the Quenda *Giardia*. Accession numbers for the SSU-rDNA sequences are as follows: Quenda (AY309064); Assemblage A (group I, X52949; group II, AF199446); Assemblage B (AF199447); Assemblage C (AF199443); Assemblage D (AF199449); Assemblage E (AF199448); Assemblage F (AF199444); Assemblage G (AF199450); *G. ardeae* (Z17210); *G. muris* (X65063); *G. psittaci* (AF473853) and *G. microti* (AF006676, AF006677). Accession numbers for these *ef1α* sequences are as follows: Quenda (AY309065); Assemblage A (group I, D14342; group II, AF069573); Assemblage B (group III, AF069569; group IV, AF069570); Assemblage C (AF069574); Assemblage D (AF069575); Assemblage E (AF069571); Assemblage F (AF069572); Assemblage G (AF069568); *G. ardeae* (AF069567); *G. muris* (AF069566).

These sequences were aligned using the computer software package Clustal X (Thompson *et al.*, 1997) and the alignments manually adjusted by eye as required. Subsequent phylogenetic analysis of these sequences was performed using MEGA 2.1 (Kumar *et al.*, 2001). Based on the results of Monis *et al.* (1999), which found congruence between distance-based, parsimony and maximum likelihood analyses for *Giardia ef1α* and SSU-rDNA sequences, only distance-based analysis was conducted. Distances were estimated using the Tamura-Nei model with pair-wise deletion of missing data. Trees were constructed using the Neighbour Joining method and support for nodes was estimated by bootstrap analysis using 1,000 replicates.

4.3 RESULTS

4.3.1 Diagnosis and Morphology

Giardia cysts were detected in five of the 1230 faecal samples collected from both feral cats and a range of native mammals throughout Western Australia. *Giardia* infections were detected in one cat (*Felis catus*), one dingo (*Canis lupus dingo*), one pale field rat (*Rattus tunneyi*), one western chestnut mouse (*Pseudomys nanus*) and one quenda (*Isoodon obesulus*). The cat was collected from the Dragon Rocks Nature Reserve, whilst the dingo, *R. tunneyi* and *P. nanus* were all collected from Lake Argyle. The quenda was one of 72 collected from the south west region of Western Australia (Figure 2.1).

Measurements of 63 quenda *Giardia* cysts at ×1,000 magnification showed a size range of 10.4 to 14.3 µm in length with an average length of 12.5±0.87 µm. Cyst width varied from 6.9 to 8.8 µm with an average width of 7.7±0.47 µm. No trophozoites were detected. Attempts to culture the isolated quenda *Giardia* cysts using the methods outlined by Hopkins *et al.* (1997) failed. Whether this was due to the *Giardia* cysts being non-viable or due to the inability of the isolate to grow under the specified culture conditions is unknown.

4.3.2 Sequence Confirmation and Alignment

Sequence profiles were obtained for the *Giardia* isolates collected from the cat, dingo and quenda. However, despite multiple attempts, amplification of *Giardia* DNA at both the SSU-rDNA and *ef1α* loci was unsuccessful for the two cases of *Giardia* infection in the *R. tunneyi* and *P. nanus* collected from Lake Argyle. Therefore, despite

microscopic confirmation of *Giardia* infection in these two animals, speciation of the isolates was not possible.

Sequence data obtained from the cat, dingo and quenda were confirmed to be *Giardia* SSU-rDNA via a BLAST search of the GenBank database. Speciation and characterisation of the cat and dingo isolates, via alignment at the SSU-rDNA locus with the published sequence information available from GenBank (see section 4.2.5 for accession numbers), found the cat and dingo were harbouring *G. duodenalis* infections. Further characterisation of these two isolates at the 5' end of the SSU-rDNA identified the *G. duodenalis* present in the cat as belonging to Assemblage A and the *G. duodenalis* in the dingo belonging to Assemblage D. Sequence analysis of the *Giardia* isolate collected from the quenda at the 5' end of the SSU-rDNA identified it as a novel genotype of *Giardia* not previously reported and warranted further investigation.

DNA sequences were obtained for the quenda *Giardia* isolate for both the entire SSU-rDNA and *ef1α* loci. The sequence data obtained from the quenda *Giardia* was confirmed to be *Giardia* SSU-rDNA and *ef1α* sequence via a BLAST search of the GenBank database. Phylogenetic analysis of the SSU-rDNA (Figure 4.1) and the *ef1α* (Figure 4.2) sequences identified the quenda isolate as a novel genotype of *Giardia* not previously reported. In both cases it was placed as a lineage external to all of the previously described assemblages of *G. duodenalis*. In the case of the SSU-rDNA data, which has more published sequences for a greater range of *Giardia* species, the quenda isolate was placed external to the cluster including *G. duodenalis*, *G. microti* and *G. psittaci*. The placement of the quenda isolate in both analyses was highly supported by bootstrap analysis (100% for SSU-rDNA, 85% for *ef1α*).

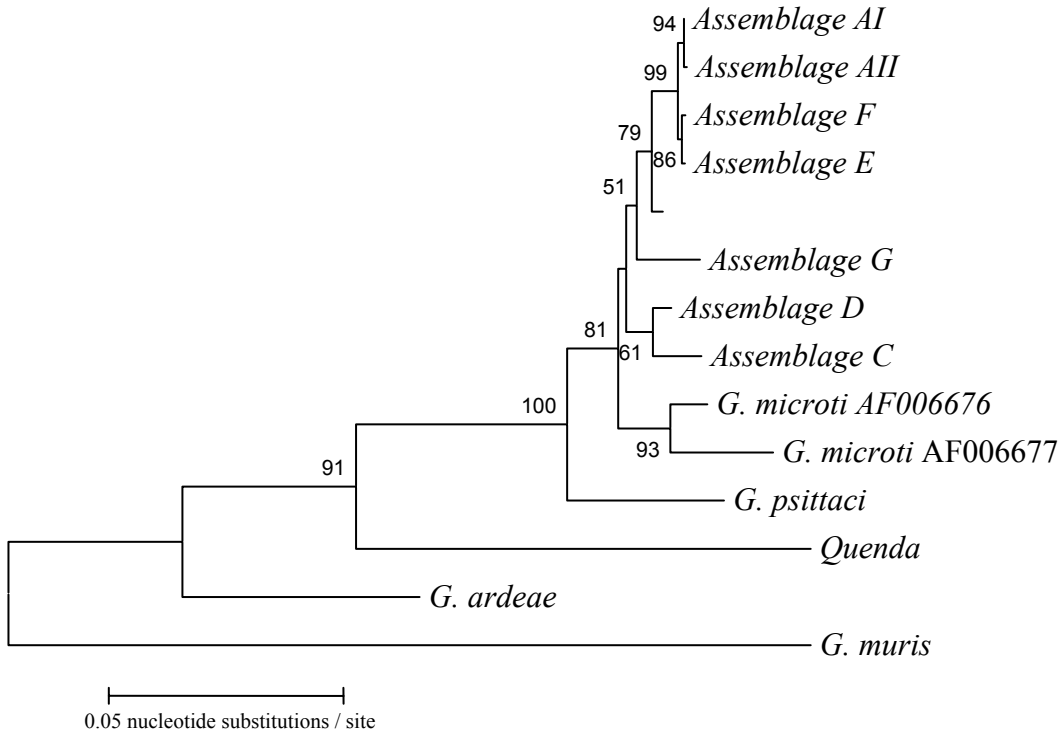

Figure 4.1 Phylogenetic relationships of *Giardia* isolates inferred by distance-based analysis of SSU-rDNA sequences. Bootstrap support (>50% for 1,000 replicates) is indicated at eachnode.

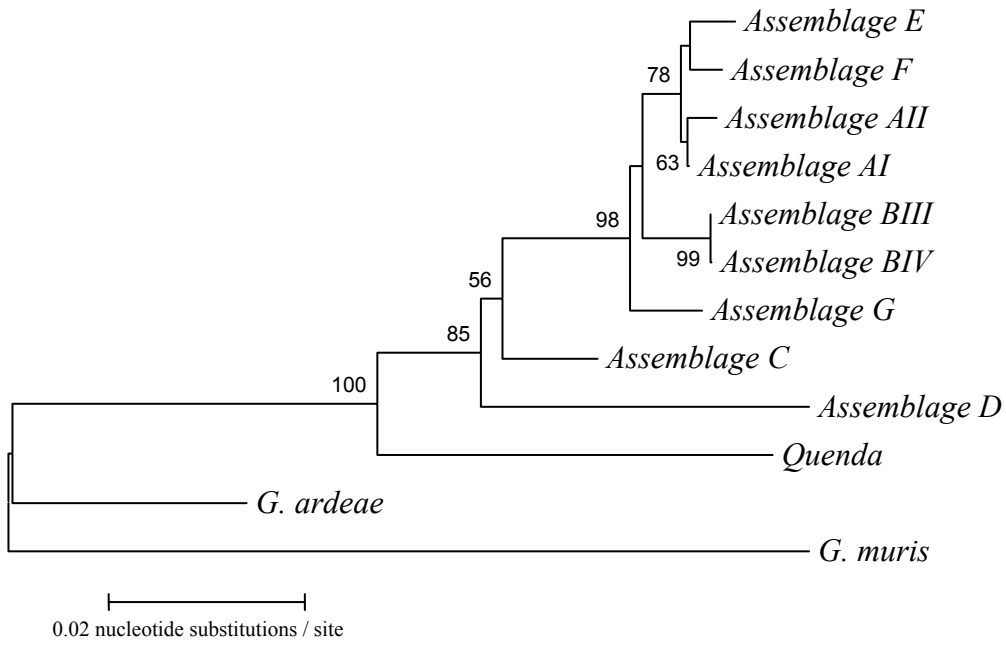

Figure 4.2 Phylogenetic relationships of *Giardia* isolates inferred by distance-based analysis of *ef1α* sequences. Bootstrap support (>50% for 1,000 replicates) is indicated at each node.

4.4 DISCUSSION

4.4.1 Epidemiology of Giardia *in Australia*

The detection of *Giardia* in only five of 1230 (0.4%) faecal samples from both native and introduced fauna suggests an extremely low prevalence of this parasite in Western Australia. However, McGlade *et al.* (2003) showed that detection of *Giardia* infections in domestic cats via microscopy was highly insensitive when compared to PCR or coproantigen detection methods. Presumably this finding is unaffected by host species as the shedding of *Giardia* cysts from an infected host is typically intermittent and repeat sampling of individuals is generally required to obtain an accurate assessment of prevalence (O'Handley *et al.*, 1999; Meloni *et al.*, 1993). It has been estimated that between 15% and 50% of *Giardia* infections can go undetected if only one stool sample is examined per individual for the presence of cysts (Goka *et al.*, 1990; Danciger and Lopez, 1975). For this study it was not possible to obtain repeat samples from individual animals and as such it is not unreasonable to assume that the prevalence of *Giardia* infections in both native and introduced animals in Western Australia would be higher than reported in this study.

The investigation of *Giardia* occurrence in Australian wildlife has been largely limited to the study of native fauna, dogs and cats in Tasmania, primarily due to the potential risk zoonotic transmission poses to the tourist industry (Kettlewell *et al.*, 1998). A survey for *Giardia* infections by Bettiol *et al.* (1997) on 295 native animals collected across Tasmania found an average prevalence of 21%. The higher *Giardia* prevalence detected in Tasmanian marsupials by Bettiol *et al.* (1997) in comparison to the present study can be attributed to their use of a coproantigen detection test (Hopkins *et al.*, 1993) in addition to microscopy, which helps overcome the detection problems

associated with the intermittent nature of cyst shedding and low infection levels in infected hosts (McGlade *et al.*, 2003).

Bettiol *et al.* (1997) and Kettlewell *et al.* (1998) showed that *Giardia* appears to be a common parasite occurring in both native and introduced animals in Tasmania. Bettiol *et al.* (1997) detected the highest prevalence of *Giardia* in bandicoots (*Isoodon obesulus* and *Perameles gunnii*), with 16 of 26 animals infected. In the present study, only one of 77 quenda examined was found to be shedding *Giardia* cysts, though this difference in prevalence may be due to intermittent shedding as previously discussed. Additionally, Bettiol *et al.* (1997) demonstrated that eastern barred bandicoots (*Perameles gunnii*) were capable of becoming infected with and excreting cysts of human source *G. duodenalis*. These bandicoots displayed no clinical symptoms, nor suffered from diarrhoea or weight loss, however the ease with which these animals became infected with *Giardia* of human origin together with the apparent prevalence of *Giardia* in Tasmania's native animals raises concern over the potential zoonotic role wildlife may play in *Giardia* transmission.

The role of wildlife as a reservoir of human giardiasis has been demonstrated as occurring with beavers in a waterborne outbreak of *G. duodenalis* in Canada (Isaac-Renton *et al.*, 1993), with the greatest zoonotic risk believed to be from *Giardia* belonging to Assemblage A, and to a lesser extent Assemblage B (Monis and Thompson, 2003; Thompson, 2000). These genotypes have been found to occur in farm animals, pets and some wild animals, though whether animals act as reservoir hosts for humans or vice versa is unclear (van Keulen *et al.*, 2002; O'Handley *et al.*, 2000; Thompson *et al.*, 2000a). However, animal-specific genotypes appear to be host-

adapted, and so far have been restricted to livestock, dogs, cats and rodents, and have not been detected in humans (Thompson *et al.*, 2000a; van Keulen *et al.*, 1998; Ey *et al.*, 1997).

Epidemiological studies of human giardiasis suggests that humans are most likely to be the main reservoir for infection and that person to person contact is a major route of transmission and most likely plays a more important role than zoonotic transmission in human infection (Robertson *et al.*, 2000; Schantz, 1991). However, studies have demonstrated that dogs and cats regularly harbour *Giardia* infective to humans (Hopkins *et al.*, 1997; Monis *et al.*, 1996; Mayrhofer *et al.*, 1995; Meloni *et al.*, 1995). This is exemplified by the finding of *G. duodenalis* in a cat from Dragon Rocks which was shown to belong to Assemblage A, the genotype most commonly found infecting humans and considered the greatest zoonotic risk (Thompson *et al.*, 2000a; Thompson *et al.*, 2000b).

In contrast, the *G. duodenalis* isolate found in a dingo from Lake Argyle in the north of Western Australia belonged to Assemblage D, which is commonly found infecting dogs (Thompson *et al.*, 2000a; Monis *et al.*, 1998). Both Assemblage A and Assemblage D genotypes have been reported to occur frequently in dogs from remote Aboriginal communities in the north of Western Australia (Hopkins *et al.*, 1997), though experimental evidence suggests that the Assemblage D genotype will, by competitive exclusion, out-compete other genotypes from Assemblage A, thus preventing their colonisation of the dog intestine (Thompson *et al.*, 1996). Therefore the diagnosis of the Assemblage D genotype of *G. duodenalis* in a dingo from Lake Argyle is not surprising given the frequency with which Aboriginal camp dogs in this region are

infected by *Giardia* (Hopkins *et al.*, 1997; Meloni *et al.*, 1995) and the potential for interaction between both dingoes and dogs in this environment. Clearly, cats and dogs may act as carriers of *Giardia* not only for humans, but may also provide opportunities for infection of native animals due to their encroachment on native habitat (Kettlewell *et al.*, 1998).

The investigation of cross-transmission of *Giardia* infections ideally requires the examination of the dynamics of *Giardia* transmission between hosts living in the same geographical area (Hopkins *et al.*, 1997; Thompson and Lymbery, 1996), as the occurrence of similar genotypes of *Giardia* in different host species is not by itself conclusive evidence that zoonotic transmission is taking place (Thompson, 2000). As such, the detection of *Giardia* in *R. tunneyi* and *P. nanus* collected from the same region as the dingo mentioned above was of great interest. However, despite microscopic confirmation of the presence of *Giardia* cysts in the faecal samples collected from these two rodents, molecular characterisation was not possible. Due to the small quantity of faecal material obtained from *R. tunneyi* and *P. nanus*, salt/sucrose gradient purification (Meinema, unpublished) could not be applied, and it is presumed that PCR inhibitors, common in faecal samples (Ghosh *et al.*, 2000), combined with the small quantity of faecal material contributed to the failure to extract *Giardia* DNA of sufficient quality to allow PCR amplification.

4.4.2 Novel Genotype or New Species?

Sequence analysis of the quenda *Giardia* isolate at the SSU-rDNA and *ef1α* loci show it to be clearly distinct from the sequences of all the other *G. duodenalis* isolates from humans and other mammals (Figure 4.1, 4.2). Similarly, the quenda *Giardia* isolate is

also distinct from the other recognised species *G. microti*, *G. psittaci*, *G. ardeae* and *G. muris* at the SSU-rDNA locus as well as being distinct from the species *G. ardeae* and *G. muris* at the *ef1α* locus. Therefore, the sequence information obtained from this isolate clearly demonstrates that the quenda *Giardia* isolate is more distantly related to *G. duodenalis* than either *G. microti* or *G. psittaci*, and should be classified as a new species of *Giardia*.

Cyst measurements of the quenda *Giardia* isolate (10.4 to 14.3 μm) fall within those previously recorded for *G. duodenalis* (Garcia, 1998). However, the size of *Giardia* cysts (and presumably the size of trophozoites as well) in both humans and dogs have been shown to vary significantly from day to day according to host diet and different environmental conditions (Tsuchiya, 1931, 1930). It has also been argued that whilst cyst morphology may be able to distinguish between populations of *Giardia* in two hosts of the same species, it is inadvisable to use differences in size alone as a means of species distinction within the genus *Giardia* (Filice, 1952). Indeed, it is the lack of reliably consistent differences between the *G. duodenalis* assemblages A and B that is retarding their recognition as distinct species (Thompson *et al.*, 2000b), even though it has been demonstrated that the genetic distance separating them exceeds that used to delineate other species of protozoa (Monis and Thompson, 2003; Monis *et al.*, 1996; Mayrhofer *et al.*, 1995; Andrews *et al.*, 1989). This indicates that genetic and biological data may be more reliable than morphology when delineating species of *Giardia*.

The distinctiveness of the quenda *Giardia* isolate also raises the issue of whether or not this is a potentially new zoonotic species or if it has a limited or specific host range adapted to the Australian wildlife. The latter seems more likely, given that it has never

before been isolated from humans or domestic animals and host-adapted genotypes such as the quenda *Giardia* isolate appears to be, have not been identified in humans and consequently do not appear to represent a risk to public health (Thompson *et al.*, 2000a; van Keulen *et al.*, 1998; Ey *et al.*, 1997). As such, if the quenda *Giardia* isolate is an endemic species that has evolved within the Australian fauna over millions of years, it raises the issue of whether it is the same as the *Giardia* previously found by Bettiol *et al.* (1997) in Tasmanian marsupials.

4.4.3 Epidemiological Importance of Molecular Characterisation

The dispersal of similar genotypes of *Giardia* throughout different host species is not by itself conclusive evidence that zoonotic transmission is occurring (Thompson *et al.*, 2000b). As such, selected wildlife species have been scrutinised as potential sources of *Giardia* infection for humans in North America and Canada for several years (Measures and Olson, 1999; Graczyk *et al.*, 1998; Olson *et al.*, 1997a; Wallis *et al.*, 1996; Erlandsen *et al.*, 1990; Isaac-Renton *et al.*, 1987; Monzingo and Hibler, 1987). However, the advent of molecular characterisation has shown these animals harbour host specific genotypes which are not zoonotic threats (Thompson *et al.*, 2000b; Hopkins *et al.*, 1999; Monis *et al.*, 1998; van Keulen *et al.*, 1998; Ey *et al.*, 1997; Hopkins *et al.*, 1997). Therefore, the role of wildlife in the transmission of *Giardia* is not necessarily one of a reservoir for human infection.

Kettlewell *et al.* (1998) and Bettiol *et al.* (1997) speculated that the occurrence of *Giardia* in native marsupials from Tasmania was linked to the contamination of the environment by humans and introduced animals. However, genetic characterisation of these isolates was not performed and without genotyping the *Giardia* recovered from

both native and introduced wildlife it is not possible to accurately establish a potential transmission cycle. Genotyping of *Giardia* isolates provides a powerful predictive tool for providing direct evidence of zoonotic transmission as well as determining sources of infection in outbreak conditions (Thompson *et al.*, 2000b). The use of such tools on the quenda *Giardia* isolate has demonstrated that the isolate is genetically distinct enough to be placed outside the *G. duodenalis* group, among the other recognised species of *Giardia*.

This finding is of interest from both a conservation and epidemiological viewpoint as previous reports of *Giardia* in Australian native mammals are extremely limited and more commonly attributed to genotypes of *G. duodenalis* (Kettlewell *et al.*, 1998; Bettiol *et al.*, 1997). The information reported here supports the hypothesis of this being a distinct species of *Giardia* that has persisted in the Australian native fauna and possibly evolved with it over the last 40 million years since Australasia split from Gondwanaland. If this is the case, it could be extremely helpful in understanding the origin and evolution of *Giardia* (Simpson *et al.*, 2002). It also highlights our lack of knowledge concerning the Australian fauna and its parasites (Adlard and O'Donoghue, 1998), as research into the transmission of *Giardia* and similar parasites within Australia to date has focussed primarily on domestic and companion animals in regard to zoonotic transmission (O'Handley *et al.*, 2000; Olson *et al.*, 1997b; Meloni *et al.*, 1993; Collins *et al.*, 1987; Swan and Thompson, 1986). Hopefully the discovery of this new species will encourage further investigation into the taxonomy, infection and transmission of *Giardia* in Australian wildlife.

Chapter 5 –Occurrence and Distribution of *Toxoplasma gondii* in Feral Cats and Native Mammals from Western Australia: Comparison of Serology and PCR Detection Methods

5.1 INTRODUCTION

5.1.1 What is Toxoplasma gondii*?*

Toxoplasma gondii is a ubiquitous, obligate intracellular coccidian parasite that occurs in most areas of the world and is of both veterinary and medical importance worldwide due to its implication in abortion and congenital disease in its intermediate hosts (Dubey *et al.*, 1998; Dubey and Beattie, 1988). Virtually all species of warm-blooded animals including humans can be infected by *T. gondii*, though only cats and other members of the family Felidae are the definitive hosts (Dubey and Lappin, 1998). There are three infectious stages: sporozoites in oocysts, generally found as a contaminant of food or water; tachyzoites, the actively multiplying stage present in intermediate host tissue; and bradyzoites, the slowly multiplying stage enclosed in tissue cysts (Tenter *et al.*, 2000; Dubey and Lappin, 1998). Oocysts are excreted in faeces only by members of the Felidae, whereas tachyzoites and bradyzoites can be found in tissues of both definitive and intermediate hosts (Dubey and Lappin, 1998).

5.1.2 Toxoplasma gondii *Life Cycle*

The life cycle of *T. gondii* can be divided into two stages, the enteroepithelial life cycle (occurring only in the definitive feline host) and the extraintestinal life cycle (occurring in all hosts including cats).

5.1.2.1 Enteroepithelial Life Cycle

The Enteroepithelial life cycle occurs only in members of the Felidae with most cats thought to become infected with *T. gondii* through the ingestion of infected intermediate hosts (Dubey and Lappin, 1998). Bradyzoites are released from tissue cysts into the stomach and small intestine following their ingestion by a member of the Felidae (Dubey, 1998b; Dubey and Lappin, 1998), whereby they undergo asexual proliferation followed by sexual reproduction and oocyst formation in the epithelium of the small intestine (Tenter *et al.*, 2000; Dubey *et al.*, 1998). Infected epithelial cells rupture and discharge unsporulated oocysts into the intestinal lumen, which are then excreted into the environment with the faeces (Dubey and Lappin, 1998).

Sporulation of *T. gondii* oocysts occurs in the environment within one to five days, and like other coccidial parasites is dependent on temperature and moisture conditions (Jackson and Hutchison, 1989; Dubey *et al.*, 1970). Oocyst excretion in cats generally lasts for one to three weeks (Dubey and Frenkel, 1972a) with up to 1 million oocysts shed per gram of faeces (Frenkel *et al.*, 1995). Local soil contamination can be as high as 100,000 oocysts/g of soil (Frenkel *et al.*, 1995), with sporulated *T. gondii* oocysts able to survive in the environment for up to 1.5 years (Frenkel *et al.*, 1975).

The prepatent period (time to the shedding of oocysts after initial infection) and frequency of oocyst shedding varies according to the stage of *T. gondii* ingested (Dubey, 1996; Freyre *et al.*, 1989; Dubey and Frenkel, 1976). The shortest prepatent period in cats (3 to 10 days) is experienced after infection via ingestion of tissue cysts (Dubey, 1998a), whilst production of oocysts can be delayed up to 18 days or more following ingestion of oocysts or tachyzoites (Dubey, 1996). Fewer than 30% of cats shed oocysts after ingesting tachyzoites or oocysts, whereas nearly all cats shed oocysts after ingesting tissue cysts (Dubey, 1996; Dubey and Frenkel, 1976). Tissue cysts generally persist for the life of the cat and subsequent shedding of oocysts has been reported to occur sporadically (Dubey, 1977).

5.1.2.2 Extraintestinal Life Cycle

The extraintestinal development of *T. gondii* is the same for all hosts and is not dependent on whether tissue cysts or oocysts are ingested (Dubey and Lappin, 1998). After the ingestion of oocysts, sporozoites excyst in the lumen of the small intestine and penetrate the intestinal cells where they undergo asexual development to become tachyzoites (Tenter *et al.*, 2000; Dubey and Lappin, 1998). Tachyzoites are capable of multiplying rapidly in many different types of host cells for an undetermined period of time, before initiating a second phase of development which results in the formation of tissue cysts containing bradyzoites (Dubey and Lappin, 1998). Tissue cysts may develop in intermediate hosts as early as 6-7 days after infection by either oocysts or other tissue cysts (Dubey *et al.*, 1998).

Tissue cysts have a high affinity for neural and muscular tissues and are located predominantly in the central nervous system (CNS) and the eye as well as skeletal and

cardiac muscles of infected hosts (Dubey *et al.*, 1998). They may also be found in visceral organs, such as the lungs, liver and kidneys (Tenter *et al.*, 2000; Dubey *et al.*, 1998). Tissue cysts are the terminal life cycle stage in the intermediate host and are immediately infectious. They generally persist for the life of the host, however it is believed that tissue cysts breakdown periodically in hosts, releasing bradyzoites which transform into tachyzoites to reinvade host cells and form new tissue cysts (Dubey, 1998a; Dubey *et al.*, 1998).

5.1.3 Transmission of Toxoplasma gondii

T. gondii is capable of undergoing both horizontal and vertical transmission within both intermediate and definitive hosts (Figure 5.1) (Dubey *et al.*, 1997b; Dubey *et al.*, 1995a; Dubey and Carpenter, 1993; Beverley, 1959). Because *T. gondii* can be transmitted by multiple sources, it is difficult to establish the definite mode of transmission on an individual basis. Therefore, it is currently not known which of the various routes of transmission is more important, however epidemiologic evidence suggests that cats are

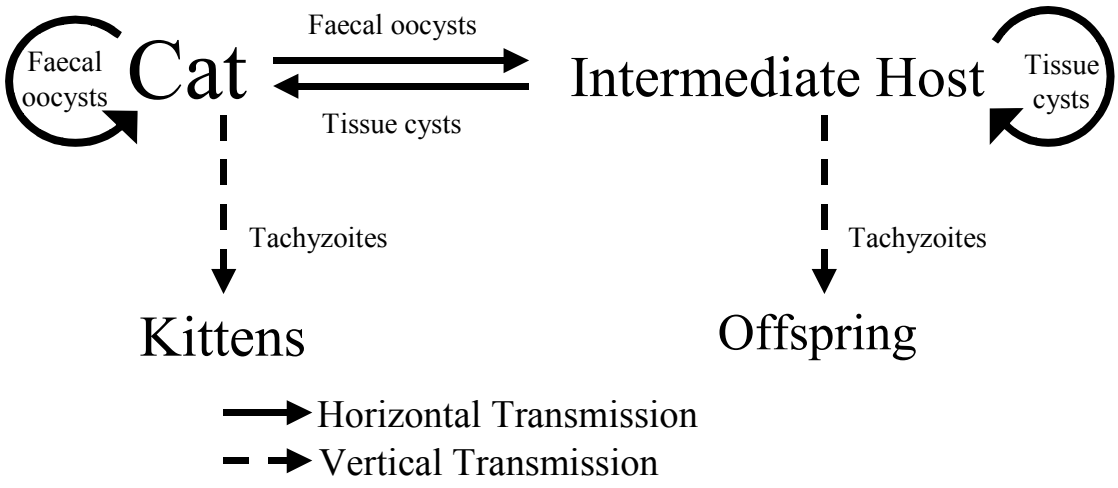

Figure 5.1 Transmission cycle of *T. gondii*.

ultimately essential for the maintenance of *T. gondii* in the environment (Dubey *et al.*, 1997a; Frenkel and Ruiz, 1981; Munday, 1972; Wallace *et al.*, 1972).

There are three infectious stages in the life cycle of *T. gondii*: tachyzoites; bradyzoites contained in tissue cysts; and sporozoites contained in sporulated oocysts. All three stages are infectious for both intermediate and definitive hosts which may acquire a *T. gondii* infection mainly via one of the following routes: a) horizontally by oral ingestion of infectious oocysts from the environment, b) horizontally by oral ingestion of tissue cysts contained in raw or undercooked meat or primary offal (viscera) of intermediate hosts, or c) vertically by transplacental transmission of tachyzoites (Dubey *et al.*, 1998; Dubey, 1991; Jackson and Hutchison, 1989; Dubey and Beattie, 1988). Additionally, transmission of tachyzoites from mothers to offspring via their milk has been observed in several hosts (Powell *et al.*, 2001; Tenter *et al.*, 2000; Jackson and Hutchison, 1989; Dubey and Beattie, 1988), whilst other minor modes of transmission involve transfusion of bodily fluids and transplantation of organs (Dubey and Beattie, 1988).

5.1.3.1 Ingestion of Oocysts

Grazing animals and birds can directly ingest oocysts as contaminants of pasture, hay, grain or water supplies. Humans are at risk through gardening in contaminated areas and through external contamination of fresh vegetable material (Dubey, 1986; Rothe *et al.*, 1985; Hartley and Munday, 1974). Insects such as flies and cockroaches can act as mechanical transport hosts by carrying oocysts on their feet and mouthparts (Hartley and Munday, 1974; Wallace, 1973, 1972, 1971).

5.1.3.2 Ingestion of Tissue Cysts

All meat eating animals, including predatory birds and humans can be infected when tissues containing viable *T. gondii* cysts are ingested (Dubey, 1986; Rothe *et al.*, 1985). This route of infection is also important in terms of human exposure (Tenter *et al.*, 2000). *T. gondii* tissue cysts are common in meat producing animals such as pigs, sheep and goats, and less frequently in poultry, rabbits and horses (Tenter *et al.*, 2000). Although *T. gondii* tissue cysts can be destroyed by heating to 70ºC for ten minutes, the mere searing of meat (rare) will not destroy the organisms (Dubey, 1986; Rothe *et al.*, 1985).

5.1.3.3 Congenital Transmission

Traditionally, toxoplasmosis is of most importance to previously non-infected women of childbearing age due to the risk of transplacental infection of the foetus during pregnancy (Jones *et al.*, 2001). Following ingestion of *T. gondii* via oocyst contamination of food or water, or the ingestion of tissue cysts, tachyzoites can multiply in the placenta and foetus (Cook *et al.*, 2000; Tenter *et al.*, 2000). Congenital toxoplasmosis is known to cause abortion, neonatal death, or foetal abnormalities with detrimental effects such as the development of blindness or mental retardation (Tenter *et al.*, 2000; Dubey and Beattie, 1988). The estimated economic burden of *T. gondii* infections in the United States, primarily from congenital toxoplasmosis, is $7.7 billion each year (Jones *et al.*, 2001). Most human infections however are sub-clinical and the majority of people postnatally infected with *T. gondii* will go through life with no adverse effects.

T. gondii infections are generally more widespread in sheep and are a major economic cost to the industry due to high levels of abortion in flocks (Dubey and Beattie, 1988). The extent of *T. gondii* infection in sheep populations varies widely, with infection rates for individual flocks of sheep ranging from 5% to 95% (Blewett, 1983). Whilst current views consider congenital transmission of *T. gondii* in sheep to be insignificant (Blewett and Watson, 1983), a recent study by Duncanson *et al.* (2001) has shown congenital transmission to occur in 61% of pregnancies in a commercial sheep flock. This study has highlighted the significance of vertical transmission in the persistence of *T. gondii* and is causing the re-evaluation of earlier studies which demonstrated the importance of vertical transmission in maintaining the parasite in populations of intermediate hosts (Owen and Trees, 1998; de Roever-Bonnet, 1969; Remington *et al.*, 1961; Beverley, 1959).

Congenital toxoplasmosis in cats is also common, with the severity of the infection varying with the stage of gestation at the time of infection. Many kittens born to queens infected with *T. gondii* during gestation become infected transplacentally or via suckling (Dubey *et al.*, 1995a; Dubey and Carpenter, 1993; Sato *et al.*, 1993). Much the same as with sheep and other hosts, *T. gondii* infections are most severe in transplacentally infected kittens, causing stillbirths as well as mortality prior to weaning (Dubey *et al.*, 1995a; Dubey and Carpenter, 1993).

5.1.4 Pathogenesis

It is not fully understood why some infected animals develop clinical toxoplasmosis whilst others do not. Age, sex, host species, strain of *T. gondii*, number of organisms and stage of the parasite ingested may all account for some of the differences, whilst

stress, concomitant illness and immunosuppression may also increase host susceptibility to *T. gondii* (Dubey and Lappin, 1998). Symptoms of acute toxoplasmosis can include fever, coughing, pneumonia, mastitis, abortion and stillbirths (Dubey, 1994). In the sub-clinical form, symptoms may be absent or inapparent with a degree of immunity being acquired by the host (Liesenfeld, 1999; Davis and Dubey, 1995; Dubey, 1995). Acute cases may produce inflammation in heart muscle, liver and skin (rash) with localised swelling of lymph nodes as well as lethargy, weight loss, vomiting and diarrhoea (Dubey and Lappin, 1998; Dubey *et al.*, 1995a; Dubey and Carpenter, 1993).

Tachyzoites are the invasive asexual forms of the parasite that require intracellular existence for replication and survival, (Carruthers, 2002; Dubey and Lappin, 1998). Cell necrosis is common at localised sites of infection and is due to the intracellular growth of *T. gondii* (Dubey and Lappin, 1998). In infections acquired after the ingestion of tissue cysts or oocysts, initial clinical signs are usually due to the necrosis of intestinal epithelium and associated lymphoid organs caused by tachyzoite proliferation (Dubey and Lappin, 1998). Tachyzoites are spread to extraintestinal organs via blood or lymph, with the brain, liver, lungs, skeletal muscle and eyes being common sites for the chronic persistence of infection (Dubey and Lappin, 1998; Dubey *et al.*, 1998).

Approximately three weeks after infection tachyzoites begin to disappear from visceral tissues and may localise as tissue cysts which persist in the host for life, though intermittent relapses may occur if the host becomes immunosuppressed or highly stressed (Dubey and Lappin, 1998). The clinical outcome of the infected host is determined by the extent of injury it sustains to its internal organs, particularly heart,

lung, liver and adrenal glands (Dubey and Lappin, 1998). Generally, most host species will recover from an infection, though acute disseminated *T gondii* infections can often be fatal (Dubey and Lappin, 1998).

5.1.5 Toxoplasma gondii *in the Australian Fauna*

Toxoplasmosis is a common cause of death in captive and wild Australian marsupials, especially macropods and bandicoots (Reddacliff *et al.*, 1993; Lenghaus *et al.*, 1990; Obendorf and Munday, 1990, 1983). Deaths due to *T. gondii* have also been reported in wild possums (Cook and Pope, 1959), captive dasyurids (Attwood *et al.*, 1975), captive wombats (Munday, 1988; Arundel *et al.*, 1977), koalas (Hartley *et al.*, 1990) and echidnas (McOrist and Smales, 1986). Clinical signs of toxoplasmosis in marsupials include sudden death, lethargy, inappetence, respiratory distress, diarrhoea and neurological disturbances (Canfield *et al.*, 1990; Obendorf and Munday, 1990). Lesions can be present in many organs, but the main changes in marsupials are usually in the brain and lungs (Munday, 1988).

Native animals have been reported in the wild as being blinded, stumbling, and dying from toxoplasmosis (Obendorf *et al.*, 1996; Obendorf and Munday, 1990). Wild animals infected with *T. gondii* may exhibit pathology including obscured vision and difficulty in walking, though these general symptoms are not specific to *T. gondii* infections (Reddacliff *et al.*, 1993; Ashton, 1979). Toxoplasmosis often occurs in captive marsupials causing outbreaks of sudden death with no apparent symptoms, though respiratory and nervous signs are sometimes observed (Reddacliff *et al.*, 1993). Whilst deaths from toxoplasmosis may constitute a major form of mortality for some

native mammals (Obendorf *et al.*, 1996), attributing mortalities exclusively to *T. gondii* infections is difficult to achieve.

Tissue cysts are often described from latent infections and their detection has previously been used to estimate the prevalence of *Toxoplasma* infection in wild populations of macropods (Gibb *et al.*, 1966). However, failure to detect them microscopically does not eliminate the possibility of infection. Often there are no gross lesions in macropods dying with acute toxoplasmosis and those that are present are generally not specific for *T. gondii* (Canfield *et al.*, 1990). In many cases it may be impossible to determine the nature of the infection on pathology alone (Reddacliff *et al.*, 1993), and as a consequence toxoplasmosis is often overlooked or misdiagnosed in Australian marsupials.

5.1.6 Toxoplasma gondii *Detection Methods*

Tachyzoites may be detected directly in various tissues and body fluids by cytology during acute illness, though they are rarely found (Dubey and Lappin, 1998). Additionally, despite a worldwide seroprevalence of over 30% in naturally infected cats, studies have estimated that less than 1% of cats shed oocysts on any given day (Dubey and Lappin, 1998; Dubey and Beattie, 1988). Because cats usually only shed *T. gondii* oocysts for 1 to 2 weeks after their first exposure, oocysts are rarely found in routine faecal examination and cats do not normally experience diarrhoea or clinical symptoms during this period of shedding either (Dubey and Lappin, 1998).

Oocysts of *T. gondii* are also morphometrically indistinguishable from oocysts of *Hammondia hammondi* and *Besnoitia darlingi*, which also occur in cats (Dubey and

Lappin, 1998). Therefore, the accurate identification and detection of *T. gondii* infection in both intermediate and definitive hosts is achieved using bioassay, serology or molecular tools, each of which has their own advantages and disadvantages. Bioassay involves the inoculation of animals or cell cultures with suspected *T. gondii* infected tissue(s) or suspected *T. gondii* oocysts (Dubey and Lappin, 1998). This method is understandably complicated, cumbersome and expensive for routine screening and was not used in the present study.

5.1.6.1 Serology

Serological testing is the most commonly used technique for supporting the clinical diagnosis of toxoplasmosis. Numerous serological tests are available for the diagnosis of *T. gondii* infections in most host species, however no single serological assay exists that can definitively confirm or refute toxoplasmosis in a host (Dubey and Lappin, 1998). The detection of different classes of antibodies against *T. gondii* show that IgM, IgG and IgA responses to infection overlap and can be detected in both healthy and clinically ill animals (Lappin, 1996). There is no definite pattern to *T. gondii*-specific IgM, IgG and IgA serological responses over time that can be used to reliably document recent infection or clinical disease using individual serum samples (Lappin, 1996).

Many animals suffering from toxoplasmosis have low-grade clinical signs and therefore may not be serologically evaluated until antibody titres have already reached maximal levels, which makes it very difficult to interpret serum antibody titres (Lappin, 1996). Because *T. gondii* specific antibodies occur in the serum of both healthy and diseased animals, results of serologic tests cannot independently prove clinical toxoplasmosis (Dubey and Lappin, 1998). *T. gondii* specific IgM is also occasionally detected in the

serum of animals with chronic or reactivated infection and does not always correlate with recent exposure (Dubey and Lappin, 1998). As such, serological test results can be interpreted in the following ways:

A positive result indicates one of four things:

1) A current infection;
2) A previous infection to which the host is now immune;
3) Cross-reaction with shared antibody from another infection; or
4) The presence of antibodies transferred from mother to young.

Whilst a negative result also indicates one of four things:

1) The individual is not and never was infected;
2) The infection is so recent that detectable antibody responses have not yet developed;
3) The host was previously infected, but immunity was short-term and antibodies are no longer present in detectable quantities; or
4) The host is or was infected but was not capable of producing antibodies against the infection (Scott, 1988).

5.1.6.2 Molecular Diagnosis

The use of PCR to amplify and subsequently detect DNA from micro-organisms in a range of tissues has proven extremely valuable, particularly for pathogens that are difficult to culture or for which sample volumes are generally small (Stiles *et al.*, 1996; MacPherson and Gajadhar, 1993). As such, molecular methods have been investigated to aid in the clinical management of toxoplasmosis. The ability to identify *T. gondii* DNA in biological samples provides direct evidence of the presence of the organism as opposed to serology, which is dependent upon a host response (Jones *et al.*, 2000). A

limitation of PCR techniques however, is that they cannot distinguish between active and chronic *T. gondii* infections, nor do they provide information on the viability of the parasite (Lee *et al.*, 1999), though the recent development of *T. gondii* quantitative PCR assays aim to overcome this obstacle (Jauregui *et al.*, 2001; Costa *et al.*, 2000; Homan *et al.*, 2000; Lin *et al.*, 2000).

The ability of PCR-based techniques to provide a sensitive answer in less than one day makes them effective tools for early diagnosis, whilst the detection of *T. gondii* in body fluids or tissues offers a more specific means of documenting infections than does serum antibody titres (Stiles *et al.*, 1996). Additionally, PCR has been used to make the definitive diagnosis of toxoplasmosis from a wide range of biological samples including: ocular fluid (Burney *et al.*, 1998); amniotic fluid (Guy *et al.*, 1996); CSF and tissues (Cingolani *et al.*, 1996; van de Ven *et al.*, 1991); bronchoalveolar lavage fluid (Bretagne *et al.*, 1993); as well as lymph and blood samples (Lamoril *et al.*, 1996; Dupon *et al.*, 1995; Ho-Yen *et al.*, 1992).

However, no single diagnostic technique is infallible and PCR, much like histopathology, can only detect an infection if *T. gondii* is present in the sample collected. The risk of false negatives is related to the sparse and focal distribution of *T. gondii* tissue cysts and low-level infections, which may result in the absence of the parasite in a sample even though it was collected from and infected individual.

5.1.7 Detection of Toxoplasma gondii *in Biological Samples*

Whilst the detection of *T. gondii* antibodies is the standard diagnostic test used for antemortem diagnosis of toxoplasmosis, the presence of antibodies does not

automatically infer disease or infection (Dubey and Lappin, 1998). Additionally, the extrapolation of serological techniques designed for human applications to veterinary medicine creates problems with sensitivity and specificity, thus compromising the reliability of these techniques (MacPherson and Gajadhar, 1993). As such, direct detection of *T. gondii* in body fluids or tissues offers a more specific and sensitive means of documenting infection than do serum antibody titres, however there is no agreement as to the preferred PCR target gene for the diagnosis of toxoplasmosis in either humans or animals.

T. gondii PCR amplification protocols have centred around three well conserved gene targets: the rDNA genes (Fazaeli *et al.*, 2000; Guay *et al.*, 1993; MacPherson and Gajadhar, 1993); the P30 gene, which codes for the major surface antigen of the tachyzoite (Lee *et al.*, 1999; Nguyen *et al.*, 1996); and the B1 gene, which is a 35-fold repetitive gene of unknown function (Muller *et al.*, 1996; Stiles *et al.*, 1996; Guy and Joynson, 1995; van de Ven *et al.*, 1991; Burg *et al.*, 1989). The B1, P30 and 18S rDNA genes are most widely used due to their well-conserved nature within the *T. gondii* species. However, the B1 gene has been shown to be highly specific and more sensitive in the detection of *T. gondii* in clinical samples than the P30 and 18S rDNA genes (Jones *et al.*, 2000; Holmdahl *et al.*, 1994).

The B1 gene has been widely researched and used for the detection of *T. gondii* (Grigg and Boothroyd, 2001; Jones *et al.*, 2000; Ellis, 1998; Owen and Trees, 1998; Pelloux *et al.*, 1996; Burg *et al.*, 1989), and has previously been shown to be comparable to traditional *T. gondii* detection methods (van de Ven *et al.*, 1991). The repetitive nature of the B1 gene allows PCR detection of as little as 50 fg of *T. gondii* DNA (Jones *et al.*,

2000), which has been estimated as the amount of DNA contained within a single *T. gondii* organism (Wright and Manos, 1990). Being well conserved, the B1 gene allows the amplification of all strains of *T. gondii*, without any cross reaction with closely related parasitic organisms, nor human, bacterial or fungal species (Jones *et al.*, 2000).

Due to the sensitive and specific nature of the B1 gene, a hemi-nested PCR protocol directed at this site has been developed (Pujol-Rique *et al.*, 1999). This protocol represents a potentially robust molecular diagnostic test for the detection of *T. gondii* infection in biological samples. This study was designed to investigate the diagnostic potential of this technique on a broad range of host species from the Australian fauna. As such, the aims of this study were to:

1. Compare the sensitivity of the molecular testing to that of diagnostic serology;
2. Determine the applicability of the molecular test as a diagnostic tool for the detection of acute and chronic *T. gondii* infections in biological samples from feral cats and Australian native fauna;
3. Use the single tube hemi-nested PCR to screen tissue samples collected from feral cats and native fauna throughout Western Australia for the presence of *T. gondii* and to investigate the potential risk toxoplasmosis poses to the native fauna.

5.2 MATERIALS AND METHODS

5.2.1 Tissue Samples and Collection

5.2.1.1 Clinical Samples

Tissue samples consisting of brain, liver, spleen, muscle, skin lesions and lymph nodes were collected from a naturally infected cat presented to the Murdoch University Veterinary Hospital suffering from acute toxoplasmosis. Brains from experimentally infected mice and purified *T. gondii* RH strain tachyzoites were provided courtesy of Professor Alan Johnson (UTS, Sydney). These samples were used to test the sensitivity and specificity of the one-tube hemi-nested PCR protocol.

Additional tissue samples were collected from a limited number of native and introduced animals that were known to have either an acute or chronic *T. gondii* infection diagnosed by either serological or histopathological detection, as well as from animals with no history of infection that were presented with possible symptoms of toxoplasmosis. Samples collected included brain, spleen, liver, lung, kidney, muscle, spinal cord, tongue, heart, lymph node and skin lesion. However, the conditions under which some of the samples were collected were such that not all tissues could be collected from all animals.

Sample animals consisted of: a ferret (*Mustela putorius furo*) submitted to the veterinary clinic suffering from suspected toxoplasmosis; a southern hairy nosed wombat (*Lasiorhinus latifrons*) from the Perth Zoo with a serological history of *T. gondii* exposure; two ravens (*Corvus coronoides*) which died after being submitted to the veterinary clinic suffering neurological disorders (one of which was confirmed as having toxoplasmosis via histopathology); a southern brown bandicoot (*Isoodon*

obesulus) recovered from a suburban backyard with no obvious cause of death; three western barred bandicoots (*Perameles bougainville*) from a captive breeding colony that died of no obvious causes; and four quokkas (*Setonix brachyurus*) from Rottnest Island displaying possible symptoms of *T. gondii* prior to death.

5.2.1.2 Serological Samples

Serum samples were collected from a combination of 70 stray and feral cats from which tissue samples were also collected. Seroprevalence of cats was determined using the latex agglutination test (LAT) (does not distinguish immunoglobulin classes), indirect immunofluorescence assay (IFA) (anti-*Toxoplasma* antibody test), direct agglutination test (DAT) (detection of IgM) and modified agglutination test (MAT) (detection of IgG). Two different commercial laboratories (VetPath, Perth and ClinPath, Hobart) performed the serological tests.

5.2.1.3 Feral Cat and Native Fauna Samples

Once the single tube hemi-nested PCR was calibrated with biological samples, it was used to screen tissue (brain) samples collected from 268 feral cats and 200 native mammals from different regions throughout Western Australia, comprising: 69 *Macropus fuliginosus*; 42 *Notomys alexis*; 39 *Macropus rufus*; 16 *Pseudomys hermannsburgensis*; 10 *Mus musculus*; 6 *Bettongia penicillata*; 5 *Pseudomys nanus*; 5 *Rattus tunneyi*; 3 *Pseudomys delicatulus*; 3 *Trichosurus vulpecula*; 1 *Isoodon obesulus*; 1 *Dasyurus geoffroii* (Figure 5.2).

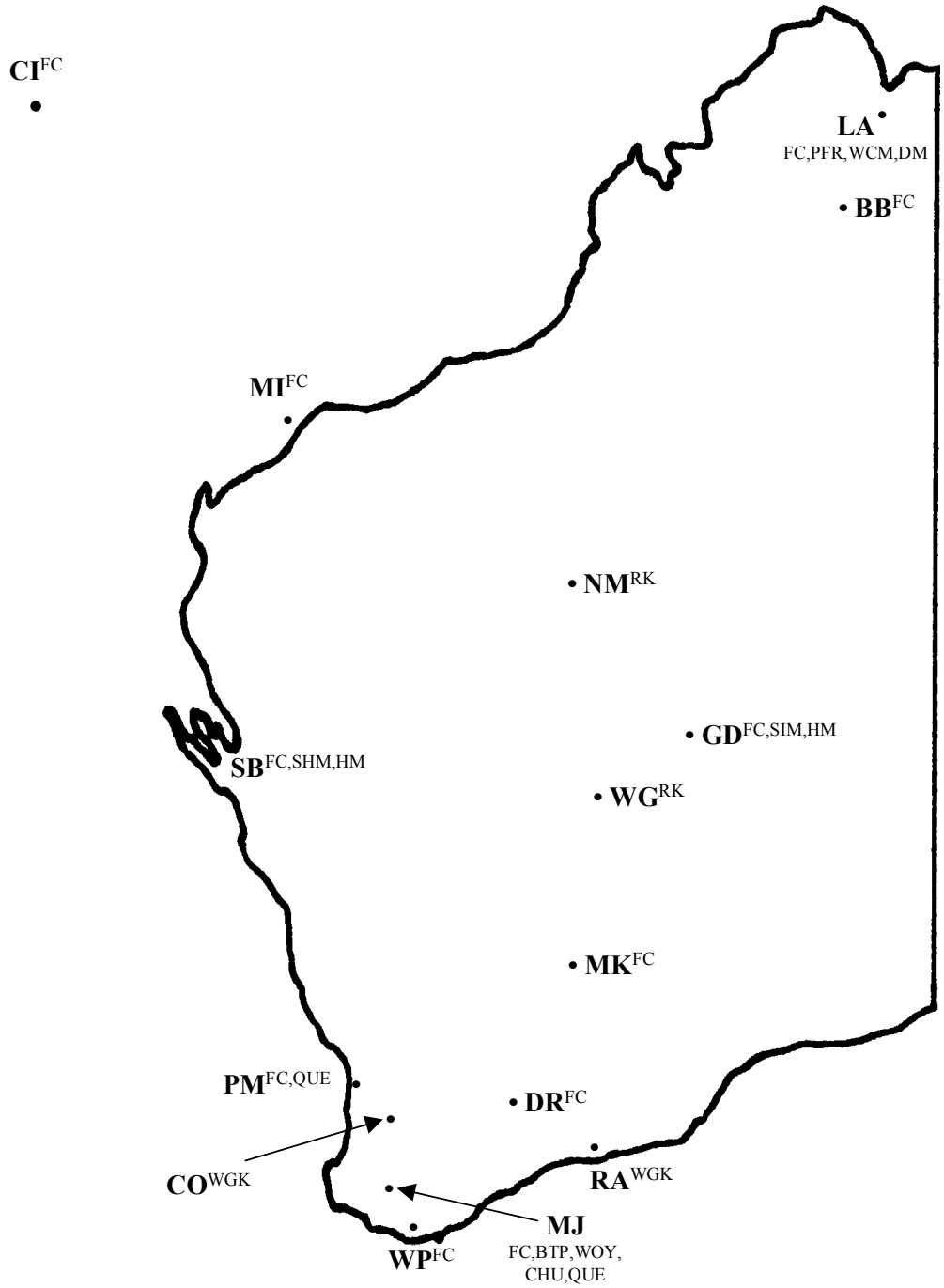

Figure 5.2 Location of tissue sampling sites for *T. gondii* within Western Australia showing mammal species collected from each site. Key to sampling site abbreviations: BB = Bungle Bungles; CI = Cocos Islands; CO = Collie; DR = Dragon Rocks; GD = Gibson Desert; LA = Lake Argyle; MI = Montebello Islands; MJ = Manjimup; MK = Mount Keith; NW = Newman; PM = Perth Metropolitan Region; RA = Ravensthorpe; SB = Shark Bay; WG = Wongawol Station; WP = Walpole. Key to species abbreviations: BTP = brushtail possum (*Trichosurus vulpecula*); FC = cat (*Felis catus*); CHU = chudich (*Dasyurus geoffroii*); DM = delicate mouse (*Pseudomys delicatulus*); HM = house mouse (*Mus musculus*); PFR = pale field rat (*Rattus tunneyi*); QUE = quenda (*Isoodon obesulus*); RK = red kangaroo (*Macropus rufus*); SHM = spinifex hopping mouse (*Notomys alexis*); SIM = sandy inland mouse (*Pseudomys hermannsburgensis*); WCM = western chestnut mouse (*Pseudomys nanus*); WGK = western grey kangaroo (*Macropus fuliginosus*); WOY = woylie (*Bettongia penicillata*).

5.2.2 DNA Extraction

Two DNA extraction protocols were used for this study, Chelex® 100 resin solution and a DNA MasterPure™ Extraction kit from Epicentre. Both extraction techniques were trialled to observe the technique best suited to quick and efficient large scale DNA extractions on biological samples. In both protocols the brain tissue from sampled animals was frozen and macerated prior to DNA extraction to provide a homogenous suspension of brain tissue and *T. gondii* parasites (if present in the brain).

5.2.2.1 Chelex® Resin Protocol

Tissue samples were macerated and the DNA extracted using Chelex® 100 resin (Walsh *et al.*, 1991). In brief, the macerated tissue was digested with Proteinase K (final concentration 150 µg/ml) at 56ºC for 24-48 hours with continuous mixing in 5% w/v Chelex® 100 resin in ddH_2O. Following incubation the Chelex® 100 resin and any remaining cellular debris was pelleted in a bench top microcentrifuge and the supernatant transferred to a clean tube. The supernatant was subsequently treated with 1 µg of RNAase A at 37ºC for 30 min before being used for PCR amplification.

5.2.2.2 Epicentre MasterPure Kit

DNA was extracted using the MasterPure kit from Epicentre, which utilises a rapid desalting process resulting in the removal of contaminating macromolecules (Miller *et al.*, 1988) and is applicable to a wide variety of biological samples. Extraction of DNA was performed following the manufacturer's instructions. In brief, approximately 50 – 100 mg of tissue sample was suspended in 300 µl of Tissue Lysis Buffer with a final concentration of 15 µg/ml Proteinase K and incubated at 56ºC with continuous mixing for 20 – 24 hours. Samples were cooled on ice then incubated with 1 µg of RNAase A

at 37ºC for 30 min. Samples were incubated on ice for a further 3–5 min before adding 150 µl of MasterPure Protein Procipitate Buffer and vortexing for 10 seconds. The resulting precipitate was spun down at maximum speed in a bench top microcentrifuge (14,000 rpm) for 10 min at 4 ºC. The supernatant was removed and transferred to a clean tube and the DNA was precipitated with 500 µl of isopropanol and gentle mixing. The DNA was pelleted by spinning at maximum speed (14,000 rpm) in a bench top microcentrifuge for 10 min. The pellet was washed twice with 70% ethanol to remove any residual salts and vacuum dried before resuspending in TE buffer (pH 7.4).

5.2.3 Single Tube Hemi-Nested PCR Amplification

Amplification of *T. gondii* B1 gene was performed using the highly sensitive one-tube hemi-nested PCR protocol and primers developed by Pujol-Rique *et al.* (1999) with modifications. In brief, 10-50 ng of extracted DNA was added to a final volume of 25 µl of PCR buffer consisting of 50 mM KCl, 15 mM Tris-HCl (pH 8.0), 1.5 mM $MgCl_2$, 50 µM each of dATP, dCTP, dGTP and dTTP, primer TM1 at 0.01 µM, primer TM2 at 0.1 µM and primer TM3 at 1 µM, 0.5 U of Taq Extender™ PCR Additive (Stratagene, Integrated Sciences, Australia) and 0.5 U of AmpliTaq Gold® (Applied Biosystems, Foster City, CA) polymerase.

Samples were amplified in a Perkin Elmer 2400 thermocycler (Perkin Elmer Cetus). The hemi-nested thermocycle program consisted of two rounds of 30 cycles each. The varying primer concentrations allowed the initial amplification of a 619 bp fragment in the first round followed by the amplification of a 362 bp fragment in the second round (Table 5.1, Figure 5.3). After an initial denaturation for 3 min at 95ºC, conditions in the first round of 30 cycles were: denaturation at 94ºC for 30 s; annealing at 65ºC for 30 s

and elongation at 72ºC for 1 min. After an additional extension period of 10 min, the second round of amplification was performed under the same conditions, except the annealing step was carried out at 55ºC. Another additional extension step of 72ºC for 10 min was included after the second round of 30 cycles followed by a holding temperature of 15ºC. A negative control (high purity H_2O for injecting) was included in every amplification reaction as was a positive control. Positive controls consisted of extracted *T. gondii* DNA from cultured RH strain tachyzoites (supplied by Professor Alan Johnson), as well as *T. gondii* DNA extracted from a clinically submitted cat suffering from confirmed acute toxoplasmosis. PCR products were visualised on 1% agarose gels stained with ethidium bromide and compared to a 100 bp DNA ladder (New England Biolabs), as previously described (section 3.2.6).

The sensitivity of the PCR protocol was determined both for pure *T. gondii* genomic DNA (serial dilutions of *T. gondii* RH tachyzoite DNA), and in the presence of a back ground of murine host tissue DNA spiked with different amounts of *T. gondii* RH tachyzoite DNA. Additionally, the screening of brains of mice experimentally infected with *T. gondii* RH tested the sensitivity of the PCR protocol. The specificity of the reaction was confirmed against the GenBank database at the network server of the National Centre for Biotechnology information, and was demonstrated by PCR analysis of the closely related protozoan *Neospora caninum*.

Table 5.1 Primers used in one-tube hemi-nested PCR.

Primer*	Sequence	PCR Product
TM1	5'-GAGAGGTCCGCCCCCACAAG-3'	…
TM2	5'-CTGCTGGTGCGACGGGAGTG-3'	619 bp[a]
TM3	5'-CAGGAGTTGGATTTTGTAGA-3'	362 bp[b]

*Primers sourced from Pujol-Rique *et al.* (1999).
[a]Produced with primers TM1 and TM2, [b]Produced with primers TM1, TM2 and TM3.

5.2.4 Sequencing

Sequencing of PCR products was carried out as previously described (section 3.2.5), using the primer pairs TM1/TM2 and TM2/TM3.

5.3 RESULTS

5.3.1 DNA Extraction

Both the Chelex® Resin and MasterPure extraction techniques were efficient at extracting genomic DNA from all types of tissue samples. However, improved sensitivity and specificity of *T. gondii* amplification was achieved using the MasterPure DNA extraction Kit which yielded cleaner DNA with less carry-over contaminants than did the Chelex® Resin technique. As such this was the DNA extraction technique used for all biological samples.

5.3.2 Histopathology

Histological examination was performed on only a limited number of samples due to the majority of samples being collected throughout Western Australia (Figure 2.1) and the vast distances involved resulting in a lack of available fresh tissue. As such, histopathology was only conducted on those animals available for "fresh" autopsy. Of those samples where histological examination was carried out (one cat, one ferret, two ravens and four Quokka), only the cat, the ferret and one of the ravens had lesions compatible with *T. gondii* infections being present. Multiple regions of encephalitis throughout the brain sections of the cat and the raven were observed though no visual confirmation of *T. gondii* was obtained. The raven also had ocular degeneration present in both eye sections. Histological examination of the quokka samples was inconclusive due to widespread autolysis from storage of the carcasses at -20ºC prior to autopsy.

5.3.3 Specificity and Sensitivity of Hemi-Nested PCR

PCR successfully amplified a single band of the expected size (362 bp) from the *T. gondii* B1 gene whose identity was confirmed by sequencing (Figure 5.3). No PCR product was generated from using *N. caninum* DNA as template. Analysis of samples from serial dilutions of *T. gondii* RH tachyzoite pure genomic DNA confirmed the detection limit of the PCR assay of 0.1 *T. gondii* organism or 0.1 pg as previously stated (Pujol-Rique *et al.*, 1999). The ability of the assay to detect *T. gondii* in the background of host tissue was assessed by PCR amplification of extracts from *T. gondii* negative tissues spiked with different amounts of *T. gondii* RH tachyzoite DNA. Thus an amount between 250 and 500 ng of host and parasite DNA combined was found to be the best amount to avoid interference with the amplification of *T. gondii* and still detect as little as 1 pg of *T. gondii* DNA.

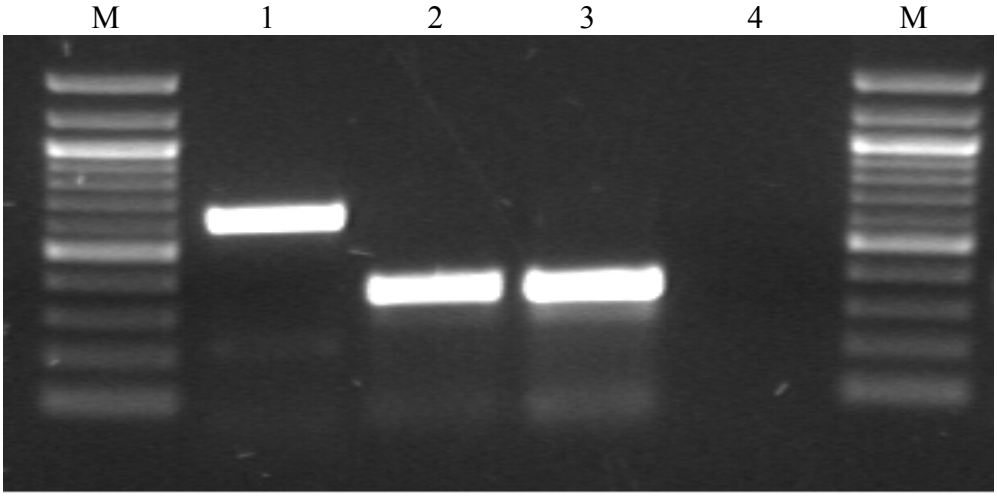

Figure 5.3 Amplification of *T. gondii* B1 gene using both single-step and one-tube hemi-nested PCR techniques. Lane M, 100 bp DNA ladder; 1, 619 bp fragment generated by single-step PCR with primers TM1 and TM2; 2, 362 bp fragment generated by single-step PCR with primers TM2 and TM3; 3, 362 bp fragment generated by single-step PCR with primers TM1, TM2 and TM3; 4, negative control.

5.3.4 PCR Detection of Toxoplasma gondii

5.3.4.1 Application to Clinical Samples

Brain samples were analysed by PCR for each animal as well as any additional tissue samples that were available. A total of 11 tissue samples collected from five animals known to be positive for *T. gondii* by serology, histopathology or both were analysed by PCR (Table 5.2). Samples from additional animals suspected of being infected with *T. gondii* were also analysed by PCR, however no visible lesions were detected in raven 2, whilst histopathology was hampered by autolysis in the four quokkas and was not conducted on the remaining samples (Table 5.2). An example of the typical PCR results obtained is shown in Figure 5.3. Among the tissue samples analysed, brain was found to be optimal for PCR detection of *T. gondii* infection. Other tissues that provided PCR positive results included spleen, lung, liver, and kidney (Table 5.2). PCR successfully amplified *T. gondii* DNA from all the tissue samples with histological lesions positive for *T. gondii* infection.

5.3.4.2 Molecular versus Serology

Serological testing of feral cat serum samples produced varying results as to the number of positive animals from each area except Shark Bay where antibodies to *T. gondii* were not detected in any of the animals tested (Table 5.3). The latex agglutination test gave positive reactions for 18 of 62 samples. *T. gondii* antibodies were detected in 16 of the 25 cats evaluated with DAT and six with MAT, with the following titres: 1:16 in 4 cats; 1:64 in 5 cats; 1:256 in 4 cats; and 1:1024 in 2 cats. The MAT titres were: 1:64 in 1 cat; 1:256 in 4 cats; and 1:1024 in 1 cat. IFA found 3 of 3 cats evaluated positive. PCR analysis detected *T. gondii* DNA in four of the 70 feral cats tested serologically.

Table 5.2 *T. gondii* one-tube hemi-nested PCR amplification results for different host species and tissue samples.

	Brain	Liver	Spleen	Muscle	Heart	Kidney	Lung	Tongue	Spinal Cord	Lymph	Skin Lesion
Cat*	+	+	+	+						+	+
Mouse*	+										
Ferret*							+			+	
SHNW*	+										
Raven1*	+										
Raven2	-	-	-		-		-				
WBB1	-	-		-							
WBB2	-	-		-							
WBB3	-	-		-							
SBB	+		+	+							
Quokka1	-	-	-	-							
Quokka2	+	-	-	+	+	+	-	-	+		
Quokka3	-	-	-	-	-	-	-	-	-		
Quokka4	-	-	-	-	-	-	-	-	-		

*Indicates animals that were known to be positive for *T. gondii* via histopathology or serology, all other animals presented with suspected symptoms of *T. gondii* infection. SHNW = Southern Hairy Nosed Wombat; WBB = Western Barred Bandicoot; SBB = Southern Brown Bandicoot

Table 5.3 Comparison of four different serological tests and the one-tube hemi-nested PCR to detect *T. gondii* in feral cats from three different regions in Western Australia.

	Serology*				PCR
	Latex	IFAT	DAT	MAT	
Shark Bay	0 (42)	-	-	-	0 (42)
Perth Metro	3 (3)	3 (3)	6 (8)	6 (8)	1 (11)
Walpole	15 (17)	-	5 (17)	5 (17)	3 (17)

*Where applicable, a titre of 1:64 or greater is deemed to be a positive reaction.
Numbers in parentheses indicate sample size.

5.3.4.3 Detection of *T. gondii* in Feral Cats and Native Mammals

PCR was positive in 13 of 268 feral cat brain samples (Table 5.4) and 13 of 200 native mammal brain samples (Table 5.5) collected throughout Western Australia. *T. gondii* was detected in cats collected from the Perth metropolitan region, Walpole, Manjimup tip, Mount Keith, the Gibson Desert and the Cocos Islands. *T. gondii* was detected in the brains of 13 of 20 red kangaroos (*Macropus rufus*) collected from Wongawol Station in the Goldfields region.

Table 5.4 Detection of *T. gondii* DNA by one-tube hemi-nested PCR in brain samples of 268 feral cats collected from eleven different regions throughout Western Australia.

	Sample Size	Positive	Prevalence
Perth Metro	18	2	11.1%
Walpole	20	4	20.0%
Manjimup	15	1	6.7%
Dragon Rocks	6	0	0.0
Mount Keith	19	1	5.3%
Shark Bay	127	0	0.0
Gibson Desert	17	4	23.6%
Lake Argyle	20	0	0.0
Bungle Bungles	4	0	0.0
Montebello Islands	3	0	0.0
Cocos Islands	19	1	5.3%
Total	268	13	4.9%

Table 5.5 Detection of *T. gondii* DNA by one-tube hemi-nested PCR in brain samples of 200 native mammals collected from eight different regions throughout Western Australia.

	Sample Size	Positive	Prevalence
Manjimup			
Bettongia penicillata	6	0	0.0
Trichosurus vulpecula	3	0	0.0
Isoodon obesulus	1	0	0.0
Dasyurus geoffroii	1	0	0.0
Collie			
Macropus fuliginosus	47	0	0.0
Ravensthorpe			
Macropus fuliginosus	22	0	0.0
Wongawol			
Macropus rufus	20	13	65.0%
Gibson Desert			
Pseudomys hermannsburgensis	16	0	0.0
Mus musculus	3	0	0.0
Newman			
Macropus rufus	19	0	0.0
Lake Argyle			
Rattus tunneyi	5	0	0.0
Pseudomys nanus	5	0	0.0
Pseudomys delicatulus	3	0	0.0
Shark Bay			
Notomys alexis	42	0	0.0
Mus musculus	7	0	0.0
Total	200	13	6.5%

5.4 DISCUSSION

5.4.1 Molecular Detection of Toxoplasma gondii

Development of a diagnostic tool that was sensitive and specific enough to detect acute and chronic cases of toxoplasmosis was crucial to this study. The highly sensitive and specific nature of the one-tube hemi-nested PCR protocol targeting the multicopy B1 gene was determined using *T. gondii* trophozoite DNA kindly supplied by Professor Alan Johnson of the University of Technology, Sydney (see section 5.3.3). Detection of *T. gondii* DNA was also possible in six different tissues collected from a naturally infected cat presented to the Murdoch University Teaching Veterinary Hospital (Table 5.2). This cat was suffering from acute toxoplasmosis and the parasite had proliferated throughout most of its tissues to a stage where tachyzoites were readily discernable in most biopsy smears. The sensitivity and specificity of the PCR technique was also confirmed using brain tissue from an experimentally infected mouse (also supplied by Professor Alan Johnson, UTS, Sydney) as well as various tissues from a ferret, southern hairy nosed wombat and a raven, all diagnosed with *T. gondii* infections (Table 5.2). The ability to detect *T. gondii* DNA from each of these different host species not only illustrates the ubiquitous nature of *T. gondii* infections, but also demonstrates the versatility of this molecular technique for the detection of *T. gondii* in different species without the risk of decreased specificity or sensitivity.

The single tube hemi-nested PCR protocol minimises the occurrence of contamination without hindering the sensitivity of the assay by eliminating the additional handling of amplified products that occurs in standard nested PCR protocols. The detection limit of this technique has previously been shown to be 0.1 *T. gondii* organism or approximately 0.2 pg (Pujol-Rique *et al.*, 1999), which was replicated in the laboratory using pure *T.*

gondii DNA extracted from trophozoites. However, in the presence of host DNA this detection limit was reduced to approximately 1 pg of *T. gondii* DNA which represents 1 to 5 tachyzoites (Savva, 1989). This detection level is similar to that reported by other authors (Hurtado *et al.*, 2001; Owen *et al.*, 1998; Guay *et al.*, 1993; MacPherson and Gajadhar, 1993; Burg *et al.*, 1989) and was deemed sufficient to detect the presence of *T. gondii* in tissues of infected hosts. Brain, spleen and muscle were most often used for analysis though *T. gondii* was also detected in kidney, liver, heart, lung and lymph nodes (Table 5.2).

5.4.2 Molecular Detection versus Serology and Histopathology

The one-tube hemi-nested PCR technique was shown to be highly sensitive and specific in detecting *T. gondii* DNA in all the tissues obtained from animals that were positive for *T. gondii* via histopathology or serology or both (Table 5.2). Whilst some of the animal carcasses sampled were subject to varying degrees of decomposition the one-tube hemi-nested PCR could still detect the presence of *T. gondii* DNA, even though histological interpretation was difficult. Therefore, autolyzed tissues from stored and frozen animal carcasses, though ineffectual for histopathological or serological examination, are still useful for PCR detection. Indeed, one of the major advantages of molecular techniques is their generally robust nature and the ability to be successfully applied to a wide variety of samples, which may not necessarily be useful for other detection methods.

The reproducibility and interpretation of serological results can vary widely in regards to sensitivity and specificity based on the test used (Hopkins *et al.*, 1993; MacPherson and Gajadhar, 1993). This is evidenced by the varying results obtained from two

commercial laboratories using two different tests on the same serum samples collected from feral cats from Walpole. Analysis of 17 feral cat sera using the LAT detected 15 positive samples, whilst analysis using both DAT and MAT detected only 5 positive samples (Table 5.3). Of the five samples positive by DAT and MAT, four had titres of 1:64 and one had a titre of 1:1024. Further detection of IgG titres on these serum samples using IFA detection by a second laboratory produced titres that were both higher and lower than those detected by MAT (results not shown). Unfortunately, the limited number of sera collected and the high costs associated with serological testing of antibody titres prevented further investigation of these discrepancies.

Molecular analysis of tissue samples using the one-tube hemi-nested PCR detected *T. gondii* in three of the five serologically positive cats from Walpole whose corresponding titres were 1:64, 1:64 and 1:1024 (Table 5.3). The least significant level of reactivity for IFA, DAT and MAT is considered to be 1:64 (Dubey and Lappin, 1998), therefore titres from these tests should be ≥1:64 before they are considered to be positive. As such, four of the five serologically positive cats are borderline positive and infection may be questionable. The detection of *T. gondii* DNA in only two of the four cats with titres of 1:64 suggests that the other two samples were actually serological false positives. However, an accurate assessment of these two techniques is not possible as the PCR involves the direct detection of *T. gondii* DNA, whilst serology measures the host response to the parasite.

The much higher number of serologically positive cats from Walpole detected by the LAT may be due to cross-reaction rather than a true positive result as the LAT has previously been shown to be less sensitive/specific than MAT in detecting *T. gondii*

antibodies (Dubey *et al.*, 1997a). In general, MAT detects *T. gondii* antibodies earlier and of greater magnitude than most other serological tests (Lappin, 1996; Dubey *et al.*, 1995b). However, MAT cannot necessarily document recent or active infection in cats as MAT antibody titres of ≥1:10,000 have been observed in healthy cats that shed *T. gondii* oocysts six years earlier and have not been re-exposed since then (Dubey, 1995). It has also been shown that some cats do not mount an antibody response despite infection with *T. gondii* (Dubey, 1986), and this process may very well occur in other host species.

PCR has an advantage over serology in its ability to directly detect *T. gondii* DNA and not have to rely upon host responses to the parasite. Also, the high levels of sensitivity and specificity afforded by PCR techniques allows the detection of *T. gondii* infections in many different host species without affecting its accuracy and can be used to detect both acute and chronic infections (Hurtado *et al.*, 2001; Jauregui *et al.*, 2001; Lin *et al.*, 2000; Pujol-Rique *et al.*, 1999; MacPherson and Gajadhar, 1993), however it cannot differentiate between them. One problem that has hindered the introduction of PCR as a routine diagnostic technique has been the occurrence of false positive and false negative results (Dubey and Lappin, 1998; Ellis, 1998).

The use of a one-tube hemi-nested PCR technique for the detection of *T. gondii* infections provides greatly increased specificity over single step PCR protocols, whilst at the same time decreasing the risk of contamination, thereby practically eliminating the risk of false positives from cross contamination and non-specific primer binding (Hurtado *et al.*, 2001; Pujol-Rique *et al.*, 1999). The occurrence of false positives in PCR is predominantly due to inhibition of the PCR (Ellis, 1998), or in regard to *T.*

gondii, due to the sparse and focal distribution of the parasite in sample tissues (da Silva and Langoni, 2001; Esteban-Redondo *et al.*, 1999).

In the present study, the risk of inhibitors creating false negatives was overcome by the use of a DNA extraction kit designed to neutralise and eliminate PCR inhibitors during the extraction of the DNA (see section 5.2.2.2). To overcome the risk of false negatives due to the potentially diffuse distribution of *T. gondii* in host tissues, samples were macerated to provide a homogeneous distribution of *T. gondii* tissue cysts throughout the sample before an aliquot was taken for DNA extraction. Whilst the multi-copy B1 gene is a highly specific target sequence (Cazenave *et al.*, 1991; Burg *et al.*, 1989), that has been shown to be up to 20 times more sensitive than the P30 and 18S rDNA genes in detecting *T. gondii* DNA (Jones *et al.*, 2000), these cannot be guaranteed to eliminate the risk of false negatives.

Whilst every effort was made to sample greater than 50% of the brain of all animals collected in a bid to ensure the detection of *T. gondii* in infected individuals, this was not always possible. The remote locations and large distances involved with many of the sampling areas meant that numerous samples were collected by volunteers and Department of Conservation and Land Management officers who were not familiar with the nature or importance of collecting these samples. This coupled with the somewhat unsavoury task of collecting the required samples resulted in less than 50% of the brain being collected for many of the samples. As such, a number of false negatives may have occurred due to inappropriate sampling, resulting in the underestimation of the prevalence of *T. gondii* in Western Australia.

Whilst PCR based methods have been used to detect *T. gondii* in many different host species (da Silva and Langoni, 2001; Hurtado *et al.*, 2001; Jauregui *et al.*, 2001; Feng and Milhausen, 1999; Dupon *et al.*, 1995), to date there have been no published reports on the use of such molecular techniques for the detection of *T. gondii* in Australian marsupials. The results of this study have shown that the single tube hemi-nested PCR targeting the multicopy B1 gene is a reliable technique for the diagnosis of *T. gondii* infection in multiple species of animals (Table 5.2, 5.5), so long as sample collection is designed to maximise the chances of sampling the *T. gondii*. The sensitivity of this technique, together with its capacity to overcome the autolytic and immunologic status of the host, makes it a valuable tool for the detection of *T. gondii* DNA. However, whilst PCR provides a potentially more sensitive tool for the diagnosis of toxoplasmosis in biological samples, it may be further enhanced as a diagnostic tool when used in conjunction with histopathological and/or serological analysis (Stiles *et al.*, 1996). Ideally, given the complex nature and our less than complete understanding of the nature of *T. gondii* infections, test results (PCR, serology or histology) should be interpreted in conjunction with any other observable or related information whenever possible.

5.4.3 Toxoplasma gondii *in Feral Cats from Western Australia*

The one-tube hemi-nested PCR indicated that 13 of the 268 feral cats (4.9%) collected throughout Western Australia contained *T. gondii* tissue cysts in their brains (Table 5.4). This is the first known study to use molecular methods for the detection of *T. gondii* in feral cats in Australia. Previous studies on feral cats from Victoria (Coman *et al.*, 1981b), Tasmania (Milstein and Goldsmid, 1997; Gregory and Munday, 1976) and southern Western Australia (Jakob-Hoff and Dunsmore, 1983) used serology to survey

the occurrence of *T. gondii* in feral cats. These surveys found the prevalence of *T. gondii* antibodies to range from 0% to 52.3% (Table 5.6). Gregory and Munday (1976) reported finding *T. gondii* antibodies in 51 of the 53 cats examined, however this included 23 cat sera that reacted at titres below 1:64 and are therefore not considered positive (Dubey and Lappin, 1998). This revised prevalence of *T. gondii* (52.8%) is comparable to the 50% prevalence detected in feral cats from Tasmania by Milstein and Goldsmid (1997).

Table 5.6 Summary of *T. gondii* surveys conducted on feral cats in Australia

State	Climate	Status	Prevalence	Method[b]	Reference
Western Australia	Temperate Temperate	Stray[a] Feral	36% (66) 0% (8)	IHA	Jakob-Hoff and Dunsmore (1983)
Victoria	Temperate Semi-Arid	Stray Feral	44% (16) 14% (59)	IHA	Coman *et al.* (1981b)
Tasmania	Temperate	Feral	52.8% (53)	IFA	Gregory and Munday (1976)
Tasmania	Temperate	Feral	50% (18)	LAT	Milstein and Goldsmid (1997)

[a]Includes both domestic and stray cats.
[b]IHA, indirect haemagglutination assay; IFA, indirect fluorescent antibody test; LAT, latex agglutination test.

The overall *T. gondii* prevalence of 4.9% detected in feral cats in the present study appears to be significantly different to previous findings in feral cats in Australia, however it is difficult to make an accurate comparison between molecular and serological assays. As mentioned previously, the relatively low overall prevalence in the present study may be an underestimation of the true prevalence of *T. gondii* in cats from Western Australia. However, the large number of cats sampled from numerous locations in this study may have more to do with the low overall prevalence than any methodology related underestimation as the local prevalence of *T. gondii* in cats from several areas is comparable to previous studies (Table 5.4, 5.6).

Jakob-Hoff and Dunsmore (1983) as well as Coman *et al.* (1981b) found a lower prevalence of *T. gondii* in cats from drier areas. This apparent preference for wetter regions has been observed in both native and introduced mammals elsewhere in Australia (O'Donoghue *et al.*, 1987; Smith and Munday, 1965; Cook and Pope, 1959), and has been attributed to the susceptibility of *T. gondii* oocysts to dessication (Dubey, 1998c; Yilmaz and Hopkins, 1972). The presence of *T. gondii* in 20% of feral cats sampled from Walpole and its absence in feral cats from Lake Argyle, the Bungle Bungles and the Montebello Islands correlates well with these previous studies. However, whilst the occurrence of *T. gondii* in the present study occurred predominantly in cats from the wetter southern region of the state, *T. gondii* was detected in cats from the Gibson Desert and Mount Keith which both experience an arid climate. Indeed, the highest prevalence of *T. gondii* in feral cats was detected in those from the Gibson Desert.

The low prevalence of *T. gondii* detected in cats from the Cocos Islands (5.3%), Manjimup (6.7%) and the Perth metropolitan area (11.1%) in comparison to the previous surveys is of interest as these cats were classified as strays due to their collection from, or in close proximity to, rubbish tips. Coman *et al.* (1981b) detected what appears to be a significantly higher occurrence of *T. gondii* antibodies in stray cats (44%) compared to feral cats (14%). Jakob-Hoff and Dunsmore (1983) found no significant difference between the prevalence of *T. gondii* antibodies in stray and domestic cats, whilst none of their feral cats had antibodies to *T. gondii*. Likewise, stray cats have also been found to have a higher prevalence of *T. gondii* antibodies (59.7%) than domestic cats (37.5%) in America (Dubey, 1973). The predominantly scavenging

diet of stray cats is considered to increase their likelihood of being exposed to infective *T. gondii* stages (Sumner and Ackland, 1999; Dubey, 1973).

Stomach content analysis of cats collected from Perth, Manjimup and the Cocos Islands indicated that these cats relied predominantly on scavenging, therefore the relatively low *T. gondii* prevalence observed might be an indication of the increased sanitation methods employed at tips as opposed to camping grounds and picnic reserves. Additionally, the isolated nature and small size of the Cocos Islands means that the majority of their meat supply is frozen, which has been shown to reduce the viability of *T. gondii* tissue cysts if not kill them (Tenter *et al.*, 2000; Kotula *et al.*, 1991). This reduced infectivity may also be responsible for the low prevalence of *T. gondii* observed in the stray cat population despite their scavenging diet.

The present study has shown that *T. gondii* infections in cats are not limited to the southern cooler regions of Western Australia where conditions are presumably more favourable for the transmission and persistence of the parasite. Instead, the distribution of *T. gondii* in cats from Western Australia does not appear to be influenced by climatic conditions and occurs in feral cats from arid regions as well as from wetter regions. However, it is unknown if the nature and origin of *T. gondii* infections in feral cats from different climatic regions of Western Australia are the same.

5.4.4 Toxoplasma gondii *in Native Mammals from Western Australia*

The collection of native species was performed so as to maximise the overlap with the sampling of feral cats, though this was not always possible. Of the eleven different

areas from which feral cats were collected, native fauna were sampled from four of them: Manjimup; Shark Bay; the Gibson Desert and Lake Argyle (Figure 5.2). The remaining four native fauna sampling sites were selected so as to be comparable to the remaining regions from where cats were collected. Given the rare and endangered status of many of Western Australia's native fauna species, the destructive sampling required for this study naturally limited both the scope and range of species studied. However, 200 native and introduced mammals encompassing 12 different species were sampled and screened using the one-tube hemi-nested PCR protocol as used for the feral cats. *T. gondii* tissue cysts were detected only in the brains of 13 red kangaroos (*Macropus rufus*) collected from Wongawol Station, situated in the arid central region of Western Australia (Table 5.5, Figure 5.2).

Although feral cats were not collected from Wongawol Station the detection of *T. gondii* in 65% of *M. rufus* sampled from this area appears to correlate with the presence of *T. gondii* in feral cats from the similarly arid sampling areas of the Gibson Desert and Mount Keith. However, large macropods such as kangaroos are not frequent prey items for feral cats and are not likely to be scavenged except when prey is hard to come by (Martin *et al.*, 1996; Catling, 1988). Therefore, the high prevalence of *T. gondii* in *M. rufus* is difficult to explain, especially considering its absence from kangaroos (*M. fuliginosus*) in the southern sampling regions of Western Australia (Table 5.5), where feral cats are more plentiful (Adams and Chavand, 2002).

As such, a more accurate indication of the prevalence of *T. gondii* in native fauna would most likely be achieved by surveying the smaller marsupial and rodent species that occur more frequently in the feral cat diet throughout Western Australia (Adams and

Chavand, 2002; Angus *et al.*, 2002; Martin *et al.*, 1996; Muir, 1982). This was achieved for Shark Bay, Lake Argyle, the Gibson Desert and Manjimup, though sample sizes obtained from each location were not always adequate. For example, the low number of medium sized mammals sampled from Manjimup (Table 5.5) means that any *T. gondii* infections detected would have been fortuitous rather than an indication of the true prevalence of the parasite. However, as previously discussed, the destructive nature of the sampling method combined with the endangered status of these native species drastically limited the extent of sampling of these species.

Additionally, *T. gondii* was not detected in 19 native and introduced rodents from the Gibson Desert, however these animals were collected in 1999 a year before the collection of feral cats. *T. gondii* was also absent from rodents collected from Shark Bay and Lake Argyle though none of the cats sampled from these areas were infected either (Table 5.4, 5.5). The absence of *T. gondii* in native fauna samples, even from those regions where the parasite was detected in feral cats, does not necessarily mean that *T. gondii* does not occur in the wildlife (Gustafsson and Uggla, 1994). The failure to detect *T. gondii* in any of the native mammals sampled from the southern regions of Western Australia may be due more to the selection of native species than the absence of *T. gondii* from these areas.

Obendorf *et al.* (1996) observed *T. gondii* related mortalities in wild eastern barred bandicoots (*Perameles gunnii*) whilst Bettiol *et al.* (2000) demonstrated the pathogenicity of the parasite in the same host species. Additionally, the high susceptibility of Australian marsupials has been observed in captive populations (Dreesen, 1990; Patton *et al.*, 1986; Jensen *et al.*, 1985; Boorman *et al.*, 1977). Perhaps

this high mortality level associated with *T. gondii* infections in the Australian wildlife is responsible for the low detection levels of the parasite, as relatively few individuals survive to develop chronic infections (de Thoisy *et al.*, 2003; Gustafsson and Uggla, 1994). A detailed serological survey of feral cats and those species considered to be most at risk of contracting *T. gondii* from the same vicinity would most likely provide a more accurate indication of whether or not this is occurring.

5.4.5 Persistence of Toxoplasma gondii *in the Environment*

Whilst Coman *et al.* (1981b) observed an apparent environmental influence in *T. gondii* infections between cats from different regions of Victoria (Table 5.6), they also suggested that the dietary habits of the cats surveyed may have had a greater impact on the prevalence of *T. gondii*. Stray cats, such as those collected from rubbish tips, picnic reserves and camping grounds by Coman *et al.* (1981b) predominantly feed on garbage and as such would be more likely to be exposed to infective *T. gondii* stages through the scavenging of human food scraps and waste (Dubey, 1973). However, this does not explain the higher prevalence rate of *T. gondii* detected in feral cats from the Gibson Desert (23.6%) compared to stray cats collected predominantly from tips in the Perth metropolitan region (11.1%) and Manjimup (6.7%) in the present study (Table 5.4).

Interestingly, of the 17 feral cats collected from the Gibson Desert, 11 were collected in September 2000 with the remaining 6 being collected in September 2001 from the same study site. Whilst juvenile mortality in feral cat populations in Australia is known to be high (Brothers *et al.*, 1985; Coman *et al.*, 1981b), the removal of 11 mature cats from the Gibson Desert sample site in September 2000 would have improved juvenile survival in the local population by creating new niches for young cats to colonise.

These juvenile cats would have originated from within as well as from outside the Gibson Desert sample site due to the natural dispersal process of young cats in the environment (Algar *et al.*, 2000). Therefore, these cats would have made up a significant portion of the cats sampled from the Gibson Desert in September 2001.

Epidemiological studies of toxoplasmosis in cats indicate that most wild/feral cats become infected with *T. gondii* soon after they are weaned through the ingestion of infective intermediate host tissues (Dubey, 1994). Whilst cats are known to shed as many as 162 million *T. gondii* oocysts following infection (Dubey, 1996; Rommel *et al.*, 1987), *T. gondii* infections are transmitted in cats much more efficiently by carnivorism than by the ingestion of oocysts (Dubey, 1996). Alternatively, oocysts are highly infective to a wide range of intermediate hosts, particularly small mammals which are widely regarded as being important for the maintenance of *T. gondii* in the environment (Dubey, 1997; Dubey *et al.*, 1995c; Dubey, 1994; Dreesen, 1990).

Several studies have demonstrated the potential of wild rodent populations to represent not only a highly prevalent, but also a persistent intermediate host reservoir for *T. gondii* in the environment (Owen and Trees, 1998; Webster, 1994b; Jackson *et al.*, 1986). Beverley (1959) showed that mice congenitally infected with *T. gondii* are able to produce their own congenitally infected offspring for up to 9 generations, whilst de Roever-Bonnet (1969) and Remington *et al.* (1961) also showed vertical transmission of *T. gondii* to occur in successive litters of rodents. More recently, high levels of vertical transmission have been shown to occur consistently in chronically infected house mice (*M. musculus*) and field mice (*A. sylvaticus*), which most likely persists for the life of

breeding females at substantially higher rates than previous studies on laboratory mice and hamsters have observed (Owen and Trees, 1998).

The Gibson Desert Nature Reserve experienced a wetter winter in 2001 than it did for several years previously (BureauofMeteorology, 2001), which resulted in an improved seed set in the local vegetation and subsequent increase in rodent numbers. Therefore, vertical transmission of *T. gondii* from chronically infected dams to their offspring during a favourable season(s) would result in the effective amplification of the parasite in the rodent population. A localised insurgence of young *T. gondii* susceptible cats in the Gibson Desert after sampling in September 2000 combined with the increasing rodent population would have provided optimal conditions for transmission of *T. gondii* to a large portion of the feral cat population. Therefore, it appears that the prevalence of *T. gondii* in feral cats from arid regions of Australia may fluctuate in relation to the climatic conditions.

Similarly, the high prevalence of *T. gondii* detected in *M. rufus* from Wongawol Station in 2002 could be due to vertical transmission within a rapidly expanding kangaroo population following on from the same favourable season(s). Although the occurrence of congenital transmission has not been investigated extensively in macropods, Dubey *et al.* (1988) identified *T. gondii* in two black-faced kangaroo joeys (*Macropus fuliginosus melanops*), of which at least one was congenitally infected. Whilst this is the only report of vertical transmission of *T. gondii* in Australian marsupials, there is no reason to suggest that it is not a common occurrence, with congenital transmission shown to occur in a wide variety of animal species including humans (Jones *et al.*, 2001; Cook *et al.*, 2000), pigs (Jungersen *et al.*, 2001), sheep (Duncanson *et al.*, 2001),

mice (Owen and Trees, 1998) rats (Freyre *et al.*, 1999; Dubey *et al.*, 1997b) and even a dolphin (Jardine and Dubey, 2002). Vertical transmission of the closely related parasite *Neospora caninum* has also been demonstrated in mice (Cole *et al.*, 1995), dogs (Barber and Trees, 1998), foxes (Schares *et al.*, 2001) and cattle (Bjorkman *et al.*, 1996).

Traditionally, vertical transmission of *T. gondii* has not been considered an important route of infection due to the development of an immunity in the host following infection, which prevents the transfer of *T. gondii* to future offspring (Dubey *et al.*, 1999; Dubey, 1995; Zenner *et al.*, 1993). Alternatively, vertical transmission is considered to be a much more important route of infection for *N. caninum*, at least in cattle, as host immunity does not occur after infection, allowing its perpetuation for successive generations (Dubey, 2003; Adrianarivo *et al.*, 2000; Paré *et al.*, 1996). However, congenital transmission of *T. gondii* has recently been reported in a newborn child whose mother had been infected 20 years earlier (Silveira *et al.*, 2003). This finding supports earlier hypotheses of the potential for reinfection and subsequent vertical transmission of *T. gondii* (Johnson, 1997). Indeed, Duncanson *et al.* (2001) detected congenital transmission in 61% of pregnancies in a single sheep flock, and theorised that vertical transmission of *T. gondii* may be sufficient to maintain the parasite in natural populations of sheep without requiring frequent new infections by oocysts excreted by cats.

The hypothesis of vertical transmission is not unreasonable, as similar results have been reported in wild rodent populations. Jackson *et al.* (1986) observed an elevated prevalence of *T. gondii* infections in recovering populations of field mice (*A. sylvaticus*) and bank voles (*Clethrionomys glareolus*) in Scotland in 1982, following a severe

winter that caused a considerable reduction in the numbers of these two rodents. Jackson *et al.* (1986) detected *T. gondii* infections in approximately 20% of the rodents trapped in 1983 following the severe winter, though only a single animal (1.9%) was infected in 1983 after a mild winter that did not result in the depletion of either population. The lack of any age or sex bias between *T. gondii* infected and uninfected animals (Jackson *et al.*, 1986), indicating that congenital transmission was responsible for the high prevalence observed in 1983. If *T. gondii* infections were being contracted from the environment via infective oocysts then the prevalence in older animals would be higher than in the younger ones because of their increased chances of exposure to infective stages through having spent more time in the environment, however this was not the case (Jackson *et al.*, 1986).

The source and mode of transmission of the *T. gondii* infections in *M. rufus* from Wongawol Station remains speculative, as cat samples were not collected from this area and the kangaroos were sampled only once. However, Jackson *et al.* (1986) have shown that the prevalence of *T. gondii* can be influenced by environmental conditions causing a cyclical variation of *T. gondii* in rodents that has also been observed in cases of human toxoplasmosis (Tizard *et al.*, 1976). Such cyclic variation could explain the change in prevalence of *T. gondii* observed in feral cats from the Gibson Desert between 2000 (0%) and 2001 (66.7%). Indeed, if sampling of cats from the Gibson Desert was not performed in 2001, it might have lead to the erroneous conclusion that *T. gondii* was absent from cats in this area, particularly given the observed lack of *T. gondii* in native fauna collected from the Gibson Desert in 1999. However, the sampling of native fauna appears to have occurred before the proliferation of *T. gondii* in this region and unfortunately further collections did not occur in 2000 or 2001. Therefore, it is not

known if *T. gondii* infections increased in native fauna in the Gibson Desert as hypothesised.

The present study indicates that *T. gondii* infections appear to fluctuate in feral cats, at least in the Gibson Desert, though this cannot necessarily be extended to include other populations of feral cats or Australian wildlife without further investigation. Whilst congenital transmission of *T. gondii* has been shown to take place in numerous host species, it is reliant upon many factors (Duncanson *et al.*, 2001; Zenner *et al.*, 1993), and the extent to which it occurs in the Australian fauna is unknown. However, it is believed that vertical transmission is an important factor in the persistence of *T. gondii* in populations of host species within Australia.

5.4.6 Effect of Toxoplasma gondii *on Host Species*

Australian marsupials along with New World monkeys and Madagascan lemurs are most susceptible to *T. gondii*, presumably because they evolved in the absence of any indigenous felids (Reddacliff *et al.*, 1993). Whilst acute toxoplasmosis in Australian marsupials has been shown to have high levels of mortality, chronic infections do occur (Bettiol *et al.*, 2000a; Obendorf *et al.*, 1996; Reddacliff *et al.*, 1993). This "latent" phase of toxoplasmosis has traditionally been considered to be asymptomatic and harmless (Havlicek *et al.*, 2001), however there is a growing realisation that *T. gondii* has the profound ability to modify the behaviour of its hosts (Webster, 2001; Yolken *et al.*, 2001; Berdoy *et al.*, 1995).

The manipulation hypothesis states that parasites may be able to alter the behaviour of their hosts for their own selective benefit, usually by increasing the intermediate host's

susceptibility to predation by a definitive host, which in turn facilitates the completion of the parasite's life cycle (Moore and Gotteli, 1990). The indirect life cycle of *T. gondii*, where members of the cat family are the only known definitive hosts, would clearly benefit from improving its transmission rate from intermediate to definitive hosts, and as such there might be strong selective pressure to evolve a mechanism to enhance this transmission (Flegr *et al.*, 1996). Given the parasite's predilection for host brain tissue, it is also situated in an ideal position in which to successfully achieve such a manipulation, and there is now a convincing body of evidence to suggest that *T. gondii* does affect the behaviour of its host (Webster, 2001).

Studies carried out under different experimental conditions suggest that *T. gondii* infected rodents behave differently for a suite of behaviours that may make them more likely to be predated by cats (Webster, 2001; Berdoy *et al.*, 2000, 1995; Hay *et al.*, 1984; Hay *et al.*, 1983a; Hay *et al.*, 1983b; Hay *et al.*, 1983c). The results of these studies suggest a significant difference in the perceived response to cat predation between uninfected rodents and infected rodents. Thus the effect of *T. gondii* appears to be specific to those behavioural categories that may increase transmission, rather than simply causing a generalised illness and/or change in the host's behaviour (Webster, 2001).

Rats and mice are not the only animals that have been shown to exhibit behavioural alterations due to latent toxoplasmosis infections. There is no doubt that congenital toxoplasmosis can reduce intellectual function (Jones *et al.*, 2001; Cook *et al.*, 2000), though recent studies also suggest that latent toxoplasmosis in humans can affect long-term concentration as well as cause personality changes in people (Havlicek *et al.*,

2001; Flegr *et al.*, 1996). Indeed, a recent study has suggested a possible link between *T. gondii* and schizophrenia in humans, with significantly higher levels of *T. gondii* antibodies reported in first-incidence schizophrenic patients than in the general public (Yolken *et al.*, 2001).

Latent toxoplasmosis, although frequently dismissed as asymptomatic and clinically unimportant in both humans and animals, does alter host behaviour (Havlicek *et al.*, 2001; Webster, 2001). Ultimately, actual predation rates by the feline definitive host upon infected and uninfected hosts are required to properly measure any proposed behavioural manipulation amongst intermediate hosts (Webster, 2001). However, the potential influence of *T. gondii* infections given their high prevalence within both human and animal populations worldwide should not be ignored nor discredited. Indeed, the apparent susceptibility of Australian native mammals to predation by introduced foxes and cats should not necessarily be attributed to a phylogenetic "dim-wittedness" (Freeland, 1994).

In some cases contraction of an introduced parasite may be sufficient to cause significant mortality of native mammals, whilst in others this is due to the incorporation of the host species into the complex life cycle of the parasite (Freeland, 1994). With regard to *T. gondii* in the Australian fauna, it appears that both these effects are true. The apparent physiological susceptibility of the Australian native fauna to *T. gondii* combined with the more subtle influences of this introduced parasite may have further perturbed an already destabilised situation. Therefore, a better understanding of the transmission of *T. gondii* in Australia is required before we can fully appreciate the effect that this ubiquitous parasite is having on our unique fauna.

Chapter 6 - General Discussion

6.1 GENERAL DISCUSSION

Introduced species are now recognised as a serious threat to Australia's unique fauna, second only to habitat clearing and fragmentation. Whilst the more obvious impacts of predation and competition are well known, the more subtle effects of foreign pathogens on the native wildlife have been less studied. Many of Western Australia's unique mammal fauna currently require at least some level of management to ensure their survival. The increasing role of captive breeding and translocation programs in the conservation of species, dictates that pathogens and their potential impact on host populations need to be recognised as important factors in conservation biology.

The detection of thirteen helminth and five protozoan genera occurring in feral cats from different geographical and climatic regions of Western Australia indicates the diverse nature of parasitic infections harboured by these introduced animals. Whilst all of these parasite genera have been previously reported in cats from Australia (Prescott, 1984), their distribution and prevalence provide a valuable insight into the ecology of the feral cat in Western Australia. Likewise, an understanding of the parasite communities of our native fauna is of increasing concern, particularly as human intervention and management of wildlife populations becomes increasingly important in ensuring the survival of species. Whilst the identification of many of the native fauna parasites in the present study was only to order or family level, even this level of classification provides a useful base of knowledge for future studies, as these groups

comprise large assemblages of diverse species (O'Donoghue and Adlard, 2000; Spratt *et al.*, 1990).

Therefore, this study was intended to provide an overview of the gastro-intestinal parasites occurring in feral cats and native mammals throughout Western Australia using both traditional and molecular techniques where applicable, and paying particular attention to the ubiquitous protozoan *Toxoplasma gondii*. In order to predict the potential for cross transmission of feline parasites to native mammals, two main lines of investigation were followed:

1. A general survey of helminth and protozoan parasites was conducted on feral cats and a number of selected native mammals throughout Western Australia based on the examination of faecal samples to:
    a. Investigate the distribution and occurrence of feral cat parasites in Western Australia;
    b. Compare the parasite communities of selected native fauna with published findings.
2. The application of molecular techniques to parasite species of particular interest from both an epidemiological and conservational view point:
    a. A PCR-RFLP was developed to identify species of *Ancylostoma* from eggs collected in the faeces of feral cats;
    b. Characterisation of *Giardia* using SSU-rDNA and *ef1α* sequence data to determine the taxonomy and potential transmission routes of infections detected in both native and introduced species;
    c. A one-tube hemi-nested PCR technique was adapted for diagnostic screening of feral cat and native fauna tissue samples to determine the

prevalence and distribution of *T. gondii* in the feral cat population and attempt to detect it in the native fauna.

6.1.1 Parasite Sharing and Interaction

The two most convincing pieces of evidence gathered from this study supporting the cross transmission of parasites between feral cats and native mammals were firstly, the presence of *T. gondii* in several native mammal species (Chapter 5) and secondly, the occurrence of the nematode *Cyathospirura dasyuridis* in a feral cat (Chapter 2). Whilst the transfer of parasites between cats and the native wildlife in Western Australia appears to be limited to these two parasite species, it would be naïve indeed to believe that the distribution and occurrence of feline pathogens is as limited as this. Dickman (1996b) reviewed the published literature on the coincidence of non-specific feline parasites recorded from Australian vertebrate fauna and found that few cat viruses, protozoan or helminth parasites have been recorded. However, the lack of feline pathogens detected in the native fauna in the present study does not exonerate the feral cat as a reservoir of disease.

Many of the parasites detected in the present study such as *Spirometra erinaceieuropaei*, *Gnathostoma spinigerum*, *Taenia taeniaeformis*, *Oncicola pomatostomi*, *Toxocara cati* and *T. gondii*, readily involve numerous species of native fauna as either intermediate or paratenic hosts. However, the detection of these intermediate stages requires highly invasive sampling techniques, as the role of the intermediate host is typically to facilitate the transmission of these stages to the definitive host (most commonly via predation). The varied composition of the feral cat diet, of which small native mammals comprise a large portion (Paltridge *et al.*, 1997;

Martin *et al.*, 1996; Jones and Coman, 1981), combined with the high prevalence of these parasites in particular regions further supports the involvement of the native fauna as intermediate hosts. However, the impact these parasites may have on the native fauna is difficult to quantify, as symptoms may only be apparent in particular components of these host populations or at certain times of year (Dickman, 1996b; Freeland, 1994). Even when infection may result in acute mortality (Bettiol *et al.*, 2000a; Bettiol *et al.*, 2000b), it is notoriously difficult to detect infection in wild populations, and when infection results in more subtle effects it is even harder to discern (Scott, 1988).

The effects at population level of introduced disease on the Australian fauna are not adequately known for any species, though the transmission of pathogens from invading species to susceptible natives has been shown to have a more profound effect than any direct impact of the invader itself (see section 1.4.3). The present study demonstrates that feral cats carry a wide range of parasitic organisms, many of which have the potential to impact on not only native fauna survival and reproduction, but also on community structure (Lyles and Dobson, 1993; Scott, 1988). This is of particular importance with regard to the captive breeding and translocation of native animals for conservation purposes. Therefore, application of the appropriate methodologies is an extremely important step for the effective investigation of parasites and their associated diseases.

Whilst the detection of a parasitic infection in an individual animal is reasonably straightforward and can be conducted with a high level of confidence, detection of the same parasitic infection in a host population can be considerably more complex. An

important characteristic of some parasites is their tendency to be over dispersed within a host population such that many individuals are uninfected or only lightly infected whilst a few individuals harbour very heavy infections (Scott, 1988). A consequence of this is that the examination of a small sample of the host population may under estimate the true prevalence of the parasite or even suggest its absence. The role parasites play in the decline of Australian mammals is largely unknown as evidence of disease causing local declines is largely anecdotal (How *et al.*, 1987; Archer, 1984; Guiler, 1961; White, 1952), and the effects of infection and disease in wild animal populations are notoriously difficult to detect, let alone demonstrate (Scott, 1988).

6.1.2 Sampling Limitations and Distribution of Parasites

Molecular techniques aid in the detection and differentiation of parasites within hosts and the environment by providing technically simple and highly sensitive tools for studying the epidemiology of parasite infection and transmission (Monis and Andrews, 1998; McManus and Bowles, 1996). The stable nature of DNA and the ability to isolate and amplify it from small amounts of tissue or other biological samples allows the collection and storage of material for molecular techniques to be less stringent than that required for many other identification methods. Similarly, the ability to detect infectious agents from crude material, such as faeces, eliminates the need for invasive sampling techniques and avoids time consuming culturing methods, which allows for the simultaneous analysis of large numbers of samples (Monis and Andrews, 1998; McManus and Bowles, 1996).

Molecular markers are a particularly attractive option for the study of parasites or parasite stages that lack useful morphological characters (Monis, 1999). Molecular

techniques not only provide definitive results, their increased levels of sensitivity also allow the distinction between species, subspecies and/or strain groups, as well as inferring their phylogenetic relationships and thus identifying routes of transmission (Monis and Andrews, 1998; McManus and Bowles, 1996). This is demonstrated by the differentiation of morphologically identical *Ancylostoma* eggs from faeces by PCR-RFLP (Chapter 3) as well as the phylogenetic characterisation of *Giardia* cysts detected in both native and introduced wildlife (Chapter 4).

The ability to directly test for the presence of parasite DNA avoids many of the ambiguities associated with indirect detection methods and is invaluable in the epidemiological study of many parasite species (Zarlenga and Higgins, 2001). For example, despite the numerous serological tests available for the detection of *T. gondii*, there is no single assay that can definitively confirm or refute the occurrence of toxoplasmosis in a host (Dubey and Lappin, 1998), whilst the direct nature of PCR detects the presence or absence of the parasite itself and as such is not reliant upon a host response (Chapter 5). However, despite the numerous advantages offered by molecular techniques, their sensitivity is still limited by the quality of the samples. In particular, the sometimes very sparse and focal distribution of *T. gondii* in host tissues can produce false negatives.

The majority of sampling for the current study was performed in conjunction with pre-existing trapping and monitoring programs conducted by the Department of Conservation and Land Management (DCLM). Whilst sample collection from feral cats was unrestricted, many of the native fauna monitoring programs were linked to the study of predominantly rare and/or endangered species and sampling was limited to the

collection of faeces only. Additionally, the overlap of sampling both feral cats and native fauna was not ideal as areas of interest for feral predator control involved the arid and semi-arid regions of Western Australia whilst native fauna monitoring was predominantly conducted in the southern regions of the state. Therefore, whilst the sampling of feral cats and native fauna was performed from most areas, it did not always occur simultaneously nor was it always possible to collect samples from the preferred species.

The destructive nature of collecting tissue samples resulted in the subsequent collection of predominantly faecal samples from native fauna due to their endangered status. This limited the ability of the current study to detect intermediate stages of parasites such as *T. gondii* and *S. erinaceieuropaei*, which are most likely to occur in the native fauna via transmission from feral cats (Dickman, 1996b). The non-invasive sampling of the native fauna also resulted in a lack of adult stages necessary for the correct identification of many of the helminth parasites detected due to the morphologically similar eggs these different species shed. However, most eggs were identified to order or family level, which provided a useful base for the comparison of helminth and protozoan communities detected with published records.

The most readily accessible species of native fauna that were amenable to large-scale tissue collection were kangaroos. These samples were collected with the aid of professional shooters and as such, the location and frequency of sampling was largely reliant upon the professional shooters. Wherever possible, tissue samples were collected from areas as close to feral cat sampling sites as possible, however this is far from an ideal method of sampling. Although *T. gondii* infections and outbreaks have

been reported in numerous species of macropod (Reddacliff *et al.*, 1993; Johnson *et al.*, 1989; Johnson *et al.*, 1988), kangaroos generally suffer very low rates of predation by feral cats. In retrospect, kangaroos would not be expected to be important in the transmission and persistence of *T. gondii* infections, however the collection of tissue samples from other native mammals species was restricted.

The comprehensive and extensive literature available in regard to feline parasites is linked to the cosmopolitan distribution of the cat. Therefore, many of the parasites encountered in feral cats in this study were readily identifiable to genus if not species level. Additionally, the carnivorous nature of the cat makes it a top-level predator and thus a definitive host for many of its parasites, again making the detection and identification of parasite infections easier. In comparison, the failure to detect many feline parasites occurring in the native fauna is again linked to the limited sample collection available from the native fauna (i.e. inability to screen for parasites or parasite stages that occur other than in the gastro-intestinal tract). This further highlights the need for a stringent sampling regime, which was an inherent problem in the present study, as the quality of the sample ultimately dictates the sensitivity of the subsequent assays.

Much of the pre-existing parasite information regarding Australia's native and introduced mammal fauna has been drawn from studies in Queensland, New South Wales, Victoria and Tasmania. Whilst the available information provides a useful basis for comparison with species from other regions of Australia, it also highlights the lack of knowledge regarding many of Western Australia's unique mammal species. Although the current study did not identify many of the parasites detected in native

mammals to species level, it did identify a number of discrepancies between the parasite communities of Western Australian mammals and published records from the other states (Chapter 2).

6.1.3 Anthropogenic Influence

Numerous studies from around the world have reported cat population densities to vary from less than one to more than 2000 cats km^{-2} in response to the availability of resources (Liberg and Sandell, 1994). It has been shown that populations of group-living cats exploiting resource-rich sites are well structured and functional rather than being *ad hoc* collections of individuals (Denny *et al.*, 2002; Kerby and Macdonald, 1994). This ability of the cat to live in a structure social system at high densities appears to be dependent upon human subsidies and occurs as a response to a rich and clumped food supply (Liberg and Sandell, 1994).

Rubbish tips and waste disposal sites provide stable, resource-rich "islands of opportunity" and are known to support high densities of cats (Denny *et al.*, 2002; Wilson *et al.*, 1994). The ubiquitous nature of these resource-rich sites in the landscape clearly enhances the number of feral cats in Australia, however rubbish tips are not the only resource-rich sites present in the environment. Pastoralism and mining activities throughout much of Australia have resulted in the construction of numerous artificial water reservoirs for stock and as a by-product of mining activities. These readily available sources of surface water attract a myriad of species and result in localised "pockets" of wildlife existing at higher densities than the surrounding areas. The wildlife attracted to these focal points represent a stable and abundant food supply for feral cats. Additionally, the increased soil moisture and humidity associated with these

sources of water form microenvironments, which ultimately facilitate the transmission of pathogens in both the feral cats and native fauna frequenting these areas.

The higher densities of cats and native fauna occurring in the vicinity of these microenvironments results in higher levels of interaction between infective stages and potential hosts, which translates to increased transmission and a higher average parasite burden per host (Scott, 1988). The existence of these microenvironments in Western Australia is exemplified by the occurrence of *Ancylostoma tubaeforme* and *S. erinaceieuropaei* in feral cats from Mount Keith (Chapter 2 and 3). Both of these parasites have very different life cycles, however neither parasite is suited to an arid climate such as that experienced at Mount Keith. Human modification of the landscape at Mount Keith in the form of pastoral and mining activities has created numerous stable microenvironments through the creation of water troughs, sewage treatment ponds and a tailings dam from the mine.

The importance of these anthropogenic water reservoirs for the persistence of *A. tubaeforme* and *S. erinaeceieuropaei* is further proven by the absence of these two parasite species from cats in the Gibson Desert. These two sampling locations experience similar climatic conditions with similar cat densities (Angus *et al.*, 2002; Algar *et al.*, 2000). However, the Gibson Desert is outside the pastoral zone and has not been subjected to mining, making surface water extremely rare and intermittent. Therefore, human activities and their subsequent modification of the environment appear to have inadvertently contributed not only to the high densities of cats observed throughout Australia, but also to the occurrence of a wide range of introduced parasite species that would not have otherwise occurred.

However, these anthropogenic effects are not only confined to the rural regions of Australia. The general pattern in most areas of the world today involves the expansion of urban areas to the detriment of natural habitats. As urban areas continue to expand, wild animal populations are being driven into smaller and smaller areas. Forcing animals to exist at these higher densities will affect virtually every aspect of the life and health of these animals, their parasite fauna being no exception (Scott, 1988). Host density, susceptibility and contact rates are important factors in the successful transmission of parasites within a host population. Contact rates are generally influenced by the behaviour of the potential host, whilst host susceptibility is influenced by many factors including genetic predisposition (O'Brien *et al.*, 1985; Wakelin, 1978), nutritional status (Ambroise-Thomas, 2000) and stress (Sedlak *et al.*, 2000). Therefore anything that influences the behaviour and/or survival of the host may also influence the persistence of their parasites (Scott, 1988).

Even apparently non-pathogenic parasites may become important in populations of native wildlife when they become stressed due to overcrowding, malnutrition, age, increased densities of infective stages or a number of other factors (Scott, 1988). When these factors are combined they may also predispose animals to other population pressures (Scott, 1988; Arundel *et al.*, 1977). Likewise, human encroachment on these already stressed wildlife populations greatly increases the potential for transmitting foreign pathogens and diseases. In particular, the protozoan parasite *T. gondii* has been identified as serious risk and contributor to mortalities in wild populations of bandicoots and other marsupial species adjacent to urban areas (Obendorf *et al.*, 1996; Freeland, 1994; Obendorf and Munday, 1990). It is important to realise that the impact of

infection and disease on host populations is only going to increase as urban areas continue to expand and the world wide movement of animals, plants and produce continues to intensify.

6.1.5 Future Directions

An improved understanding of the host-parasite relationships in the Australian wildlife is required to overcome the general paucity of information surrounding our native fauna. There is also a strict need to determine the distribution of foreign parasites within our native fauna as well as to identify their insidious impacts. A better understanding of what represents a natural parasite burden in the native fauna will further improve our management of both native and introduced parasites. Whilst we should not disregard either one or the other, we need to realise that foreign diseases have the potential to decimate host populations. Perhaps the best approach is to manage and/or control disease, rather than attempt to eliminate parasites and thus risk producing naïve and highly susceptible populations.

This is particularly important as the conservation of native species is becoming increasingly reliant upon programs of captive breeding and translocations (Atkinson, 2001; Abbott, 2000; Fischer and Lindenmayer, 2000; Short and Turner, 2000). The potential impact that parasites can have on intensive conservation practices such as these, dictates the need to combine options from the fields of ecology, evolution and veterinary medicine to effectively manage pathogens and disease in these endangered species (Lyles and Dobson, 1993).

As conservation efforts become more and more important to the survival of many of our unique species, we must realise that forcing animals into dense conditions will inevitably have a detrimental effect on the host population and disease will be one of the consequences. There is no point in conserving animal populations unless those populations are managed properly to promote their health and continued wellbeing. Many attempts are made at keeping animals free from infection, especially in zoos and captive breeding facilities. In many ways attempting to keep animals free of disease can be considered a sensible strategy as it should enable animals to survive and reproduce at rates close to maximum which would have to be good for the conservation of the species. However, the elimination of parasites and the role they play from a host population would be a shortsighted approach to the conservation of the host species.

Infectious disease is a normal, constant and continuing feature in the lives of most organisms. The ability of parasites to regulate the growth of host populations, even in the complete absence of other influences such as predation or intraspecific competition, has important implications for the overall health of host species (May and Anderson, 1978). Pathogens can and do exert important effects on host population dynamics in both endemic and epidemic forms (Gulland, 1995). However, overprotection of a species may do more to weaken the population and increase its susceptibility to infection than it does to improving survivorship (Cunningham, 1996; Lyles and Dobson, 1993; Scott, 1988). As such, any sound, long term approach to disease management requires a much better understanding of the dynamic relationship between animals and their pathogens than we currently have.

As mentioned previously, the limiting factor to research of this kind in the Australian fauna is the threatened status of many of our native mammal species. Because of this, the majority of information regarding parasite occurrence in these endangered species has generally been extrapolated from closely related species or members of the same family. Studies of native fauna have also traditionally depended on the utilisation of road kills and opportunistic sampling as major sources of material for epidemiological parasite surveys (Oakwood and Spratt, 2000; Barker *et al.*, 1989; Beveridge and Arundel, 1979). These apparent limitations can be overcome provided a systematic approach is taken to sample collection and the correct tools are applied.

The value of the molecular techniques developed and used in the current study are evident in the detection of *T. gondii*, *Giardia* and *Ancylostoma* species. Their robust nature coupled with a high level of sensitivity makes them ideal for the investigation of parasite occurrence in a wide range of host species and biological samples. The published sequence information available for a wide range of helminth and protozoan parasites and the relative ease with which these techniques can be developed and applied to different developmental stages provides new avenues for the low impact, comprehensive study of parasites in both common and endangered species with a minimum of interference.

The fluctuation of *T. gondii* detected in feral cats in the present study highlights the importance of extended sampling regimes. The ability to sample individuals and/or populations repeatedly without any adverse effects can provide a wealth of information on seasonal and temporal variation in parasite prevalence. This level of understanding of the host-parasite relationship can help predict when populations or individuals may

be most at risk of infection and allow preventative measures to be put in place, ultimately leading to the effective management of threatened or vulnerable populations.

The long-term management of native fauna in Western Australia has already addressed the direct impacts of introduced predators and competitors by attempting to control their numbers in areas of particular conservational value. With regard to the feral cat, the current study has highlighted the importance of human activities in facilitating the persistence of both high densities of feral cats and a variety of their parasites in the Australian environment. Effective control of the feral cat to minimise its potential impact on established and reintroduced populations of native fauna needs to address the presence of resource "hotspots" in the landscape, of both anthropogenic and natural origin.

Control of the definitive host only is not necessarily sufficient to break parasitic life cycles, as evidenced by the potential maintenance of *T. gondii* in host species via vertical transmission. Additionally, the correct management of potential resource-rich "islands" would eliminate or at least minimise the transmission of several parasite species such as *Ancylostoma* spp., *S. erinaceieuropaei*, and *G. spinigerum* from feral cats to the wildlife, whilst monitoring programs can help protect the native fauna from diseases such as toxoplasmosis. In particular, captive breeding and translocation programs should avoid the use of individuals that have a history of *T. gondii* infection to minimise the risk of stress-induced mortalities due to reactivated toxoplasmosis as well as preventing the potential spread of *T. gondii* to "clean" populations.

Clearly, management practices that effectively reduce or eliminate the scavenging potential for cats at rubbish tips and similar sites throughout Australia will not only help to minimise the transmission of feline pathogens in the environment, they will also enhance the control of feral cats. The importance of such management will only intensify as the number of conservation and reintroduction programs operating throughout Australia continues to increase.

REFERENCES

Abbott, I. (2000) Improving the conservation of threatened and rare mammal species through translocation to islands: case study Western Australia. *Biological Conservation*. **93**: 195-201.

Adams, P.J. and Chavand, O.L.A. (2002) Assessment of feral cat numbers and parasite burdens in the Walpole district: A Report to the Department of Conservation and Land Management. In Perth: Murdoch University, pp. 9.

Adlard, R.D. and O'Donoghue, P.J. (1998) Perspectives on the biodiversity of parasitic protozoa in Australia. *International Journal for Parasitology*. **28**: 887-897.

Adrianarivo, A.G., Rowe, J.D., Barr, B.C., Anderson, M.L., Packham, A.E., Sverlow, K.W., Choromanski, L., Loui, C., Grace, A. and Conrad, P.A. (2000) A POLYGEN-adjuvanted killed *Neospora caninum* tachyzoite preparation failed to prevent foetal infection in pregnant cattle following i.v./i.m. experimental tachyzoite challenge. *International Journal for Parasitology*. **30**: 985-990.

Algar, D. and Angus, G.J. (2000) Recommendations on a Control Strategy for Feral Cats at Peron Peninsula, Western Australia: Report to the Project Eden Management Committee. In Perth: Department of Conservation and Land Management, Woodvale, pp. 49.

Algar, D., Angus, G.J., Adams, P.J. and Chavand, O.L.A. (2000) Assessment of feral cat numbers and control options in the bait exclusion area on Mt. Keith: A Report to WMC Resources. In Perth: Department of Conservation and Land Management, pp. 9.

Algar, D., Angus, G.J. and Sinagra, J.A. (1999) Preliminary assessment of a trapping technique to measure feral cat abundance. In Perth: Department of Conservation and Land Management.

Ambroise-Thomas, P. (2000) Emerging parasite zoonoses: the role of host-parasite relationship. *International Journal for Parasitology*. **30**: 1361-1367.

Anderson, R.C. (1972) The ecological relationships of meningeal worm and native cervids in North America. *Journal of Wildlife Diseases*. **8**: 304-310.

Anderson, R.M. and May, R.M. (1979) Population biology of infectious diseases: Part I. *Nature*. **280**: 361-367.

Anderson, R.M. and May, R.M. (1978) Regulation and stability of host-parasite population interactions I. regulatory processes. *Journal of Animal Ecology*. **47**: 219-247.

Andrews, R.H., Adams, M., Boreham, P.F.L., Mayrhofer, G. and Meloni, B.P. (1989) *Giardia intestinalis*: electrophoretic evidence for a species complex. *International Journal for Parasitology*. **19**: 183-190.

Angus, G.J., Onus, M., Fuller, P.J., Liddelow, G. and Ward, B. (2002) Comparison of two aerial baiting regimes with respect to bait acceptance by introduced predators and non-target native fauna, at the Gibson Desert Nature Reserve, Western Australia: A report to the Wind Over Water Foundation. In Perth: Department of Conservation and Land Management, pp. 36.

Appels, R. and Honeycutt, R.L. (1986) rDNA: evolution over a billion years. In *DNA Systematics*. Dutta, S.K. (ed.) Boca Raton, Florida: CRC Press, pp. 81-135.

Archer, M. (1984) The status of Australian dasyurids, thylacinids and myrmecobiids. In *Vertebrate Zoology and Evolution in Australiasia*. Clayton, G. (ed.) Carlisle: Hesperion Press, pp. 1015-1021.

Arundel, J.H., Barker, I.K. and Beveridge, I. (1977) Diseases of marsupials. In *The Biology of Marsupials*. Gillmore, D. (ed.) London: MacMillan Press Ltd., pp. 141-154.

Arundel, J.H., Beveridge, I. and Presidente, P.J.A. (1979) Parasites and pathological findings in enclosed and free-ranging populations of *Macropus rufus* (Desmarest) (Marsupialia) at Menindee, New South Wales. *Australian Wildlife Research*. **6**: 361-379.

Ashton, N. (1979) Ocular toxoplasmosis in wallabies (*Macropus rufogriseus*). *American Journal of Ophthalmology*. **88**: 322-332.

Atkinson, I.A.E. (2001) Introduced mammals and models for restoration. *Biological Conservation*. **99**: 81-96.

Attwood, H.D., Woolley, P.A. and Rickard, M.D. (1975) Toxoplasmosis in Dasyurid marsupials. *Journal of Wildlife Diseases*. **11**: 543-551.

Baldock, F.C., Thompson, R.C., Kumaratilake, L.M. and Shield, J. (1985) *Echinococcus granulosus* in farm dogs and dingoes in south eastern Queensland. *Australian Veterinary Journal*. **62**: 335-337.

Barber, J.S. and Trees, A.J. (1998) Naturally occurring vertical transmission of *Neospora caninum* in dogs. *International Journal for Parasitology*. **28**: 57-64.

Barker, I.K., O'Callaghan, M.G. and Beveridge, I. (1988) *Eimeria* spp. (Apicomplexa: Eimeriidae) parasitic in the rat-kangaroos *Hypsiprymnodon moschatus*, *Potorous tridactylus*, *Aepyprymnus rufescens* and *Bettogia gaimardi* (Marsupialia: Potoroidae). *International Journal for Parasitology*. **18**: 947-953.

Barker, I.K., O'Callaghan, M.G. and Beveridge, I. (1989) Host-parasite associations of *Eimeria* spp. (Apicomplexa: Eimeriidae) in kangaroos and wallabies of the genus *Macropus* (Marsupialia: Macropodidae). *International Journal for Parasitology*. **19**: 241-263.

Barratt, D.G. (1997) Predation by house cats, *Felis catus* (L.), in Canberra, Australia. I. Prey composition and preference. *Wildlife Research*. **24**: 263-277.

Bartlett, M.S. (1960) The critical community size for measles in the United States. *Journal of the Royal Statistical Society Series A*. **123**: 37-44.

Bartlett, M.S., Harper, K., Smith, N., Verbanac, P. and Smith, J.W. (1978) Comparative evaluation of a modified zinc sulfate flotation technique. *Journal of Clinical Microbiology*. **7**: 524-528.

Barton, M.A. and McEwan, D.R. (1993) Spirurid nematodes in dogs and cats from central Australia. *Australian Veterinary Journal*. **70**: 270.

Bayly, C.P. (1978) A comparison of the diets of the red fox and the feral cat in an arid environment. *South Australian Naturalist*. **53**: 20-28.

Bayly, C.P. (1976) Observations on the food of the feral cat (*Felis catus*) in an arid environment. *South Australian Naturalist*. **51**: 22-24.

Bearup, A.J. (1948) Observations on the life cycle of *Diphyllobothrium* (*Spirometra*) *erinacei* in Australia. *Australian Journal of Science*. **10**: 183-184.

Behnke, J.M. (1991) Pathology. In *Hookworm Infections*. Ball, P.A.J. (ed.) Amsterdam: Elsevier Science Publishers B. V., pp. 51-91.

Berdoy, M., Webster, J.P. and Macdonald, D.W. (2000) Fatal attraction in *Toxoplasma*-infected rats: a case of parasite manipulation of its mammalian host. *Proceedings of the Royal Society of London Series B Biological Sciences*. **267**: 1591-1594.

Berdoy, M., Webster, J.P. and Macdonald, D.W. (1995) Parasite-altered behaviour: is the effect of *Toxoplasma gondii* on *Rattus norvegicus* specific? *Parasitology*. **111**: 403-409.

Bettiol, S.S., Kettlewell, J.S., Davies, N.J. and Goldsmid, J.M. (1997) Giardiasis in native marsupials of Tasmania. *Journal of Wildlife Diseases*. **33**: 352-354.

Bettiol, S.S., Obendorf, D.L., Nowarkowski, M. and Goldsmid, J.M. (2000a) Pathology of experimental toxoplasmosis in Eastern Barred Bandicoots in Tasmania. *Journal of Wildlife Diseases*. **36**: 141-144.

Bettiol, S.S., Obendorf, D.L., Nowarkowski, M., Milstein, T. and Goldsmid, J.M. (2000b) Earthworms as paratenic hosts of toxoplasmosis in Eastern barred Bandicoots in Tasmania. *Journal of Wildlife Diseases*. **36**: 145-148.

Beveridge, I. (2002) Australian hookworms (Ancylostomatoidea): a review of the species present, their distributions and biogeographical origins. *Parassitologia*. **44**: 83-88.

Beveridge, I. (1985) The genus *Bertiella* (Cestoda: Anoplocephalidae) from Australasian mammals: new species, new records and redescriptions. *Systematic Parasitology*. **7**: 241-289.

Beveridge, I. (1980) *Uncinaria hydromyidis* sp. n. (Nematoda: Ancylostomatidae) from the Australian water rat, *Hydromys chrysogaster*. *Journal of Parasitology*. **66**: 1027-1031.

Beveridge, I. and Arundel, J.H. (1979) Helminth parasites of grey kangaroos, *Macropus giganteus* Shaw and *M. fuliginosus* (Desmarest), in eastern Australia. *Australian Wildlife Research*. **6**: 69-77.

Beveridge, I., Chilton, N.B. and Andrews, R.H. (1993) Sibling species within *Macropostrongyloides baylisi* (Nematoda: Strongyloidea) from macropodid marsupials detected by allozyme electrophoresis. *International Journal for Parasitology*. **23**: 21-32.

Beveridge, I., Friend, S.C.E., Jeganathan, N. and Charles, J. (1998) Proliferative sparganosis in Australian dogs. *Australian Veterinary Journal*. **76**: 757-759.

Beveridge, I. and Jones, M.K. (2002) Diversity and biogeographical relationships of the Australian cestode fauna. *International Journal for Parasitology*. **32**: 343-351.

Beveridge, I., Presidente, P.J.A. and Arundel, J.H. (1978) *Gnathostoma spinigerum* infection in a feral cat from New South Wales. *Australian Veterinary Journal*. **54**: 46.

Beveridge, I., Speare, R., Johnson, P.M. and Spratt, D.M. (1992) Helminth parasite communities of Macropodoid marsupials of the genera *Hypsiprymnodon*, *Aepyprymnus*, *Thylogale*, *Onychogalea*, *Lagorchestes* and *Dendrolagus* from Queensland. *Wildlife Research*. **19**: 359-376.

Beveridge, I. and Spratt, D.M. (1996) The helminth fauna of Australasian marsupials: Origins and evolutionary biology. *Advances in Parasitology*. **37**: 135-254.

Beverley, J.K.A. (1959) Congenital transmission of toxoplasmosis through successive generations of mice. *Nature*. **183**: 1348-1349.

Bjorkman, C., Johansson, O., Stenlund, S., Holmdahl, O.J. and Uggla, A. (1996) *Neospora* species infection in a herd of dairy cattle. *Journal of the American Veterinary Medical Association*. **208**: 1441-1444.

Blake, R.T. and Overend, D.J. (1982) The prevalence of *Dirofilaria immitis* and other parasites in urban pound dogs in north-eastern Victoria. *Australian Veterinary Journal*. **58**: 111-114.

Blewett, D.A. (1983) The epidemiology of ovine toxoplasmosis. I. The interpretation of data for the prevalence of antibody in sheep and other host species. *British Veterinary Journal*. **139**: 537-545.

Blewett, D.A. and Watson, W.A. (1983) The epidemiology of ovine toxoplasmosis. II. Possible sources of infection in outbreaks of clinical disease. *British Veterinary Journal*. **139**: 546-555.

Bonsall, M.B. and Hassell, M.P. (1997) Apparent competition structures ecological assemblages. *Nature*. **388**: 371-373.

Boorman, G.A., Kollias, G.V. and Taylor, R.F. (1977) An outbreak of toxoplasmosis in wallaroos (*Macropus robustus*) in a California zoo. *Journal of Wildlife Diseases*. **13**: 64-68.

Bowles, J. and McManus, D.P. (1993) Rapid discrimination of *Echinococcus* species and strains using a polymerase chain reaction-based RFLP method. *Molecular and Biochemical Parasitology*. **57**: 231-240.

Bowman, D.D., Hendrix, C.M., Lindsay, D.S. and Barr, S.C. (2002) *Feline Clinical Parasitology*. Ames: Iowa Sate University Press.

Bradley, D.J. (1974) Stability in host-parasite systems. In *Ecological Stability*. Williamson, M.H. (ed.) London: Chapman and Hall, pp. 71-87.

Breckwoldt, R. (1988) *A Very Elegant Animal, the Dingo*. North Ryde, NSW: Angus & Robertson.

Bretagne, S., Costa, J.M., Vidaud, M., Nhieu, J.T.V. and Fleury-Feith, J. (1993) Detection of *Toxoplasma gondii* by competitive DNA Amplification of bronchoalveolar lavage samples. *Journal of Infectious Diseases*. **168**: 1585-1588.

Brothers, N.P. (1984) Breeding, distribution and status of burrow-nesting petrels at Macquarie Island. *Australian Wildlife Research*. **11**: 113-131.

Brothers, N.P., Skira, I.J. and Copson, G.R. (1985) Biology of the feral cat, *Felis catus* (L.), on Macquarie Island. *Australian Wildlife Research*. **12**: 425-436.

Bundy, D.A.P. and Keymer, A.E. (1991) The epidemiology of hookworm infection. In *Hookworm Infections*. Ball, P.A.J. (ed.) Amsterdam: Elsevier Science Publishers B. V., pp. 157-178.

Burbidge, A.A. and McKenzie, N.L. (1989) Patterns in the modern decline of Western Australia's vertebrate fauna: causes and conservation implications. *Biological Conservation*. **50**: 143-198.

BureauofMeteorology (2001) Interannual rainfall difference of Western Australia, September 2001-September 2000, http://www.bom.gov.au. In: Commonwealth of Australia.

Burg, J.L., Grover, C.M., Pouletty, P. and Boothroyd, J.C. (1989) Direct and sensitive detection of a pathogenic protozoan, *Toxoplasma gondii*, by polymerase chain reaction. *Journal of Clinical Microbiology*. **27**: 1787-1792.

Burney, D.P., Chavkin, M.J., Dow, S.W., Potter, T.A. and Lappin, M.R. (1998) Polymerase chain reaction for the detection of *Toxoplasma gondii* within aqueous humor of experimentally-inoculated cats. *Veterinary Parasitology*. **79**: 181-186.

Buxton, D. (1998) Protozoan infections (*Toxoplasma gondii*, *Neospora caninum* and *Sarcocystis* spp.) in sheep and goats: recent advances. *Veterinary Research*. **29**: 289-310.

Campbell, A.J., Gasser, R.B. and Chilton, N.B. (1995) Differences in a ribosomal DNA sequence of *Strongylus* species allows identification of single eggs. *International Journal for Parasitology*. **25**: 359-365.

Canfield, P.J., Hartley, W.J. and Dubey, J.P. (1990) Lesions of toxoplasmosis in Australian marsupials. *Journal of Comparative Pathology*. **103**: 159-167.

Carruthers, V.B. (2002) Host cell invasion by the opportunistic pathogen *Toxoplasma gondii*. *Acta Tropica*. **81**: 111-122.

Cassis, G. (1998) Biodiversity loss: a human health issue. *Medical Journal of Australia*. **169**: 568-569.

Catling, P.C. (1988) Similarities and contrasts in the diets of foxes, *Vulpes vulpes*, and cats, *Felis catus*, relative to fluctuating prey populations and drought. *Australian Wildlife Research*. **15**: 307-317.

Cazenave, J., Cheyrou, A. and Blouin, P. (1991) Use of polymerase chain reaction to detect *Toxoplasma*. *Journal of Clinical Pathology*. **44**: 1037.

Chandler, A.C. (1925) The helminthic parasites of cats in Calcutta and the relation of cats to human helminthic infections. *Indian Journal of Medical Research*. **13**: 213.

Chilton, N.B., Beveridge, I. and Andrews, R.H. (1992) Detection by allozyme electrophoresis of cryptic species of *Hypodontus macropi* (Nematoda: Strongyloidea) from macropodid marsupials. *International Journal for Parasitology*. **22**: 271-279.

Chilton, N.B., Beveridge, I. and Andrews, R.H. (1993) Electrophoretic comparison of *Rugopharynx longibursaris* Kung and *Rugopharynx omega* Beveridge (Nematoda: Strongyloidea), with the description of *R. sigma* n.s.p. from the red-legged pademelon, *Thylogale stigmatica*. *Systematic Parasitology*. **26**: 159-169.

Chilton, N.B. and Gasser, R.B. (1999) Sequence differences in the internal transcribed spacers of DNA among four species of hookworm (Ancylostomatoidea: *Ancylostoma*). *International Journal for Parasitology*. **29**: 1971-1977.

Chowdhury, A.B. and Schad, G.A. (1972) *Ancylostoma ceylanicum*: a parasite of man in Clacutta and environs. *American Journal of Tropical Medicine and Hygiene*. **21**: 300-301.

Christensen, P. and Burrows, N. (1994) Project desert dreaming: experimental reintroduction of mammals to the Gibson Desert, Western Australia. In *Reintroduction Biology of Australian and New Zealand Fauna*. Serena, M. (ed.) Chipping Norton: Surrey Beatty & Sons, pp. 199-207.

Cingolani, A., Luca, A.D., Ammassari, A., Murri, R., Linzalone, A., Grillo, R. and Antinori, A. (1996) PCR detection of *Toxoplasma gondii* DNA in CSF for the differential diagnosis of AIDS-related focal brain lesions. *Journal of Medical Microbiology*. **45**: 472-476.

Cleaveland, S. and Dye, C. (1995) Maintenance of a microparasite infecting several host species: rabies in the Serengeti. *Parasitology*. **111**: S33-47.

Cleaveland, S., Thirgood, S. and Laurenson, K. (1999) Pathogens as allies in island conservation? *Trends in Ecology and Evolution*. **14**: 83-84.

Cockburn, A. (1980) The diet of the New Holland mouse (*Pseudomys navaehollandiae*) and the house mouse (*Mus musculus*) in a Victorian coastal heathland. *Australian Mammalogy*. **3**: 31-34.

Cole, R.A., Lindsay, D.S., Blagburn, B.L. and Dubey, J.P. (1995) Vertical transmission of *Neospora caninum* in mice. *Journal of Parasitology*. **81**: 730-732.

Collins, G.H., Emslie, D.R., Farrow, B.R.H. and Watson, A.D.J. (1983) Sporozoa in dogs and cats. *Australian Veterinary Journal*. **60**: 289-290.

Collins, G.H., Pope, S.E., Griffin, D.L., Walker, J. and Connor, G. (1987) Diagnosis and prevalence of *Giardia* spp. in dogs and cats. *Australian Veterinary Journal*. **64**: 89-90.

Coman, B.J. (1973a) The diet of red foxes, *Vulpes vulpes* L. in Victoria. *Australian Journal of Zoology*. **21**: 391-401.

Coman, B.J. (1972a) Helminth parasites of the dingo and feral dog in Victoria with some notes on the diet of the host. *Australian Veterinary Journal*. **48**: 456-460.

Coman, B.J. (1973b) Helminth parasites of the fox (*Vulpes vulpes*) in Victoria. *Australian Veterinary Journal*. **49**: 378-384.

Coman, B.J. (1972b) A survey of the gastro-intestinal parasites of the feral cat in Victoria. *Australian Veterinary Journal*. **48**: 133-136.

Coman, B.J. and Brunner, H. (1972) Food habits of the feral house cat in Victoria. *Journal of Wildlife Management*. **36**: 848-853.

Coman, B.J., Jones, E.H. and Driesen, M.A. (1981a) Helminth parasites and arthropods of feral cats. *Australian Veterinary Journal*. **57**: 324-327.

Coman, B.J., Jones, E.H. and Westbury, H.A. (1981b) Protozoan and viral infections of feral cats. *Australian Veterinary Journal*. **57**: 319-323.

Cook, A.J., Gilbert, R.E., Buffolano, W., Zufferey, J., Petersen, E., Jenum, P.A., Foulon, W., Semprini, A.E. and Dunn, D.T. (2000) Sources of *Toxoplasma* infection in pregnant women: European multicentre case-control study. European Research Network on Congenital Toxoplasmosis. *British Medical Journal*. **321**: 142-147.

Cook, I. and Pope, J.H. (1959) *Toxoplasma* in Queensland, III. A preliminary survey of animal hosts. *Australian Journal of Experimental Biology and Medical Science*. **37**: 253-262.

Costa, J.M., Pautas, C., Ernault, P., Foulet, F., Cordonnier, C. and Bretagne, S. (2000) Real-time PCR for diagnosis and follow-up of *Toxoplasma* reactiviation after allogenic stem cell transplantation using

fluorescence resonance energy transfer hybridization probes. *Journal of Clinical Microbiology*. **38**: 2929-2932.

Costanza, R., d'Arge, R., de Groots, R., Farber, S., Grasso, M., Hannon, B., Limburg, K., Naeem, S., O'Neill, R.V., Paruelo, J., Raskin, R.G., Sutton, P. and van den Belt, M. (1997) The value of the world's ecosystem services and natural capital. *Nature*. **387**: 253-260.

Croft, J.D. and Hone, L.J. (1978) The stomach contents of foxes, *Vulpes vulpes* collected in New South Wales. *Australian Wildlife Research*. **5**: 85-92.

Croll, N.A. (1972) Feeding and lipid synthesis of *Ancylostoma tubaeforme* preinfective larvae. *Parasitology*. **64**: 369-378.

Croll, N.A., Matthews, B.E. and Smith, J.M. (1975) Hookworm behaviour: larval movement patterns after entering hosts. *International Journal for Parasitology*. **5**: 551-556.

Crompton, D.W. (2000) The public health importance of hookworm disease. *Parasitology*. **121**: S39-50.

Cross, J. (1990) The feral cat - justification for its control. In *Associate Diploma in Applied Science thesis* Riverina: Charles Sturt University.

Cunningham, A.A. (1996) Disease risks of wildlife translocations. *Conservation Biology*. **10**: 349-353.

Cunningham, A.A. and Daszak, P. (1998) Extinction of a species of land snail due to infection with a microsporidian parasite. *Conservation Biology*. **12**: 1139-1141.

da Silva, A.V. and Langoni, H. (2001) The detection of *Toxoplasma gondii* by comparing cytology, histopathology, bioassay in mice, and the polymerase chain reaction (PCR). *Veterinary Parasitology*. **97**: 193-200.

Daengsvang, S., Sermswatsri, B. and Yongyi, P. (1969) Spontaneous cure of the natural and induced *Gnathostoma spinigerum* infection in cats. *Annals of Tropical Medicine and Parasitology*. **63**: 489-491.

Danciger, M. and Lopez, M. (1975) Numbers of *Giardia* in the faeces of infected children. *American Journal of Tropical Medicine and Hygiene*. **24**: 237-242.

Dards, J.L. (1983) The behaviour of dockyard cats: interactions of adult males. *Applied Animal Ethology*. **10**: 133-153.

Dards, J.L. (1978) Home ranges of feral cats in Portsmouth Dockyard. *Carnivora Genetics Newsletter*. **3**: 242-255.

Daszak, P. and Cunningham, A.A. (1999) Extinction by infection. *Trends in Ecology and Evolution*. **14**: 279.

Daszak, P., Cunningham, A.A. and Hyatt, A.D. (2001) Anthropogenic environmental change and the emergence of infectious diseases in wildlife. *Acta Tropica*. **78**: 103-116.

Daszak, P., Cunningham, A.A. and Hyatt, A.D. (2000) Emerging infectious diseases of Wildlife- threats to biodiversity and human health. *Science*. **287**: 443-449.

Davis, S.W. and Dubey, J.P. (1995) Mediation of immunity to *Toxoplasma gondii* oocyst shedding in cats. *Journal of Parasitology*. **81**: 882-886.

de Roever-Bonnet, H. (1969) Congenital *Toxoplasma* infections in mice and hamsters infected with avirulent and virulent strains. *Tropical and Geographical Medicine*. **21**: 443-450.

de Thoisy, B., Demar, M., Aznar, C. and Carme, B. (2003) Ecologic correlates of *Toxoplasma gondii* exposure in free-ranging neotropical mammals. *Journal of Wildlife Diseases*. **39**: 456-459.

Delvinquier, B.L.J. and Freeland, W.J. (1988) Protozoan parasites of the Cane Toad, *Bufo marinus*, in Australia. *Australian Journal of Zoology*. **36**: 301-316.

Denny, E., Yakovlevich, P., Eldridge, M.D.B. and Dickman, C.R. (2002) Social and genetic analysis of a population of free-living cats (*Felis catus* L.) exploiting a resource-rich habitat. *Wildlife Research*. **29**: 405-413.

Dickman, C.R. (1996a) Impact of exotic generalist predators on the native fauna of Australia. *Wildlife Biology*. **3**: 165-175.

Dickman, C.R. (1996b) *Overview of the Impacts of Feral Cats on Australian Native Fauna*. Canberra: Australian Nature Conservation Agency.

Dobson, A.P. and Carper, E.R. (1996) Infectious diseases and human population history. *Bioscience*. **46**: 115-126.

Dobson, A.P. and Crawley, M. (1994) Pathogens and the structure of plant communities. *Trends in Ecology and Evolution*. **9**: 393-398.

Dobson, A.P. and May, R.M. (1986) Disease and conservation. In *Conservation Biology: The Science of Scarcity and Diversity*. Soule, M. (ed.) Mass: Sinauer Associates, pp. 345-365.

Dobson, A.P. and Meagher, M. (1996) The population dynamics of brucellosis in the Yellowstone National Park. *Ecology*. **77**: 1026-1036.

Dreesen, D.W. (1990) *Toxoplasma gondii* infections in wildlife. *Journal of the American Veterinary Medical Association*. **196**: 274-276.

Dubey, J.P. (1998a) Advances in the life cycle of *Toxoplasma gondii*. *International Journal for Parasitology*. **28**: 1019-1024.

Dubey, J.P. (1997) Distribution of tissue cysts in organs of rats fed *Toxoplasma gondii* oocysts. *Journal of Parasitology*. **83**: 755-757.

Dubey, J.P. (1995) Duration of immunity to shedding of *Toxoplasma gondii* oocysts by cats. *Journal of Parasitology*. **81**: 410-415.

Dubey, J.P. (1973) Feline toxoplasmosis and coccidiosis: a survey of domiciled and stray cats. *Journal of the American Veterinary Medical Association*. **162**: 873-877.

Dubey, J.P. (1996) Infectivity and pathogenicity of *Toxoplasma gondii* oocysts for cats. *Journal of Parasitology*. **82**: 957-961.

Dubey, J.P. (1977) Persistence of *Toxoplasma gondii* in the tissues of chronically infected cats. *Journal of Parasitology*. **63**: 156-157.

Dubey, J.P. (1998b) Refinement of pepsin digestion method for isolation of *Toxoplasma gondii* from infected tissues. *Veterinary Parasitology*. **74**: 75-77.

Dubey, J.P. (2003) Review of *Neospora caninum* and neosporosis in animals. *Korean Journal of Parasitology*. **41**: 1-16.

Dubey, J.P. (1998c) *Toxoplasma gondii* oocyst survival under defined temperatures. *Journal of Parasitology*. **84**: 862-865.

Dubey, J.P. (1994) Toxoplasmosis. *Journal of the American Veterinary Medical Association*. **205**: 1593-1598.

Dubey, J.P. (1986) Toxoplasmosis in cats. *Feline Practice*. **16**: 12-45.

Dubey, J.P. (1991) Toxoplasmosis--an overview. *Southeast Asian Journal of Tropical Medicine and Public Health*. **22 Suppl**: 88-92.

Dubey, J.P., Barr, B.C., Barta, J.R., Bjerkas, I., Bjorkman, C., Blagburn, B.L., Bowman, D.D., Buxton, D., Ellis, J.T., Gottstein, B., Hemphill, A., Hill, D.E., Howe, D.K., Jenkins, M.C., Kobayashi, Y., Koudela, B., Marsh, A.E., Mattsson, J.G., McAllister, M.M., Modry, D., Omata, Y., Sibley, L.D., Speer, C.A., Trees, A.J., Uggla, A., Upton, S.J., Williams, D.J. and Lindsay, D.S. (2002) Redescription of *Neospora caninum* and its differentiation from related coccidia. *International Journal for Parasitology*. **32**: 929-946.

Dubey, J.P. and Beattie, C.P. (1988) *Toxoplasmosis of animals and man*. Boca Raton, FL: CRC Press.

Dubey, J.P. and Carpenter, J.L. (1993) Neonatal toxoplasmosis in littermate cats. *Journal of the American Veterinary Medical Association*. **203**: 1546-1549.

Dubey, J.P., Carpenter, J.L., Speer, C.A., Topper, M.J. and Uggla, A. (1988) Newly recognised fatal protozoan disease of dogs. *Journal of the American Veterinary Medical Association*. **192**: 1269-1285.

Dubey, J.P. and Frenkel, J.K. (1972a) Cyst-induced toxoplasmosis in cats. *Journal of Protozoology*. **19**: 155-177.

Dubey, J.P. and Frenkel, J.K. (1972b) Extra-intestinal stages of *Isospora felis* and *Isospora rivolta* (Protozoa: Eimeriidae) in cats. *Journal of Protozoology*. **19**: 89-92.

Dubey, J.P. and Frenkel, J.K. (1976) Feline toxoplasmosis from acutely infected mice and the development of *Toxoplasma* cysts. *Journal of Protozoology*. **23**: 537-546.

Dubey, J.P. and Lappin, M.R. (1998) Toxoplasmosis and Neosporosis. In *Infectious Diseases of the Dog and Cat*. Greene, C.E. (ed.) Philadelphia: W. B. Saunders, pp. 493-509.

Dubey, J.P., Lappin, M.R. and Thulliez, P. (1995a) Diagnosis of induced toxoplasmosis in neonatal cats. *Journal of the American Veterinary Medical Association*. **207**: 179-185.

Dubey, J.P., Lappin, M.R. and Thulliez, P. (1995b) Long-term antibody responses of cats fed *Toxoplasma gondii* tissue cysts. *Journal of Parasitology*. **81**: 887-893.

Dubey, J.P., Lindsay, D.S. and Speer, C.A. (1998) Structures of *Toxoplasma gondii* tachyzoites, bradyzoites, and sporozoites and biology and development of tissue cysts. *Clinical Microbiology Reviews*. **11**: 267-299.

Dubey, J.P., Miller, N.L. and Frenkel, J.K. (1970) The *Toxoplasma gondii* oocyst from cat feces. *Journal of Experimental Medicine*. **132**: 636-662.

Dubey, J.P., Rollor, E.A., Smith, K., Kwok, O.C. and Thulliez, P. (1997a) Low seroprevalence of *Toxoplasma gondii* in feral pigs from a remote island lacking cats. *Journal of Parasitology*. **83**: 839-841.

Dubey, J.P., Shen, S.K., Kwok, O.C. and Thulliez, P. (1997b) Toxoplasmosis in rats (*Rattus norvegicus*): congenital transmission to first and second generation offspring and isolation of *Toxoplasma gondii* from seronegative rats. *Parasitology*. **115**: 9-14.

Dubey, J.P., Shen, S.K., Kwok, O.C.H. and Frenkel, J.K. (1999) Infection and immunity with the RH strain of *Toxoplasma gondii* in rats and mice. *Journal of Parasitology*. **85**: 657-662.

Dubey, J.P., Weigel, R.M., Siegel, A.M., Thulliez, P., Kitron, U.D., Mitchell, M.A., Mannelli, A., Mateus-Pinilla, N.E., Shen, S.K., Kwok, O.C. and Todd, K.S. (1995c) Sources and reservoirs of *Toxoplasma gondii* infection on 47 swine farms in Illinois. *Journal of Parasitology*. **81**: 723-729.

Duncanson, P., Terry, R.S., Smith, J.E. and Hide, G. (2001) High levels of congenital transmission of *Toxoplasma gondii* in a commercial sheep flock. *International Journal for Parasitology*. **31**: 1699-1703.

Dunsmore, J.D. and Shaw, S.E. (1990) *Clinical Parasitology of Dogs. Veterinary Review 31*. Sydney: The University of Sydney Post-Graduate Foundation in Veterinary Science.

Dupon, M., Cazenave, J., Pellegrin, J., Ragnaud, J., Cheyrou, A., Fischer, I., Leng, B. and Lacut, J. (1995) Detection of *Toxoplasma gondii* by PCR and tissue culture in cerebrospinal fluid and blood of human immunodeficiency virus-seropositive patients. *Journal of Clinical Microbiology*. **33**: 2421-2426.

Ellis, J.T. (1998) Polymerase chain reaction approaches for the detection of *Neospora caninum* and *Toxoplasma gondii*. *International Journal for Parasitology*. **28**: 1053-1060.

Engels, W.R. (1993) Contributing software to the Internet: the *Amplify* program. *Trends in Biochemical Sciences*. **18**: 448-450.

Epe, C., Samson-Himmelstjerna, G.V. and Schnieder, T. (1997) Differences in a ribosomal DNA sequence of lungworm species (Nematoda:Dictyocaulidae) from fallow deer, cattle, sheep and donkeys. *Research in Veterinary Science*. **62**: 17-21.

Erlandsen, S.L., Sherlock, L.A., Bemrick, W.J., Ghobrial, H. and Jakubowski, W. (1990) Prevalence of *Giardia* spp. in beaver and muskrat populations in northeastern states and Minnesota: detection of intestinal trophozoites at necropsy provides greater sensitivity than detection of cysts in faecal samples. *Applied and Environmental Microbiology*. **56**: 31-36.

Esteban-Redondo, I., Maley, S.W., Thomson, K., Nicoll, S., Wright, S., Buxton, D. and Innes, E.A. (1999) Detection of *T. gondii* in tissues of sheep and cattle following oral infection. *Veterinary Parasitology*. **86**: 155-171.

Ey, P.L., Andrews, R.H. and Mayrhofer, G. (1993) Genetic differentiation of major genotypes of *Giardia intestinalis* using polymerase chain reaction anlysis of a gene encoding a specific trophozoite surface antigen. *Parasitology*. **106**: 347-356.

Ey, P.L., Mansouri, M., Kulda, J., Nohynkova, E., Monis, P.T., Andrews, R.H. and Mayrhofer, G. (1997) Genetic analysis of *Giardia* from hoofed farm animals reveals Articodactyl-specific and potentially zoonotic genotypes. *Journal of Eukaryotic Microbiology*. **44**: 626-635.

Fazaeli, A., Carter, P.E. and Pennington, T.H. (2000) Intergenic spacer (IGS) polymorphism: a new genetic marker for differentiation of *Toxoplasma gondii* strains and *Neospora caninum*. *Journal of Parasitology*. **86**: 716-723.

Feely, D.E., Gardner, M.D. and Hardin, E.L. (1991) Excystation of *Giardia muris* induced by a phosphate-bicarbonate medium: localization of acid phosphatase. *Journal of Parasitology*. **77**: 441-448.

Feng, X.C. and Milhausen, M. (1999) Localization of *Toxoplasma gondii* in feline intestinal tissue using PCR. *Journal of Parasitology*. **85**: 1041-1046.

Filice, F.P. (1952) Studies on the cytology and life history of a *Giardia* from the laboratory rat. *University of California Publications in Zoology*. **57**: 53-146.

Fischer, J. and Lindenmayer, D.B. (2000) An assessment of the published results of animal relocations. *Biological Conservation*. **96**: 1-11.

Flegr, J., Zitkova, S., Kodym, P. and Frynta, D. (1996) Induction of changes in human behaviour by the parasitic protozoan *Toxoplasma gondii*. *Parasitology*. **113**: 49-54.

Frappel, P.B. and Rose, R.W. (1986) A radiographic study of the gastrointestinal tract of *Potorous tridactylus*, with a suggestion as to the role of the foregut and hindgut in potoroine marsupials. *Australian Journal of Zoology*. **34**: 463-477.

Freeland, W.J. (1994) Parasites, pathogens and the impacts of introduced organisms on the balance of nature in Australia. In *Conservation Biology in Australia and Oceania*. Kikkawa, J. (ed.) Sydney: Surrey Beatty & Sons, pp. 171-180.

Freeland, W.J. and Boulton, W.J. (1992) Coevolution of food webs: Parasites, predators and plant secondary compounds. *Biotropica*. **24**: 309-327.

Freeland, W.J., Delvinquier, B.L.J. and Bonnin, B. (1986) Decline of cane toad, *Bufo marinus*, populations: Status of urban populations. *Australian Wildlife Research*. **13**: 597-601.

Frenkel, J.K. and Dubey, J.P. (1972) Rodents as vectors for the feline coccidia, *Isospora felis* and *Isospora rivolta*. *Journal of Infectious Diseases*. **125**: 69-72.

Frenkel, J.K., Hassanein, K.M., Hassanein, R.S., Brown, E., Thulliez, P. and Quintero-Nunez, R. (1995) Transmission of *Toxoplasma gondii* in Panama City, Panama: a five-year prospective cohort study of children, cats, rodents, birds, and soil. *American Journal of Tropical Medicine and Hygiene*. **53**: 458-468.

Frenkel, J.K. and Ruiz, A. (1981) Endemicity of toxoplasmosis in Costa Rica, transmission between cats, soil, intermediate hosts and humans. *American Journal of Epidemiology*. **113**: 254-269.

Frenkel, J.K., Ruiz, A. and Chinchilla, M. (1975) Soil survival of *Toxoplasma* oocysts in Kansas and Costa Rica. *American Journal of Tropical Medicine and Hygiene*. **24**: 439-443.

Freyre, A., Dubey, J.P., Smith, D.D. and Frenkel, J.K. (1989) Oocyst-induced *Toxoplasma gondii* infections in cats. *Journal of Parasitology*. **75**: 750-755.

Freyre, A., Falcon, J., Correa, O., El Elhou, S., Mendez, J. and Gedda, C. (1999) Congenital transmission of experimental chronic toxoplasmosis in rats. *Journal of Parasitology*. **85**: 746-748.

Garcia, L.S. (1998) Giardiasis. In *Parasitology*. Wakelin, D. (ed.) London: Arnold, pp. 193-202.

Gardiner, M.R. and Fraser, D. (1960) Canine hookworm disease in south western Australia. *Australian Veterinary Journal*. **36**: 405-407.

Garnett, G.P. and Holmes, E.C. (1996) The ecology of emergent infectious disease. *Bioscience*. **46**: 127-135.

Gasser, R.B., Chilton, N.B., Hoste, H. and Beveridge, I. (1993) Rapid sequencing of rDNA from single worms and eggs of parasitic helminths. *Nucleic Acids Research*. **21**: 2525-2526.

Gasser, R.B., Monti, J.R., Bao-Zhen, Q., Polderman, A.M., Nansen, P. and Chilton, N.B. (1998) A mutation scanning approach for the identification of hookworm species and analysis of population variation. *Molecular and Biochemical Parasitology*. **92**: 303-312.

Gasser, R.B., Stewart, L.E. and Speare, R. (1996) Genetic markers in ribosomal DNA for hookworm identification. *Acta Tropica*. **62**: 15-21.

Ghosh, S., Debnath, A., Sil, A., De, S., Chattopadhyay, D.J. and Das, P. (2000) PCR detection of *Giardia lamblia* in stool: targeting intergenic spacer region of multicopy rRNA gene. *Molecular and Cellular Probes*. **14**: 181-189.

Gibb, D.G.A., Kakulas, B.A., Perret, D.H. and Jenkyn, D.J. (1966) Toxoplasmosis in the Rottnest Quokka (*Setonix brachyurus*). *Australian Journal of Experimental Biology and Medical Science*. **44**: 665-672.

Gibson, D.F., Lundie-Jenkins, G., Langford, D.G., Cole, J.R., Clarke, D.E. and Johnson, K.A. (1994) Predation by feral cats, *Felis catus*, on the Rufous Hare-Wallaby, *Lagorchestes hirsutus*, in the Tanami Desert. *Australian Mammalogy*. **17**: 103-107.

Goka, A.K.J., Rolston, D.D.K., Mathan, V.I. and Farthing, M.J.G. (1990) The relative merits of faecal and duodenal juice microscopy in the diagnosis of giardiasis. *Transactions of the Royal Society of Tropical Medicine and Hygiene*. **84**: 66-67.

Gordon, H.M.L., Forsyth, B.A. and Robinson, M. (1954) Sparganosis in feral pigs in New South Wales. *Australian Veterinary Journal*. **30**: 135-138.

Graczyk, T.K., Fayer, R., Trout, J.M., Lewis, E.J., Farley, C.A., Sulaiman, I. and Lal, A.A. (1998) *Giardia* sp. cysts and infectious *Cryptosporidium parvum* oocysts in the feces of migratory Canada Geese (*Branta canadensis*). *Applied and Environmental Microbiology*. **64**: 2736-2738.

Grainger, H.J. and Jenkins, D.J. (1996) Transmission of hydatid disease to sheep from wild dogs in Victoria, Australia. *International Journal for Parasitology*. **26**: 1263-1270.

Gregory, G.G. and Munday, B.L. (1976) Internal parasites of feral cats from the Tasmanian Midlands and King Island. *Australian Veterinary Journal*. **52**: 317-320.

Gregory, G.G., Munday, B.L., Beveridge, I. and Rickard, M.D. (1975) Studies on *Anoplotaenia dasyuri* Beddard, 1911 (cestoda: taeniidae), a parasite of the Tasmanian Devil: life cycle and epidemiology. *International Journal for Parasitology*. **5**: 187-191.

Grenfell, B. and Harwood, J. (1997) (Meta)population dynamics of infectious diseases. *Trends in Ecology and Evolution*. **12**: 395-399.

Grice, R.L. and Prociv, P. (1993) *In vitro* embryonation of *Syphacia obvelata* eggs. *International Journal for Parasitology*. **23**: 257-260.

Grigg, M.E. and Boothroyd, J.C. (2001) Rapid identification of virulent type I strains of the protozoan pathogen *Toxoplsma gondii* by PCR-restriction fragment length polymorphism analysis at the *B1* gene. *Journal of Clinical Microbiology*. **39**: 398-400.

Guay, J.M., Dubois, D., Morency, M.J., Gagnon, S., Mercier, J. and Levesque, R.C. (1993) Detection of the pathogenic parasite *Toxoplasma gondii* by specific amplification of ribosomal sequences using comultiplex polymerase chain reaction. *Journal of Clinical Microbiology*. **31**: 203-207.

Guiler, E.R. (1961) The former distribution and decline of the Thylacine. *Australian Journal of Science*. **23**: 207-210.

Gulland, F.M.D. (1995) The impact of infectious diseases on wild animal populations - a review. In *The Ecology of Infectious Diseases in Natural Populations*. Dobson, A.P. (ed.) Cambridge: Cambridge University Press, pp. 20-51.

Gustafsson, K. and Uggla, A. (1994) Serologic survey for *Toxoplasma gondii* infection in the brown hare (*Lepus europaeus* P.) in Sweden. *Journal of Wildlife Diseases*. **30**: 201-204.

Guthrie, R.D. (1984) Mosaics, allelochemicals and nutrients: An ecological theory of late Pleistocene megafaunal extinctions. In *Quaternary Extinctions*. Klein, R.G. (ed.) Tucson: University of Arizona Press, pp. 259-298.

Guy, E.C. and Joynson, D.H.M. (1995) Potential of the polymerase chain reaction in the diagnosis of active *Toxoplasma* infection by detection of parasite in blood. *Journal of Infectious Diseases*. **172**: 319-322.

Guy, E.C., Pelloux, H., Lappalainen, M., Aspock, H., Habl, A., Melby, K.K., Holberg-Petterson, M., Peterson, E., Simon, J. and Ambroise-Thomas, P. (1996) Interlaboratory comparison of polymerase chain reaction for the detection of *Toxoplasma gondii* DNA added to samples of amniotic fluid. *European Journal of Clinical Microbiology and Infectious Diseases*. **15**: 836-839.

Hartley, W.J., Dubey, J.P. and Spielman, D.S. (1990) Fatal toxoplasmosis in Koalas (*Phascolarctos cinereus*). *Journal of Parasitology*. **76**: 271-272.

Hartley, W.J. and Munday, B.L. (1974) Felidae in the dissemination of toxoplasmosis to man and other animals. *Australian Veterinary Journal*. **50**: 224-228.

Havlicek, J., Gasova, Z.G., Smith, A.P., Zvara, K. and Flegr, J. (2001) Decrease of psychomotor performance in subjects with latent 'asymptomatic' toxoplasmosis. *Parasitology*. **122**: 515-520.

Hawdon, J.M. (1996) Differentiation between the human hookworms *Ancylostoma duodenale* and *Necator americanus* using PCR-RFLP. *Journal of Parasitology*. **82**: 642-647.

Hay, J., Aitken, P.P., Hair, D.M., Hutchison, W.M. and Graham, D.I. (1984) The effect of congenital *Toxoplasma* infection on mouse activity and relative preference for exposed areas over a series of trials. *Annals of Tropical Medicine and Parasitology*. **78**: 611-618.

Hay, J., Aitken, P.P., Hutchison, W.M. and Graham, D.I. (1983a) The effect of congenital and adult-acquired *Toxoplasma* infections on the motor performance of mice. *Annals of Tropical Medicine and Parasitology*. **77**: 261-277.

Hay, J., Hutchison, W.M. and Aitken, P.P. (1983b) Congenital *Toxoplasma* infection and response to novelty in mice. *Annals of Tropical Medicine and Parasitology*. **77**: 437-439.

Hay, J., Hutchison, W.M., Aitken, P.P. and Graham, D.I. (1983c) The effect of congenital and adult-acquired *Toxoplasma* infections on activity and responsiveness to novel stimulation in mice. *Annals of Tropical Medicine and Parasitology*. **77**: 483-495.

Healy, G.R. (1990) Giardiasis in perspective: the evidence of animals as a source of human *Giardia* infections. In *Giardiasis*. Meyer, E.A. (ed.) Amsterdam: Elsevier Science Publishers B. V., pp. 305-313.

Hendrix, C.M., Bruce, H.S., Kellman, N.J., Harrelson, G. and Bruhn, B.F. (1996) Cutaneous larva migrans and enteric hookworm infections. *Journal of the American Veterinary Medical Association*. **209**: 1763-1767.

Heydon, G.M. (1929) Creeping eruption or larva migrans in north Queensland and a note on the worm *Gnathostoma spinigerum* (Owen). *Medical Journal of Australia*. **1**: 583-591.

Heydon, G.M. and Bearup, A.J. (1963) *Ancylostoma braziliense* and *A. ceylanicum*. *Transactions of the Royal Society of Tropical Medicine and Hygiene*. **57**: 76.

Hill, D.R. (1993) Giardiasis. Issues in diagnosis and management. *Infectious Disease Clinics of North America*. **7**: 503-525.

Hillis, D.M. and Davies, S.K. (1986) Evolution of the ribosomal DNA: fifty million years of recorded history of the frog genus *Rana*. *Evolution*. **40**: 1275-1288.

Holmdahl, O.J.M., Mattsson, J.G., Uggla, A. and Johansson, K.-E. (1994) The phylogeny of *Neospora caninum* and *Toxoplasma gondii* based on ribosomal RNA sequences. *FEMS Microbiology Letters*. **119**: 187-192.

Holt, R.D. and Lawton, J.H. (1994) The ecological consequences of shared natural enemies. *Annual Review of Ecology and Systematics*. **25**: 495-520.

Homan, W.L., Vercammen, M., De Braekeleer, J. and Verschueren, H. (2000) Identification of a 200- to 300-fold repetitive 529 bp DNA fragment in *Toxoplasma gondii*, and its use for diagnostic and quantitative PCR. *International Journal for Parasitology*. **30**: 69-75.

Hopkins, R.M., Constantine, C.C., Groth, D.A., Wetherall, J.D., Reynoldson, J.A. and Thompson, R.C.A. (1999) PCR-based DNA fingerprinting of *Giardia duodenalis* isolates using the intergenic rDNA spacer. *Parasitology*. **118**: 531-539.

Hopkins, R.M., Deplazes, P., Meloni, B.P., Reynoldson, J.A. and Thompson, R.C.A. (1993) A field and laboratory evaluation of a commercial ELISA for the detection of *Giardia* coproantigens in humans and dogs. *Transactions of the Royal Society of Tropical Medicine and Hygiene*. **87**: 39-41.

Hopkins, R.M., Meloni, B.P., Groth, D.M., Wetherall, J.D., Reynoldson, J.A. and Thompson, R.C.A. (1997) Ribosomal RNA sequencing reveals differences between the genotypes of *Giardia* isolates recovered from humans and dogs living in the same locality. *Journal of Parasitology*. **83**: 44-51.

Horton, D.R. (1984) Red kangaroos: Last of the Australian megafauna. In *Quaternary Extinctions*. Klein, R.G. (ed.) Tucson: University of Arizona Press, pp. 639-680.

Hotez, P. and Pritchard, D.I. (1995) Hookworm infection. *Scientific American*. **272**: 42-48.

Hotez, P.J. and Cerami, A. (1983) Secretion of a proteolytic anticoagulant by *Ancylostoma* hookworms. *Journal of Experimental Medicine*. **157**: 1594-1603.

How, R.A., Dell, J. and Humphreys, W.F. (1987) The ground vertebrate fauna of coastal areas between Busselton and Albany, Western Australia. *Records of the Western Australian Museum*. **13**: 553-574.

Howkins, A.B. (1966a) Epidemiology of hydatid disease in eastern New South Wales. 1. Geographical distribution and incidence in sheep. *Australian Veterinary Journal*. **42**: 238-240.

Howkins, A.B. (1966b) Epidemiology of hydatid disease in eastern New South Wales. 2. Incidence in macropodidae and other possible intermediate hosts. *Australian Veterinary Journal*. **42**: 313-314.

Ho-Yen, D.O., Joss, A.W.L., Balfour, A.H., Smyth, E.T.M., Baird, D. and Chatterton, J.M.W. (1992) Use of the polymerase chain reaction to detect *Toxoplasma gondii* in human blood samples. *Journal of Clinical Pathology*. **45**: 910-913.

Hu, M., Chilton, N.B., Zhu, X. and Gasser, R.B. (2002) Single-strand conformation polymorphism-based analysis of mitochondrial cytochrome *c* oxidase subunit 1 reveals significant substructuring in hookworm populations. *Electrophoresis*. **23**: 27-34.

Hudson, P.J., Dobson, A.P. and Newborn, D. (1998) Prevention of population cycles by parasite removal. *Science*. **282**: 2256-2258.

Hudson, P.J. and Greenman, J. (1998) Competition mediated by parasites: biological and theoretical progress. *Trends in Ecology and Evolution*. **13**: 387-390.

Hume, I.D. (1982) *Digestive Physiology and Nutrition of Marsupials*. London: Cambridge University Press.

Hurtado, A., Aduriz, G., Moreno, B., Barandika, J. and Garcia-Perez, A.L. (2001) Single tube nested PCR for the detection of *Toxoplasma gondii* in fetal tissues from naturally aborted ewes. *Veterinary Parasitology*. **102**: 17-27.

Hyde, J.E. (1990) *Molecular Parasitology*. Milton Keynes: Open University Press.

Isaac-Renton, J.L., Cordeiro, C., Sarafis, K. and Shahriari, H. (1993) Characterization of *Giardia duodenalis* isolates from a waterborne outbreak. *Journal of Infectious Diseases*. **167**: 431-440.

Isaac-Renton, J.L., Moricz, M.M. and Proctor, E.M. (1987) A *Giardia* survey of fur-bearing water mammals in British Columbia, Canada. *Journal of Environmental Health*. **50**: 80-83.

Islam, A. (1990) Giardiasis in developing countries. In *Giardiasis*. Meyer, E.A. (ed.) Amsterdam: Elsevier Science Publishers B. V., pp. 235-266.

Izawa, M. and Doi, T. (1993) Flexibility of the social system of the feral cat, *Felis catus*. *Physiology and Ecology Japan*. **29**: 237-247.

Izawa, M., Doi, T. and Ono, Y. (1982) Grouping patterns of feral cats (*Felis catus*) living on a small island in Japan. *Japanese Journal of Ecology*. **32**: 373-382.

Jackson, M.H. and Hutchison, W.M. (1989) The prevalence and source of *Toxoplasma* infection in the environment. *Advances in Parasitology*. **28**: 55-105.

Jackson, M.H., Hutchison, W.M. and Siim, J.C. (1986) Toxoplasmosis in a wild rodent population of central Scotland and a possible explanation of the mode of transmission. *Journal of Zoology*. **209**: 549-557.

Jakob-Hoff, R.M. and Dunsmore, J.D. (1983) Epidemiological aspects of toxoplasmosis in southern Western Australia. *Australian Veterinary Journal*. **60**: 217-218.

Jardine, J.E. and Dubey, J.P. (2002) Congenital toxoplasmosis in a Indo-Pacific bottlenose dolphin (*Tursiops aduncus*). *Journal of Parasitology*. **88**: 197-199.

Jauregui, L.H., Higgins, J., Zarlenga, D., Dubey, J.P. and Lunney, J.K. (2001) Development of a real-time PCR assay for detection of *Toxoplasma gondii* in pig and mouse tissues. *Journal of Clinical Microbiology*. **39**: 2065-2071.

Jensen, J.M., Patton, S., Wright, B.G. and Loeffler, D.G. (1985) Toxoplasmosis in marsupials in a zoological collection. *Journal of Zoo Animal Medicine*. **16**: 129-131.

Johnson, A.M. (1997) Speculation on possible life cycles for the clonal lineages in the genus *Toxoplasma*. *Parasitology Today*. **13**: 393-397.

Johnson, A.M., Roberts, H. and Munday, B.L. (1988) Prevalence of *Toxoplasma gondii* antibody in wild macropods. *Australian Veterinary Journal*. **65**: 199-201.

Johnson, A.M., Roberts, H., Statham, P. and Munday, B.L. (1989) Serodiagnosis of acute toxoplasmosis in macropods. *Veterinary Parasitology*. **34**: 25-33.

Johnston, J. and Gasser, R.B. (1993) Copro-parasitological survey of dogs in southern Victoria. *Australian Veterinary Practitioner*. **23**: 127-131.

Jones, A. and Bray, R.A. (1994) Family Davaineidae Braun, 1900. In *Keys to the Cestode Parasites of Vertebrates*. Bray, R.A. (ed.) Wallingford: CAB International, pp. 407-441.

Jones, C.D., Okhravi, N., Adamson, P., Tasker, S. and Lightman, S. (2000) Comparison of PCR detection methods for B1, P30, and 18S rRNA genes of *T. gondii* in aqueous humor. *Investigative Ophthalmology and Visual Science*. **41**: 634-644.

Jones, E. (1988) Feral cat. In *The Australian Museum Complete Book of Australian Mammals*. Strahan, R.A. (ed.) Sydney: Angus & Robertson, pp. 489.

Jones, E.H. and Coman, B.J. (1981) Ecology of the feral cat, *Felis catus* (L.), in south-eastern Australia I. Diet. *Australian Wildlife Research*. **8**: 537-547.

Jones, E.H. and Coman, B.J. (1982) Ecology of the feral cat, *Felis catus* (L.), in south-eastern Australia III. Home ranges and population ecology in semi-arid north-west Victoria. *Australian Wildlife Research*. **9**: 409-420.

Jones, H.I. (1983) *Abbreviata* (Nematoda: Physalopteroidea) in lizards of the *Varanus gouldii* complex (Varanidae) in Western Australia. *Australian Journal of Zoology*. **31**: 285-298.

Jones, J.E., Lopez, A., Wilson, M., Schulkin, J. and Gibbs, R. (2001) Congenital toxoplasmosis: a review. *Obstetrical and Gynecological Survey*. **56**: 296-305.

Jungersen, G., Bille-Hansen, V., Jensen, L. and Lind, P. (2001) Transplacental transmission of *Toxoplasma gondii* in minipigs infected with strains of different virulence. *Journal of Parasitology*. **87**: 108-113.

Kelly, J.D. (1977) *Canine Parasitology*. Sydney: The University of Sydney Post-Graduate Foundation in Veterinary Science.

Kelly, J.D. and Ng, B.K.Y. (1975) Helminth parasites of dogs and cats. II. Prevalence in urban environments in Australasia. *Australian Veterinary Practitioner*. **5**: 133-141.

Kerby, G. and Macdonald, D.W. (1994) Cat society and the consequences of colony size. In *The Domestic Cat: the Biology of its Behaviour*. Bateson, P. (ed.) Cambridge: Cambridge University Press, pp. 67-81.

Kettlewell, J.S., Bettiol, S.S., Davies, N., Milstein, T. and Goldsmid, J.M. (1998) Epidemiology of giardiasis in Tasmania: a potential risk to residents and visitors. *Journal of Travel Medicine*. **5**: 127-130.

Khalil, L.F., Jones, A. and Bray, R.A. (eds.) (1994) *Keys to the Cestode Parasites of Vertebrates*. Wallingford: CAB International.

Kotula, A.W., Dubey, J.P., Sharar, A.K., Andrews, C.D., Shen, S.K. and Lindsay, D.S. (1991) Effect of freezing on infectivity of *Toxoplasma gondii* tissue cysts in pork. *Journal of Food Protection*. **54**: 687-690.

Kumar, S., Tamura, K., Jakobsen, I.B. and Nei, M. (2001) MEGA2: molecular evolutionary genetics analysis software. *Bioinformatics*. **17**: 1244-1245.

Lamoril, J., Molina, J.-M., de Gouvello, A., Garin, Y.J., Deybach, J.-C., Modai, J. and Derouin, F. (1996) Detection by PCR of *Toxoplasma gondii* in blood in the diagnosis of cerebral toxoplasmosis in patients with AIDS. *Journal of Clinical Pathology*. **49**: 89-92.

Lappin, M.R. (1996) Feline toxoplasmosis: interpretation of diagnostic test results. *Seminars in Veterinary Medicine and Surgery*. **11**: 154-160.

Laundre, J. (1977) The daytime behaviour of domestic cats in a free-roaming population. *Animal Behaviour*. **25**: 990-998.

Lee, P.Y.C., Mangan, J., Holliman, R.E. and Butcher, P.D. (1999) Quantitation of *Toxoplasma gondii* DNA in a competitive nested polymerase chain reaction. *Journal of Clinical Pathology*. **52**: 61-64.

Lenghaus, C., Obendorf, D.L. and Wright, F.H. (1990) Veterinary aspects of *Perameles gunnii* biology with special reference to species conservation. In *Management and Conservation of Small Populations*. Seebeck, J.H. (ed.) Chicago: Chicago Zoological Society, pp. 89-108.

Leyhausen, P. (1965) The communal organisation of solitary mammals. *Symposia of the Zoological Society of London*. **14**: 249-263.

Liberg, O. and Sandell, M. (1994) Spatial organisation and reproductive tactics in the domestic cat and other felids. In *The Domestic Cat: the Biology of its Behaviour*. Bateson, P. (ed.) Cambridge: Cambridge University Press, pp. 83-98.

Liesenfeld, O. (1999) Immune responses to *Toxoplasma gondii* in the gut. *Immunobiology*. **201**: 229-239.

Lin, M.H., Chen, T.C., Kuo, T.T., Tseng, C.C. and Tseng, C.P. (2000) Real-time PCR for quantitative detection of *Toxoplasma gondii*. *Journal of Clinical Microbiology*. **38**: 4121-4125.

Low, T. (1999) *Feral Future: The Untold Story of Australia's Exotic Invaders*. Ringwood: Viking.

Lunney, D. and Leary, T. (1988) The impact on native mammals of land-use changes and exotic species in the Bega district, New South Wales, since settlement. *Australian Journal of Ecology*. **13**: 67-92.

Lyles, A.M. and Dobson, A.P. (1993) Infectious disease and intensive management: population dynamics, threatened hosts, and their parasites. *Journal of Zoo and Wildlife Medicine*. **24**: 315-326.

Lymbery, A.J., Thompson, R.C. and Hobbs, R.P. (1990) Genetic diversity and genetic differentiation in *Echinococcus granulosus* (Batsch, 1786) from domestic and sylvatic hosts on the mainland of Australia. *Parasitology*. **101 Pt 2**: 283-289.

MacPherson, J.M. and Gajadhar, A.A. (1993) Sensitive and specific polymerase chain reaction detection of *Toxoplasma gondii* for veterinary and medical diagnosis. *Canadian Journal of Veterinary Research*. **57**: 45-48.

Mahy, B.W.J. and Brown, C.C. (2000) Emerging zoonoses: crossing the species barrier. *Revue Scientifique et Techniques*. **19**: 33-40.

Majewska, A.C. (1994) Successful experimental infection of a human volunteer and Mongolian gerbils with *Giardia* of animal origin. *Transactions of the Royal Society of Tropical Medicine and Hygiene*. **88**: 360-362.

Marck, C. (1988) 'DNA Strider': a 'C' program for the fast analysis of DNA and protein sequences for the Apple Macintosh family of computers. *Nucleic Acids Research*. **16**: 1829-1836.

Marti, M., Li, Y., Schraner, E.M., Wild, P., Kohler, P. and Hehl, A.B. (2003a) The secretory apparatus of an ancient eukaryote: protein sorting to separate export pathways occurs before formation of transient golgi-like compartments. *Molecular Biology of the Cell*. **14**: 1433-1447.

Marti, M., Regos, A., Li, Y., Schraner, E.M., Wild, P., Muller, N., Knopf, L.G. and Hehl, A.B. (2003b) An ancestral secretory apparatus in the protozoan parasite *Giardia intestinalis*. *Journal of Biological Chemistry*. **278**: 24837-24848.

Martin, G.R., Twigg, L.E. and Robinson, D.J. (1996) Comparison of the diet of feral cats from rural and pastoral Western Australia. *Wildlife Research*. **23**: 475-484.

Matthews, B.E. (1985) The influence of temperature and osmotic stress on the development and eclosion of hookworm eggs. *Journal of Helminthology*. **59**: 217-224.

Mawson, P.M. (1974) The genus *Potorostrongylus* Johnson and Mawson (Nematoda: Trichonematidae) from macropod marsupials. *Transactions of the Royal Society of South Australia*. **98**: 135-138.

Mawson, P.M. (1960) Nematodes belonging to the Trichostrongylidae, Subuluridae, Rhabdiasidae, and Trichuridae from bandicoots. *Australian Journal of Zoology*. **8**: 261-284.

Mawson, P.M. (1968) Two species of Nematoda (Spirurida: Spiruridae) from Australian dasyurids. *Parasitology*. **58**: 75-78.

May, R.M. (1988) Conservation and disease. *Conservation Biology*. **2**: 28-30.

May, R.M. and Anderson, R.M. (1979) Population of infectious diseases: Part II. *Nature*. **280**: 455-461.

May, R.M. and Anderson, R.M. (1978) Regulation and stability of host-parasite population interactions II. destabilizing processes. *Journal of Animal Ecology*. **47**: 249-267.

May, S.A. and Norton, T.W. (1996) Influence of fragmentation and disturbance on the potential impact of feral predators on native fauna in Australian forest ecosystems. *Wildlife Research*. **23**: 387-400.

Mayrhofer, G., Andrews, R.H., Ey, P.L. and Chilton, N.B. (1995) Division of *Giardia* isolates from humans into two genetically distinct assemblages by electrophoretic analysis of enzymes encoded at 27 loci and comparison with *Giardia muris*. *Parasitology*. **111**: 11-17.

McCallum, H. and Dobson, A.P. (1995) Detecting disease and parasite threats to endangered species and ecosystems. *Trends in Ecology and Evolution*. **10**: 190-194.

McGlade, T.R., Robertson, I.D., Elliot, A.D. and Thompson, R.C.A. (2003) High prevalence of *Giardia* detected in cats by PCR. *Veterinary Parasitology*. **110**: 197-205.

McIntyre, L., Hoang, L., Ong, C.S.L., Lee, P. and Isaac-Renton, J.L. (2000) Evaluation of molecular techniques to biotype *Giardia duodenalis* collected during an outbreak. *Journal of Parasitology*. **86**: 172-177.

McManus, D.P. and Bowles, J. (1996) Molecular genetic approaches to parasite identification: their value in diagnostic parasitology and systematics. *International Journal for Parasitology*. **26**: 687-704.

McOrist, S. and Smales, L. (1986) Morbidity and mortality of free-living and captive echidnas, *Tachyglossus aculeatus* (Shaw), in Australia. *Journal of Wildlife Diseases*. **22**: 375-380.

McRoberts, K.M., Meloni, B.P., Morgan, U.M., Marano, R., Binz, N., Erlandsen, S.L., Halse, S.A. and Thompson, R.C.A. (1996) Morphological and molecular characterization of *Giardia* isolated from the Straw-Necked Ibis (*Threskiornis spinicollis*) in Western Australia. *Journal of Parasitology*. **82**: 711-718.

Measures, L.N. and Olson, M.E. (1999) Giardiasis in Pinnipeds from Eastern Canada. *Journal of Wildlife Diseases*. **35**: 779-782.

Meek, P.D., Jenkins, D.J., Morris, B., Ardler, A.J. and Hawksby, R.J. (1995) Use of two humane leg-hold traps for catching pest species. *Wildlife Research*. **22**: 733-739.

Meloni, B.P., Lymbery, A.J. and Thompson, R.C.A. (1995) Genetic characterization of isolates of *Giardia duodenalis* by enzyme electrophoresis: implications for reproductive biology, population structure, taxonomy, and epidemiology. *Journal of Parasitology*. **81**: 368-383.

Meloni, B.P., Thompson, R.C.A., Hopkins, R.M., Reynoldson, J.A. and Gracey, M. (1993) The prevalence of *Giardia* and other intestinal parasites in children, dogs and cats from Aboriginal communities in the Kimberley. *Medical Journal of Australia*. **158**: 157-159.

Menkhorst, P. and Knight, F. (2001) *A Field Guide to the Mammals of Australia*. Melbourne: Oxford University Press.

Migasena, S. and Gilles, H.M. (1991) Clinical features and diagnosis. In *Hookworm Infections*. Ball, P.A.J. (ed.) Amsterdam: Elsevier Science Publishers B. V., pp. 179-193.

Miller, S.A., Dykes, D.D. and Polesky, H.F. (1988) A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Research*. **16**: 1215.

Milstein, T.C. and Goldsmid, J.M. (1997) Parasites of feral cats from southern Tasmania and their potential significance. *Australian Veterinary Journal*. **75**: 219-219.

Mirmovitch, V. (1995) Spatial organisation of urban feral cats (*Felis catus*) in Jerusalem. *Wildlife Research*. **22**: 299-310.

Miyazaki, I. (1960) On the genus *Gnathostoma* and human gnathostomiasis, with special reference to Japan. *Experimental Parasitology*. **9**: 338-370.

Monis, P.T. (1999) The importance of systematics in parasitological research. *International Journal for Parasitology*. **29**: 381-388.

Monis, P.T. and Andrews, R.H. (1998) Molecular epidemiology: assumptions and limitations of commonly applied methods. *International Journal for Parasitology*. **28**: 981-987.

Monis, P.T., Andrews, R.H., Mayrhofer, G. and Ey, P.L. (1999) Molecular systematics of the parasitic protozoan *Giardia intestinalis*. *Molecular Biology and Evolution*. **16**: 1135-1144.

Monis, P.T., Andrews, R.H., Mayrhofer, G., Mackrill, J., Kulda, J., Isaac-Renton, J.L. and Ey, P.L. (1998) Novel lineages of *Giardia intestinalis* identified by genetic analysis of organisms isolated from dogs in Australia. *Parasitology*. **116**: 7-19.

Monis, P.T., Mayrhofer, G., Andrews, R.H., Homan, W.L., Limper, L. and Ey, P.L. (1996) Molecular genetic analysis of *Giardia intestinalis* isolates at the glutamate dehydrogenase gene. *Parasitology*. **112**: 1-12.

Monis, P.T. and Thompson, R.C.A. (2003) *Cryptosporidium* and *Giardia* - zoonoses: fact or fiction? *Infection Genetics and Evolution*. **(In Press)**.

Monti, J.R., Chilton, N.B., Bao-Zhen, Q. and Gasser, R.B. (1998) Specific amplification of *Necator americanus* or *Ancylostoma duodenale* DNA by PCR using markers in ITS-1 rDNA, and its implications. *Molecular and Cellular Probes*. **12**: 71-78.

Monzingo, D.L. and Hibler, C.P. (1987) Prevalence of *Giardia* sp. in a beaver colony and the resulting environmental contamination. *Journal of Wildlife Diseases*. **23**: 576-585.

Moore, J. and Gotteli, N.J. (1990) Phylogenetic perspective on the evolution of altered host behaviours: a critical look at the manipulation hypothesis. In *Parasitism and Host Behaviour*. Behnke, J.M. (ed.) London: Taylor and Francis, pp. 193-230.

Morgan, U.M. and Thompson, R.C.A. (1998) Molecular detection of parasitic protozoa. *Parasitology*. **117**: S73-S85.

Morton, S.R. (1990) The impact of European settlement on the vertebrate animals of arid Australia: a conceptual model. *Proceedings of the Ecological Society of Australia*. **16**: 201-213.

Mueller, J., Ellenburger, B., Fusio, A.C., Salafsky, B. and Siddiqui, A.A. (1989) A simple method for the collection of *Necator americanus* larvae. *Journal of Helminthology*. **63**: 77-78.

Muir, B.G. (1982) Cats in Western Australian national parks. *Australian Ranger Bulletin*. **2**: 8.

Muller, N., Zimmermann, V., Hentrich, B. and Gottstein, B. (1996) Diagnosis of *Neospora caninum* and *Toxoplasma gondii* infection by PCR and DNA hybridization immunoassay. *Journal of Clinical Microbiology*. **34**: 2850-2852.

Munday, B.L. (1988) Marsupial diseases. *Proceedings of the Postgraduate Committee in Veterinary Science*. **104**: 299-365.

Munday, B.L. (1972) Serological evidence of *Toxoplasma* infection in isolated groups of sheep. *Research in Veterinary Science*. **13**: 100-102.

Munday, B.L., Hartley, W.J., Harrigan, K.E., Presidente, P.J.A. and Obendorf, D.L. (1979) *Sarcocystis* and related organisms in Australian wildlife: II. survey findings in birds, reptiles, amphibians and fish. *Journal of Wildlife Diseases*. **15**: 57-73.

Munday, B.L., Hartley, W.J., Presidente, P.J.A. and Obendorf, D.L. (1978) *Sarcocystis* and related organisms in Australian wildlife: I. survey findings in mammals. *Journal of Wildlife Diseases*. **14**: 417-433.

Munday, B.L. and Mason, R.W. (1980) *Sarcocystis* and related organisms in Australian wildlife: III. *Sarcocystis murinotechis* sp. N. life cycle in rats (*Rattus, Pseudomys* and *Mastocomys* spp.) and tiger snakes (*Notechis ater*). *Journal of Wildlife Diseases*. **16**: 83-87.

Munday, B.L., Smith, D.D. and Frenkel, J.K. (1980) *Sarcocystis* and related organisms in Australian wildlife: IV. studies on *Sarcocystis cuniculi* in European rabbits (*Oryctolagus cuniculus*). *Journal of Wildlife Diseases*. **16**: 201-204.

Myers, N., Mittermeier, R.A., Mittermeier, C.G., da Fonseca, G.A.B. and Kent, J. (2000) Biodiversity hotspots for conservation priorities. *Nature*. **403**: 853-858.

Newsome, A.E. (1991) Feral cats: an overview. In *The Impact of Feral Cats on Native Wildlife*. Potter, C. (ed.) Canberra: Endangered Species Unit Australian National Parks and Wildlife Service, pp. 7-13.

Newton, L.A., Chilton, N.B., Monti, J.R., Bjorn, H., Varady, M., Christensen, C.M. and Gasser, R.B. (1997) Rapid PCR-based delineation of the porcine nodular worms, *Oesophagostomum dentatum* and *O. quadrispinulatum*. *Molecular and Cellular Probes*. **11**: 149-153.

Nguyen, T.D., De Kesel, M., Bigaignon, G., Hoet, P., Pazzaglia, G., Lammens, M. and Delmee, M. (1996) Detection of *Toxoplasma gondii* tachyzoites and bradyzoites in blood, urine, and brains of infected mice. *Clinical and Diagnostic Laboratory Immunology*. **3**: 635-639.

Nizeyi, J.B., Mwebe, R., Nanteza, A., Cranfield, M.R., Kalema, G.R.N.N. and Graczyk, T.K. (1999) *Cryptosporidium* sp. and *Giardia* sp. infections in mountain gorillas (*Gorilla gorilla beringei*) of the Bwindi Impenetrable National Park, Uganda. *Journal of Parasitology*. **85**: 1084-1088.

Norris, D.E. (1971) The migratory behaviour of the infective-stage larvae of *Ancylostoma braziliense* and *Ancylostoma tubaeforme* in rodent paratenic hosts. *Journal of Parasitology*. **57**: 998-1009.

Norton, T.W. (1987) The ecology of small mammals in north-eastern Tasmania, II. *Pseudomys novaehollandiae* and introduced *Mus musculus*. *Australian Wildlife Research*. **14**: 435-441.

Nwosu, A.B. (1978) Investigations into the free-living phase of the cat hookworm life cycle. *Zeitschrift fur Parasitenkunde*. **56**: 243-249.

Oakwood, M. and Spratt, D.M. (2000) Parasites of the northern quoll, *Dasyurus hallucatus* (Marsupialia: Dasyuridae) in tropical savanna, Northern Territory. *Australian Journal of Zoology*. **48**: 79-90.

Obendorf, D.L. and Munday, B.L. (1990) Toxoplasmosis in wild eastern barred bandicoots, *Perameles gunnii*. In *Bandicoots and Bilbies*. Kemper, C.M. (ed.) Sydney: Surrey Beatty & Sons, pp. 193-197.

Obendorf, D.L. and Munday, B.L. (1983) Toxoplasmosis in wild Tasmanian wallabies. *Australian Veterinary Journal*. **60**: 62.

Obendorf, D.L., Statham, P. and Driessen, M. (1996) Detection of agglutinating antibodies to *Toxoplasma gondii* in sera from free-ranging eastern barred bandicoots (*Perameles gunnii*). *Journal of Wildlife Diseases*. **32**: 623-626.

O'Brien, S.J. and Evermann, J.F. (1988) Interactive influence of infectious disease and genetic diversity in natural populations. *Trends in Ecology and Evolution*. **3**: 254-259.

O'Brien, S.J., Goldman, D., Merril, C.R. and Bush, M. (1983) The cheetah is depauperate in genetic variation. *Science*. **221**: 459-462.

O'Brien, S.J., Roelke, M.E., Marker, L., Newman, A., Winkler, C.A., Meltzer, D., Colley, L., Evermann, J.F., Bush, M. and Wildt, D.E. (1985) Genetic basis for species vulnerability in the cheetah. *Science*. **227**: 1428-1434.

O'Callaghan, M.G. and Beveridge, I. (1996) Gastro-intestinal parasites of feral cats in the Northern Territory. *Transactions of the Royal Society of South Australia*. **120**: 175-176.

O'Callaghan, M.G. and Moore, E. (1986) Parasites and serological survey of the common brushtail possum (Trichosurus vulpecula) from Kangaroo Island, South Australia. *Journal of Wildlife Diseases*. **22**: 589-591.

O'Callaghan, M.G., Moore, R. and Ford, G.E. (1984) Helminth and arthropod parasites from dogs and cats in South Australia. *Australian Veterinary Practitioner*. **14**: 159-161.

O'Donoghue, P.J. and Adlard, R.D. (2000) Catalogue of protozoan parasites recorded in Australia. *Memoirs of the Queensland Museum*. **45**: 1-163.

O'Donoghue, P.J., Riley, M.J. and Clarke, J.F. (1987) Serological survey for *Toxoplasma* infections in sheep. *Australian Veterinary Journal*. **64**: 40-45.

O'Handley, R.M., Cockwill, C., McAllister, T., Jelinski, M., Morck, D.W. and Olson, M.E. (1999) Duration of naturally acquired giardiosis and cryptosporidiosis in dairy calves and their association with diarrhea. *Journal of the American Veterinary Medical Association*. **214**: 391-396.

O'Handley, R.M., Olson, M.E., Fraser, D., Adams, P.J. and Thompson, R.C.A. (2000) Prevalence and genotypic characterisation of *Giardia* in dairy calves from Western Australia and Western Canada. *Veterinary Parasitology*. **90**: 193-200.

Olds, R.J. (1952) *Gnathostoma spinigerum* (Owen, 1836) in cats in north Queensland. *Australian Veterinary Journal*. **28**: 124-126.

Olson, M.E., Roach, P.D., Stabler, M. and Chan, W. (1997a) Giardiasis in Ringed Seals from the Western Arctic. *Journal of Wildlife Diseases*. **33**: 646-648.

Olson, M.E., Thorlakson, C.L., deselliers, L., Morck, D.W. and McAllister, T.A. (1997b) *Giardia* and *Cryptosporidium* in Canadian farm animals. *Veterinary Parasitology*. **68**: 375-381.

Ortega, Y.R. and Adam, R.D. (1997) *Giardia*: overview and update. *Clinical Infectious Diseases*. **25**: 545-550.

Owen, M.R., Clarkson, M.J. and Trees, A.J. (1998) Diagnosis of *Toxoplasma* abortion in ewes by polymerase chain reaction. *Veterinary Record*. **142**: 445-448.

Owen, M.R. and Trees, A.J. (1998) Vertical transmission of *Toxoplasma gondii* from chronically infected house (*Mus musculus*) and field (*Apodemus sylvaticus*) mice determined by polymerase chain reaction. *Parasitology*. **116**: 299-304.

Owen-Smith, N. (1987) Pleistocene extinctions: The pivotal role of megaherbivores. *Palaeobiology*. **13**: 351-362.

Paltridge, R., Gibson, D. and Edwards, G. (1997) Diet of the feral cat (*Felis catus*) in central Australia. *Wildlife Research*. **24**: 67-76.

Paré, J., Hietala, S.K. and Thurmond, M.C. (1996) Congenital *Neospora caninum* infection in dairy cattle and the associated calfhood mortality. *Canadian Journal of Veterinary Research*. **60**: 133-139.

Paton, D.C. (1993) Impacts of domestic and feral cats on wildlife. In *Proceedings of Cat Management Workshop*. Owens, C. (ed.) Brisbane: Queensland Department of Environment and Heritage, pp. 9-15.

Patton, S., Johnson, S.L., Loeffler, D.G., Wright, B.G. and Jensen, J.M. (1986) Epizootic of toxoplasmosis in kangaroos, wallabies, and potaroos: possible transmission via domestic cats. *Journal of the American Veterinary Medical Association*. **189**: 1166-1169.

Pavlov, P.M. and Howell, M.J. (1977) Helminth parasites of Canberra cats. *Australian Veterinary Journal*. **53**: 599-600.

Pawlowski, Z.S., Schad, G.A. and Stott, G.J. (1991) *Hookworm Infection and Anaemia*. Geneva: World Health Organisation.

Pelloux, H., Weiss, J., Simon, J., Muet, F., Fricker-Hidalgo, H., Goullier-Fleuret, A. and Ambroise-Thomas, P. (1996) A new set of primers for the detection of *Toxoplasma gondii* in amniotic fluid using polymerase chain reaction. *FEMS Microbiology Letters*. **138**: 11-15.

Pichelin, S., Thomas, P.M. and Hutchinson, M.N. (1999) A checklist of helminth parasites of Australian reptiles. *South Australian Museum Monograph Series*. **5**: 1-61.

Pimm, S.L. and Raven, P. (2000) Biodiversity. Extinction by numbers. *Nature*. **403**: 843-845.

Powell, C.C., Brewer, M. and Lappin, M.R. (2001) Detection of *Toxoplasma gondii* in the milk of experimentally infected lactating cats. *Veterinary Parasitology*. **102**: 29-33.

Pozio, E. (2000) Factors affecting the flow among domestic, synanthropic and sylvatic cycles of *Trichinella*. *Veterinary Parasitology*. **93**: 241-262.

Prescott, C.W. (1984) *Parasitic Diseases of the Cat in Australia*. Sydney: The University of Sydney.

Prociv, P. and Croese, J. (1996) Human enteric infection with *Ancylostoma caninum*: hookworms reappraised in the light of a "new" zoonosis. *Acta Tropica*. **62**: 23-44.

Prommas, C. and Daengsvang, S. (1937) Feeding experiments on cats with *Gnathostoma spinigerum* larvae obtained from the second intermediate host. *Journal of Parasitology*. **23**: 115-116.

Pujol-Rique, M., Derouin, F., Garcia-Quintanilla, A., Valls, M.E., Miro, J.M. and Anta, M.T.J.d. (1999) Design of a one-tube hemi-nested PCR for detection of *Toxoplasma gondii* and comparison of three DNA purification methods. *Journal of Medical Microbiology*. **48**: 857-862.

Pullar, E.M. (1946) A survey of Victorian canine and vulpine parasites. *Australian Veterinary Journal*. **22**: 40-48.

Ralls, K., Brugger, K. and Ballou, J. (1979) Inbreeding and juvenile mortality in small populations of ungulates. *Science*. **206**: 1101-1103.

Ransom, B.H. (1907) Tapeworm cysts (*Dithyridium cynocephali* n. sp.) in the muscles of a marsupial wolf (*Thylacinus cynocephalus*). *Transactions of the American Microbiological Society*. **27**: 31-32.

Rausch, R.L. (1994) Family Taeniidae Ludwig, 1886. In *Keys to the Cestode Parasites of Vertebrates*. Bray, R.A. (ed.) Wallingford: CAB International, pp. 665-672.

Rausch, R.L. (1985) Parasitology: retrospect and prospect. *Journal of Parasitology*. **71**: 139-151.

Read, J. and Bowen, Z. (2001) Population dynamics, diet and aspects of the biology of feral cats and foxes in arid South Australia. *Wildlife Research*. **28**: 195-203.

Reddacliff, G.L., Hartley, W.J., Dubey, J.P. and Cooper, D.W. (1993) Pathology of experimentally-induced, acute toxoplasmosis in macropods. *Australian Veterinary Journal*. **70**: 4-6.

Remington, J.S., Jacobs, L. and Melton, M.L. (1961) Congenital transmission of toxoplasmosis from mother animals with acute and chronic infections. *Journal of Infectious Diseases*. **108**: 163-173.

Risbey, D.A., Calver, M.C. and Short, J. (1999) The impact of cats and foxes on the small vertebrate fauna of Heirisson Prong, Western Australia. I. Exploring potential impact using diet analysis. *Wildlife Research*. **26**: 621-630.

Risbey, D.A., Calver, M.C., Short, J., Bradley, J.S. and Wright, I.W. (2000) The impact of cats and foxes on the small vertebrate fauna of Heirisson Prong, Western Australia. II. A field experiment. *Wildlife Research*. **27**: 223-235.

Roberts, D., Gracey, M. and Spargo, R.M. (1988) Growth and morbidity in children in a remote Aboriginal community in north-west Australia. *Medical Journal of Australia*. **148**: 68-71.

Robertson, I.D., Irwin, P.J., Lymbery, A.J. and Thompson, R.C.A. (2000) The role of companion animals in the emergence of parasitic zoonoses. *International Journal for Parasitology*. **30**: 1369-1377.

Rommel, M., Schnieder, T., Krause, H.D. and Westerhoff, J. (1987) Trials to suppressthe formation of oocysts and cysts of *Toxoplasma gondii* in cats by medication of the feed with toltrazuril. *Veterinary Medical Review*. **2**: 141-153.

Romstad, A., Gasser, R.B., Monti, J.R., Polderman, A.M., Nansen, P., Pit, D.S. and Chilton, N.B. (1997) Differentiation of *Oesophagostomum bifurcum* from *Necator americanus* by PCR using genetic markers in spacer ribosomal DNA. *Molecular and Cellular Probes*. **11**: 169-176.

Rothe, J., McDonald, P. and Johnson, A.M. (1985) Detection of *Toxoplasma* cysts and oocysts in an urban environment in a developed country. *Pathology*. **17**: 497-499.

Ryan, G.E. (1976a) Gastro-intestinal parasites of feral cats in New South Wales. *Australian Veterinary Journal*. **52**: 224-227.

Ryan, G.E. (1976b) Helminth parasites of the fox (*Vulpes vulpes*) in New South Wales. *Australian Veterinary Journal*. **52**: 126-131.

Sandars, D.F. (1953) A study of Diphyllobothriidae (Cestoda) from Australian hosts. *Proceedings of the Royal Society of Queensland*. **63**: 65-70.

Sato, K., Iwamoto, I. and Yoshiki, K. (1993) Experimental toxoplasmosis in pregnant cats. *Journal of Veterinary Medical Science*. **55**: 1005-1009.

Savini, G., Dunsmore, J.D., Robertson, I.D. and Seneviratna, P. (1992a) The epidemiology of *Sarcocystis* spp. in cattle of Western Australia. *Epidemiology and Infection*. **108**: 107-113.

Savini, G., Dunsmore, J.D., Robertson, I.D. and Seneviratna, P. (1992b) *Sarcocystis* spp. in Western Australian cattle. *Australian Veterinary Journal*. **69**: 201-202.

Savini, G., Dunsmore, J.D., Robertson, I.D. and Seneviratna, P. (1993) *Sarcocystis* spp. in Western Australian sheep. *Australian Veterinary Journal*. **70**: 152-154.

Savva, D. (1989) Isolation of a potential DNA probe for *Toxoplasma gondii*. *Microbios*. **58**: 162-172.

Schad, G.A. (1991) The parasite. In *Hookworm Infections*. Ball, P.A.J. (ed.) Amsterdam: Elsevier Science Publishers B. V., pp. 15-49.

Schad, G.A. and Banwell, J.G. (1990) Hookworms. In *Tropical and Geographical Medicine*. Mahmoud, A.A.F. (ed.) New York: McGraw-Hill Book Co., pp. 379-393.

Schantz, P.M. (1991) Parasitic zoonoses in perspective. *International Journal for Parasitology*. **21**: 161-170.

Schares, G., Wenzel, U., Muller, T. and Conraths, F.J. (2001) Serological evidence for naturally occurring transmission of *Neospora caninum* among foxes (*Vulpes vulpes*). *International Journal for Parasitology*. **31**: 418-423.

Schmidt, G.D. (1983) What is *Echinorhynchus pomatostomi* Johnston and Cleland, 1912? *Journal of Parasitology*. **69**: 397-399.

Schmitz, O.J. and Nudds, T.D. (1994) Parasite-mediated competition in deer and moose: how strong is the effect of meningeal worm on moose? *Ecological Applications*. **4**: 91-103.

Schrag, S.J. and Wiener, P. (1995) Emerging infectious disease: what are the relative roles of ecology and evolution? *Trends in Ecology and Evolution*. **10**: 319-324.

Scott, M.E. (1988) The impact of infection and disease on animal populations: implications for conservation biology. *Conservation Biology*. **2**: 40-56.

Seddon, H.R. (1967) *Diseases of Domestic Animals in Australia. Part 1. Helminth Infestations*. Canberra: Commonwealth of Australia, Department of Health.

Sedlak, K., Literak, I., Faldyna, M., Toman, M. and Benak, J. (2000) Fatal toxoplasmosis in brown hares (*Lepus europaeus*): possible reasons of their high susceptibility to the infection. *Veterinary Parasitology*. **93**: 13-28.

Setasuban, P. and Waddell, A.H. (1973) Hookworms in cats and dogs in Queensland. *Australian Veterinary Journal*. **49**: 110.

Shaw, J., Dunsmore, J. and Jakob-Hoff, R. (1983) Prevalence of some gastrointestinal parasites in cats in the Perth area. *Australian Veterinary Journal*. **60**: 151-152.

Short, J. and Smith, A. (1994) Mammal decline and recovery in Australia. *Journal of Mammalogy*. **75**: 288-297.

Short, J. and Turner, B. (2000) Reintroduction of the burrowing bettong *Bettongia lesueur* (Marsupialia: Potoroidae) to mainland Australia. *Biological Conservation*. **96**: 185-196.

Silveira, C., Ferreira, R., Muccioli, C., Nussenblatt, R. and Belfort, R. (2003) Toxoplasmosis transmitted to a newborn from the mother infected 20 years earlier. *American Journal of Ophthalmology*. **136**: 370-371.

Simpson, A.G., Roger, A.J., Silberman, J.D., Leipe, D.D., Edgcomb, V.P., Jermiin, L.S., Patterson, D.J. and Sogin, M.L. (2002) Evolutionary history of "early-diverging" eukaryotes: the excavate taxon Carpediemonas is a close relative of *Giardia*. *Molecular Biology and Evolution*. **19**: 1782-1791.

Smales, L.R. and Mawson, P.M. (1978) Nematode parasites of the Kangaroo Island Wallaby, *Macropus eugenii* (Desmarest) I. seasonal and geograpical distribution. *Transactions of the Royal Society of South Australia*. **102**: 9-15.

Smith, A.P. and Quin, D.G. (1996) Patterns and causes of extinction and decline in Australian conilurine rodents. *Biological Conservation*. **77**: 243-267.

Smith, I.D. and Munday, B.L. (1965) Observations on the incidence of *Toxoplasma gondii* in native and introduced feral fauna in Eastern Australia. *Australian Veterinary Journal*. **41**: 285-286.

Soulsby, E.J.L. (1982) *Helminths, Arthropods and Protozoa of Domesticated Animals*. London: Bailliére Tindall.

Spratt, D.M. (1987) Helminth communities in small mammals in southeastern New South Wales. *International Journal for Parasitology*. **17**: 197-202.

Spratt, D.M., Beveridge, I. and Walter, E.L. (1990) A catalogue of Australasian monotremes and marsupials and their recorded helminth parasites. *South Australian Museum Monograph Series*. **1**: 1-105.

Sprent, J.F.A. (1970) *Baylissascaris tasmaniensis* sp. nov. in marsupial carnivores: Heirloom or souvenir? *Parasitology*. **61**: 75-86.

Stephenson, L.S., Latham, M.C. and Ottesen, E.A. (2000) Malnutrition and parasitic helminth infections. *Parasitology*. **121**: S23-38.

Stevenson, L.A., Chilton, N.B. and Gasser, R.B. (1995) Differentiation of *Haemonchus placei* from *H. contortus* (Nematoda: Trichostrongylidae) by the ribosomal DNA second internal transcribed spacer. *International Journal for Parasitology*. **25**: 483-488.

Stewart, L. (1994) The taxonomy of *Ancylostoma* species in the Townsville region of North Queensland. In Cairns: James Cook University of North Queensland.

Stiles, J., Prade, R. and Greene, C. (1996) Detection of *Toxoplasma gondii* in feline and canine biological samples by use of the polymerase chain reaction. *American Journal of Veterinary Research*. **57**: 264-267.

Strahan, R. (1983) *The Australian Museum Complete Book of Australian Mammals*. Sydney: Angus and Robertson.

Strong, B.W. and Low, W.A. (1983) Some observations of feral cats (*Felis catus*) in the southern Northern Territory. In Alice Springs: Conservation Commission of the Northern Territory, pp. 12.

Sumner, B. and Ackland, M.L. (1999) *Toxoplasma gondii* antibody in domestic cats in Melbourne. *Australian Veterinary Journal*. **77**: 447-449.

Swan, J.M. and Thompson, R.C.A. (1986) The prevalence of *Giardia* in dogs and cats in Perth, Western Australia. *Australian Veterinary Journal*. **63**: 110-112.

Swerczek, T.W., Nielsen, S.W. and Helmboldt, C.F. (1971) Transmammary passage of *Toxocara cati* in the cat. *American Journal of Veterinary Research*. **32**: 89-92.

Taylor, R.H. (1979) How the Macquarie Island parakeet became extinct. *New Zealand Journal of Ecology*. **2**: 42-45.

Tenter, A.M., Heckeroth, A.R. and Weiss, L.M. (2000) *Toxoplasma gondii*: from animals to humans. *International Journal for Parasitology*. **30**: 1217-1258.

Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F. and Higgins, D.G. (1997) The CLUSTAL-X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Research*. **25**: 4876-4882.

Thompson, R.C., Lymbery, A.J., Pearce, D.A., Finn, K.C., Reynoldson, J.A. and Meloni, B.P. (1996) *Giardia duodenalis*: exposure to metronidazole inhibits competitive interactions between isolates of the parasite *in vitro*. *Journal of Parasitology*. **82**: 679-683.

Thompson, R.C., Meloni, B.P., Hopkins, R.M., Deplazes, P. and Reynoldson, J.A. (1993a) Observations on the endo- and ectoparasites affecting dogs and cats in aboriginal communities in the north-west of Western Australia. *Australian Veterinary Journal*. **70**: 268-270.

Thompson, R.C., Reynoldson, J.A. and Mendis, A.H. (1993b) *Giardia* and giardiasis. *Advances in Parasitology*. **32**: 71-160.

Thompson, R.C.A. (2001) Echinococcosis. In *Principles and Practice of Clinical Parasitology*. Pearson, R.D. (ed.) Sussex: Wiley, pp. 595-612.

Thompson, R.C.A. (2000) Giardiasis as a re-emerging infectious disease and its zoonotic potential. *International Journal for Parasitology*. **30**: 1259-1267.

Thompson, R.C.A., Constantine, C.C. and Morgan, U.M. (1998) Overview and significance of molecular methods: what role for molecular epidemiology? *Parasitology*. **117**: S161-S175.

Thompson, R.C.A., Hopkins, R.M. and Homan, W.L. (2000a) Nomenclature and genetic groupings of *Giardia* infecting mammals. *Parasitology Today*. **16**: 210-213.

Thompson, R.C.A. and Lymbery, A.J. (1996) Genetic variability in parasites and host-parasite interactions. *Parasitology*. **112**: S7-S22.

Thompson, R.C.A., Morgan, U.M., Hopkins, R.M. and Pallant, L.J. (2000b) Enteric protozoan infections. In *Molecular Epidemiology of Infectious Diseases*. Thompson, R.C.A. (ed.) London: Arnold, pp. 194-209.

Tidemann, S.C., McOrist, S., Woinarski, J.C. and Freeland, W.J. (1992) Parasitism of wild Gouldian finches (*Erythrura gouldiae*) by the air-sac mite *Sternostoma tracheacolum*. *Journal of Wildlife Diseases*. **28**: 80-84.

Tizard, I.R., Fish, N.A. and Quinn, J.P. (1976) Some observations on the epidemiology of toxoplasmosis in Canada. *Journal of Hygiene*. **77**: 11-21.

Tompkins, D.M. and Begon, M. (1999) Parasites can regulate wildlife populations. *Parasitology Today*. **15**: 311-313.

Triggs, B., Brunner, H. and Cullen, J.M. (1984) The food of fox, dog and cat in Croajingalong national park, south-eastern Victoria. *Australian Wildlife Research*. **11**: 491-499.

Trueman, K.F. and Ferris, P.B.C. (1977) Gnathostomiasis in three cats. *Australian Veterinary Journal*. **53**: 498-499.

Tsuchiya, H. (1931) Changes in morphology of *Giardia canis* as affected by diet. *Proceedings of the Society for Experimental Biology*. **28**: 708-709.

Tsuchiya, H. (1930) A comparative study of two diverse strains of *Giardia lamblia* Stiles, 1915. *American Journal of Hygiene*. **12**: 467-491.

Uhazy, L.S., Holmes, J.C. and Stelfox, J.G. (1973) Lungworms in the Rocky Mountain bighorn sheep of western Canada. *Canadian Journal of Zoology*. **51**: 817-824.

van de Ven, E., Melchers, W., Galama, J., Camps, W. and Meuwissen, J. (1991) Identification of *Toxoplasma gondii* infections by BI gene amplification. *Journal of Clinical Microbiology*. **29**: 2120-2124.

van Keulen, H., Freely, D.E., Macechko, P.T., Jarroll, E.L. and Erlandsen, S.L. (1998) The sequence of *Giardia* small subunit rRNA shows that voles and muskrats are parasitized by a unique species *Giardia microti*. *Journal of Parasitology*. **84**: 294-300.

van Keulen, H., Macechko, P.T., Wade, S., Schaaf, S., Wallis, P.M. and Erlandsen, S.L. (2002) Presence of human *Giardia* in domestic, farm and wild animals, and environmental samples suggests a zoonotic potential for giardiasis. *Veterinary Parasitology*. **108**: 97-107.

van Riper, C. and van Riper, S.G. (1986) The epizootiology and ecological significance of malaria in Hawaiian land bird. *Ecological Monographs*. **56**: 327-344.

Viggers, K.L., Lindenmayer, D.B. and Spratt, D.M. (1993) The importance of disease in reintroduction programmes. *Wildlife Research*. **20**: 687-698.

Visco, R.J., Corwin, R.M. and Selby, L.A. (1978) Effect of age and sex on the prevalence of intestinal parasitism in cats. *Journal of the American Veterinary Medical Association*. **172**: 797-800.

Vitousek, P.M., D'Antonio, C.M., Loope, L.L. and Westbrooks, R. (1996) Biological invasions as global environmental change. *American Scientist*. **84**: 468-478.

Vitousek, P.M., Mooney, H.A., Lubchenco, J. and Melillo, J.M. (1997) Human domination of Earth's ecosystems. *Science*. **277**: 494-499.

Vizoso, A.D. (1968) A red squirrel disease. *Symposium of the Zoological Society of London*. **24**: 29-38.

von Samson-Himmelstjerna, G., Woidtke, S., Epe, C. and Schnieder, T. (1997) Species-specific polymerase chain reaction for the differentiation of larvae from *Dictyocaulus viviparus* and *Dictyocaulus eckerti*. *Veterinary Parasitology*. **68**: 119-126.

Wakelin, D. (1978) Genetic control of susceptibility and resistance to parasitic infection. *Advances in Parasitology*. **16**: 219-308.

Wallace, G.D. (1972) Experimental transmission of *Toxoplasma gondii* by cockroaches. *Journal of Infectious Diseases*. **126**: 545-547.

Wallace, G.D. (1971) Experimental transmission of *Toxoplasma gondii* by filth-flies. *American Journal of Tropical Medicine and Hygiene*. **20**: 411-413.

Wallace, G.D. (1973) Intermediate and transport hosts in the natural history of *Toxoplasma gondii*. *American Journal of Tropical Medicine and Hygiene*. **22**: 456-464.

Wallace, G.D., Marshall, L. and Marshall, M. (1972) Cats, rats, and toxoplasmosis on a small Pacific island. *American Journal of Epidemiology*. **95**: 475-482.

Wallis, P.M., Erlandsen, S.L., Isaac-Renton, J.L., Olson, M.E., Robertson, W.J. and Keulen, H.v. (1996) Prevalence of *Giardia* cysts and *Cryptosporidium* oocysts and characterization of *Giardia* spp. isolated from drinking water in Canada. *Applied and Environmental Microbiology*. **62**: 2789-2797.

Walsh, P.S., Metzger, D.A. and Higuchi, R. (1991) Chelex 100 as a medium for simple extraction of DNA for PCR-based typing from forensic material. *BioTechniques*. **10**: 506-513.

Warner, L.R. (1998) Australian helminths in Australian rodents: an issue of biodiversity. *International Journal for Parasitology*. **28**: 839-846.

Watts, C.H.S. and Braithwaite, R.W. (1978) The diet of *Rattus lutreolus* and five other rodents in southern Victoria. *Australian Wildlife Research*. **5**: 47-57.

Webster, J.P. (1994a) The effect of *Toxoplasma gondii* and other parasites on activity levels in wild and hybrid *Rattus norvegicus*. *Parasitology*. **109**: 583-589.

Webster, J.P. (1994b) Prevalence and transmission of *Toxoplasma gondii* in wild brown rats, *Rattus norvegicus*. *Parasitology*. **108**: 407-411.

Webster, J.P. (2001) Rats, cats, people and parasites: the impact of latent toxoplasmosis on behaviour. *Microbes and Infection*. **3**: 1037-1045.

Webster, J.P., Brunton, C.F. and MacDonald, D.W. (1994) Effect of *Toxoplasma gondii* upon neophobic behaviour in wild brown rats, *Rattus norvegicus*. *Parasitology*. **109**: 37-43.

White, S.R. (1952) The occurrence of the quokka in the south-west. *Western Australian Naturalist*. **3**: 101-103.

Wilson, P.M., Tidemann, C.R. and Meischke, H.R.C. (1994) Are cats on rubbish dumps a problem? In *Urban Animal Management*. Paxton, D.W. (ed.) Sydney: Australian Veterinary Association, pp. 163-174.

Wilson-Hanson, S.L. and Prescott, C.W. (1982) A survey for parasites in cats. *Australian Veterinary Journal*. **59**: 194.

Wolfe, M.S. (1990) Clinical symptoms and diagnosis by traditional methods. In *Giardiasis*. Meyer, E.A. (ed.) Amsterdam: Elsevier Science Publishers B. V., pp. 175-185.

Wright, D.K. and Manos, M.M. (1990) Sample preparation from paraffin-embedded tissues. In *PCR Protocols. A Guide to Methods and Applications*. Sninsky, J.J. (ed.) San Diego: Harcourt, Brace, Jovanovich, pp. 154-156.

Yamane, A., Ono, Y. and Doi, T. (1994) Home range size and spacing pattern of a feral cat population on a small island. *Journal of the Mammalogical Society of Japan*. **19**: 9-20.

Yilmaz, S.M. and Hopkins, S.H. (1972) Effects of different conditions on duration of infectivity of Toxoplasma gondii oocysts. *Journal of Parasitology*. **58**: 938-939.

Yolken, R.H., Bachmann, S., Ruslanova, I., Lillehoj, E., Ford, G., Torrey, E.F., Schroeder, J. and Rouslanova, I. (2001) Antibodies to *Toxoplasma gondii* in individuals with first-episode schizophrenia. *Clinical Infectious Diseases*. **32**: 842-844.

Yoshida, Y. (1971) Comparative studies on *Ancylostoma brasiliense* and *Ancylostoma ceylanicum*. I. The adult stage. *J Parasitol*. **57**: 983-989.

Yoshida, Y., Okamoto, K. and Chiu, J.-K. (1971) Experimental infection of man with *Ancylostoma ceylanicum* (Looss, 1911). *Chinese Journal of Microbiology*. **4**: 157-167.

Zarlenga, D.S. and Higgins, J. (2001) PCR as a diagnostic and quantitative technique in veterinary parasitology. *Veterinary Parasitology*. **101**: 215-230.

Zenner, L., Darcy, F., Cesbron-Delauw, M.F. and Capron, A. (1993) Rat model of congenital toxoplasmosis: rate of transmission of three *Toxoplasma gondii* strains to fetuses and protective effect of a chronic infection. *Infection and Immunity*. **61**: 360-363.