

Copyright is owned by the Author of the thesis. Permission is given for a copy to be downloaded by an individual for the purpose of research and private study only. The thesis may not be reproduced elsewhere without the permission of the Author.

**Prevalence of selected infectious diseases in
Samoan dogs**

A thesis presented in partial fulfilment of the requirements for the degree
of

Master of Veterinary Science

at Massey University, Manawatu,

New Zealand

Rosalind Jane Carslake

2013

Abstract

Samoa has a tropical island climate ideally suited to many infectious diseases, and vectors for some infectious diseases are known to be present. Dogs are very commonly owned in Samoa with 88% of households owning an average of two dogs. Many canine infectious diseases are zoonotic and there is limited preventative medicine available for dogs in Samoa. There are very few studies into the presence of zoonotic pathogens in Samoa or other South Pacific islands, and the role of dogs as a reservoir for zoonotic diseases is unknown.

The prevalence of selected infectious diseases was evaluated in 242 dogs undergoing surgical sterilisation in Samoa in July 2010 and August 2011. Data were obtained from dogs' owners by interview, including age, environment and any previous preventative medication. Serum and faecal samples were collected, and the skin examined for external parasites. Seroprevalence of *Leishmania infantum*, *Anaplasma phagocytophilum*, *Ehrlichia canis*, *Borrelia burgdorferi* and *Dirofilaria immitis* were assessed using point of care qualitative ELISA assays. Faecal flotation was performed on fresh faecal samples to screen for intestinal parasites. Ninety-three faecal samples were also tested for *Giardia* and *Cryptosporidium* spp.

The median age of dogs was one year, with a range of four months to eight years and 73.3% were male. The vast majority of dogs were owned, the remaining were stray animals. Prevalence of *D. immitis* was 46.8% and *A. phagocytophilum* seroprevalence was 8.4%. All serum samples tested negative for *E. canis*, *B. burgdorferi* and *L. infantum*. Prevalence of hookworm was 92.6%. *Trichuris vulpis*, *Dipylidium caninum*, *Toxocara canis* and *Capillaria* spp. were also detected. Prevalence of *Giardia* spp. was 29.0% while no *Cryptosporidium* was detected. Fleas were found on 83.7% of the dogs, ticks on 42.1% and lice on 8.1%. Identified ticks were *Rhipicephalus sanguineus*, with no *Ixodes* spp. found.

The results indicate a very high prevalence of hookworm, *D. immitis*, and external parasites in Samoan dogs. This study provides valuable information on canine health and suggests dogs could play a role in the spread of some zoonoses in Samoa. Further studies are required to review the public health implications of this study.

Acknowledgements

Firstly I would like to thank my supervisors, Els Acke, Kate Hill and Debbie Prattley who have consistently offered me the encouragement, guidance and support needed to enable me to complete this study and thesis; this all in spite of me being so far away and often preoccupied with moving houses, pregnancy or maternity leave.

The staff of the Animal Protection Society of Samoa deserve many thanks for allowing and enabling us to collect data during the Samoan BVSc final year electives of 2010 and 2011. A full schedule of sterilisation clinics was laid on in some truly stunning Samoan locations, ensuring a steady supply of dogs to sample and they were always there to help us overcome any obstacles we met in the field. Without this the study could not have happened.

This study would also not have been possible without the support from IDEXX laboratories in donating all the ELISA kits required. Many thanks to John Stamaris at IDEXX for making it happen.

I would like to extend my gratitude to all those involved in helping with data collection and laboratory analysis of the samples: Kim Sjölander, Alison Harland and the final year veterinary students on the 2010 and 2011 Samoan electives with their help in data collection, and Kim's help with the laboratory analysis of the 2011 samples; Barbara Adlington and the Massey University parasitology department for their provision of equipment and teaching time; Niluka Velathanthiri, Anthony Pita and Magnus Beckman for their assistance with the *Giardia* and *Cryptosporidium* laboratory work.

I am hugely grateful to my family; without your babysitting and childcare I would never have had the time to get this written. And lastly, but by no means least, I am wholeheartedly thankful to my husband, Harry, for his endless encouragement, support and belief in me throughout this project.

This study design was approved by the Massey University Animal Ethics Committee.

Table of contents

Abstract	ii
Acknowledgements	iii
Table of contents	iv
List of figures and tables	vi
Abbreviations	ix
Chapter 1 Introduction and aims	1
Chapter 2 Literature review	3
2.1 Samoa: Location, climate and demographics	3
2.2 Canine health, husbandry and disease in Samoa	5
2.2.1 Dogs and public attitudes to dogs in Samoa	5
2.2.2 Canine disease and health in Samoa and the Pacific	7
2.3 Intestinal parasites	8
2.3.1 Canine hookworm	8
2.3.2 Other intestinal parasitic helminths	14
2.3.3 Enteric protozoa: <i>Giardia duodenalis</i>	17
2.3.4 Enteric protozoa: <i>Cryptosporidium</i> spp.	22
2.4 Ectoparasites: Tick, fleas and lice	25
2.5 Vector-borne diseases	27
2.5.1 <i>Dirofilaria immitis</i> : Canine heartworm	27
2.5.2 <i>Ehrlichia canis</i>	34
2.5.3 <i>Anaplasma phagocytophilum</i>	40
2.5.4 <i>Anaplasma platys</i>	47
2.5.5 <i>Borrelia burgdorferi</i>	50
2.5.6 <i>Leishmania infantum</i>	56
Chapter 3 Materials and methods	64
3.1 Sample population	64
3.2 Sample collection	64
3.3 Serologic testing	65
3.4 Faecal flotation	66
3.5 Detection of <i>Cryptosporidium</i> and <i>Giardia</i> by direct IFA coproscopy	67
3.6 Statistical analysis	67

Chapter 4 General results	68
4.1 Geographical distribution of samples	68
4.2 Questionnaire results	68
Chapter 5 Prevalence of selected external and intestinal parasites in Samoan dogs ..	72
5.1 Results	72
5.1.1 Faecal examination results	72
5.1.2 Skin examination results	74
5.2 Discussion	75
Chapter 6 Prevalence of selected vector-borne diseases in Samoan dogs	83
6.1 Results	83
6.1.1 Descriptive results	83
6.1.2 Association between prevalence of canine heartworm and age and area	85
6.1.3 Association between seroprevalence of <i>Anaplasma</i> spp. and area .	87
6.2 Discussion	88
Chapter 7 General discussion	95
References	99
Appendix 1: Questionnaire completed by the owner of the dog	122

List of figures and tables

Fig 2.1	Map of Samoa	3
Fig 2.2	The geographical regions used in the 2011 Samoa census: Apia Urban Area, North West Upolu, Rest of Upolu and Savai'i	4
Fig 2.3	A selection of Samoan dogs	6
Tab 2.1	Results of a study investigating selected canine infectious diseases (Martin, 1999)	7
Fig 2.4	Life cycle of canine hookworm	11
Fig 2.5	Hookworm egg (x400)	12
Fig 2.6	Life cycle of <i>Toxocara canis</i>	15
Tab 2.2	Assemblages and host range of isolates of <i>Giardia duodenalis</i>	18
Fig 2.7	Life cycle of <i>Dirofilaria immitis</i>	30
Tab 2.3	Summary of clinical signs of canine heartworm disease	31
Fig 2.8	Approximate worldwide geographic distribution of four <i>Ixodes</i> spp. tick vectors of <i>Anaplasma phagocytophilum</i> and their overlapping regions	41
Fig 2.9	The lifecycle of <i>Ixodes scapularis</i>	51
Fig 2.10	Map of the global distribution of canine leishmaniasis due to <i>Leishmania infantum</i> and human visceral leishmaniasis	56
Fig 2.11	Life cycle of <i>Leishmania infantum</i>	59
Fig 4.1	Map of Samoa marking the villages from which the 242 dogs sampled to study selected infectious diseases originated. The number of dogs sampled from each area is denoted by numbers in parentheses	68
Fig 4.2	Map of the Apia urban area marking the villages from which dogs sampled to study infectious diseases originated. The number of dogs sampled from each area is denoted by the number in parentheses	69
Tab 4.1	The household location of 242 dogs sampled to study selected infectious diseases compared to Samoan household distribution from the 2011 Samoa census (Reupena, 2012b) and the household location of 327 respondents to a questionnaire concerning attitudes to dogs in Samoa (Farnworth et al., 2012)	69

Tab 4.2	The demographics and tests performed on 242 dogs in the study of selected infectious disease of dogs in Samoa. The number (n) and percentage (%) of dogs sampled for each variable of interest are provided	71
Tab 5.1	Faecal examination data from the study of 242 dogs in Samoa sampled for selected infectious diseases. The number of positives/total tested (n), prevalence and 95% confidence intervals for faecal examination results are provided	73
Fig 5.1	Prevalence of intestinal parasites in faecal examination from 242 dogs from Samoa	73
Tab 5.2	Skin examination data from the study of 242 dogs in Samoa sampled for selected infectious diseases. Number of positives/total tested (n), prevalence and 95% confidence intervals for external parasites detected on skin examination are provided	74
Fig 5.2	Prevalence of external parasites on 221 dogs from Samoa	74
Tab 5.3	Number of positives/total tested (n), prevalence and 95% confidence intervals for <i>Rhipicephalus sanguineus</i> infestations of dogs for each area. Data from the study of 242 dogs in Samoa sampled for selected infectious diseases	75
Tab 6.1	Number (N) and percentage (%) of 237 dogs sampled in the study of selected infectious disease of dogs in Samoa for each variable of interest, and number and percentage (%) of samples with a positive ELISA test result for heartworm antigen and <i>Anaplasma</i> antibody for each variable	84
Fig 6.1	The seroprevalence of selected vector borne diseases by ELISA in 237 Samoan dogs. <i>Dirofilaria immitis</i> (Di), <i>Anaplasma</i> (Ap), <i>Borrelia burgdorferi</i> (Bb), <i>Ehrlichia canis</i> (Ec) and <i>Leishmania infantum</i> (Li)	84
Tab 6.2	Number of positives/total tested (n), prevalence and 95% confidence intervals for 237 Samoan dogs with positive heartworm antigen tests within the four age categories	85
Fig 6.2	The seroprevalence of heartworm antigen in 237 Samoan dogs by age with 95% error bars	85
Tab 6.3	Number of positives/total tested (n), prevalence and 95% confidence intervals for 237 Samoan dogs with positive heartworm antigen tests within three Samoan geographical areas	86

Fig 6.3	The prevalence of heartworm in 237 dogs in three main areas of Samoa with 95% error bars	86
Fig 6.4	The prevalence of heartworm in 237 dogs in three main areas of Samoa, divided into age groups	87
Tab 6.4	Number of positives/total tested (n), prevalence and 95% confidence intervals for 237 Samoan dogs with positive <i>Anaplasma</i> ELISA tests within three Samoan geographical areas	88

List of abbreviations

The following abbreviations are used within the main text and are defined in full when first used.

AHS	American Heartworm Society
AIDS	Acquired immune deficiency syndrome
APS	Animal Protection Society of Samoa
BVSc	Bachelor of Veterinary Science
CGA	Canine granulocytotropic anaplasmosis
CLM	Cutaneous larva migrans
CME	Canine monocytotropic ehrlichiosis
ELISA	Enzyme-linked immunosorbent assay
HDU	Heartworm development units
HGA	Human granulocytic anaplasmosis
HrCLM	Hookworm-related cutaneous larva migrans
ICAM	International Companion Animal Management Coalition
IFA	Immunofluorescent antibody
L1	Stage 1 larvae
L2	Stage 2 larvae
L3	Stage 3 larvae
L4	Stage 4 larvae
OLM	Ocular larva migrans
PCR	Polymerase chain reaction
PCR-RFLP	Polymerase chain reaction-restriction fragment length polymorphism
VLM	Visceral larva migrans
WB	Western immunoblotting
ZSCT	Zinc sulphate centrifugation test

Chapter 1 Introduction and aims

The aim of this study was to investigate the prevalence of selected infectious diseases in dogs in Samoa. The opportunity for this research arose as result of Massey University trialling a student elective as part of the Bachelor of Veterinary Science (BVSc) final year in July 2010. For this elective, a group of final year veterinary students and a veterinarian from the Massey University Veterinary Teaching Hospital collaborated with the Animal Protection Society (APS) of Samoa, a non-profit organisation founded in 1994 to improve the welfare of companion animal species in Samoa. For a four week period, the APS advertised a widespread free canine and feline spay and castration mobile clinic which would travel to many remote areas on both of the main islands. On investigation prior to this inaugural elective, it was apparent that there was very little information in the literature regarding canine health and disease in Samoa, and yet it was clear that dog ownership was very common with dogs living in close contact with people in town and village communities. A lack of population control meant that canine overpopulation was a visible problem (Farnworth et al., 2012).

Large, free-roaming populations of dogs are known to increase the prevalence of canine disease and associated risk of zoonotic infection (Acosta-Jamett et al., 2010; Farnworth et al., 2012). Dogs are important reservoirs for certain zoonotic vector-borne diseases, such as leishmaniasis (Michel et al., 2011) and potentially anaplasmosis (Doudier et al., 2010). Knowing the prevalence of infectious diseases in a canine population can help to give an indication of risk to public health. Dogs living within communities also defaecate in living areas of the towns and villages and this can increase the risk of zoonotic diseases such as hookworm related cutaneous larva migrans (HrCLM) (Hochedez and Caumes, 2007) and toxocariasis (Rubinsky-Elefant et al., 2010). The condition of large populations of free-roaming dogs is often relatively poor (Farnworth et al., 2012). This can be due to lack of veterinary preventative care and treatment and high infectious disease rates, and can comprise a major animal welfare issue.

By investigating the prevalence or seroprevalence rates of selected canine infectious diseases, this study aimed to establish which of these diseases are present in the Samoan canine population, within the confines of the study population examined. In addition, by providing prevalence data on several zoonotic diseases, the results aimed to assess which of these diseases people in direct contact with dogs, or sharing the same environment as dogs, could be at risk of contracting. The study also aimed to investigate what current canine preventative

health care treatments are commonly used by Samoan dog owners for their dogs. This would form the basis for recommendations regarding preventative health care for dogs in Samoa, particularly with regard to reducing the potential risk of zoonotic disease transmission to people living or holidaying in close proximity to dogs. This study would also give baseline prevalence levels for monitoring the results of any preventative measures implemented.

Chapter 2 Literature Review

2.1 Samoa: Location, climate and demographics

Samoa, previously known as Western Samoa, is situated in the South Pacific and consists of two main islands, Upolu and Savai'i, and a number of smaller islets. The surface area is 2785 km² (Figure 2.1).

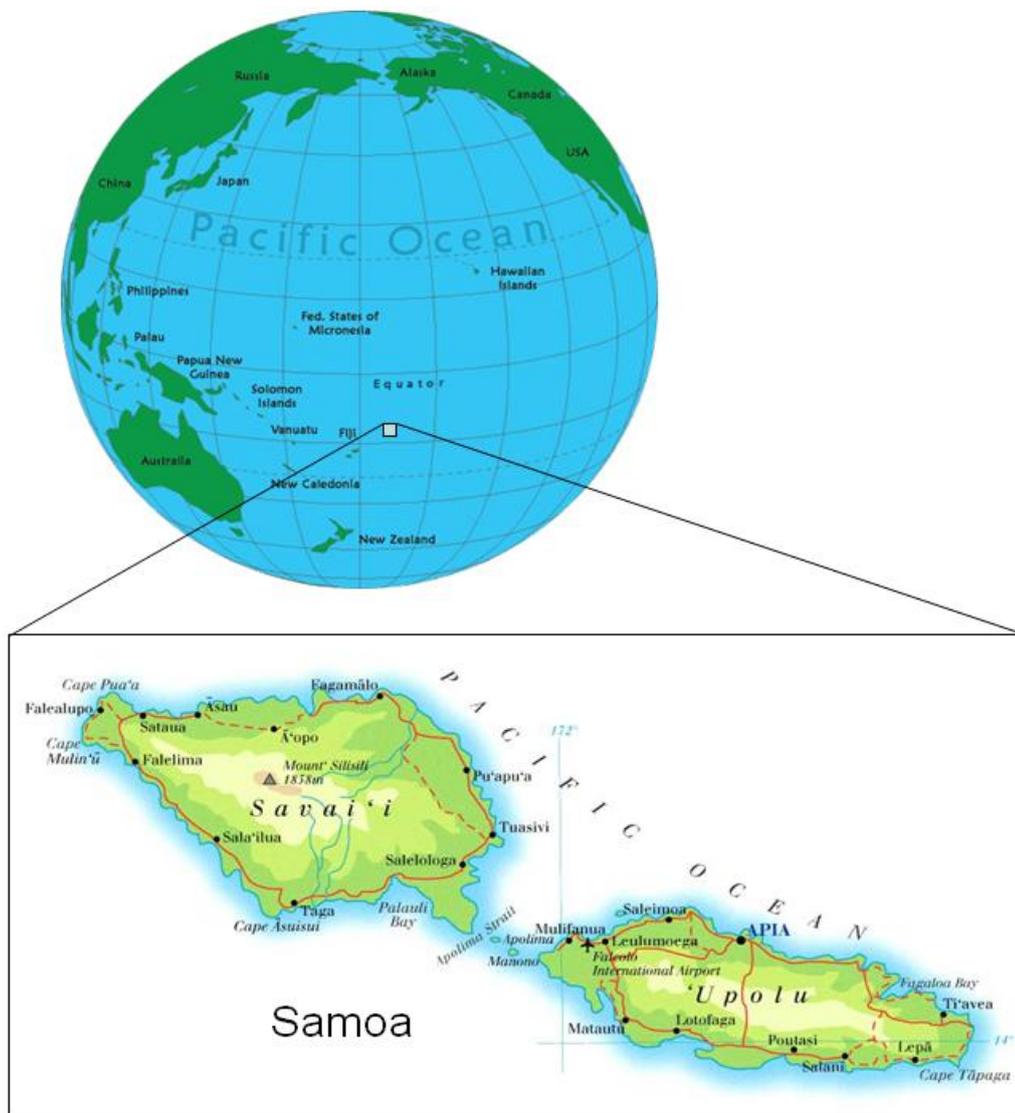


Figure 2.1: Map of Samoa

The climate is tropical with precipitation varying from 2,540 mm annually on the northern and western coasts to 7,620 mm inland. Temperatures vary little with an average of 27 °C (range 23 to 30 °C) throughout the year (Anonymous, 2012a) .

The last official census of Samoa was carried out on 11th November 2011. On this date the population of Samoa was 187,820 and showed annual growth of 0.63% per annum when compared with the census of 2001. The total number of households was 26,205 with a mean of 7.2 persons per household (Reupena, 2012b). For analysis of the census data, the population was divided into four groups according to geographical area (Figure 2.2). The Apia urban area, contained 19% of the Samoan population and was the most densely populated region. The other three regions were classified as rural: northwest Upolu contained 33% of population, the rest of Upolu 24%, and Savaii 23% (Reupena, 2012b).

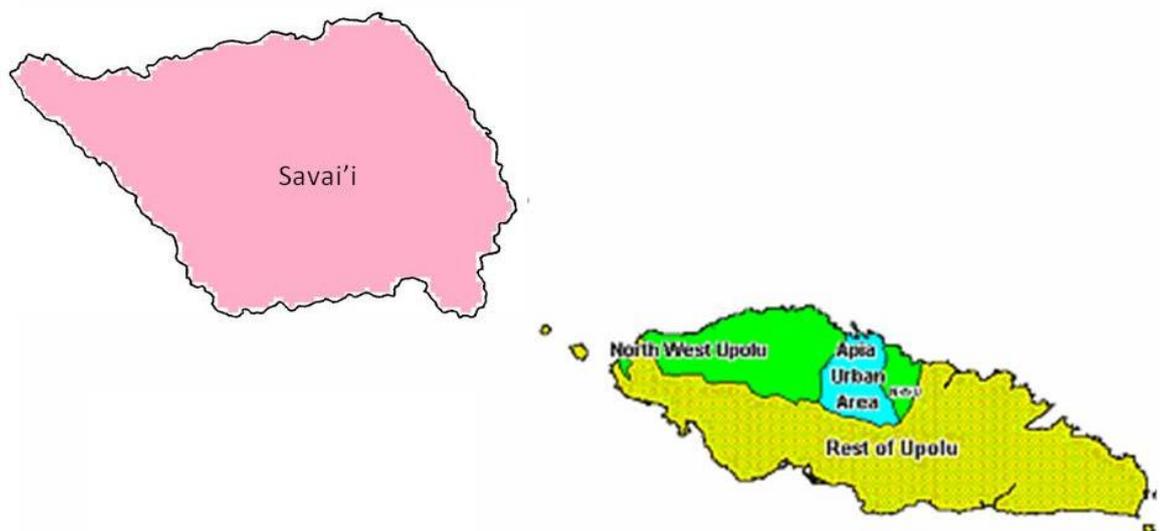


Figure 2.2: The geographical regions used in the 2011 Samoa census: Apia Urban Area, North West Upolu, Rest of Upolu and Savai'i (Reupena, 2012b).

The Samoan economy is dependent on tourism, which represents the largest single employment activity in the country. Tourism arrivals are increasing each year, with 127,600 arrivals in 2011, contributing over US \$120.8 million to the economy. New Zealand is the dominant source country for visitors to Samoa, with a total of 54,900 visitors from New Zealand in 2011 (Reupena, 2012a).

2.2 Canine health, husbandry and disease in Samoa

2.2.1 Dogs and public attitudes to dogs in Samoa

It is not known exactly how many dogs there are in Samoa, however they are very commonly owned. In a questionnaire based study conducted in 2009 which interviewed people from rural and urban communities on both main islands, 88% of 327 respondents were dog owners and the median number of dogs per household was two (Farnworth et al., 2012). This can be considered a very high rate of dog ownership. The vast majority of dog owners (79%) stated that the primary reason for dog ownership was for protection and as a result the relationship of Samoans towards their dogs is very different to that of most western cultures. The vast majority of dogs are free-roaming, with few households fenced in, and there is no system of dog registration or licensing in the country. This large and free-roaming canine population is very visible in both urban and rural Samoa. In the aforementioned study the majority of people believed dogs should be fenced in (67%), however only 5% of respondents had their property fenced in such a way that a dog could be contained (Farnworth et al., 2012). Tourists frequently comment on this apparently stray dog population, and there are anecdotal reports of tourists being bitten by aggressive dogs. Traveller advice includes carrying sticks or stones to scare off any dogs that may approach (Anonymous, 2007b, 2012e). Tourists might increase their risk of being bitten by trying to approach or feed stray dogs (Anonymous, 2010b). One quarter of Samoans themselves admit that they or a family member have been bitten by a dog, most often by one they themselves own (Farnworth et al., 2012). Abuse and killing of dogs appears to be common and was considered acceptable by 26% of people asked (Farnworth et al., 2012). It is generally well accepted that education is one of the most important routes by which improvements in dog welfare and changes in social attitudes to dogs can be achieved (Anonymous, 2007a), and in Samoa rates of dog-specific education is very low (16%) (Farnworth et al., 2012).

Anecdotal reports suggest in many communities it is common practice to control the dog population by killing female puppies in a litter at birth. This is reflected in a highly skewed sex ratio with male dogs representing 71% of the canine population (Farnworth et al., 2012). Over 80% of dogs are entire and the remaining 20% would have been sterilised by the APS. There are currently no private veterinarians in Samoa. The only veterinary care for companion animals comes from the APS, based in Apia, who run free sterilisation clinics in an attempt to help control the canine population. The APS also provides preventative health care, with

vaccines, anthelmintics and parasiticides sold at cost price from the clinic in Apia, but for many Samoans their dog's health is not a priority and so very few dogs are vaccinated, de-wormed or de-fleaed regularly. For other dog owners the clinic is not accessible, especially if their dogs are unapproachable and they are unable to transport them to the clinic. For some, the need for preventative health care is not understood.

In the South Pacific region, dogs were introduced with human settlers. The dogs today are as a result of multiple introductions, estimated to have begun approximately 2000 years before the present (Anderson, 2009). The Samoan dog type is a mixed breed, tending to be medium sized, with a short to medium hair-coat (Figure 2.3).



Figure 2.3: A selection of Samoan dogs (author's own photographs)

Compared with dogs, cats are much less visible in Samoan communities. It is assumed that feline population control is largely due to the large numbers of dogs in these areas. There is even less information available on feline populations and ownership in Samoa than there is for dogs.

2.2.2 Canine disease and health in Samoa and the Pacific

Very few previous studies have been carried out into animal disease status in Samoa, especially in small animal medicine. A serological study of animal health status in Samoa conducted in 1997 (Martin, 1999) concentrated mainly on production animals, although ten dogs were also sampled. The results confirmed the presence of *Ehrlichia canis* and canine parvovirus (Table 2.1).

Table 2.1: Results of a study investigating selected canine infectious diseases (Martin, 1999). Serum agglutination test (SAT), Indirect fluorescent antibody test (IFAT), Serum neutralisation test (SNT), Haemagglutination inhibition test (HIT).

Canine disease	Serological test	No. of samples	
		Tested	Positive
Canine parvovirus	SAT	10	8
<i>Ehrlichia canis</i>	IFAT	10	6
Canine distemper virus	SNT	7	0
<i>Brucella canis</i>	HIT	10	0
Japanese encephalitis virus	HIT	1	0

This study also confirmed that leptospirosis was endemic in Samoan bovine, equine and porcine populations. No canine samples were tested for leptospirosis. Samples from commercial and village pig and cattle populations, and village horse populations were tested against 21 *Leptospira* serovars. Of the 316 bovine samples tested, 40% were positive to one or more serovars, with *hardjo*, *australis*, *szwajizak* and *medanesis* being most prevalent. Of the 161 porcine sera tested, 23% were positive, with *copenhageni*, *australis*, *canicola* and *swajizak* being the most prevalent serovars. Forty-four percent of the 63 equine sera tested were positive, with *copenhageni* and *canicola* being most prevalent (Martin, 1999).

Internal and external parasites identified in the dog included *Ancylostoma caninum*, *Toxocara canis*, *Trichuris vulpis*, *Dipylidium caninum*, *Dirofilaria immitis*, *Ctenocephalides canis*, *Ctenocephalides felis*, *Demodex canis*, *Trichodectes canis*, *Rhipicephalus sanguineus*, *Giardia* spp. and *Coccidia* (Martin, 1999).

Unpublished anecdotal evidence, recorded by veterinarians working for the APS, also indicates the presence of canine parvovirus and canine distemper, all the parasites listed above and *Sarcoptes scabiei*. To the authors knowledge, there are no reports of Ixodes ticks being present in Samoa, and rabies has never been recorded (Rupprecht and Shlim, 2012).

2.3 Intestinal parasites

Many of the most common canine intestinal parasites have a worldwide distribution, however, prevalence varies according to region and the lifestyle and veterinary care of the population investigated (Bugg et al., 1999; Fontanarrosa et al., 2006; Hackett and Lappin, 2003; Itoh et al., 2009; Joffe et al., 2011; Little et al., 2009; Overgaauw et al., 2009; Traub et al., 2005). Knowledge of local prevalence helps veterinarians to make recommendations to dog owners on anthelmintic use, and given the zoonotic potential of some canine endoparasites (Schantz, 1994), this information is also valuable to human health care providers. The parasites discussed in detail below are the most common intestinal parasites of potential zoonotic importance, found in both tropical and temperate regions worldwide.

2.3.1 Canine hookworm

Aetiology

Worldwide, the most common hookworms of dogs are *Ancylostoma caninum*, *Ancylostoma braziliense*, *Ancylostoma ceylanicum* and *Uncinaria stenocephala*. Canine hookworm can cause HrCLM and eosinophilic enteritis in humans, with *A. braziliense* and *A. caninum* most commonly implicated, respectively (Prociv, 2003).

Epidemiology

The exact geographical distribution of each hookworm species is not known, as ranges overlap, hosts can harbour more than one species simultaneously and the *Ancylostoma* spp. eggs are morphologically indistinct, with only the larger *U. stenocephala* egg being identifiably different. Distribution is determined by the presence of suitable hosts and environmental conditions which support transmission (Prociv, 2003).

A. caninum, *A. braziliense* and *A. ceylanicum* are found in tropical and subtropical climates where the conditions are ideal for hookworm survival, whereas *U. stenocephala* is more commonly found in the colder climates of North America, Europe, Australia and New Zealand (Bowman et al., 2010; Prociv, 2003). The most common species of canine hookworm worldwide is *A. caninum* which is found throughout the warmer regions of the world (Bowman et al., 2010; Landmann and Prociv, 2003; Prociv, 2003). *A. braziliense* has been reported from warmer regions of the USA, the Gulf of Mexico and the Caribbean, regions of South America, Africa, Australia and Asia (Bowman et al., 2010; Lucio-Forster et al., 2012; Palmer et al., 2007; Traub et al., 2007), and *A. ceylanicum* has been reported from many regions in Asia, Australia and South America (Bowman et al., 2010; Mahdy et al., 2012; Palmer et al., 2007; Traub et al., 2007; Traub et al., 2008). Prevalence of infection varies depending on the density of dogs, climate and conditions, and anthelmintic use in dog populations. Hookworm prevalence from canine faecal samples has been reported as high as 98% (Traub et al., 2004). This highest reported prevalence was from dogs from a rural tea growing region of India with a sub-tropical climate, using a combination of polymerase chain reaction (PCR) and conventional microscopy.

There are very few data on canine hookworm species present in the Pacific Islands. Anecdotally, canine hookworm is known to be present in Samoa, and has been assumed to be *A. caninum* (Martin, 1999), although there is no evidence that any speciation has been carried out.

Pathogenesis and lifecycle

The main cause of morbidity in dogs with hookworm infection is blood loss due to sucking adults in the small intestine. *A. caninum* causes by far the greatest blood loss with up to 1-2mls of blood loss caused by each adult worm per day (Georgi et al., 1969; Traub et al., 2004). *A. braziliense* and *A. ceylanicum* cause significantly less blood loss and are rarely associated with disease as a direct result of blood loss (Miller, 1966b; Traub et al., 2004). *U. stenocephala* is

not haematophagic, but can cause diarrhoea in heavy infections as a result of a protein losing enteropathy (Prociv, 2003).

Eggs are produced by adult female worms feeding in the lumen of the small intestine, and are passed in the faeces. Under the right environmental conditions the stage 1 larvae (L1) hatch in 1-2 days (Figure 2.4). Larvae continue to grow and develop in the soil or faeces, moulting twice until after 5-10 days they develop into the infective stage 3 larvae (L3). These infective larvae can survive 3-4 weeks in the environment in favourable conditions (Anonymous, 2012d; Prociv, 2003). On contact with the host skin, L3 is able to invade either by directly penetrating the skin (*A. braziliense*) or by gaining access via hair follicles (other hookworm species) (Prociv, 2003). L3 is then carried to the heart and lungs via blood vessels. In the lungs, larvae penetrate the pulmonary alveoli and climb the bronchial tree to the pharynx where they are swallowed. Larvae reach the small intestine where they develop into adults and lay more eggs (Anonymous, 2012d). Infection may also be acquired by the ingestion of L3, either directly from the environment or by ingestion of paratenic host tissue. In this case, larvae may develop directly into adult worms in the intestine, without tracheal migration (Prociv, 2003).

In pregnant bitches following parturition, dormant *A. caninum* larvae are able to migrate to the mammary tissue and lead to neonatal infection via the milk, although this has not been shown in other species. Transplacental infection occurs only rarely and is not considered an important route of infection (Stone and Girardeau, 1968).

The prepatent period varies from 14-29 days following percutaneous infection, though this may be longer in older dogs, and shorter when L3 is directly ingested (Miller, 1966a; Prociv, 2003; Yutuc, 1954). Adult worms attain their maximal size after 30 days and live an average of 6 months with individual females producing as many as 28,000 eggs a day at their peak (Prociv, 2003).

Clinical presentation

A. caninum is the most important hookworm in dogs and is associated with blood loss and haemorrhagic enteritis (Miller, 1966a). Disease in puppies can result in diarrhoea, weakness, pallor, dehydration and poor growth. Infection can cause a blood loss anaemia, which may occur as a rapid, potentially fatal anaemia or a chronic iron deficiency anaemia (Okewole and Oduye, 2000). *A. ceylanicum*, *A. braziliense* and *U. stenocephala* cause little or no blood loss (Prociv, 2003).

Hookworm dermatitis can occur in dogs when the larvae of *A. braziliense*, *A. caninum* or *U. stenocephala* enter the skin of areas in direct contact with the ground. The skin on the feet, sternum, ventral abdomen, tail and posterior thighs are usually affected, with red papules developing into erythematous, thickened, alopecic lesions that can be pruritic (Campbell, 2005).

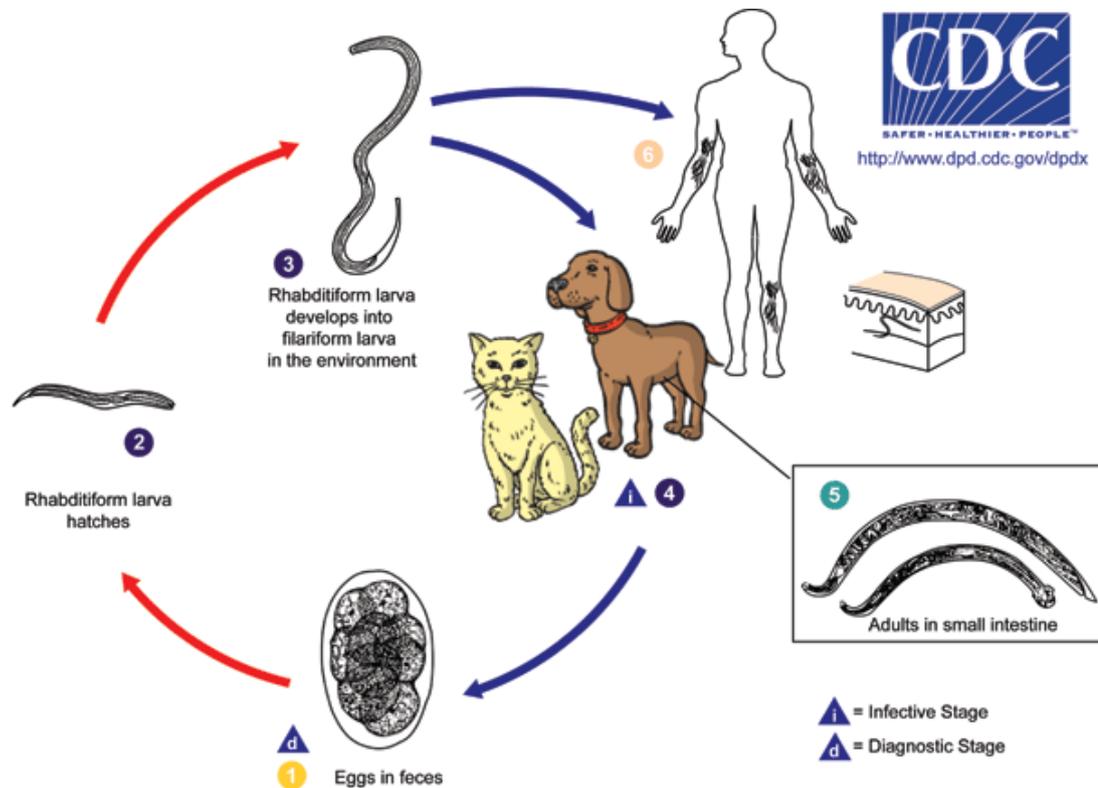


Figure 2.4: Life cycle of canine hookworm: Eggs are passed in the faeces ① and larvae hatch in 1-2 days. The larvae develop in the faeces and/or soil ② into the infective L3 larvae ③. On contact with the animal host ④, the L3 larva penetrates the skin, is carried to the heart then lungs via blood vessels. Larvae penetrate the pulmonary alveoli, ascend the bronchial tree to the pharynx, where they are swallowed. Larvae reach the small intestine where they develop into adults ⑤. Humans can become infected when L3 penetrates the skin but usually larvae do not develop further and migrate aimlessly within the epidermis ⑥. (Anonymous, 2012d)

Diagnosis

Diagnosis of hookworm infection is made by demonstration of the ova in faeces. Faecal concentration methods such as faecal flotation allow concentration of any parasite eggs and cysts, while removing debris. The principle of any flotation method is that parasitic material is less dense than the flotation fluid in which the faeces are suspended and so float to the top.

This top layer can then be collected for microscopic evaluation. Saturated solutions of sodium chloride, sodium nitrate, magnesium sulphate and zinc sulphate all float common helminth eggs including hookworm (Zajak and Conboy, 2012).

Faecal flotation is inexpensive and relatively easy to perform, however it is not possible to distinguish between the eggs of different hookworm species by this method alone, as grossly, the eggs appear very similar. Figure 2.5 depicts the appearance of a hookworm egg. By measuring the eggs, it is possible to distinguish between *Ancylostoma* spp. and *U. stenocephala*, as the latter are slightly larger, but it has been widely accepted that distinguishing between *Ancylostoma* spp. is not possible from light microscopy alone (Traub et al., 2004). A recent study has shown a difference between the size of *A. braziliense* and *A. caninum* eggs, as measured by light microscopy, with *A. braziliense* eggs significantly smaller than *A. caninum* eggs (Lucio-Forster et al., 2012), however the study only evaluated a small number of eggs from very few dogs all from the same area.



Figure 2.5: Hookworm egg (x400)

Cultured larvae of all hookworm species are also morphologically very similar, so historically, accurate speciation has required the examination of adult worms, obtained either from faeces following treatment, or from necropsy (Prociv, 2003).

Recent advances in DNA technology have meant that increasingly PCR can be used to detect hookworm DNA in canine faeces, and PCR-restriction fragment length polymorphism (PCR-RFLP)

techniques have been described to accurately differentiate between *A. caninum*, *A. braziliense* and *A. ceylanicum*. PCR-RFLP has been shown to be highly sensitive, detecting *Ancylostoma* spp. from microscopy-negative samples (Traub et al., 2004).

Cutaneous lesions in dogs are usually diagnosed based on a combination of clinical signs and a history of contact with ova-containing faeces or contaminated soil (Campbell, 2005).

Public health

Hookworm larval infection in humans can lead to HrCLM with *A. braziliense* most commonly implicated as the cause (Bowman et al., 2010; Prociw, 2003). As such, the occurrence of HrCLM seems to mirror the geographical distribution of this species, with infections most frequently occurring in tropical and subtropical countries in Southeast Asia, Africa, South America, the Caribbean and south-eastern areas of the USA (Hochedez and Caumes, 2007). Most reported cases have been tourists who frequented beaches in regions where *A. braziliense* is endemic in cats and dogs (Hochedez and Caumes, 2007). The lesions characteristic of HrCLM are itchy and persistent, long serpiginous tracts in the skin and occur as a result of aimless migration of the larvae in the epidermis after gaining percutaneous access (Feldmeier and Schuster, 2012; Prociw, 2003). Infections occur after contact with moist soil, sand or canine faeces contaminated with L3 stage hookworm larvae, and so lesions usually occur on skin that has been exposed to these, frequently feet and hands (Hochedez and Caumes, 2007). HrCLM is eventually self limiting, however it is often treated due to discomfort and potential secondary bacterial infection, with albendazole and ivermectin being the treatments of choice (Feldmeier and Schuster, 2012; Hochedez and Caumes, 2007).

U. stenocephala is also thought to cause HrCLM as autochthonous cases have been reported in parts of Europe where other hookworm species do not occur (UK and Germany) (Diba et al., 2004; Klose et al., 1996). It is known that *U. stenocephala* can infect humans percutaneously, resulting in serpiginous lesions (Bowman et al., 2010), though in reported cases no larvae were identified (Diba et al., 2004; Klose et al., 1996).

A. caninum can also cause HrCLM lesions in people, however, these are milder and more transient, follicular, papular and pustular lesions (Caumes et al., 2002). Myositis has been associated with *A. caninum* larvae (Little et al., 1983), which have also been suspected as a potential cause of diffuse unilateral subacute neuroretinitis, a form of ocular larva migrans

which can lead to loss of vision and retinal lesions (Bowman et al., 2010). It has also been known for this species to reach adulthood in humans resulting in an eosinophilic enteritis, although this is uncommon (Landmann and Prociw, 2003). However it is well recognised that *A. ceylanicum* is able to develop into adults in humans, and can result in abdominal pain (Bowman et al., 2010; Carroll and Grove, 1986; Prociw, 2003). Eosinophilic pneumonitis associated with *Ancylostoma* spp. larvae has been reported, often following the development of HrCLM (Hochedez and Caumes, 2007).

2.3.2 Other intestinal parasitic helminths

Toxocara canis

Toxocara canis is a helminth parasite of dogs with a worldwide distribution. It has been reported in dogs in Samoa (Martin, 1999). The dog and fox are the definitive hosts of *T. canis*, although it is able to complete its lifecycle in other canids, and every species of mammal and bird has the potential to be a paratenic host (Lloyd, 1998a).

Infection occurs when an adult dog ingests embryonated eggs or an infected paratenic host (Figure 2.6). In the gastrointestinal tract the infective eggs hatch and the larvae penetrate the gut wall. In young dogs, larvae migrate to the lungs, ascend the bronchial tree and trachea and are swallowed to return to the intestine and develop into adult worms. In adult dogs, the majority of larvae migrate to the somatic tissues where the larvae encyst, although some larvae may undergo tracheal migration and proceed to develop in the intestinal tract. Encysted larvae can remain in tissues for several years, and in late pregnancy, these larvae are activated and migrate across the placenta to the foetus. Puppies are also infected by the transmammary route. Larvae passed onto puppies, by either route, develop into adults in the small intestine (Anonymous, 2012f). Faecal egg counts in puppies can reach 100,000 per gram of faeces and so puppies are a major source of environmental egg contamination (Lloyd, 1998a). Humans can become accidental hosts by ingesting eggs in contaminated soil or infected paratenic hosts (Rubinsky-Elefant et al., 2010).

In the environment, eggs develop to the infective embryonated stage at temperatures above 10°C, and can survive in favourable conditions for at least 6-12 months. Only certain disinfectants (Verocai et al., 2010), heat greater than 30-35°C and desiccation kills the eggs in the environment (Lloyd, 1998a).

In adult dogs, clinical signs and pathology are usually absent, but *T. canis* can cause severe illness and even death in young, heavily infected puppies. Damage by adult worms to the intestine can result in villous atrophy, malabsorption and poor growth, and large numbers migrating through the lungs can damage tissue and result in respiratory signs (Lloyd, 1998a). A diagnosis of *T. canis* infection is usually quite easily made by faecal flotation methods, as faecal egg output is usually high in affected animals (Lloyd, 1998a).

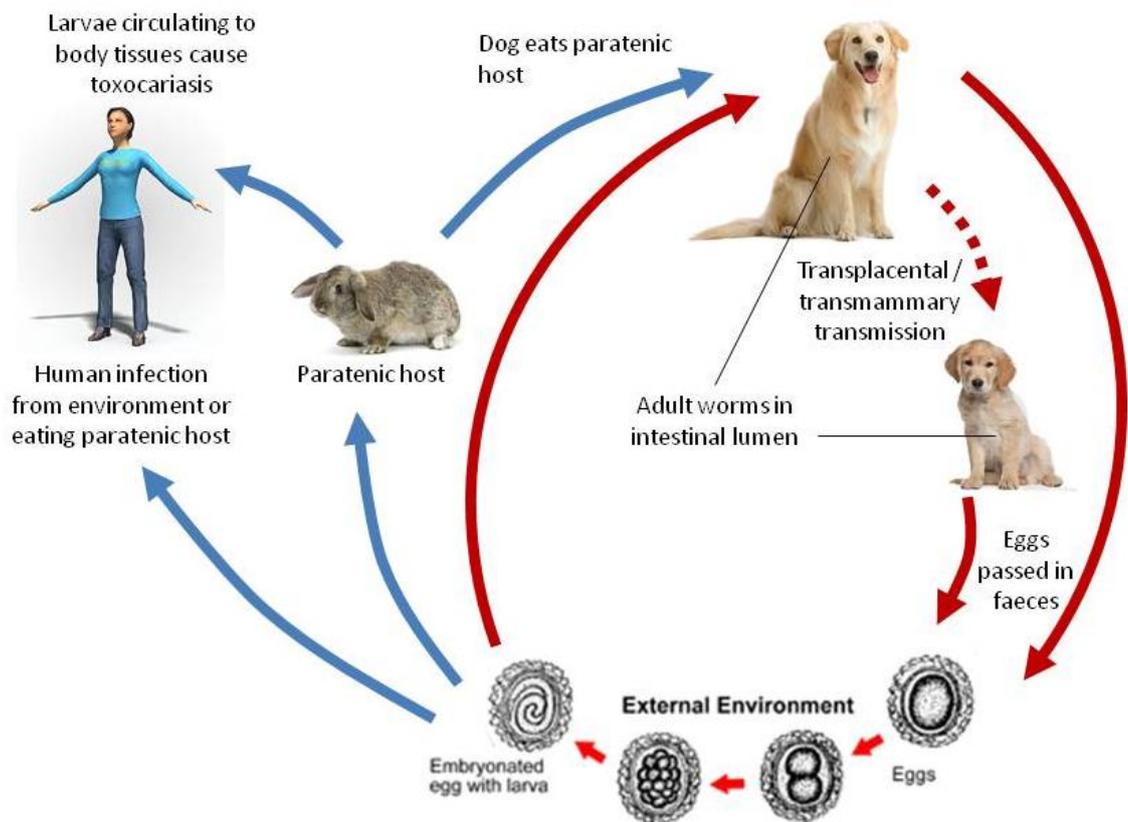


Figure 2.6: Life cycle of *Toxocara canis* (adapted from www.dpd.cdc.gov) (Anonymous, 2012f).

Infection with *T. canis* in humans results in toxocariasis, one of the most common zoonotic infections worldwide (Rubinsky-Elefant et al., 2010). Most infections remain asymptomatic, however when symptoms do occur they can result in permanent ocular or neurological damage. The two main presentations of toxocariasis are visceral larva migrans (VLM) and ocular larva migrans (OLM). After ingestion, the eggs hatch and the larvae penetrate the intestinal wall and are carried by the circulation to a wide variety of tissues (heart, lungs, liver,

brain, muscle, eyes). The larvae do not develop any further in these locations, but it is the severe local immune responses to the larvae that result in toxocariasis (Rubinsky-Elefant et al., 2010). Fever, abdominal pain, respiratory signs and hepatomegaly may occur in cases of VLM. Permanent blindness may be the outcome from OLM. Risk factors for disease include geophagia, rural residence, low level education, overcrowding and poverty. Pet ownership has not been convincingly linked to toxocariasis, but this may just be an indicator of the degree of environmental contamination outside their households (Lee et al., 2010b).

Dipylidium caninum

The cestode *Dipylidium caninum* is the most common tapeworm found in dogs (Conboy, 2009) and has been reported previously in Samoa (Martin, 1999). The life cycle involves the flea, *Ctenocephalides felis* or *Ctenocephalides canis*, and the dog louse, *Trichodectes canis*, as an intermediate host (Conboy, 2009). Proglottids are passed from the anus of an infected dog or cat. These are then eaten by the flea larvae and develop to mature cysticercoids in the flea. Infected adult fleas are then eaten by cat or dog during grooming. *D. caninum* prevalences tend to follow the prevalence of the intermediate host flea i.e. usually higher in tropical developing countries, and in stray cat or dog populations (Lloyd, 1998b). Infection in dogs is well tolerated. Faecal flotation has poor detection sensitivity, but may demonstrate eggs or egg packets (Conboy, 2009). Human *D. caninum* infection can occur after ingestion of an infected flea, mainly in young children, though causes minimal clinical signs of disease (Conboy, 2009).

Trichuris vulpis

The canine whipworm, *Trichuris vulpis*, is a common cause of large bowel diarrhoea in dogs. Transmission is by the faecal-oral route. After ingestion of the infective embryonated egg, it passes into the small intestine where it hatches. Larvae then migrate to the caecum and colon where they attach to the mucosa to feed, develop to adulthood and start producing eggs (Washabau and Holt, 2005). The prepatent period is 8-12 weeks (Traversa, 2011).

Eggs remain viable and infective for years in the environment, with heat, cold, desiccation and sunlight having little effect on viability. Eggs are a constant source of infection and reinfection for dogs living in contaminated environments. This, coupled with the fact that there is no transmammary or transplacental transmission, and a long prepatent period, means the incidence and parasite burden tends to be higher in older dogs (Traversa, 2011).

Affected dogs tend to show mild clinical signs of colitis, although some dogs may have a pseudo-Addisonian presentation, with clinical signs and laboratory findings suggestive of hypoadrenocorticism, but a normal response to an ACTH-stimulation test. Diagnosis can usually be made by finding eggs with routine faecal flotation, however intermittent shedding may result in false-negative results (Washabau and Holt, 2005).

The zoonotic potential of *T. vulpis* is being debated. In the past it has been reported to cause VLM and patent intestinal infections in humans, however few cases have been described and only very rarely a diagnosis of *T. vulpis* definitively confirmed. Further studies are required, but until definitively proven to be so, *T. vulpis* cannot be considered to be a parasite of zoonotic importance (Traversa, 2011).

2.3.3 Enteric protozoa: *Giardia duodenalis*

Aetiology

Giardia duodenalis (syn. *Giardia intestinalis*, *Giardia lamblia* and *Giardia entarica*) is an enteric protozoa that infects a variety of mammals (Payne and Artzer, 2009). The species complex comprises of eight assemblages, from A to H, based on genotypes determined by various molecular factors (Caccio and Ryan, 2008; Lasek-Nesselquist et al., 2010; Xiao and Fayer, 2008). Some assemblages are considered largely species-specific, while others can infect humans and animals. Dogs primarily harbour the species specific C and D, although assemblage D has also rarely been isolated from cat faeces (Palmer et al., 2008b). Assemblages A and B, mainly human isolates, have also been amplified from dog faeces (Table 2.2) (Lebbad et al., 2010; Li et al., 2012; Upjohn et al., 2010; Xiao and Fayer, 2008). Whereas assemblage A has been shown to have a modest to high prevalence in some studies, assemblage B is rarely found in dogs (Xiao and Fayer, 2008).

Epidemiology

Giardia has a worldwide distribution with many prevalence studies and case reports published in dogs (Becker et al., 2012; Itoh et al., 2005; Joffe et al., 2011; Little et al., 2009; Mircean et al., 2012; Overgaauw et al., 2009; Papini et al., 2005; Silva et al., 2012; Traub et al., 2009; Upjohn et al., 2010). Reported prevalence ranges from very low to as high as 55.2%, with the higher prevalences tending to occur in studies examining shelter and kennelled dogs (Itoh et al., 2005; Papini et al., 2005; Upjohn et al., 2010). Infection is also more common in younger animals

(Fontanarrosa et al., 2006; Gates and Nolan, 2009b; Little et al., 2009), and there is a trend for higher prevalence rates in winter (Fontanarrosa et al., 2006). A variety of techniques were employed to detect *Giardia* cysts or antigen in these studies, and this coupled with different inclusion criteria make the prevalences hard to compare. The prevalence of each of the eight assemblages, in all affected species, varies considerably from country to country (Caccio and Ryan, 2008).

In a study of intestinal parasites of cats and dogs in Australia, *Giardia* was found to be the most prevalent parasite in dogs (Palmer et al., 2008a). *Giardia* is also present in cats and dogs in New Zealand (Tonks et al., 1991). Elsewhere in the Pacific region data on *Giardia* presence or prevalence are scarcer. In Papua New Guinea, although there are no records of *Giardia* infection in dogs, cats or other species, human infections have been recorded (Owen, 2005). This also the case for New Caledonia (Germani et al., 1994). There are no case reports or prevalence studies of giardiasis from Samoa in the literature in any species, however *Giardia* infection is known to occur in dogs in Samoa (Martin, 1999). The paucity of data in this region is probably more due to lack of surveillance studies than an indicator of the absence of this pathogen.

Table 2.2: Assemblages and host range of isolates of *Giardia duodenalis* (Lasek-Nesselquist et al., 2010; Palmer et al., 2008b; Xiao and Fayer, 2008)

Assemblage	Primary hosts species
A	Humans, primates, cattle, cats, dogs, rodents, wild mammals
B	Humans, primates, dogs, cattle, horses
C	Dog
D	Dog (cats)
E	Artiodactyls
F	Cats
G	Rodents
H	Grey seals, gulls

Pathogenesis and lifecycle

Giardia spp. are transmitted by the faecal-oral route either by direct transmission or indirect ingestion of contaminated food, water or fomites (Scorza and Lappin, 2012b). After the cysts are ingested by a susceptible host, excystation occurs in the duodenum, after exposure to gastric acid and pancreatic enzymes. The two trophozoites contained in the cyst mature and attach to the brush border of the villous epithelium, start feeding and establish an infection, mainly in the duodenum and ileum in dogs (Gallego et al., 2007). In the intestinal tract, trophozoites multiply by binary fission and subsequently encyst on passage through the intestinal tract. Encystment is an adaptation for survival outside the host (Midlej and Benchimol, 2009). These cysts are then passed in the faeces, with a prepatent period of 1-2 weeks in dogs. Infection can last from 24 hours to months (Thompson et al., 2008). Young dogs shed an average of 2000 cysts per gram of faeces, but the degree of shedding can vary widely and tends to be intermittent (Tangtrongsup and Scorza, 2010). Cysts can survive for several months outside the host in wet, cold conditions, but are susceptible to desiccation in hot, dry conditions (Scorza and Lappin, 2012b).

A combination of hypersecretion and malabsorption are the primary mechanisms causing *Giardia* diarrhoea. The exact pathogenesis of *Giardia* is not fully understood; however there appears to be a T-lymphocyte mediated pathogenesis leading to a diffuse loss of the microvillus brush border with or without villous atrophy. This results in reduced disaccharidase activity and malabsorption of electrolytes, nutrients and water, ultimately resulting in diarrhoea (Buret, 2005). Induction of intestinal epithelial cell apoptosis and disruption of the tight junctions results in increased permeability and this with hypersecretion of electrolytes adds to the diarrhoea (Troeger et al., 2007).

Clinical presentation

The main clinical signs of giardiasis are diarrhoea and weight loss. The diarrhoea is usually mucoid, pale and soft with a strong odour, and blood is uncommon. Most infected dogs are afebrile, do not vomit and serum biochemistry and complete blood count results are within normal ranges (Scorza and Lappin, 2012b). However, most dogs shedding *Giardia* cysts do not show any clinical signs. Younger, immunosuppressed animals and animals living in a crowded environment are most at risk of showing clinical signs (Thompson et al., 2008).

Diagnosis

Giardia is one of the most commonly over- and under-identified parasites (Dryden et al., 2006). Cyclical shedding of cysts mean that repeated faecal analysis may be necessary for detection, and cysts can deteriorate in faecal floatation solutions, leading to false negative results. False positives can result from misidentification of pseudoparasites or yeasts (Dryden et al., 2006). There are a number of detection methods available in dogs which can be used alone or in combination, and although specificity can be excellent in some tests, there is no one test with 100% sensitivity on a single faecal sample (Scorza and Lappin, 2012b).

Giardia trophozoites can be observed in direct smears of unstained fresh faecal specimens using light microscopy. This method works best with diarrhoeic faeces or faecal mucus (Scorza and Lappin, 2012b). Although a quick, cheap and easy test, the sensitivity of this method is low, 30% in one study, and therefore should not be relied upon to rule out *Giardia* infection (Scorza and Lappin, 2012b).

Faecal concentration techniques such as Sheather's sugar centrifugation and zinc-sulphate centrifugation technique (ZSCT) can be employed to detect *Giardia* cysts (Tangtrongsup and Scorza, 2010). Passive flotation methods without centrifugation detected only 14.7% of *Giardia* cysts compared to the ZSCT (Gates and Nolan, 2009a). Because of intermittent shedding of cysts, it is recommended to evaluate three samples over a period of one week before ruling out the presence of *Giardia*. The ZSCT performed on three faecal samples is considered by some as the gold standard technique for diagnosis of *Giardia* (Payne and Artzer, 2009).

Immunochemical detection using a monoclonal antibody-based direct immunofluorescent antibody (IFA) assay can be used to detect *Giardia* spp. cysts in faeces. Because this assay gives immunologic confirmation by fluorescence and morphologic evaluation (size and shape), and therefore false positives are less likely, the results from this assay are sometimes considered the gold standard in test comparison studies (Rishniw et al., 2010). This test can only be performed in laboratories equipped with a fluorescent microscope.

There are multiple commercially available *Giardia* antigen enzyme-linked immunosorbent assay (ELISA) tests for use on dog and cat faeces. In a study that used the direct IFA assay as the gold standard, the *Giardia* antigen ELISA was estimated to have a moderate sensitivity of 77% and a specificity of 92%, although at a lower "real world" prevalence level the positive predictive value dropped making it a less reliable test when used alone, but similar to a single

ZSCT (Rishniw et al., 2010). The Companion Animal Parasite Council recommends that faecal antigen tests are used as an addition to faecal floatation and only in the evaluation of dogs and cats with diarrhoea, as it is unknown what the zoonotic impact of an antigen-positive but cyst-negative healthy pet might be, and it is also unknown how long the *Giardia* antigen assay remains positive after the resolution of diarrhoea (Anonymous, 2011).

PCR can be used to amplify *Giardia* DNA from faeces and is a useful tool for determining the assemblage, however results can be inconsistent when different single genes are used for amplification; i.e. an isolate can be genotyped as a host specific assemblage by one gene but as a potentially zoonotic assemblage by another (Caccio et al., 2008). For this reason when genotyping is to be done, multilocus genotyping is recommended (Scorza and Lappin, 2012b).

Public health

Giardia is the most common parasitic infection affecting humans worldwide, with the majority of infections acquired by drinking contaminated water sources. Children are most at risk of contracting the infection. As with dogs, many human infections are asymptomatic. When symptoms do occur they can range from an acute to intermittent to chronic non-bloody diarrhoea. Abdominal cramping, nausea, vomiting, steatorrhoea, anorexia and weight loss may also occur (Thompson, 1998b). However, humans are only infected with assemblages A and B, whereas as stated above the majority of canine infections are assemblages C and D. Some studies have shown dogs to be infected with assemblage A and, less frequently, assemblage B, but there are very few studies that actually assess the zoonotic potential of *Giardia* from cats and dogs (Ballweber et al., 2010; Xiao and Fayer, 2008). In a limited number of studies where there has been simultaneous sample collection from companion animals and humans, there is some evidence that dogs, cats and humans living in close proximity may have the same *Giardia* genotypes or subtypes (Ballweber et al., 2010). However, small sample sizes and a scarcity of cases with cats, dogs and humans having the same genotypes preclude detailed categorical conclusions. Further research into this field is required to resolve the role of cats and dogs as a source of human giardiasis.

2.3.4 Enteric protozoa: *Cryptosporidium* spp.

Aetiology

Cryptosporidium is a ubiquitous protozoan pathogen that inhabits the gastrointestinal and respiratory epithelium in mammals, birds and reptiles (Scorza and Lappin, 2012a). Most *Cryptosporidium* species are fairly host specific and the host-specific *Cryptosporidium canis* is the most commonly reported species in dogs. *Cryptosporidium parvum*, which infects mainly cattle, sheep, goats and humans, has also been isolated from the faeces of naturally infected dogs (Scorza and Lappin, 2012a). Many similarities in epidemiology, pathogenesis, disease and public health concerns exist between *Giardia duodenalis* and *Cryptosporidium* spp. infections (Xiao and Fayer, 2008).

Epidemiology

Studies investigating the prevalence of *Cryptosporidium* spp. infections in dogs have demonstrated a worldwide distribution, with prevalences ranging up to 45% in countries in North America, South America, Europe, Asia, Australia and Africa (Batchelor et al., 2008; Bugg et al., 1999; Hackett and Lappin, 2003; Lallo and Bondan, 2006; Scorza and Lappin, 2012a; Shukla et al., 2006). Prevalence rates vary greatly depending on the study population and diagnostic techniques used. Few studies have genetically characterised the *Cryptosporidium* isolates from dogs and cats (Xiao and Fayer, 2008).

Cryptosporidium spp. infections are transmitted by the faecal-oral route, and in the environment *Cryptosporidium* oocysts are highly resistant, persisting in wet cool conditions for six months or longer (Xiao and Fayer, 2008). They can also resist many common disinfectants (Gookin et al., 2002) although are susceptible to ultraviolet light.

Pathogenesis and lifecycle

Infection is acquired by ingesting sporulated oocysts from contaminated water or food sources, or by grooming oocysts from contaminated fur. Infection may also occur when dogs or cats ingest infected prey species (Scorza and Lappin, 2012a). The oocysts excyst in the gastrointestinal tract, releasing infective sporozoites that invade epithelial cells and occupy the area between the cell membrane and the cytoplasm. In this position the parasites undergo asexual and then sexual multiplication resulting in the development of male and female sexual stages. Upon fertilisation of the female by the male stage, an oocyst is formed. Some oocysts

auto-infect, but the majority are excreted as infective sporulated oocysts in faeces (Anonymous, 2010a; Xiao and Fayer, 2008). The prepatent period in dogs is between 2 to 14 days, and patent periods in puppies last up to a month (Scorza and Lappin, 2012a).

Cryptosporidium and *Giardia* spp. have very similar pathogenic mechanisms in humans, although the pathogenesis in dogs and cats has not been thoroughly investigated. In humans *Cryptosporidium* is highly infective with as few as 100 oocysts able to precipitate disease (Martins and Guerrant, 1995). Diarrhoea is thought to be caused by a combination of intestinal malabsorption of electrolytes and nutrients with hypersecretion of chloride ions and water. Malabsorption and maldigestion are caused by the loss of the epithelial brush border and microvillus shortening that is mediated by the host activated T lymphocytes. Tight junctions are also disrupted, increasing intestinal permeability (Gookin et al., 2002).

Clinical presentation

Infected dogs often do not show any clinical signs and host immunity is likely important in the development of infection and clinical illness. Younger and immunocompromised animals are thought to be at increased risk of developing clinical signs and co-infections with *Giardia* and other intestinal diseases (parvovirus, distemper, parasitism, lymphoma) may occur in clinically affected dogs (Scorza and Lappin, 2012a). When signs do occur, there is primarily small bowel diarrhoea accompanied by weight loss. There may be fresh blood in the diarrhoea, tenesmus and discomfort in chronic cases (Scorza and Lappin, 2012a).

Diagnosis

As there is a relatively high prevalence rate of infection in healthy dogs, the positive predictive value of all assays testing for *Cryptosporidium* spp. is fairly low. Shedding of the parasite can be intermittent and dogs generally only shed 1000 oocysts per gram faeces (Scorza and Lappin, 2012a), therefore false negatives are possible when looking for the oocysts in faeces.

Unconcentrated direct faecal smears can only detect *Cryptosporidium* spp. oocysts at a threshold of 10^6 oocysts per gram faeces, and so concentration techniques such as Sheather's sugar floatation and ZSCT are required to increase sensitivity (Webster et al., 1996). However, because of the low levels shed by dogs, even concentration techniques are considered insensitive for detecting infection (Scorza and Lappin, 2012a).

Direct IFA detection methods can be more sensitive and specific than cytologic staining techniques (e.g. with modified Ziehl-Neelsen acid-fast staining) and have the added benefit of

being easier for inexperienced laboratory personnel to interpret (Weber et al., 1991). However costs are higher than other staining techniques and a fluorescent microscope is required. In one human study the average detection limit using direct IFA was 5×10^4 oocysts per gram faeces, and the sensitivity of the IFA was 100% when compared to the ELISA (Weber et al., 1991). However this test has not been completely validated in cats and dogs, and as dogs shed lower numbers of oocysts than humans, false negative results may be expected (Scorza and Lappin, 2012a).

A faecal ELISA for the detection of *Cryptosporidium* spp. antigens has been developed for use in humans. But the sensitivity and specificity for use in dog faeces has not been determined. In general, human trials have shown the ELISA to have a lower specificity than direct IFA testing (Scorza and Lappin, 2012a).

PCR testing has been widely described to detect *Cryptosporidium* spp. DNA from faeces and water supplies. Some studies suggest greater sensitivity of PCR compared with other techniques in both humans and animals, with oocysts detected in samples with very low oocyst numbers (Webster et al., 1996). In addition, PCR allows identification of the particular *Cryptosporidium* species present. However there are minimal standards between laboratories and levels of quality control can vary widely, and as with any highly sensitive test, a positive result does not necessarily mean that cryptosporidiosis is the cause of any diarrhoea. For this reason, routine screening by this method is not recommended for dogs with diarrhoea (Scorza and Lappin, 2012a).

Histological evidence of infection from intestinal biopsies has been used in clinical cases to reach a diagnosis (Scorza and Lappin, 2012a). However, the costs and invasiveness of such a procedure preclude its use as a routine diagnostic or screening tool.

Public health

Cryptosporidiosis in humans was not recognised until 1976 (Martins and Guerrant, 1995). Initially it was well documented among veterinarians and animal workers, because many case reports described the transmission of *Cryptosporidium* spp. to farm workers and veterinary students. Following the acquired immune deficiency syndrome (AIDS) epidemic in the early 1980s there was a dramatic increase in the number of reported human cryptosporidiosis cases (Scorza and Lappin, 2012a). Immunodeficient individuals and young children are most at risk of clinical disease; however immunocompetent adults can also have clinical disease (Martins and Guerrant, 1995).

In immunocompetent adults, signs can last from 2-26 days and may include a profuse watery diarrhoea, low grade fever, vomiting and abdominal discomfort. The disease tends to be self-limiting. Infants and older adults may have a more chronic persistent diarrhoea and dehydration. In immunodeficient individuals, especially those with AIDS, the severity of cryptosporidiosis increases and diarrhoea becomes chronic, debilitating and potentially fatal (Martins and Guerrant, 1995).

Transmission is by direct contact with infected humans or animals or by drinking contaminated water or food (Martins and Guerrant, 1995). *C. parvum* and *Cryptosporidium hominis* are the predominant species detected in human outbreaks (Xiao and Fayer, 2008), however *C. canis* has been detected in humans, albeit infrequently (Xiao et al., 2007). Several studies report the role of dogs and cats in the transmission of human cryptosporidiosis to be minimal, and contact with dogs and cats has not been shown to be a risk factor for the disease (Goh et al., 2004; Scorza and Lappin, 2012a; Xiao and Fayer, 2008). However, immunocompromised individuals may be more at risk of contracting the disease from these animals and because there is no effective treatment for cryptosporidiosis, prevention and risk reduction is important, especially in this group.

2.4 Ectoparasites: Ticks, fleas and lice

Parasitic arthropods are efficient vectors of a large number of bacterial, rickettsial, viral and parasitic diseases affecting humans, dogs and other animals worldwide. Worldwide, ticks have been shown to be competent vectors of many important diseases, such as ehrlichiosis, anaplasmosis, Lyme borreliosis, babesiosis, Rocky Mountain spotted fever, hepatozoonosis and cytauxzoonosis (Blagburn and Dryden, 2009; Dumler et al., 2001; Nadelman and Wormser, 1998). Most of these diseases are only transmissible by certain tick species, and the range of the disease often mirrors that of the distribution of its tick vector.

In recent years it has become apparent that the frequency of some vector-borne diseases is increasing and the range of these diseases is spreading. This is evident both in Europe and the USA (Beugnet and Marie, 2009; Otranto et al., 2009b). Various factors have been attributed to the changing epidemiology: climate change can affect arthropod vector density, geographical distribution and vectoral capacity; increased transport by road, sea and plane of production animals provides ideal conditions for the circulation of pathogens and spread of vectors;

holiday travel to distant locations, and an increased propensity for owners to travel with their pets can result in diseases being spread to non-endemic regions (Blagburn and Dryden, 2009).

Rhipicephalus sanguineus, is the only tick that has been reported as infesting dogs in Samoa in the literature (Martin, 1999), although the presence of other tick species cannot be ruled out. *R. sanguineus* is the vector of numerous important pathogens, including *Ehrlichia canis*, *Babesia Canis*, *Anaplasma platys*, *Rickettsia coronii* and *Rickettsia rickettsii* (Dantas-Torres, 2008). It has also recently been investigated as a vector for *Leishmania infantum*, and although the tick can become infected with the organism by feeding off infected dogs, it has not yet been proven to transmit disease to naive dogs (Quinnell and Courtenay, 2009). Dogs are the primary host for *R. sanguineus*, and as a consequence, the tick is widely distributed in tropical and temperate regions, wherever dogs are found. *R. sanguineus* preferentially feeds on dogs in all three life stages (larvae, nymphs and adult), although immature life stages can be detected on rodents and other small mammals. Rarely adults can be found on other mammals such as humans and cats (Dantas-Torres, 2008). The adult tick feeds for 5-21 days after which mating occurs, then the female drops off and deposits up to 4000 eggs. The eggs hatch within 20-30 days and the life cycle can be completed in as little as 63-91 days. As with many hard ticks, unfed stages can persist in the environment unfed for prolonged periods; unfed larvae, nymphs and adults can survive for up to 8, 6 and 19 months respectively (Blagburn and Dryden, 2009).

Other ectoparasites reported on dogs in Samoa are *Ctenocephalides canis*, *Ctenocephalides felis* and *Trichodectes canis* (Martin, 1999). The cat flea, *C. felis*, in particular, has been shown to be implicated in the transmission of canine pathogens *Bartonella henselae* and other *Bartonella* spp. as well as carrying certain species of cestode including *Dipylidium caninum* (Conboy, 2009). Flea infestations are common in dogs worldwide, in both tropical and temperate regions, and fleas regularly appear in large numbers causing a nuisance to the animal. As well as potentially transmitting disease, fleas themselves commonly cause flea allergic dermatitis and in heavy infestations, especially in young puppies, can result in iron deficiency anaemia or even death in some cases (Blagburn and Dryden, 2009).

2.5 Vector-borne diseases

2.5.1 *Dirofilaria immitis*: Canine heartworm

Aetiology

Dirofilaria immitis, the causative agent of heartworm infection and disease in dogs, is a nematode of the Onchocercidae family and is transmitted by mosquitoes. More than 60 species of mosquito worldwide are known to become infected with the larvae and transmit infection (Bowman and Atkins, 2009). The definitive hosts are dogs and wild canids, although a wide variety of other animals can become infected, including cats, horses and humans (Bowman and Atkins, 2009; Nelson, 2012).

Epidemiology

D. immitis is widely distributed throughout the world and is well recognised in temperate and tropical areas. The first report of canine heartworm dates back to 1626, in hunting dogs from the Po river valley in northern Italy, as observed by Birago (Nelson, 2012).

Transmission of the disease requires a reservoir of microfilaraemic dogs or wild canine hosts, suitable mosquitoes and a climate that allows incubation and development within the mosquito (Genchi et al., 2009). Prediction models can be applied using the 'heartworm development unit' (HDU), of which one unit is 24 hours at 1°C over the threshold temperature of 14°C at which development stops. Development of *D. immitis* from L1 to L3 in the mosquito require 130 HDUs (Genchi et al., 2009) i.e. development at a constant 24°C (10 HDUs) would take 13 days. This is well within the mosquito's life expectancy of 30 days.

Canine heartworm is considered endemic in North America, much of South and Central America, Southern Europe, and increasingly parts of Northern Europe, many parts of Asia, Africa and Australia (Carlisle and Atwell, 1984; Genchi et al., 2011b; Labarthe and Guerrero, 2005; Nelson, 2012; Tzipory et al., 2010). The majority of published data are from Western Europe and North America where awareness of the disease is high, and as a result, prophylaxis is common. In poorer countries awareness of the disease in dogs may be increasing, but monthly prophylaxis is often financially prohibitive for pet owners (Labarthe and Guerrero, 2005).

Heartworm is considered endemic in all 50 states of the United States, with an overall 1.4% of dogs testing positive for heartworm antigen. The prevalence is highest in south-eastern states (3.9%) where the climate is optimal for parasite development (Bowman et al., 2009). Awareness is generally high, especially in the Southeast, and prophylaxis widespread. However, the geographical range of canine heartworm in North America has expanded since the 1950's, when there were considered to be only a few hyperendemic hotspots such as Mississippi river area. This is thought to be due to an increased movement of dogs across the country and an increased awareness of the disease (Bowman and Atkins, 2009). In Europe, the geographical range of *D. immitis* is also increasing. As well as increased dog movement and increased veterinary awareness, climate change is thought to have played a role (Genchi et al., 2011a; Genchi et al., 2011b). Traditionally heartworm endemic areas were in southern Europe, especially the Mediterranean regions. However increasingly autochthonous infections are being seen in northern and eastern Europe, in countries and areas where the disease has not been considered endemic in the past such as Germany (Pantchev et al., 2009) and parts of Italy (Otranto et al., 2009a). In addition, human dirofilariasis is starting to become known as an emerging zoonosis in these regions where it has not previously been recognised (Genchi et al., 2009; Kartashev et al., 2011).

D. immitis is also known to be present in much of coastal Africa (Nelson, 2012), although published data to confirm this are scarce. Excepting a recent study showing an overall prevalence of 24% in Algeria, epidemiological status remains largely unknown in Africa (Meriem-Hind and Mohamed, 2009). *D. immitis* is considered exotic in South Africa (Verster et al., 1991). In South America canine heartworm disease is well documented in Brazil, Argentina, Colombia and Mexico (Labarthe and Guerrero, 2005). The disease is also recognised through many parts of Asia, and very well studied and documented in Japan (Genchi et al., 2001).

In the Pacific area, *D. immitis* is considered exotic in New Zealand (McKenna, 2009) although there are known to be mosquito species abundant in New Zealand, especially the North Island, that are capable vectors of *D. immitis* (Derraik and Slaney, 2005). With a climate suitable for development of the parasite in mosquitoes, only strict importation requirements for foreign dogs prevent endemic infestation (McSporran, 1994) and the disease has been diagnosed in imported dogs in the past even with strict regulations in place (Thompson, 1998a). In Australia, canine heartworm has long been recognised and cases are seen throughout most of the country (Carlisle and Atwell, 1984). A study conducted over 1982 to 1984 in Papua New Guinea detected that 86% of dogs were infected with heartworm on post mortem examination (Hamir

and Onaga, 1986). In New Caledonia, a South Pacific island with a slightly cooler climate than Samoa, 22.4% of 49 stray dogs tested positive for *D. immitis* antigen (Watier-Grillot et al., 2011) and in Hawaii in 1966 prevalence of canine heartworm was estimated at 32% in a survey of 666 dogs (Gubler, 1966). Surveys to evaluate the prevalence of *D. immitis* elsewhere in the Pacific Islands are limited, but where it has been looked for the parasite has been found, and prediction models based on the climate would support the presence of the disease.

There are no published data on canine heartworm in Samoa, but the disease is known to be endemic. *D. immitis* infections of two *Aedes* species of mosquito in Samoa were observed from 1978 to 1980 as part of a study into lymphatic filariasis in people caused by the parasitic nematode *Wuchereria bancrofti* (Samarawickrema et al., 1992). More recently, the prevalence of *D. immitis* infection in *Aedes* spp. mosquito populations in neighbouring American Samoa were 1.06% and 1.77% by dissection and PCR respectively (Chambers et al., 2009). Anecdotally, clinical cases of canine heartworm have been seen by veterinarians at the APS but the prevalence in the canine population is unknown.

Life cycle and Pathogenesis

Microfilariae (or stage L1 larvae) circulate in the peripheral blood of the definitive host and are taken up by the female mosquito during a blood meal (Figure 2.7). The larvae subsequently develop to the infective L3 stage in the mosquito, usually within 8 -17days. The moults from L1 to L3 are temperature dependant and will take longer in cooler temperatures (Bowman and Atkins, 2009). In cooler climates, the lifespan of the mosquito (estimated at 30 days) may not be long enough to complete this stage (Genchi et al., 2011b). The infective L3 stage is introduced to the host by the mosquito during another blood meal. The larvae undergo a further two moults to L4 and adult in the definitive canine host before the adult worms migrate to the heart and lungs to mature and mate, preferably in the pulmonary arteries. Adult female worms can reach up to 30cm in length, can live up to 5 -7 years and are capable of producing microfilariae for their entire life. The pre-patent period is a minimum of about 180 days, typically taking 6-7 months after infection for the host to become microfilaraemic (Atkins, 2005; Bowman and Atkins, 2009).

Heartworm is a misnomer, as the adult worms preferably reside in the pulmonary arteries, not the heart, and the clinical signs of heartworm disease are primarily due to damage to these vessels and the lungs. Physical obstruction of the arteries by the worms does not tend to be the problem so much as the worm induced inflammation and fibrosis of pulmonary arteries

and resulting pulmonary hypertension. Right sided heart failure may be the outcome of prolonged pulmonary hypertension and increased afterload. Dead worms may also cause thromboemboli resulting in obstruction of pulmonary vessels and an even more severe inflammatory reaction causing vasoconstriction, thrombosis and granulomatous reactions (Bowman and Atkins, 2009).

Glomerulonephritis resulting in proteinuria is a common complication of heartworm disease due to the antigen antibody complexes formed in response to infection (Bowman and Atkins, 2009) and aberrant migration to the muscle, brain, spinal cord and eye have also been recorded (Bowman and Atkins, 2009; Carastro et al., 1992; Cooley et al., 1987; Shires et al., 1982).

Severe burdens may result in retrograde migration of the worms from pulmonary arteries to the heart, with worms residing in the right ventricle, right atrium and even the vena cava. In these cases, heart valve function may be seriously compromised by the presence of worms and heartworm caval syndrome may result. This is a severe complication with a poor prognosis (Bove et al., 2010).

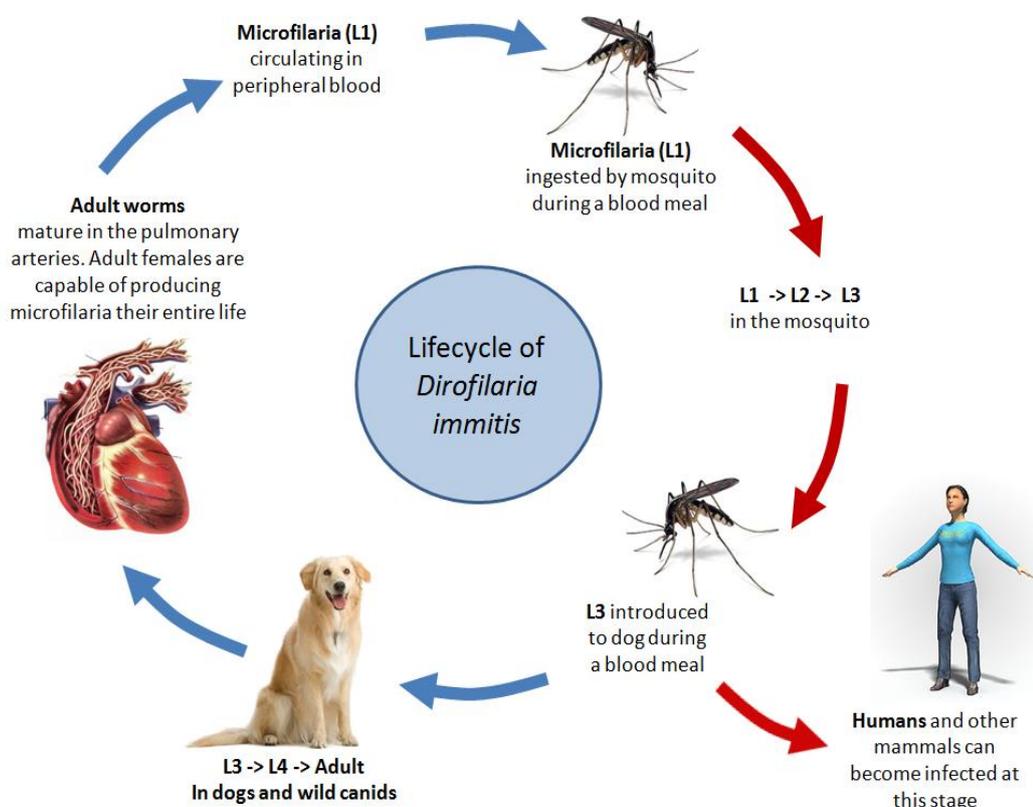


Figure 2.7: Life cycle of *Dirofilaria immitis* (adapted from www.cdc.gov)

Clinical Presentation

Clinical signs of heartworm disease depend on the duration and severity of infection and the majority of cases in dogs are subclinical, especially if the dog is sedentary. Clinical signs develop gradually, with a cough often being the only sign of mild disease. Moderate to severely affected dogs may also show signs of weight loss, exercise intolerance, lethargy and poor condition. In severe cases, as the disease progresses and pulmonary pathology worsens, signs of right sided heart failure secondary to pulmonary hypertension may develop. At any stage of the disease, exercise results in an exacerbation of clinical signs (Atkins, 2005; Nelson, 2012). Caval syndrome, a potentially lethal manifestation of heartworm disease, results in sudden onset of lethargy and weakness as the heart valves become severely compromised (Nelson, 2012). Table 2.3 describes the four classes of heartworm disease and their associated clinical signs.

Table 2.3. Summary of clinical signs of canine heartworm disease, by Nelson, 2012

Early infection	Class 1	No signs
Mild disease	Class 1	Cough
Moderate disease	Class 2	Cough, exercise intolerance, abnormal lung sounds
Severe disease	Class 3	Cough, exercise intolerance, dyspnoea, abnormal heart and lung sounds, hepatomegaly, syncope, ascites and death
Caval syndrome	Class 4	Sudden onset severe lethargy and weakness accompanied by haemoglobinaemia and haemoglobinurina

Diagnosis:

Traditionally, the diagnosis and surveillance of canine heartworm disease has relied upon microfilarial testing. Three main techniques have been described: 1. examination by light microscopy of a direct blood smear for microfilariae, 2. modified Knott's test, which involves looking for microfilariae in the layer above the buffy coat of a microhaematocrit tube, and 3. examination of microfilariae after millipore filtration. The latter two techniques are most

useful as they have the added benefit of concentrating the microfilariae and therefore increasing the chances of detection. However none of these techniques can rule out heartworm as amicrofilaraemic infections occur in as many as 5-67% of dogs (and typically 10-20%) (Atkins, 2005).

In recent years microfilarial testing has been largely supplanted by the use of heartworm antigen testing e.g. by ELISA. There are many commercial test kits on the market which are popular due to high sensitivity and specificity and their relative ease and speed of use. In a comparison of three heartworm antigen test kits, in dogs with low adult worm burden (≤ 4 worms present), specificity of all three tests was 97%, and sensitivity ranged from 78-84%. However with a burden of only one female adult worm, sensitivity was lower; as low as 58% in one test. Sensitivity in all three tests increased with worm burden, and the IDEXX Snap Heartworm RT test (IDEXX Laboratories, Westbrook, ME) performed best overall (Atkins, 2003). Canine heartworm disease has a long prepatent period, and the earliest antigen can be detected is 5 months post infection, and in some dogs it may be as long as 7 months. For this reason dogs under the age of 7 months old should not be tested with either microfilariae or antigen tests (Nelson, 2012).

Recently, PCR assays have been developed for molecular identification of *Dirofilaria* species. As with other PCR assays, these are highly sensitive and specific, even at very low microfilaraemic levels. Currently the main application of PCR is to differentiate *D. immitis* from *Dirofilaria repens*, a subcutaneous parasite, in microfilaraemic dogs in co-endemic regions. It can also be used to confirm *D. repens* infection, as currently there is no commercially available serological antigen test by which this can be done, and morphological identification is technically difficult (Gioia et al., 2010).

Human dirofilariasis and public health

The first case of human dirofilariasis was reported as early as 1887, when adult worms were found in the left ventricle of a boy in Brazil (Miyoshi et al., 2006). Pulmonary dirofilariasis was first reported in the USA in 1941 (Lee et al., 2010a) and in Japan in 1970 (Miyoshi et al., 2006).

D. immitis, along with *D. repens*, is one of the two main causative agents of zoonotic dirofilariasis. There are two main forms of human dirofilariasis; a pulmonary form, usually caused by *D. immitis*, and a subcutaneous and ocular form, usually caused by *D. repens*. The mosquito vectors for *D. immitis* feed indiscriminately on dogs and other animal hosts, as well as humans. Therefore the infective L3 can be spread to humans by a mosquito bite in the same

way the definitive hosts are infected. In humans, larvae are usually removed by the immune system, but where this fails the larva follows a similar path to that in the dog, and comes to rest in the pulmonary arteries. The nematode is almost always unable to develop to maturity and it is therefore usually a pre-adult worm that is responsible for lesions. In the pulmonary form, the parasite forms an embolus where it lodges and, subsequently, forms a pulmonary granuloma. These are usually solitary nodular pulmonary lesions in humans (radiographically known also as coin lesions), though multiple nodular lesions have been reported (Simon et al., 2005). As the adults cannot reach sexual maturity, humans do not become microfilaraemic. Affected humans are usually free of any symptoms and pulmonary dirofilariasis is most commonly detected accidentally by thoracic radiography carried out for another reason. When symptoms do occur they include cough, chest pain, fever, haemoptysis and pleural effusion (Miyoshi et al., 2006).

Nodular pulmonary lesions detected by radiology, both single and multiple, raise the suspicion of malignant tumours, tuberculosis, fungal infections and other serious diseases and may initiate an invasive and expensive diagnostic work up to reach a definitive diagnosis, as traditionally diagnosis could only be made by biopsy and histopathology, often requiring a thoracotomy (Theis, 2005). There is currently no non-invasive diagnostic test for *D. immitis* infection in humans; however, recently, serological testing for human heartworm has been used as an aide to diagnosis. Infected humans tend to mount a huge antibody response to infections involving very few worms, and this response can be easily detected serologically. However this test has low specificity resulting in a high proportion of false positives and no serologic test is currently available commercially. Serology results must be interpreted with historical information such as living in or travel to an area endemic for heartworm and radiological features indicative of the disease. In this way it is possible for a diagnosis to be made without biopsy. When pulmonary dirofilariasis is diagnosed the recommended course of action is to “wait and see” as in humans the disease is fairly benign and lesions do not progress (Simon et al., 2005).

New cases of human dirofilariasis are being reported in the literature with increasing frequency. Retrospective reviews of cases indicate that around 300 cases of pulmonary and 800 cases of subcutaneous and ocular dirofilariasis have been reported to date (Simon et al., 2009). This may be in part due to increasing interest in and awareness of the disease and the higher rates of subcutaneous and ocular disease reported may simply be because this is a more visible form and is noticed by the patient. However, an increase may in part be due to an

increase in travel to endemic areas, or due to the spread of animal dirofilariasis into areas previously considered non endemic (Lee et al., 2010a; Otranto et al., 2009a). The majority of reported cases of human pulmonary dirofilariasis from *D. immitis* are from the United States, Japan and Australia, where interest and awareness of the disease has been highest, however a total of 15 countries have reported cases (Simon et al., 2005). Human *Dirofilaria* infections in the Old World are much more commonly due to *D. repens* (Pampiglione et al., 2009).

Studies suggest that the actual prevalence of human pulmonary dirofilariasis may in fact be much higher than previously thought, given the difficulty with diagnosis. In an endemic area of western Spain, *D. immitis* infection in dogs is estimated at 33%, and in humans in the same area seroprevalence was 21%. A follow up study examining chest radiographs of 50,000 people from the same population found 8 cases of human dirofilariasis, suggesting the disease is under diagnosed (Muro et al., 1999; Simon et al., 2005). In Grand Canaria, a recent study showed that the seroprevalence of *D. immitis* in humans is directly related to infection rates dogs in regions with differing prevalence i.e. the higher the infection rate in dogs, the higher the seroprevalence in humans (Montoya-Alonso et al., 2011). In the same study, seroprevalence was significantly highest in the oldest age group (>60 years) and there were no antibodies against *D. immitis* detected in people under 20 years old. This suggests that in areas where *D. immitis* is considered endemic, especially where there is a lack of preventative measures in the dog population and prevalence is high, local inhabitants and visitors are at an increased risk of this disease, especially the middle aged to older section of the population.

To the authors' knowledge there is no information available on human dirofilariasis in Samoa or other Pacific Islands. However human cases have been reported in Australia and it can be expected that Samoa would have a similar incidence to that of countries with similar levels of canine heartworm infection and with a similar climate.

2.5.2 Ehrlichia canis

Aetiology

Ehrlichia canis is a tick borne obligate intracellular parasite that infects the monocytes of dogs (Dumler et al., 2001) and is the cause of canine monocytotropic ehrlichiosis (CME), previously known as canine tropical pancytopenia (Dumler et al., 2001; Huxsoll, 1976). It is a small pleomorphic gram negative coccoid bacterium classified in the order Anaplasmataceae

(Dumler et al., 2001). The *Ehrlichia* organisms of most importance in dogs are *E. canis*, *Ehrlichia chaffeensis*, and *Ehrlichia ewingii* (Beall et al., 2012). However, because of its worldwide distribution and because it causes most severe clinical disease in dogs, *E. canis* can be considered the most important member of this group for canines (Neer et al., 2002).

Epidemiology

E. canis was first identified in 1935 in Algeria and was the first *Ehrlichia* species found to infect dogs (Donatien and Lestoquard, 1937). Nowadays it is widely recognised, and has been shown to be present in most temperate and tropical regions of the world, as has the primary vector *Rhipicephalus sanguineus* (Dumler et al., 2001; Groves et al., 1975). *E. canis* is now considered enzootic throughout Africa with prevalence studies in various countries demonstrating seroprevalence rates in dogs as high as 67.8% (Davoust et al., 2006; Kelly et al., 2004; M'Ghirbi et al., 2009; Ndip et al., 2007).

A large scale prevalence study of *E. canis* in domestic dogs in the USA showed evidence of exposure to the disease in almost all states. The highest regional rates were in the south-eastern states, where 1.3% of dogs were seropositive. Other regions were lower (0.3-0.6%), but included some foci of infection where seroprevalence ranged from 2-11%. This study used a commercial ELISA kit (Snap 4Dx, IDEXX Laboratories, Westbrook, ME) (Bowman et al., 2009). There is serological and molecular evidence from many South and Central American countries to suggest *E. canis* is also enzootic throughout this continent (Gutierrez et al., 2008; Lopez et al., 1999; Ramos et al., 2010; Scorza et al., 2011; Vargas-Hernandez et al., 2012; Vinasco et al., 2007).

Ehrlichiosis in dogs is also well recognised in southern Europe, with studies in Italy and Spain showing seroprevalences as high as 50% (Amusatogui et al., 2008; Trotta et al., 2009). In the last ten years there have been increasing reports from some northern European countries of dogs with disease or positive antibody titres (Hamel et al., 2011; Hirsch and Pantchev, 2008). This may be due to an increase in animal movement across Europe and the geographical extension of *R. sanguineus* all over continental Europe (Beugnet and Marie, 2009). The United Kingdom is considered free of *E. canis* and the vector *R. sanguineus* is not endemic, however cases are being increasingly diagnosed in travelling dogs. Relaxation of quarantine regulations has seen increased movement of pets between the UK and many other countries. This brings the risk of importation of the tick vector and with it diseases exotic to the UK such as *E. canis* (Bates, 2008; Jameson and Medlock, 2011). Due to the long incubation period of *E. canis* and

potential silent carriers in the subclinical stage, it would be possible for disease to be picked up in one area and detected in another area months to years after exposure (Harrus et al., 1998b).

As in the UK, *E. canis* and *R. sanguineus* are considered exotic to New Zealand. Strict quarantine measures for dogs coming into the country, including checks for ticks and serology for *E. canis* with IFA test, have prevented the arrival of the disease in this country. However, *R. sanguineus* is intermittently intercepted at these checks and there is a concern that the tick could become established in the north of New Zealand or survive in heated houses (Bingham, 2010a; Hill, 1999; Stone, 2005). Australia appears to be free of *E. canis*, despite having *R. sanguineus* commonly found in the tropical north. Stringent quarantine measures and IFA testing of all imported dogs (except those from the UK or New Zealand) help to keep this status, despite the disease being endemic throughout Southeast Asia (Irwin, 2001; Masona et al., 2001).

A study into animal health in Samoa conducted in 1997 tested ten dogs for *E. canis* (by IFA test). Six of these dogs tested positive and the vector, *R. sanguineus*, was proven to be present in Samoa (Martin, 1999). Elsewhere in the Pacific there is little or no evidence for the presence of the pathogen, although this may be due to lack of surveillance. Since 1980, Ministry of Agriculture surveillance has intercepted *R. sanguineus* in New Zealand entering from Vanuatu, Fiji, New Caledonia, the Solomon Islands, Hawaii and Papua New Guinea (Bingham, 2010b, 2012; Fairley and Heath, 1997), suggesting that the tick vector is widespread in the Pacific Islands. Dogs undergoing the importation procedure from Vanuatu have also tested positive for *E. canis* antibodies using an IFA, although this may be explained by cross reactivity with other *Ehrlichia* species (Kittelberger, 2012). There was no clinical disease noted in the dogs with positive titres, however subclinical disease was not ruled out (Kittelberger, 2012).

Pathogenesis and lifecycle

The vector of *E. canis* is *R. sanguineus*, the brown dog tick, and transmission is transstadial i.e. the ticks become infected during the larval or nymphal stages by feeding on an infected dog with circulating rickettsiae (Groves et al., 1975). Transmission is completed when the tick, at a later time, injects saliva containing the organism into a non-infected animal while taking a blood meal. Transmission does not occur transovarially and it is chronically infected dogs that are considered to be the main reservoir of *E. canis*, not the ticks (Groves et al., 1975). Once infected, transmission of the disease to dogs can occur at any stage of a tick's life cycle, for as long as 155 days after infection. In temperate climates, this allows overwintering of affected

ticks resulting in infection of susceptible dogs in the spring (Lewis et al., 1977). Iatrogenic infection is also possible during blood transfusion with an infected donor (Harrus et al., 1998b).

Natural infection of *E. canis* has an incubation period of 8-20 days, followed by an acute stage lasting 2-4 weeks (Harrus et al., 1998b). In this time the organisms are phagocytosed by macrophages and multiply within these cells in morulae which may be seen as inclusions within affected cells. Each morula grows in size as the organisms multiply until it fuses with the outer membrane of the infected cell and the rickettsial contents are released into circulation. These are then phagocytosed by more monocytes. Infected mononuclear cells travel throughout the body spreading infection to the spleen, liver, lymph nodes and the vascular endothelium. (Woody and Hoskins, 1991).

If treated appropriately at the acute stage, most dogs recover completely (Neer et al., 2002). Left untreated, dogs may then enter a subclinical stage, where the dog seems clinically healthy, but there can be a persistence of the organism in mononuclear cells throughout the body for months to years, especially in the spleen which seems to be the last organ to harbour infection before elimination (Harrus et al., 1998b). Seroconversion does not offer protection from reinfection or permanent immunity and re-infection is in fact likely in endemic areas with a high tick density and disease prevalence (Neer et al., 2002). At the subclinical stage, CME can go one of three ways. Immunocompetent animals may successfully eliminate the rickettsiae and undergo a full recovery. Others may either remain persistent carriers or enter the chronic stage of disease (Harrus et al., 1998b).

Not all dogs go on to develop the chronic phase however, and the reasons for development of this stage remain unclear. Some breeds, such as German shepherd dogs seem to be particularly prone to progressing to a severe chronic CME with high probability of death. In German shepherd dogs this is thought to be due to a depressed cellular immune response when compared with beagles (Nyindo et al., 1980).

Hyperimmune mechanisms are thought to play a role in the pathogenesis of CME. These include extensive plasma cell infiltration of bone marrow and parenchymal organs; polyclonal hypergammaglobulinaemia in excess of that which can be explained by *E. canis* antibodies alone; positive Coomb's tests; antiplatelet antibody production; and the presence of circulating immune complexes (Harrus et al., 2001; Harrus et al., 1999).

Evasion of the host immune system allows the organism to persist within host cells. Constant alteration of the organism's surface antigens and the expression of different protein variants

help it to evade recognition. Immune evasion may also be achieved by down regulation of major histocompatibility complex class II receptor which in turn impairs host immune functions (Harrus et al., 2003; Mavromatis et al., 2006).

Clinical presentation

E. canis infection in dogs can be acute, chronic or subclinical. Clinical signs reflect the organs affected and are multisystemic.

In the acute stage of disease common clinical signs include fever, anorexia, depression, and haemorrhagic tendencies. Bleeding as a result of thrombocytopaenia may occur in acute disease and presents commonly as petechiae, ecchymoses and epistaxis (Woody and Hoskins, 1991). Ocular signs, such as hyphaema, anterior uveitis, and retinal detachment and haemorrhage, may be seen in CME (Harrus et al., 1998a; Komnenou et al., 2007), and meningitis or meningeal bleeding may result in a whole range of neuromuscular signs from seizures and stupor, to vestibular disease and anisocoria (Maretzki et al., 1994; Meinkoth et al., 1989). Opportunistic secondary infections may also occur as a result of immunosuppression. On physical examination, lymphadenopathy and splenomegaly may be found in 20% and 25% of patients respectively (Woody and Hoskins, 1991). Co-infections with *Babesia canis vogeli* and *Hepatozoon canis* may also occur as these diseases are transmitted by the same vector (Ramos et al., 2010). A moderate to severe thrombocytopaenia, with megathrombocytosis, is typically found, as is a mild anaemia and leukopaenia (Neer et al., 2002; Woody and Hoskins, 1991).

Dogs which receive no or insufficient treatment may then enter the subclinical phase. In this phase, dogs remain clinically healthy, although a mild thrombocytopaenia, with concomitant increase in platelet size, is usually present. These dogs will also have persistently high antibody titres to *E. canis* (Waner et al., 1997).

The chronic form of CME is characterised by severe pancytopenia, occurring as a result of bone marrow hypoplasia. Clinical signs again are multisystemic and are similar to the acute stage. Death may then occur due to haemorrhage or secondary infections (Harrus et al., 2012).

Diagnosis

The diagnosis of CME is usually based upon suggestive history, physical examination and haematologic abnormalities, coupled with serological findings. PCR is now also available for

diagnosis in a clinical setting (Harrus et al., 2012; Neer et al., 2002). Demonstration of morulae within monocytes from blood smears or tissue aspirates can be used to diagnose CME, however they can be difficult and time consuming to find (Harrus et al., 2012; Mylonakis et al., 2003).

Serology is a commonly used diagnostic tool. As with other *Ehrlichia* species, positive serological findings only indicate exposure to *E. canis*. Experimental infection with *E. canis* results in the development of IgM and IgA antibodies within about 4-7 days, with IgG antibodies generally appearing by 15 days post-infection (Waner et al., 2001) although in some dogs this can take as long as 28 days, by which time clinical signs of diseases are already apparent (Neer et al., 2002). It is these IgG antibodies that are detected in most serology tests available. In untreated dogs, serum antibodies peak at 3-5 months post infection before starting to fall again, however in some dogs serum antibodies may remain elevated for as long as three years or even life in some cases (Bartsch and Greene, 1996; Harrus et al., 1998b; Perille and Matus, 1991).

IFA test is considered the gold standard in serological testing for *E. canis*. An IFA test, usually carried out at a commercial laboratory, gives a quantitative antibody titre. Titres at or above 1:80 can be considered positive (Neer et al., 2002), and it is a very sensitive test when titres are greater than 1:160 (O'Connor et al., 2006). However, there is variable cross-reactivity between *E. canis* and *Neorickettsia risticii*, *Anaplasma platys*, *Anaplasma phagocytophilum* and granulocytic *Ehrlichia* spp such as *E. ewingii* and *E. chaffeensis* (Neer et al., 2002; O'Connor et al., 2006; Suksawat et al., 2000). Some low positives (1:40 or 1:80) may therefore be false due to cross reactivity with antibodies to these and other less pathogenic rickettsiae (O'Connor et al., 2006). For acutely ill dogs where clinical suspicion of disease is high but IFA titres are low or negative, it is recommended that serology is retested in 2-3 weeks in case the dog has not yet seroconverted (Neer et al., 2002). In addition, the *E. canis* IFA test is not standardised between commercial laboratories, therefore variation in technique and result in significant variations between laboratories (O'Connor et al., 2006).

Commercial ELISA tests are available as easy to use point of care kits. The *E. canis* part of the SNAP 4Dx (IDEXX Laboratories, Westbrook, ME) test is calibrated to be positive at titres greater than 1:160 (Harrus et al., 2012) so at lower titres is less sensitive than IFA. It uses synthetic peptides that duplicate immunodominant regions of *E. canis* surface proteins (O'Connor et al., 2006). When compared with IFA, the SNAP 4Dx test has a sensitivity of 96.2% and specificity of 100% (Chandrashekar et al., 2010), however as with the IFA, cross reactivity

of the analyte with *E. chaffeensis* and *E. ewingii* is known to occur and may result in false positives (O'Connor et al., 2006).

Western immunoblotting (WB) and PCR can be used to help distinguish between infections where cross reactivity is a possibility. Both can be used to detect *E. canis* antigen, and WB can be used to detect *E. canis* antibody as well (Neer et al., 2002). Both can be useful in distinguishing between infections with *E. canis*, *E. ewingii* and *E. chaffeensis* which can all show as positive on IFA test (Harrus et al., 2012; Neer et al., 2002). In addition, PCR has been shown to be a sensitive method for detecting early disease in experimental infection, often within 4-10 days post infection, before seroconversion has occurred, although this has not been shown in naturally infected animals (McBride et al., 1996). However, some studies have shown PCR to have poor correlation with IFA test results. This may indicate insensitivity of PCR or because of clearance of the organism following exposure (Seaman et al., 2004; Suksawat et al., 2000). There seems to be good correlation between *E. canis* antibodies detected by IFA, ELISA and WB at high titres greater than 1:320, but poor correlation at lower titres (1:80-1:160) (O'Connor et al., 2006).

Public health

During the late 1980's *E. canis* came under scrutiny for its purported ability to infect people. However in 1991 *E. chaffeensis* was discovered as a new member of the *Ehrlichia* group and that which is responsible for human monocytic ehrlichiosis. Its distribution is restricted to the United States (Anderson et al., 1991) and dogs act as a reservoir for disease in humans in endemic areas (Neer et al., 2002).

In 1991 a new variant or subspecies of *E. canis* was isolated from a human, the cause of Venezuelan human ehrlichiosis (Perez et al., 1996), but to date equivalent reports from elsewhere in the world have not been described. Today, *E. canis* is considered of little zoonotic importance worldwide (Harrus et al., 2012; Neer et al., 2002).

2.5.3 *Anaplasma phagocytophilum*

Aetiology

Anaplasma phagocytophilum, previously categorised as *Ehrlichia phagocytophila* and *Ehrlichia equi*, is an obligate intracellular bacteria which infects mammalian neutrophils. It is the

causative agent of canine granulocytotropic anaplasmosis (CGA) and human granulocytic anaplasmosis (HGA). Rickettsiae of the family *Anaplasmataceae* are gram-negative, non-motile, pleomorphic organisms and are obligate anaerobes. All species in the genus *Anaplasma* reside within vacuoles present in haematopoietic cells of mammalian hosts, and their vectors are arthropods (Dumler et al., 2001).

Epidemiology

The organism was first described as a veterinary pathogen after its identification in leukocytes of sheep in Scotland in the 1930's (Gordon et al., 1932). Since then the organism has been shown to be endemic in parts of North America, North Africa, Europe and Asia, and in endemic areas seroprevalence can be quite high (Bowman et al., 2009; Cao et al., 2000; Diniz and Breitschwerdt, 2012; Jiang et al., 2011; M'Ghirbi et al., 2009; Pusterla et al., 1998; Ravnik et al., 2009). The vector for *A. phagocytophilum* is *Ixodes* ticks, and as such the geographic distribution of the disease is determined by the range of these ticks (Figure 2.8) (Swanson et al., 2006; Telford et al., 1996). In Asia and Russia *Dermacentor silvarum* is also a vector (Jiang et al., 2011).

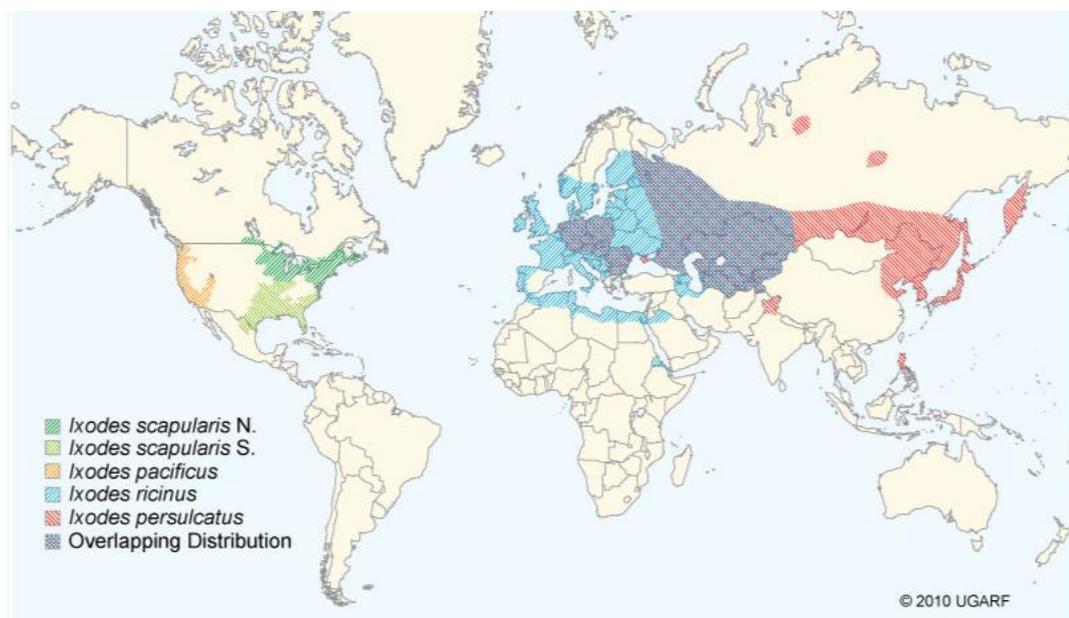


Figure 2.8: Approximate worldwide geographic distribution of four *Ixodes* spp. tick vectors of *Anaplasma phagocytophilum* and their overlapping regions (Art by Thel Melton © 2010 University of Georgia Research Foundation) (Diniz and Breitschwerdt, 2012)

A. phagocytophilum can infect a wide range of mammalian hosts, however clinical illness has only been documented in dogs, horses, cattle, cats, humans and some ruminants. A significant number of rodents and deer are implicated as natural reservoirs (Diniz and Breitschwerdt, 2012; Telford et al., 1996), and migratory birds may play an important role in the geographic distribution and spread of infected ticks (Bjoersdorff et al., 2001). Wildlife hosts are regarded as the primary reservoir, whereas dogs and humans are considered to be accidental hosts, as bacteraemia in these species is only short-lived (Bakken and Dumler, 2008; Carrade et al., 2009).

In the United States, *A. phagocytophilum* infection predominates in western, mid-western and north-eastern states (Bowman et al., 2009). Seroprevalence studies in the USA have ranged from 1.1% to 67.4%, though inclusion criteria, such as whether dogs were sick or healthy, make the studies difficult to compare (Beall et al., 2008; Bowman et al., 2009; Diniz and Breitschwerdt, 2012). A large scale survey showed an overall prevalence of 4.8% (Bowman et al., 2009). Canada appears to have very low seroprevalence, with a recent large scale survey detecting an overall seroprevalence of 0.19% (Diniz and Breitschwerdt, 2012). *A. phagocytophilum* has not yet been found in wild or domestic animals in South America.

In Europe, *A. phagocytophilum* has been recognised in many countries, and as in North America, prevalence studies vary greatly, with seroprevalence ranging from 1.2% to 70.5% (Diniz and Breitschwerdt, 2012; Pusterla et al., 1998; Ravnik et al., 2009). The higher prevalences are from studies sampling from a sick dog population.

A recent study showed evidence of *A. phagocytophilum* infection in dogs in Tunisia (M'Ghirbi et al., 2009), but other than this there are no reports of infection in Africa. The organism has been detected in ticks from Russia and China, but to date there are no epidemiologic studies in dogs in these countries (Cao et al., 2000; Jiang et al., 2011).

A limitation of seroprevalence studies is that antibodies to *A. phagocytophilum* cross react with those of *Anaplasma platys* (Bowman et al., 2009), so seropositivity may not necessarily reflect previous exposure to *A. phagocytophilum*. Whereas *A. phagocytophilum* infection seems to predominate in the Northern Hemisphere, *A. platys* seems to be the prevalent species in the dog population in South America (Abarca et al., 2007; Santos et al., 2009), Africa (Sanogo et al., 2003), Asia (Inokuma et al., 2002; Pinyoowong et al., 2008) and the Pacific region (Brown et al., 2001). To date, no cases of *A. phagocytophilum* have been reported in the Asia-Pacific region.

Pathogenesis and lifecycle

Infection is introduced to the host mammal while an infected tick feeds. Transmission between ticks is transstadial and as *Ixodes* spp. are three host ticks, feeding once at each life-stage, only nymphs and adults are potentially carriers (Macleod and Gordon, 1933; Ogden et al., 2007). Seasonality of infection is determined by the feeding habits of the tick vectors in endemic areas (Ogden et al., 2007). A minimum feeding time of 24 to 48 hours is required for transmission to occur (Katavolos et al., 1998). As the vector is *Ixodes* ticks, coinfection with *Borrelia burgdorferi* is common (Jaderlund et al., 2007; Swanson et al., 2006). Disease incubation is usually 1 to 2 weeks from first exposure (Carrade et al., 2009).

Once in the host bloodstream the organisms are taken up by neutrophils via endocytosis, after binding to cell surface receptors (Goodman et al., 1999). Once internalised in the neutrophil, *A. phagocytophilum* modulates the cell function in order to optimise its own intracellular survival, and prolong the lifespan of the cell (Rikihisa, 2006). Normal neutrophil lifespan is only 10-12 hours, but *A. phagocytophilum* inhibits endothelial adherence (Choi et al., 2003), translocation and apoptosis, therefore inducing a persistent bacteraemia and optimising uptake by uninfected ticks (Rikihisa, 2006; Yoshiie et al., 2000). Inside the intracellular membrane-bound vacuoles the organisms replicate by binary fission and form morulae. These morulae are classic of ehrlichial disease and can be visualised in infected cells by light microscopy. Eventually the infected cell ruptures, releasing the bacteria into circulation to infect other cells (Popov et al., 1998).

The exact mechanism by which *A. phagocytophilum* causes disease is not known. The immune response to infection and the immunomodulatory effect that *A. phagocytophilum* has on host neutrophils are thought to play a role. The interference with neutrophil microbicidal activity may predispose the host to secondary opportunistic infections and *A. phagocytophilum* may potentially augment neutrophil responses resulting in inflammatory tissue injury (Martin et al., 2001). Antiplatelet antibodies have been detected in dogs and humans, suggesting an immune mediated mechanism (Kohn et al., 2008).

Clinical presentation

Widespread serological evidence of infection in endemic areas in dogs with no history of illness suggests that many naturally infected dogs remain healthy (Beall et al., 2008). Disease in dogs is non-specific and primarily acute. The most common clinical signs reported are fever, lethargy and anorexia, which the majority of infected dogs present with (Egenvall et al., 1998;

Greig et al., 1996; Kohn et al., 2008). Musculoskeletal problems are also commonly reported, and in some dogs lameness results from a neutrophilic polyarthrititis (Kohn et al., 2008). Less commonly polydipsia, pale mucous membranes, vomiting, diarrhoea and haemorrhage have been reported (Greig et al., 1996; Kohn et al., 2008). On physical examination lymphadenopathy and splenomegaly may be detected (Greig et al., 1996). Infection appears to be self-limiting in dogs, and chronic disease has not been described. To date there are no case reports documenting a fatal outcome in dogs. Co-infections with *Borrelia burgdorferi* may be the cause of more severe clinical signs, possibly because of the immunomodulating effects of *A. phagocytophilum* (Beall et al., 2008).

Thrombocytopenia is the most consistent laboratory finding in affected dogs, ranging from mild to severe, and is the result of increased destruction of platelets (Egenvall et al., 1998; Greig et al., 1996; Kohn et al., 2008; Lilliehook et al., 1998). The majority of dogs also have lymphopenia, and mild hypoalbuminaemia, high serum alkaline phosphatase, hyperglobulinaemia and mild anaemia may be present in some cases (Egenvall et al., 1998; Greig et al., 1996; Kohn et al., 2008).

Diagnosis

Confirming the diagnosis of HGA requires either the identification of morulae in a blood smear combined with a single antibody titre to *A. phagocytophilum* of $\geq 1:80$; the serological evidence of a fourfold increase in antibody titre to *A. phagocytophilum* within 4 weeks; or the detection of *A. phagocytophilum* DNA by PCR, along with a supporting history, clinical and laboratory data (Bakken and Dumler, 2008). These same criteria could be applied to dogs.

Morulae are detectable only in the acute phase of disease and only transiently. They can be detected as early as 4 days after inoculation in experimentally infected dogs, and persist for between 4 and 8 days (Egenvall et al., 1998). In dogs where morulae are detected in the acute stage, between 7 to 32% of neutrophils have inclusions (Egenvall et al., 1997; Poitout et al., 2005). Morulae cannot be distinguished from those of other *Ehrlichia* species.

Serology is commonly used for the diagnosis of anaplasmosis in dogs. Experimental studies have documented that antibodies can be detected from 2 to 5 days following the appearance of morulae in the blood, or as early as 8 days following experimental inoculation (Chandrashekar et al., 2010; Egenvall et al., 1998). Serum antibody levels may remain high for over 1 year, or may revert to low or undetectable by 6 to 7 months post infection (Chandrashekar et al., 2010; Egenvall et al., 1997). When antibody levels drop, dogs are

susceptible to re-infection the following tick season (Egenvall et al., 1997). High antibody levels for periods of 1 year or more may be due to persistent infection. Persistent infection has not been verified following natural infection, although results of some studies suggest this may occur (Chandrashekar et al., 2010; Egenvall et al., 2000). Seropositivity is only an indication of exposure and antibody levels may remain high for a period of time following natural recovery (Egenvall et al., 1997). Where serology is used alone to confirm diagnosis, paired rising titres must be proven.

IFA test is a commonly used serology test for the diagnosis of canine anaplasmosis, and can be performed in many commercial laboratories. Limitations of an IFA include the lack of standardisation between IFAs performed in different laboratories, and the existence of cross-reactive antigens between *A. phagocytophilum* and other *Ehrlichia* and *Anaplasma* organisms. IFA tests for *A. phagocytophilum* will detect antibodies against both *A. phagocytophilum* and *A. platys* and for dogs in areas where both organisms are endemic, the difference can only be distinguished with PCR (Chandrashekar et al., 2010).

A commercially available point of care ELISA (Snap 4Dx, IDEXX Laboratories, Westbrook, ME) for the detection of antibodies to *A. phagocytophilum* gives quick, accurate serologic results without the need for trained laboratory staff or equipment. The ELISA uses peptides derived from the immunodominant p44 proteins of *A. phagocytophilum* and has a sensitivity and specificity of 99.1% and 100% respectively when compared with IFA. However, this test also cross-reacts with *A. platys* antibodies (Chandrashekar et al., 2010), and again can only be used to determine exposure, not current infection. Developed primarily as a screening test, it should not be relied on for diagnosis alone.

Western blot assays have also been developed for the detection of *A. phagocytophilum* antibodies, however these are not commonly used in a commercial setting (Diniz and Breitschwerdt, 2012).

PCR is a more sensitive diagnostic tool than visualising morulae in a blood smear. Dogs infected with *A. phagocytophilum* have positive PCR results before morulae are visible and may remain positive for several weeks after the acute phase (Chandrashekar et al., 2010; Egenvall et al., 2000). PCR also allows for a species-specific diagnosis, accurately distinguishing between DNA of *A. Phagocytophilum*, *A. platys* and other *Ehrlichia* spp. (Carrade et al., 2009). It is important to note, that although accurate, PCR is still subject to false-positive and false-negative results as with any other diagnostic test. Results may vary depending on the target

genes used and the laboratory technique and quality control measures employed (Diniz and Breitschwerdt, 2012).

Public health

A. phagocytophilum is the agent responsible for HGA. The disease was first identified in 1990 in a resident of Wisconsin, USA, who died after a severe febrile illness following a tick bite (Chen et al., 1994). In the following two years a further 12 human cases with similar clinical signs and intra-neutrophilic inclusions were identified in the same region (Bakken et al., 1994; Dumler et al., 2005). Since its first description, clinical cases of HGA have steadily increased in both North America and Europe, and it is currently considered an emerging disease in human medicine (Doudier et al., 2010).

In humans, as with other mammals, infection of the neutrophils with the bacterium results in immunodeficiency diseases. Symptoms may vary from a mild and self limiting fever to death, with signs requiring hospitalisation observed in half of all symptomatic patients (Bakken et al., 1996). The most common signs of HGA are fever, malaise, headache and myalgia. Less commonly arthralgia, vomiting, diarrhoea, coughing and confusion may occur (Dumler et al., 2005). Death may occur as a result of HGA, although deaths have usually been a result of complications due to immunocompromise and opportunistic secondary infections (Dumler et al., 2005). As with dogs, serologic studies suggest that many infections go unrecognised, with as many as 15% to 36% of people in endemic areas of the United States being seropositive (Aguero-Rosenfeld et al., 2002; Dumler et al., 2005). In Europe, sero-surveys have shown a similar level of seroconversion (Remy et al., 2003), though relatively few human cases are reported here. This could possibly be because some variants are more pathogenic in humans than others (Doudier et al., 2010).

As with other mammals infection is associated with *Ixodes* tick exposure and as such disease will usually only occur in tick endemic areas with a wildlife reservoir population. Most clinical cases are reported in spring and summer and occupational or recreational activities related to wooded areas are major risk factors for anaplasmosis (Doudier et al., 2010). It is not known what the potential zoonotic risk of domestic species such as dogs is to humans as a reservoir for *A. phagocytophilum*. Most commonly wildlife hosts such as small rodents and deer are implicated as the main maintenance reservoir.

2.5.4 *Anaplasma platys*

Aetiology

Anaplasma platys, previously named *Ehrlichia platys*, is the aetiological agent in infectious cyclic thrombocytopaenia of dogs (Harvey et al., 1978). It is a small rickettsial parasite of platelets and genetically it is very similar to *Anaplasma phagocytophilum* (Harvey, 2012; Harvey et al., 1978).

Epidemiology

First reported in Florida in 1978 (Harvey et al., 1978), *A. platys* has since been reported in Southern Europe (Beaufils et al., 2002; de la Fuente et al., 2006; Kontos et al., 1991; Sainz et al., 1999; Sparagano et al., 2003), Africa (Sanogo et al., 2003), Asia (Chang et al., 1996; Motoi et al., 2001; Suksawat et al., 2001a; Suksawat et al., 2001b), the Caribbean (Georges et al., 2008), Australia (Brown et al., 2001) and South America (Suksawat et al., 2001a). Whereas *A. phagocytophilum* infection seems to predominate in the Northern Hemisphere, *A. platys* seems to be the prevalent species in the dog population in South America, Africa, Asia and the Pacific region (Diniz and Breitschwerdt, 2012). Serologic studies in the United States have shown that as many as a third of thrombocytopaenic dogs in Florida and Louisiana have positive titres to *A. platys* and over 50% of dogs seropositive for *Ehrlichia canis* also have positive antibody titres to *A. platys* (Harvey, 2012; Hoskins et al., 1988). The prevalence by PCR is as high as 43% in dogs in Aboriginal communities in Australia (Brown et al., 2006).

Pathogenesis and lifecycle

The exact mechanism for transmission has not yet been established. The tick *Rhipicephalus sanguineus* has been implicated but not confirmed as the main vector of *A. platys* (Harrus et al., 1997; Woody and Hoskins, 1991). The organism has been repeatedly detected in the tick by PCR methods (Harvey, 2012; Motoi et al., 2001; Sparagano et al., 2003), although attempts to transmit infection with these ticks have not been successful (Simpson et al., 1991). *A. platys* has also been detected in *Dermacentor auratus*, *Haemaphysalis longicornis* and *Ixodes persulcatus* ticks in Asia (Kim et al., 2006; Parola et al., 2003) and the dog chewing louse *Heterodoxus spiniger* in Australia (Brown et al., 2005). *R. sanguineus* is the common vector for a number of tick-borne pathogens including *E. canis* and *Babesia canis*, and as a result co-infections are common in endemic areas (Hoskins et al., 1988; Suksawat et al., 2001b).

After experimental infection, the incubation period is 8 to 15 days (Chandrashekar et al., 2010; Harvey et al., 1978). The parasite enters the mammalian cell in a similar way to other *Anaplasma* and *Ehrlichia* spp., by adhering to the platelet surface and being taken up by endocytosis (Goodman et al., 1999; Harvey, 2012). Replication is by binary fission inside intracellular vacuoles, forming morulae within infected platelets (Harvey, 2012).

Approximately four days after the first appearance of organisms in the platelets, the platelet count drops precipitously, however recovery is as rapid with values returning to normal within another 3 to 4 days (Harvey et al., 1978; Hoskins et al., 1988). Parasitism of the platelets appears to be cyclical, with subsequent thrombocytopenic episodes occurring at 1 to 2 week intervals (Harvey, 2012). At the first episode of thrombocytopenia the percentage of parasitized platelets is high (31-67%), but this decreases with subsequent parasitaemias to as low as 1%, although the thrombocytopenia is as severe (Harvey et al., 1978).

Clinical presentation

Most infected dogs seem to remain clinically healthy, with no apparent clinical signs (Harvey et al., 1978). There may be a mild fever, and uveitis, petechiae and ecchymoses have been reported (Harvey, 2012). Despite platelet counts as low as $2-15 \times 10^9/L$ being recorded in infected dogs (Harvey et al., 1978), spontaneous bleeding is rarely reported as a clinical sign, although bleeding may occur following trauma or surgery during a thrombocytopenic episode (Harvey, 2012).

Some strains may be more pathogenic than others and more severe signs, such as high fever, anorexia, depression, weight loss have been reported outside of the USA, with haemorrhagic signs resembling acute *E. canis* infection such as intermittent epistaxis, petechiae, ecchymosis and anaemia (Harrus et al., 1997; Kontos et al., 1991). In some cases co-infections with other tick borne diseases may explain these signs (Gaunt et al., 2010). There is some evidence that dogs with simultaneous *A. platys* and *E. canis* infections suffer from a more pronounced thrombocytopenia than seen with single infections with either (Gaunt et al., 2010). Co-infections may also alter host immunocompetence and precipitate clinical signs in *E. canis* – *A. platys* infected dogs (Harvey, 2012).

Diagnosis

A diagnosis of *A. platys* can be confirmed by visualising the organisms inside the parasitized platelets, however false-negative results may occur if this method is relied on alone due to the

cyclical nature of the disease and that the parasite may be present in very low numbers (Harrus et al., 1997). In experimental infection, morulae or inclusions can be seen in platelets, by light microscopy of a blood smear, from 7 to 17 days post infection (Eddlestone et al., 2007).

Seroconversion occurs within 8 to 15 days of infection, with or shortly after parasitaemia (Chandrashekar et al., 2010; French and Harvey, 1983). In dogs co-infected with *E. canis*, seroconversion to *A. platys* may take as long as 35 days post infection (Gaunt et al., 2010). Serum antibody levels peak about 75 days post infection and may persist for months, with most dogs seronegative by 420 days post infection (Gaunt et al., 2010). As with other *Ehrlichia* spp., serology only indicates exposure to the parasite, not active infection. An IFA test to detect antibodies to *A. platys* is commercially available and is considered positive at titres of 1:80 to 1:100 or greater (Baker et al., 1987; Hoskins et al., 1988), but cross-reactivity with *A. phagocytophilum* does occur. There is no cross reactivity with *E. canis* antibodies (Harvey, 2012; Hoskins et al., 1988). A point-of-care ELISA test kit (Snap 4Dx, IDEXX Laboratories, Westbrook, ME) against *A. phagocytophilum* also reliably cross reacts with *A. platys* and can be used to aid diagnosis in endemic areas (Chandrashekar et al., 2010). When screening for exposure, an IFA titre of approximately 1:100 or greater will be positive with this test (IDEXX, 2012).

A. platys and *A. phagocytophilum* infection tend to occur in geographically distinct areas, and so a positive result can be interpreted with knowledge of what diseases are known to be endemic in that region (Diniz and Breitschwerdt, 2012). However, for dogs that may have been exposed to both organisms or in co-endemic areas, PCR is the only reliable method to distinguish between infections (Harvey, 2012). PCR may also be a more sensitive way to detect acute active *A. platys* infection, detecting infection earlier than is possible with serology. Experimentally infected dogs have positive PCR results as early as 3-4 days following inoculation (Chandrashekar et al., 2010; Eddlestone et al., 2007; Gaunt et al., 2010), with a peak in the relative level of *A. platys* DNA at 10 days post experimental inoculation (Eddlestone et al., 2007). However these DNA levels then quickly taper off, with experimentally infected dogs testing intermittently PCR negative from as early as 17 days post inoculation (Eddlestone et al., 2007), a point at which serology is generally positive. PCR results then may be transiently positive, with negative results corresponding to the periods of marked thrombocytopenia when the *A. platys* organism is temporarily cleared from the circulation (Eddlestone et al.,

2007). Infection is completely cleared by 160 days post experimental infection (Gaunt et al., 2010).

Public health

Currently, *A. platys* is thought to be of low zoonotic relevance (Otranto et al., 2009b) and there are no reported cases of human infection with this organism in the literature to date.

2.5.5 *Borrelia burgdorferi*

Aetiology

Borrelia burgdorferi sensu lato is the spirochete bacteria responsible for Lyme borreliosis in dogs and humans. It is unable to survive in the environment and is transmitted between vertebrate reservoir hosts (mammals, birds and lizards) and tick vectors (mainly *Ixodes* spp.) (Nadelman and Wormser, 1998).

Epidemiology

Lyme borreliosis was first described in people with symptoms of infectious polyarthritits in the 1970's. It takes its name from the town it was first discovered in Connecticut, USA (Nadelman and Wormser, 1998). In general, it occurs in the Northern hemisphere in temperate, cooler climate conditions and its distribution is determined by the presence of the tick vector. Lyme disease in dogs was first described in 1984 (Lissman et al., 1984).

The main vectors are ticks of the genus *Ixodes*. In North America, *Ixodes scapularis*, *Ixodes pacificus* and *Ixodes neotomae* are the vectors, and in Europe *Ixodes ricinus* is the most common vector, with *Ixodes persulcatus* also a vector in Eastern Europe and Northern Asia (Greene et al., 2012). The spirochete has been isolated from other tick species, fleas, flies, mites and mosquitoes, although vector competency is uncertain and their relevance compared to *Ixodes* ticks insignificant (Greene et al., 2012; Piesman and Happ, 1997).

Ixodes ticks have a two year life cycle, feeding on small mammals and birds in the larval and nymphal stages and on larger mammals (deer, dogs or humans) in the adult stage (Figure 2.9). Infection is maintained by infected ticks overwintering and having a wildlife reservoir (Keirans et al., 1996). It is adult ticks that appear to have the highest rate of infectivity, although

nymphs may be more likely to transmit infection in humans as they are less likely to be detected and removed due to their small size (Greene et al., 2012).

Several species of mice, voles and rats are important reservoir hosts. Squirrels, hedgehogs, shrews and birds are also involved in the maintenance of the infection cycle (Greene et al., 2012). Larger mammals such as deer are important in the lifecycle for adults to feed off, but the level of infection in these animals is insufficient to transmit infection to a tick making them unsuitable as a reservoir (Jaenson and Talleklint, 1992).

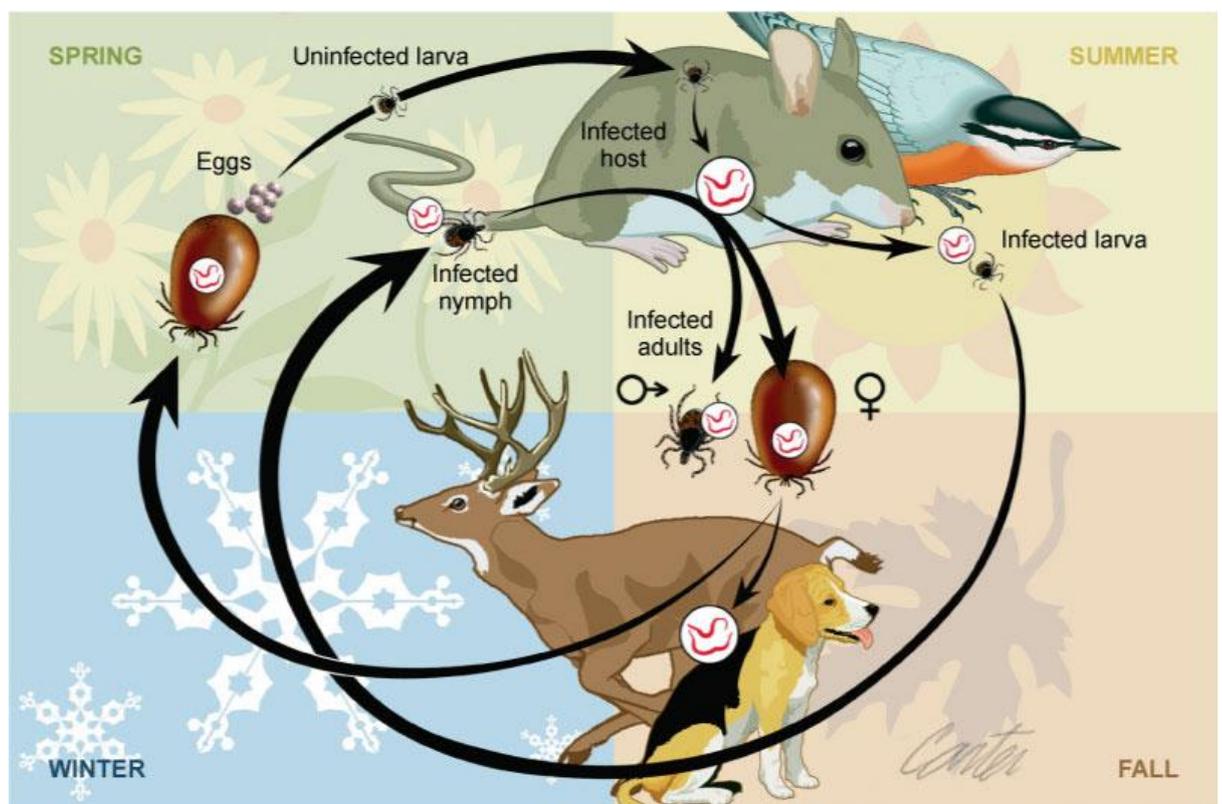


Figure 2.9: The lifecycle of *Ixodes scapularis* lasting 2 years. Eggs are oviposited in the spring and emerge approximately 1 month later. They feed once in the summer on birds or small mammals, and then overwinter. The following spring, larvae moult into nymphs, which then feed in late spring or early summer. Nymphs feed on small mammals, birds and larger mammals and humans. Nymphs moult into adults in the fall and the adults feed on larger mammals where they mate. The females die after laying their eggs. (Greene et al., 2012)

In the United States of America, the majority of Lyme disease cases are seen in the mid-Atlantic to New England coastal states, northeastern states and far western regions (Bowman

et al., 2009; Nadelman and Wormser, 1998). Seroprevalence rates in dogs are highest in the northeastern states with an average of 11.6%, compared with only 1% of dogs seropositive in Southern states (Bowman et al., 2009).

In Europe, most *B. burgdorferi* infections have been reported from central Europe and Scandinavian countries, where temperatures and humidity are moderate, although the disease can be considered endemic in most regions of Europe (Greene et al., 2012; Nadelman and Wormser, 1998), and infected ticks have been found on the other side of the Mediterranean in Northern Africa (Sarih et al., 2003).

In Northern Asia, Lyme borreliosis is recognised in Japan, China, Korea and Russia, among other countries, with many case reports, sero-surveys and tick-infection surveys confirming this (Azuma et al., 1994; Korenberg et al., 2010; Lim et al., 2010; Liu et al., 2012; Xia et al., 2012).

Although Lyme-type disease has been reported in Australia, *B. burgdorferi* has still not been isolated from patients or ticks, and so the disease cannot be considered endemic there (Cestnick, 1998; Russell et al., 1994). To date neither Lyme borreliosis nor any known competent tick vector have been reported in New Zealand, or in any other Pacific islands, including Samoa.

Pathogenesis and lifecycle

For successful transmission from vector to mammal, the tick must feed for a minimum of 50 hours. In this time, the organism multiplies within the tick gut, and spreads to the salivary glands infecting the host through the tick's saliva (Ohnishi et al., 2001). After inoculation into the host, a combination of spirochete outer surface proteins and tick saliva proteins are able to block clearance of the spirochete and allow their dissemination by evading the host immune system (Hovius et al., 2008). From the site of the tick bite the organisms spread throughout the skin and connective tissues, in time colonising many tissues including joints (Greene et al., 2012). Most animals bitten by infected ticks do not go on to develop Lyme disease, and studies have shown in endemic areas where seropositivity may be quite high (up to 75% in endemic areas in the USA (Magnarelli et al., 1987)), clinical cases are much lower. Host immunity is likely to be involved in preventing these infections (Levy and Magnarelli, 1992). Once in the body, *B. burgdorferi* is a persistent pathogen. The host immune system is able to reduce spirochete numbers to almost undetectable levels within a few weeks of infection, but by

existing extracellularly and changing immunoreactive proteins and even its shape, *B. burgdorferi* is able to evade host antibodies (Xu et al., 2008).

Clinical illness results from the host's inflammatory responses to the organism. Cytokine release to regulate the inflammatory response and reduce the parasite burden may also result in damage to surrounding tissues. In joints this is likely an important mechanism in producing arthritis (Straubinger et al., 1997). In nervous system tissues, immune responses against specific borrelial antigens stimulate an inflammatory response that may even target neural self antigens (Kuenzle et al., 2007). The glomerulonephritis that is often seen in seropositive dogs is thought to be related to the deposition of circulating immune complexes, as spirochetes are rarely found in renal lesions (Hutton et al., 2008).

Clinical presentation

The majority of dogs exposed to *B. burgdorferi* will seroconvert but remain clinically normal (Levy and Magnarelli, 1992). In clinical disease, systemic signs of fever, shifting leg lameness, joint swelling, lymphadenopathy, inappetence and general lethargy are the most common acute signs of disease. All these are responsive to antimicrobials (Greene et al., 2012; Levy and Magnarelli, 1992; Littman et al., 2006). However, it can be difficult to determine that this is due to Lyme borreliosis as these signs have been observed in dogs with and without *B. burgdorferi* specific antibodies with equal frequency, and there is no test that proves that illness is as a result of *B. burgdorferi* infection (Littman et al., 2006). In endemic areas exposure and seropositivity may be as high as 75% (Magnarelli et al., 1987). In one study only 5% of seropositive dogs went on to develop clinical signs of Lyme disease, however 5% of seronegative dogs also went on to develop similar signs (Levy and Magnarelli, 1992).

Polyarthritis is the most consistent syndrome in both naturally and experimentally infected dogs. The first limb affected is usually closest to the tick bite and lameness often persists in that limb for a few days before disappearing or shifting to another limb (Appel et al., 1993; Littman et al., 2006; Straubinger et al., 1997).

An acute, progressive renal failure due to a protein losing glomerulonephropathy has been described in naturally infected dogs in Lyme endemic areas. Sudden onset anorexia, vomiting and lethargy coupled with an azotaemia and proteinuria characterised these cases, and most dogs died or were euthanised as a result of renal failure. Vasculitis, peripheral oedema, and thromboembolic disease has also been associated with this presentation (Dambach et al., 1997).

Myocarditis and meningitis as a result of Lyme borreliosis have also been described, although these are usually based on circumstantial evidence (Greene et al., 2012). It has not yet been proven definitively that any syndrome other than polyarthrititis is as a direct result of *B. burgdorferi* infection.

Co-infections are common, and clinical illness may be precipitated or compounded by concurrent infection with pathogens such as *Anaplasma phagocytophilum* (Beall et al., 2008).

Diagnosis

There is no definitive test for Lyme borreliosis. Diagnosis is based on a compatible history, including tick exposure in an endemic area; clinical signs; laboratory findings, including the presence of *B. burgdorferi* specific antibodies; response to antimicrobial treatment; with the exclusion of other diseases (Littman et al., 2006).

Serology provides evidence of exposure to the organism, but does not prove that it is the cause of illness. However the diagnosis of Lyme borreliosis is currently a serological one because of difficulties in the detection of the organism in body fluids or tissues by culture, cytology or PCR (Greene et al., 2012; Littman et al., 2006).

Early serology tests were made using whole spirochetes, and experimentally infected dogs were shown to develop IgG-ELISA positive titres by 4-6 weeks after exposure (Appel et al., 1993). Titres reached their highest at 3 months and last for at least 2 years (Straubinger et al., 2000). The disadvantages of these tests were mainly to do with cross reactivity with vaccine induced antibodies, thereby making it impossible to distinguish between vaccinated and naturally infected dogs. Immunoblotting was required to distinguish between infected and vaccinated animals (Littman et al., 2006).

More recently tests have been developed to detect antibodies to the immunodominant protein, VlsE. This protein is highly conserved and expressed only during natural infection of mammalian hosts (Liang et al., 2000). The synthetically produced peptide C₆, derived from the VlsE antigen, has largely taken the place of whole cell preparations and is used in modern day serology tests (Littman et al., 2006). The presence of antibodies against the C₆ antigen proves natural exposure. In addition, the C₆ antibody response is quicker to rise, with positive titres developing within 3 to 5 weeks of experimental infection, and in untreated dogs titres remaining high for over 1 year (Liang et al., 2000). In a comparison with immunoblotting, the

sensitivity and specificity of the C₆ point of care ELISA (Snap 4Dx, IDEXX Laboratories, Westbrook, ME) was 98.8% and 100% respectively (Chandrashekar et al., 2010).

Public health

Lyme disease was described in humans 10 years before *B. Burgdorferi* was suggested to be the cause of clinical signs in dogs. Unlike dogs, most people exposed to *B. Burgdorferi* will go on to show clinical signs (Nadelman and Wormser, 1998). Symptoms can start from several days to one month after a tick bite and risk is greatest when the tick has attached for greater than 72 hours (Sood et al., 1997). Acute flu-like symptoms may accompany a ring-like erythematous skin lesion in the vicinity of the tick bite in the early stages of disease. Subsequent disease includes joint swelling and joint and muscle pain, and possibly cardiac, neurologic or chronic skin changes may occur (Nadelman and Wormser, 1998). In endemic areas, children under 14 and adults over 30 are most at risk of disease (Greene et al., 2012; Nadelman and Wormser, 1998). In the early stages of disease, human infection can be easily treated with antibiotics, however in chronic infections antimicrobial therapy is no longer effective (Klempner et al., 2001). Studies have shown that a single dose of doxycycline taken within 72 hours of a tick being removed can prevent Lyme disease in people (Nadelman et al., 2001).

Lyme borreliosis is classified as a zoonosis, although dogs do not appear to be a source of infection to humans. There is no evidence to prove that infected dogs pose a risk to humans other than the risk of introducing ticks into a household, although ticks cannot survive for long indoors and only usually feed once at each life stage (Greene et al., 2012). As with other large mammals, dogs do not act as a reservoir for *B. burgdorferi*, as the levels of infection generally are too low to transmit to ticks (Greene et al., 2012; Kurtenbach et al., 1998). Domestic dogs may be useful as sentinels for monitoring human disease risk in endemic areas. In these areas dogs have a higher risk of exposure due to their greater likelihood of coming into contact with the tick vector (Hamer et al., 2009).

There is some risk of infection to people removing infected ticks from dogs; if the tick is crushed and the contents of the mid-gut released and exposed to cuts or abrasions on that person's hands, infection may be transmitted (Day, 2011).

2.5.6 *Leishmania infantum*: Canine Leishmaniasis

Aetiology

Leishmania infantum is a protozoa of the family Trypanosomatidae, and the cause of canine leishmaniasis (Baneth and Solano-Gallego, 2012). The natural lifecycle requires a vertebrate host and phlebotomine sand fly vector, of the genus *Phlebotomus* or *Lutzomyia*. In the vertebrate host, *Leishmania* is detected in macrophages as amastigotes, the non-flagellate form. Within the sand fly, amastigotes transform into the extracellular, flagellate promastigote form and replicate. *L. infantum* is zoonotic, and in parts of the world where it is the cause of human visceral leishmaniasis, dogs are considered to be the main reservoir (Baneth and Solano-Gallego, 2012).

Epidemiology

Canine leishmaniasis caused by *L. infantum* is endemic in many countries, predominantly in the Mediterranean basin, central and southwest Asia and South America (Figure 2.10) (Baneth and Solano-Gallego, 2012).

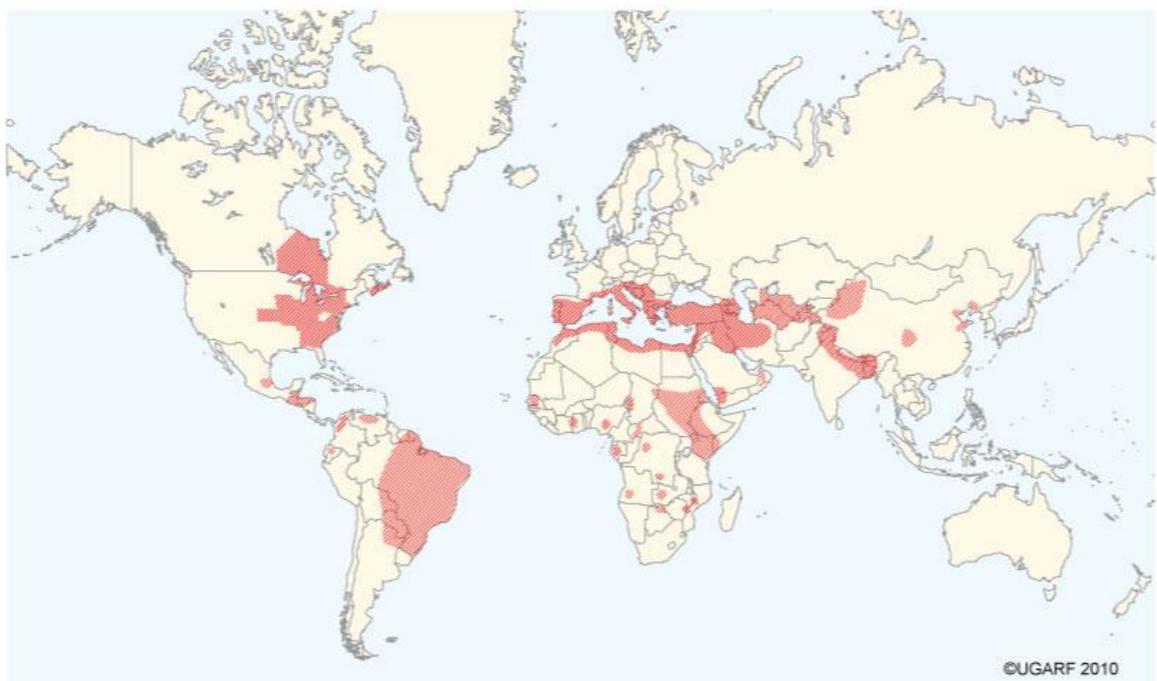


Figure 2.10: Map of the global distribution of canine leishmaniasis due to *Leishmania infantum* and human visceral leishmaniasis (Art by Thel Melton © 2010 University of Georgia Research Foundation) (Baneth and Solano-Gallego, 2012)

Risk of infection is determined by the presence and behaviour of local sand fly species and the presence of an infected canine reservoir. Approximately 70 of 1000 known sand fly species are able to transmit leishmaniasis (Murray et al., 2005). Transmission of *L. infantum* from domestic dogs by the bite of sand flies was first demonstrated in the 1930s (Adler and Theodor, 1932). Sand flies are predominantly present year round in tropical countries and are active during the warm months of the year in temperate countries. The activity of adult sand flies is crepuscular and nocturnal. The temperature range at which sand flies are active is between 15 and 28 °C, and activity is always associated with high humidity and the absence of wind or rain (Killick-Kendrick, 1999; Solano-Gallego et al., 2009). Both clinically and subclinically infected dogs are infectious to feeding sand flies, although infectiousness is greater in dogs with clinical infections (Quinnell and Courtenay, 2009).

In endemic areas, there is a high prevalence of canine infection, involving as much as 63-80% of the population when using PCR to detect, although the majority of dogs remain subclinical with a much lower apparent rate of clinical disease (Berrahal et al., 1996; Solano-Gallego et al., 2001). Based on seroprevalence studies in France, Spain, Italy and Portugal, it has been estimated that 2.5 million dogs in these countries are infected with *L. infantum*, and infection is spreading north in Europe (Baneth and Solano-Gallego, 2012; Otranto et al., 2009a).

When favourable conditions exist, with high sand fly-vector and canine-host densities, the infection spreads rapidly and extensively through the dog population (Baneth et al., 2008). In a study where a group of naive dogs were exposed to three consecutive transmission seasons in Naples, Italy, 97.3% of the dogs were PCR positive and 75.7% were seropositive by the end of the study. This extremely high transmission rate was attributed to a high density of *Phlebotomus perniciosus* vectors and a lack of any control measures to protect the dogs (Oliva et al., 2006).

A large number of studies have investigated *L. infantum* infection in other domestic and wild animal populations, as possible alternative reservoirs for infection (Quinnell and Courtenay, 2009). There have been studies demonstrating a high prevalence in domestic cats (Machado da Silva et al., 2008; Martin-Sanchez et al., 2007), and infections have also been reported in domestic horses (Fernandez-Bellon et al., 2006) and pigs (Moraes-Silva et al., 2006). *L. infantum* infection has also been reported in a wide range of wild carnivores and rodents in both the New and Old World. However there is no clear evidence for any important reservoir of zoonotic visceral leishmaniasis other than the domestic dog, without further investigation (Quinnell and Courtenay, 2009).

Sporadic cases of canine leishmaniasis have been reported in non endemic areas, such as the Netherlands, the United Kingdom and Sweden, as a result of importation of an infected dog (Baneth and Solano-Gallego, 2012). Recently, autochthonous transmission of the disease has been described in the United States and Canada in Foxhound populations, where the disease is now considered endemic in this breed, with seroprevalence rates of 8.9% to 13.5% (Petersen, 2009).

There has been renewed interest recently in potential transmission routes other than the sand fly. Infections in Foxhounds in the United States have occurred in regions of low sand fly density or where their competence as a vector is deemed to be poor. Vertical transmission is considered to be the most likely means of transmission in these dogs (Petersen, 2009). Congenital transmission has been confirmed, as has infection from blood transfusion with an infected donor and sexual transmission in dogs, though the effect of these modes of transmission is thought to be minimal (Quinnell and Courtenay, 2009). The possibility of non sand fly vectors has also been considered, and it has been suggested that the tick *Rhipicephalus sanguineus* may be able to transmit leishmaniasis. Studies have shown that it is possible for this tick, and some species of flea, to become infected from feeding on infected dogs (Coutinho et al., 2005; Quinnell and Courtenay, 2009). However the biological development of *L. infantum* within ticks and fleas, and subsequent transmission to a naive dog has not yet been proven (Quinnell and Courtenay, 2009).

In most maps showing the global distribution of *Leishmania*, Southeast Asia, Australia, New Zealand and the Pacific are depicted free of the parasite (Figure 2.10). However recently there have been reports to suggest that *Leishmania* distribution may be wider than previously thought (Thompson and Conlan, 2011). In 2004, the first autochthonous case of *Leishmania* was reported in kangaroos in Australia, involving a novel species of the parasite (Rose et al., 2004). There have also been cases reported in Thailand and East Timor, raising the possibility of vectors in this area (Thompson and Conlan, 2011). There have been no reported cases of human or canine leishmaniasis in Samoa or the surrounding Pacific region, and this region is assumed to be free of the disease. However this may be due to a lack of surveillance studies investigating the presence of the disease in dogs, humans or any other species.

Lifecycle and pathogenesis

Motile *Leishmania* promastigotes are transmitted to the host when an infected sand fly feeds and these are regurgitated into the host's skin through the saliva. The promastigotes are taken

up by macrophages in the skin, and transform within the cell into non-flagellate amastigotes. Amastigotes are protected by the host defences in phagolysosomes within the host macrophage and are able to multiply by binary fission until the cell bursts, releasing the amastigotes which are then taken up by more macrophages. Infected macrophages disseminate throughout the body via the lymphatic system, spreading mainly to the lymph nodes, spleen, liver, bone marrow and distant skin locations. The life cycle is completed when an uninfected sand fly feeds on the skin of the infected host, taking in an infected macrophage with the blood meal. The cell ruptures, releasing the amastigotes which then transform into motile promastigotes and replicate in the sand fly (Figure 2.11) (Baneth and Solano-Gallego, 2012).

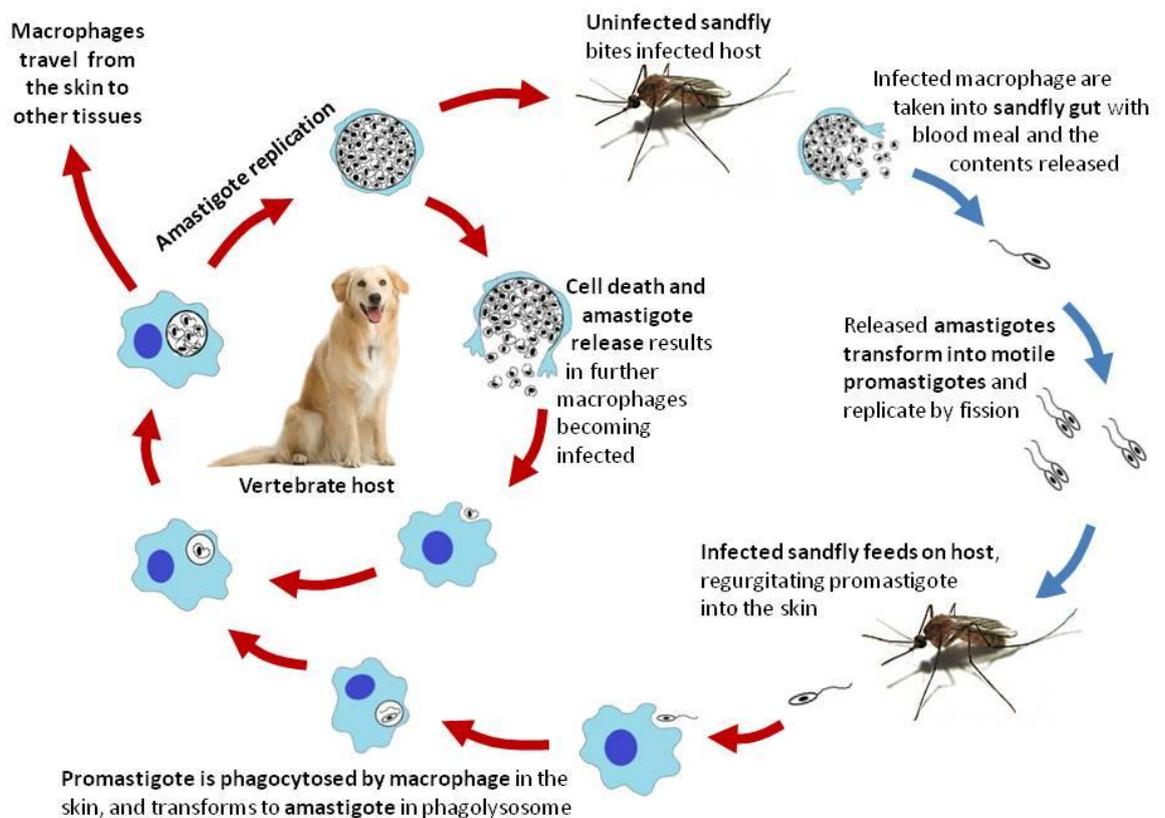


Figure 2.11: Life cycle of *Leishmania infantum* (adapted from Baneth and Solano-Gallego, 2012)

Not every dog that is infected with *Leishmania* goes on to develop clinical disease (Killick-Kendrick et al., 1994), in fact the majority of dogs remain subclinically affected (Solano-Gallego

et al., 2009). The progression to clinical disease depends on the immune response generated by the individual at the time of infection and thereafter. To resist infection, the immune system must halt amastigote replication, and either completely eliminate the parasite, or keep replication restricted and remain subclinical for long periods. These dogs are considered clinically resistant. However, the subclinical state is not always permanent and for some dogs a period of immunosuppression or concurrent disease may result in clinical disease developing (Cabral et al., 1998; Solano-Gallego et al., 2009).

Canine leishmaniasis is a chronic condition, with clinical signs developing anywhere from three months to seven years post-infection (Baneth and Solano-Gallego, 2012). Proliferation of B lymphocytes, plasma cells, macrophages and histiocytes within lymphoid tissue results in generalised lymphadenomegaly and splenomegaly. In addition T-lymphocytes become depleted, affecting the body's ability to mount a competent cell mediated response to the disease. Excessive antibody production from B lymphocytes results in hyperglobulinaemia, circulating immune complexes and in some cases autoantibody production, which in turn may lead to glomerulonephritis and renal failure, vasculitis, polyarthritis or immune-mediated thrombocytopenia. The vasculitis is an important feature, as this results in tissue necrosis and the skin, visceral and ocular lesions that are associated with the disease (Baneth and Solano-Gallego, 2012; Cortadellas et al., 2006; Font et al., 2004).

Clinical presentation

Classically, canine leishmaniasis is associated with skin lesions, often generalised, however, disease is almost always disseminated and may involve any tissue or organ in the body with a subsequent huge range of clinical signs (Baneth and Solano-Gallego, 2012). There is also a high prevalence of subclinical disease, with roughly 90% of infected dogs in an endemic area being clinically healthy (Solano-Gallego et al., 2009).

Skin lesions are seen in the majority of clinical cases (81-89%), with a range of abnormalities occurring from an exfoliative dermatitis, crusting and ulcerative lesions, to skin nodules, papules and mucocutaneous lesions. Lymphadenomegaly is also a common finding (62-90%) (Baneth and Solano-Gallego, 2012; Ciaramella et al., 1997; Koutinas et al., 1999).

Other signs include decreased activity, weight loss, lethargy, decreased appetite, polyuria and polydipsia, splenomegaly, ocular lesions, epistaxis, lameness and nail abnormalities. A serious consequence of disease is a progressive chronic renal failure, which is the main cause of death

in dogs with leishmaniasis (Baneth et al., 2008; Baneth and Solano-Gallego, 2012; Ciaramella et al., 1997).

The most consistent serum biochemistry finding in dogs with clinical disease is a polyclonal hyperglobulinaemia with a hypoalbuminaemia, and a persistent renal proteinuria. Elevated liver enzymes, renal azotaemia, mild anaemia, leukocytosis or leukopaenia may also be detected (Baneth and Solano-Gallego, 2012; Ciaramella et al., 1997; Solano-Gallego et al., 2009).

Diagnosis

Diagnosis of *L. infantum* infection is carried out for two main reasons: 1) to confirm disease is present in individuals with clinical signs indicative of canine leishmaniasis and 2) to screen a healthy population or individual for the presence of infection for epidemiological studies, or for the purpose of detecting subclinically infected dogs, either in endemic or non-endemic areas (Miro et al., 2008). The optimal choice of diagnostic technique is determined by the type of sample population.

When trying to establish the prevalence of *L. infantum* in a population, it must be taken into consideration that the incubation period before any clinical signs appear can be very long, up to seven years, and that the majority of dogs infected actually remain subclinical carriers. These subclinically infected dogs are still a reservoir for infection to others. For mass field surveillance, the ideal test would be quick and simple to use, economical and with a high sensitivity and specificity in both clinical and subclinical animals.

Diagnosis of *L. infantum* must involve either the demonstration of the parasite or parasite DNA in blood or tissue, or the detection of serum anti-leishmanial antibodies (Gomes et al., 2008). Detection of amastigotes by cytology, histology, immunohistochemistry or culture, although very specific, is time consuming, often invasive and has a lower sensitivity than PCR or serology in asymptomatic infections. These may be useful techniques in confirming diagnosis in clinical cases, but for detection of subclinical infection a more sensitive test is required (Gomes et al., 2008).

Canine leishmaniasis is most commonly diagnosed by detecting high levels of anti-leishmanial antibodies in serum. There are several serological tests available: indirect IFA test, ELISA, direct agglutination test. Serological tests, as well as being suitable for diagnosing individual cases, are useful for epidemiological surveys. Serology methods that use crude or partially purified

promastigote antigens to detect anti-leishmanial antibody are prone to cross reactivity with other closely related parasites, such as *Trypanosome cruzi*, in areas where these occur concurrently (Miro et al., 2008). In an attempt to reduce this cross reactivity in these areas, the use of recombinant polypeptides containing specific epitopes, have been adapted for use in serology tests (Miro et al., 2008). WB has proved to be more sensitive than IFA test, and its use in the routine diagnosis of canine leishmaniasis has been suggested, however WB is more laborious, requiring trained technicians, and its use is currently limited to research laboratories (Ferroglia et al., 2007). Regardless of which serological method is used, sensitivity is lower in clinically well, infected dogs, as seropositivity is evident in only 30-66% of subclinically infected dogs, compared with seropositivity of 88-100% in clinical cases (Gomes et al., 2008; Solano-Gallego et al., 2001).

PCR has greatly improved the sensitivity and specificity of parasitological diagnosis of canine leishmaniasis and assays based on kinetoplast DNA rather than genomic DNA seems to be most sensitive (Gomes et al., 2008; Miro et al., 2008). PCR on bone marrow, lymph node or spleen is most sensitive, though usually too invasive and not suitable for use in widespread epidemiological studies, whereas PCR on whole blood and urine is less sensitive (Solano-Gallego et al., 2009).

One study comparing the prevalence of leishmaniasis in an endemic area as determined by clinical disease, serology and PCR of skin, conjunctiva and bone marrow, detected an overall prevalence of 67%, with a clinical disease prevalence of 13%, a seroprevalence of 26% and positive PCR in 63% of dogs examined (Solano-Gallego et al., 2001).

It is clear that currently there is no one perfect test for surveillance and detection of subclinically infected dogs.

Public health

Leishmaniasis is a disease of major human significance and its management remains a severe public health problem (Desjeux, 2004). Human leishmaniasis is caused by several *Leishmania* species which are mostly zoonotic. The two most common diseases are visceral leishmaniasis (VL), caused by *L. infantum* and *L. donovani*, and cutaneous leishmaniasis (CL), caused by a number of other *Leishmania* species. VL is a chronic, potentially fatal condition that affects the internal organs, whereas CL affects the skin and mucocutaneous junctions, and although signs may resolve spontaneously, infection may result in extensive scarring and disability (Desjeux, 2004; Murray et al., 2005). The lifecycle in humans is as for in dogs (Figure 2.11), and clinical

infection in humans represents only a relatively small proportion of infected individuals, with asymptomatic subclinical infection being most common (Michel et al., 2011).

Leishmaniasis in people is endemic in 88 countries worldwide putting more than 350 million people at risk of the disease (Desjeux, 2004). The estimated incidence of the disease in humans is 2 million cases per year, with a quarter of these being VL, resulting in an estimated 59,000 deaths a year (Desjeux, 2004). However, incidence is not uniformly distributed in endemic areas, with 90% of VL cases occurring in poor rural and suburban areas of only five countries (Bangladesh, India, Nepal, Sudan and Brazil) (Desjeux, 2004; Gramiccia and Gradoni, 2005). VL cases occurring in the Indian subcontinent, Asia and Africa are caused by *L. donovani*. VL caused by *L. infantum* occurs in the Mediterranean region, Southwest and Central Asia and South America, primarily affecting infants, young children and immunosuppressed adults (Murray et al., 2005).

Typical clinical signs of human VL infection are fever, pallor, wasting, hepatomegaly and often a striking splenomegaly and with time, untreated clinical disease can result in death. Where treatment is available there is a 90% cure rate (Murray et al., 2005).

Domestic dogs are considered the main reservoir for *L. infantum* and canine prevalence rates may be as high as 80% in affected areas (Berrahal et al., 1996; Michel et al., 2011). Globally, canine leishmaniasis is considered one of the major zoonoses. Dogs are a common companion animal, in both developing and developed countries, living in close proximity to their human owners (Solano-Gallego et al., 2009). Infected dogs living in the vicinity of humans is associated with transmission of infection, although only one study in Iran has shown dog ownership a risk factor for human infection (Gavvani et al., 2002). In general, it is accepted that the risk of infection in humans is increased in areas where there is a high density of infected dogs, but not necessarily directly linked to dog ownership (Solano-Gallego et al., 2009).

Chapter 3 Materials and methods

3.1 Sample population

This was a pilot study, with samples collected from dogs in July 2010 (n = 50) and August 2011 (n = 187) during two periods of 4 weeks. At this time the APS was operating a free sterilisation campaign as part of their population control program. This involved mobile clinics in villages across both main Samoan Islands, and at the APS clinic in the Apia urban area.

Dogs estimated to be 10 kg or over which presented to the APS for surgical sterilisation were selected for sampling. As the blood sample volume to be collected was 10ml, a lower bodyweight limit of 10kg was decided upon to avoid sampling a large volume from small dogs or young puppies. Owner consent was required for owned dogs. A small number of stray dogs were presented for sterilisation and were included in the study.

During the initial study period in July 2010, the first 5 dogs to fit the inclusion criteria each day were sampled until a total of 50 dogs was reached. During the August 2011 sampling period every dog that fitted the inclusion criteria was sampled. Dogs which were severely obtunded or emaciated were excluded from the study to avoid risks associated with blood sampling. The study design was approved by the Massey University Animal Ethics Committee, New Zealand.

3.2 Sample Collection

For each owned dog estimated to weigh 10 kg or over, consent was sought from the owner to participate in the study and a questionnaire (Appendix 1) was completed by the APS staff with information provided by the owner. The form contained questions relating to the area in which the dog lived, its husbandry (indoor, free roaming), age and breed if known and any previous vaccination or antiparasitic treatments administered. The sex and estimated weight was recorded and for dogs whose age was not known, this was estimated following examination. For stray dogs questionnaire information was not available, but the area where they were found was recorded, along with their sex and estimated age and weight. A thorough visual examination for external parasites was also performed by a veterinarian or final year veterinary student.

For the purposes of statistical analysis the islands were divided into three areas based on the areas used by the Samoa Bureau of Statistics for the census - the urban Apia area, rural Upolu and Savai'i. In addition, ages recorded were placed in one of four categories: less than 12 months old, one year or older, but less than two years old, two years or older but less than three years old and three years or older.

3.3 Serologic testing

A 10ml blood sample was collected into plain and EDTA blood tubes, via jugular venipuncture at the time of general anaesthesia. Samples were placed in a chilled container immediately. The serum was separated, and both serum and EDTA samples were stored at -20°C within 8 hours of collection, pending analysis following transport to New Zealand.

Serum samples were thawed, brought up to room temperature and centrifuged prior to testing. A commercially available in-clinic ELISA (SNAP 4Dx test kit, IDEXX Laboratories, Westbrook, Me) for the simultaneous detection of *Anaplasma phagocytophilum*, *Borrelia burgdorferi* and *Ehrlichia canis* antibody and *Dirofilaria immitis* antigen was used according to the manufacturer's instructions. A commercially available in-clinic ELISA (SNAP Leishmania test kit, IDEXX Laboratories, Westbrook, Me) for the detection of *Leishmania infantum* antibody was used according to the manufacturer's instructions. Serum samples were processed within 60 days of collection.

A. phagocytophilum assay - This ELISA detects antibodies against a synthetic peptide derived from the major surface protein of *A. phagocytophilum* (the immunodominant p44 protein). Relative to IFA, sensitivity and specificity for the detection of *A. phagocytophilum* are 99.1% and 100% respectively. However, dogs experimentally infected with *Anaplasma platys* can also generate cross-reactive antibodies to this synthetic peptide, resulting in a positive ELISA, and it is possible that the IFA has similar cross reactivity (Chandrashekar et al., 2010). Therefore a positive *Anaplasma* result may be due to either *A. phagocytophilum* or *A. platys* exposure and consideration should be given to the presence of the vectors in that area when drawing conclusions.

B. burgdorferi assay – This ELISA detects antibodies generated against the C₆ peptide derived from the *Borrelia* membrane protein VlsE. Sensitivity and specificity are 98.8% and 100% respectively, relative to IFA (Chandrashekar et al., 2010).

***E. canis* assay** – This ELISA detects antibodies generated against peptides from the p30 and p30-1 proteins of *E. canis*. Compared with IFA, sensitivity and specificity are 96.2% and 100% respectively. It is possible for some *Ehrlichia chaffeensis* infections to produce cross reacting antibodies to these same proteins and therefore exposure can result in a positive result for both IFA and ELISA against *E. canis* (Chandrashekar et al., 2010).

***D. immitis* assay** – This analyte is derived from antibodies specific to the heartworm antigen. Sensitivity and specificity were 84% and 97% respectively, in a study investigating dogs with low heartworm burdens, when compared with necropsy (Atkins, 2003).

***L. infantum* assay** – This ELISA detects antibodies generated against *L. infantum* promastigotes. Using IFA as the reference standard, sensitivity and specificity are 91.1% and 99.2% respectively (Ferroglia et al., 2007).

3.4 Faecal floatation

Faecal samples were collected directly from the rectum at the time of general anaesthesia. They were stored at 4°C and faecal floatation performed within 48 hours of collection. Samples were processed by simple bench-top floatation technique using a saturated sodium chloride solution (NaCl, specific gravity 1.2) in July 2010 and a saturated sodium nitrate solution (NaNO₃ specific gravity 1.33) in August 2011, for a qualitative worm burden analysis. The species or genus of parasite was recorded, including *Trichuris vulpis*, *Dipylidium caninum*, *Toxocara canis*, *Cappilaria* spp. and *Sarcocystis* spp. Hookworm eggs were identified just as ‘hookworm’, as visual identification between the *Ancylostoma* spp. and *Uncinaria* spp. is not possible without measurement (Traub et al., 2004).

Approximately 3-5 grams of faeces were mixed with the pre-made floatation solution, and strained to remove any large particles. The solution was transferred to a 15 ml test tube. Additional floatation solution was added, if required, to bring the volume up to 15 ml, and to create a positive meniscus onto which a coverslip was placed. The solution was allowed to stand for a minimum of 10 minutes. The coverslip was removed and placed on a glass slide for examination by light microscopy as described elsewhere (Zajak and Conboy, 2012). The entire coverslip area was examined at 100x magnification, and 400x magnification used to aid identification. Samples were considered positive if one or more eggs were seen. Any remaining

faeces were then stored at -20°C pending analysis for *Giardia* and *Cryptosporidium*, following transportation to New Zealand.

3.5 Detection of *Cryptosporidium* and *Giardia* by direct IFA coproscopy

Direct IFA coproscopy was performed to detect *Giardia* and *Cryptosporidium* spp. cysts in faeces. Approximately 1 g of faeces was homogenized with 700 µl of 1 x phosphate-buffered saline in a 1.5 ml tube. This was vortexed at high speed for 30 seconds and left to stand for 10 minutes. A 50 µl aliquot was taken from this sample, pipetted onto a microscope slide and placed into a 37°C incubator until dry. To the slide, 50 µl of methanol was added and left to air dry for 10 minutes. The sample was then stained with 50 µl fluorescein isothiocyanate (FITC) stain (Aqua-Glo G/C, Waterborne Inc), placed in a humidity chamber and incubated at 37°C for 30 minutes. The slide was washed with 50 µl 1 x Phosphate Buffered Saline, and covered with mounting medium and cover slip. Detection was completed using a UV epi-fluorescent microscope with scanning at 200x magnification. Samples were considered positive if one or more cysts were seen.

3.6 Statistical analysis

Prevalence was calculated along with 95% confidence intervals for the individual diseases, using a binomial exact confidence interval. A Fishers exact test was used to test for differences in prevalence between geographical areas and age groups. This was chosen over a Chi-squared test due to the low number of positives in some groups. A *p*-value cut off of 0.05 was used to determine significance. The computer software R (<http://www.R-project.org/>) was used for all calculations.

Chapter 4 General results

4.1 Geographical distribution of samples

Of the 242 dogs enrolled on the study, 76 (31.4%) were from the Apia urban area, 110 (45.5%) were from rural Upolu and 56 (23.1%) were from Savai'i (Figures 4.1, 4.2 and Table 4.1).

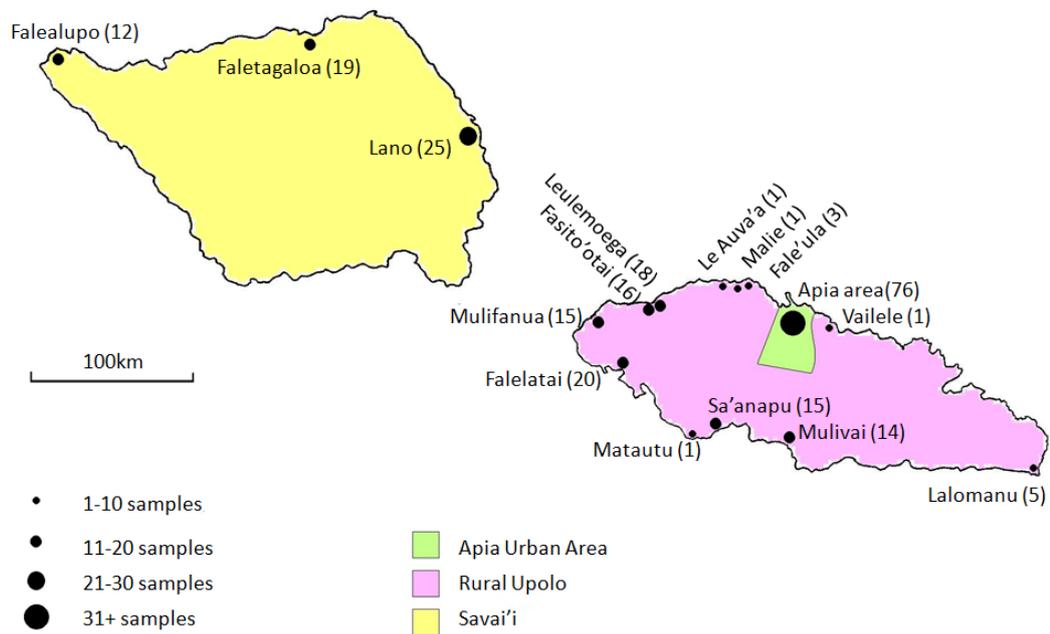


Figure 4.1: Map of Samoa marking the villages from which the 242 dogs sampled to study selected infectious diseases originated. The number of dogs sampled from each area is denoted by numbers in parentheses.

4.2 Questionnaire results

The study population totalled 242 dogs. There were 176 male dogs and 64 female dogs, and two dogs of unrecorded sex within the sample population. All the dogs sampled were the medium sized mixed breed "Samoan" dog type predominantly seen in Samoa. There were 12 stray dogs, with the remaining 230 dogs being owned. All owned dogs were stated to live outdoors. Age was known or estimated in 223 dogs with a range of four months to eight years, and 19 dogs were of unknown age. There were 56 dogs of less than 12 months of age, 70 dogs

were one year or older but less than two years old, 61 dogs were two years or over but less than three years, and 36 dogs were three years or over. The median age was one year.



Figure 4.2: Map of the Apia urban area marking the villages from which dogs sampled to study infectious diseases originated. The number of dogs sampled from each area is denoted by the number in parentheses.

Table 4.1: The household location of 242 dogs sampled to study selected infectious diseases compared to the Samoan household distribution from the 2011 Samoa census (Reupena, 2012b) and the household location of 327 respondents to a questionnaire concerning attitudes to dogs in Samoa (Farnworth et al., 2012).

Location	% in this study	% in 2011 Census	% in Farnworth study
Apia	31	20	15
Rural Upolu	46	57	53
Savai'i	23	23	23
Unknown			9

Only ten dogs from the study population of 242 had ever previously visited the APS veterinary clinic in Apia, and nine of these were from the Apia urban area, with the tenth dog from a nearby village. Nine of these dogs had visited for vaccination and worm treatment, with the remaining dog having presented for an illness. Only 14 dogs had ever received an anthelmintic treatment, and less than half of these had received it within the previous six months. All but two of these dogs were from within the Apia urban area. Ten dogs were reported to have ever received a flea treatment, although most owners did not know what product had been used or when. Four dogs were reported to have been treated two to three months previously. For all other dogs it was either unknown or reported as “long ago”. No dogs from Savai’i had ever been presented to a veterinarian, been vaccinated or treated with any anthelmintic or ectoparasiticide treatment.

Of the 242 dogs enrolled on the study, blood samples were collected from 237 dogs, faecal samples were collected for faecal flotation from 204 dogs and the skin and coat of 221 dogs was examined (Table 4.2).

Table 4.2: The demographics and tests performed on 242 dogs in the study of selected infectious disease of dogs in Samoa. The number and percentage (%) of dogs sampled for each variable of interest are provided.

Variable		Faecal sample examined (%) n=204	Skin examined (%) n=221	Total (%) n=242
Age	<1 year	46 (22.5)	53 (24.0)	56 (23.1)
	1 <2 years	59 (28.9)	60 (27.1)	70 (28.9)
	2 <3 years	52 (25.5)	56 (25.3)	61 (25.2)
	≥3 years	31 (15.2)	33 (14.9)	36 (14.9)
	Unknown	16 (7.8)	19 (8.6)	19 (7.9)
Sex	Male	149 (73.0)	161 (72.9)	176 (72.7)
	Female	53 (26.0)	58 (26.2)	64 (26.4)
	Unknown	2 (1.0)	2 (0.9)	2 (0.8)
Area	Apia urban area	66 (32.4)	65 (29.4)	76 (31.4)
	Rural Upolu	89 (43.6)	102 (46.2)	110 (45.5)
	Savai'i	49 (24.0)	54 (24.4)	56 (23.1)
Lifestyle	Stray	5 (2.5)	12 (5.4)	12 (5.0)
	Owned	199 (97.5)	209 (94.6)	230 (95.0)
Previous	Ever visited vet	8 (3.9)	9 (4.1)	10 (4.1)
Veterinary	Ever vaccinated	7 (3.4)	8 (3.6)	9 (3.7)
Treatments	Ever dewormed	12 (5.9)	13 (5.9)	14 (5.8)

Chapter 5 Prevalence of selected external and intestinal parasites in Samoan dogs

5.1 Results

5.1.1 Faecal examination results

Of the 242 dogs enrolled on the study, 204 had faecal samples collected and analysed by faecal flotation. In the remaining dogs either the faecal sample obtained was too small to test or there were no faeces in the rectum to collect. Ninety-three of the collected faecal samples were then frozen, transported to New Zealand and analysed for *Giardia* and *Cryptosporidium* spp. at a later date. In the remaining 111 cases, the faecal sample collected was only large enough to complete faecal flotation, or was too liquid to transport.

Faecal examination was positive in 190 of the 204 samples tested (93.1%). Hookworm eggs were detected in 185 dogs, giving an overall prevalence of 90.7% (95% CI=85.6-94.2). *Giardia* was the next most common parasite detected, with 27 dogs positive, giving a prevalence of 29% (95% CI=20.1-39.4). *Trichuris vulpis* and *Dipylidium* spp. eggs, coccidial oocysts, *Toxocara canis* and *Capillaria* spp. eggs, and *Sarcoystis* sporocysts were also detected in some dogs. There were no positive results for *Cryptosporidium* spp. (Table 5.1 and Figure 5.1).

Table 5.1: Faecal examination data from the study of 242 dogs in Samoa sampled for selected infectious diseases. The number of positives/total tested (n), prevalence and 95% confidence intervals for faecal examination results are provided.

Parasite	Positive /n	Prevalence	Confidence Interval (95%)
Hookworm	185/204	90.7%	85.6-94.2%
<i>Trichuris vulpis</i>	14/204	6.9%	3.9-11.5%
<i>Dipylidium caninum</i>	9/204	4.4%	2.2-8.5%
Oocysts	9/204	4.4%	2.2-8.5%
<i>Toxocara canis</i>	7/204	3.4%	1.5-7.2%
<i>Capillaria spp.</i>	4/204	2.0%	0.6-5.3%
<i>Sarcocystis sporocyst</i>	1/204	0.5%	0.0-3.1%
<i>Giardia spp.</i>	27/93	29.0%	20.1-39.4%
<i>Cryptosporidium spp.</i>	0/93	0%	0-3.9%

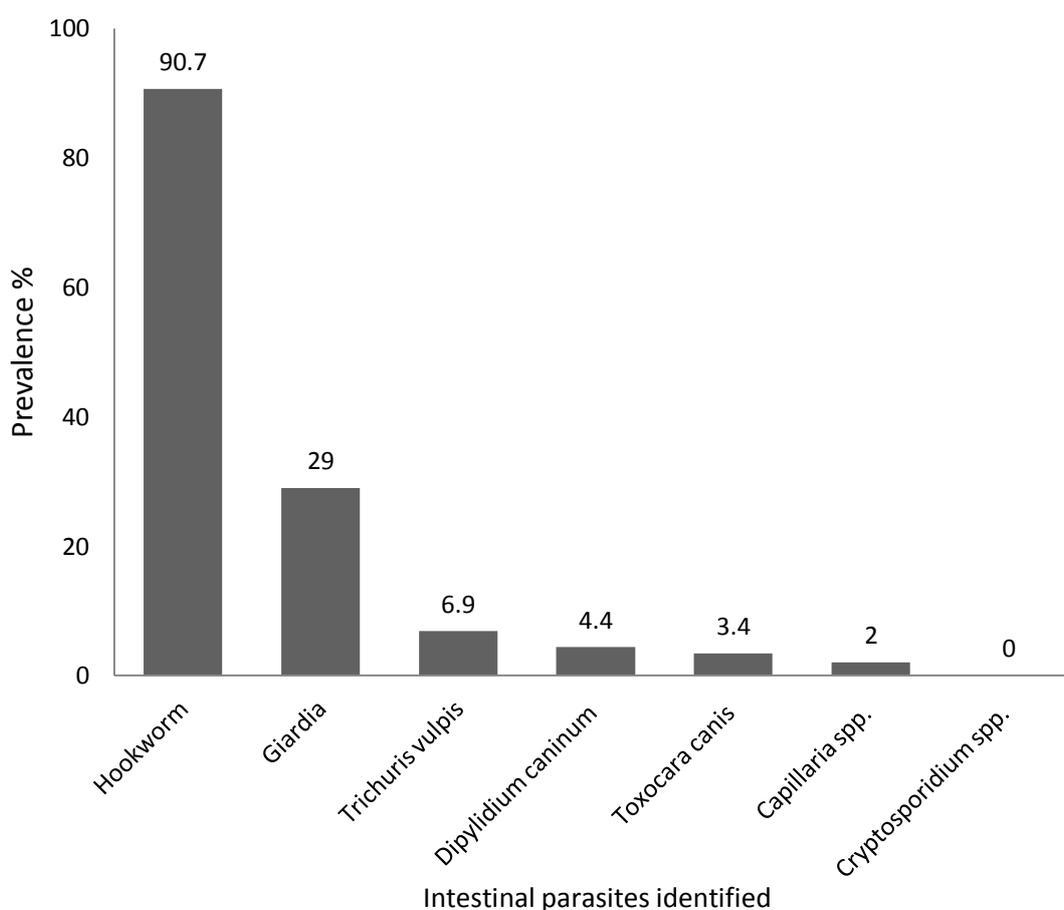


Figure 5.1: Prevalence of intestinal parasites in faecal examination from 242 dogs from Samoa.

5.1.2 Skin examination results

Of the 242 dogs in the study, 221 (91.3%) had a skin examination. In 210 dogs examined, external parasites were detected (95.0%). Fleas were present on 185/221 dogs (83.7%) and lice were detected on 18/221 dogs (8.1%) (Table 5.2 and Figure 5.2). The lice species identified was *Trichodectes canis*. Ticks were present on 93/221 dogs (42.1%) and where a positive identification was made, the species identified was *Rhipicephalus sanguineus*. In a few cases no identification was made. No *Ixodes* spp. ticks were found.

Table 5.2: Skin examination data from the study of 242 dogs in Samoa sampled for selected infectious diseases. Number of positives/total tested (n), prevalence and 95% confidence intervals for external parasites detected on skin examination are provided.

Parasite	Positive/n	Prevalence	Confidence Interval (95%)
Ticks	93/221	42.1%	35.5-48.9%
Fleas	185/221	83.7%	78.2-88.3%
Lice	18/221	8.1%	4.9-12.6%

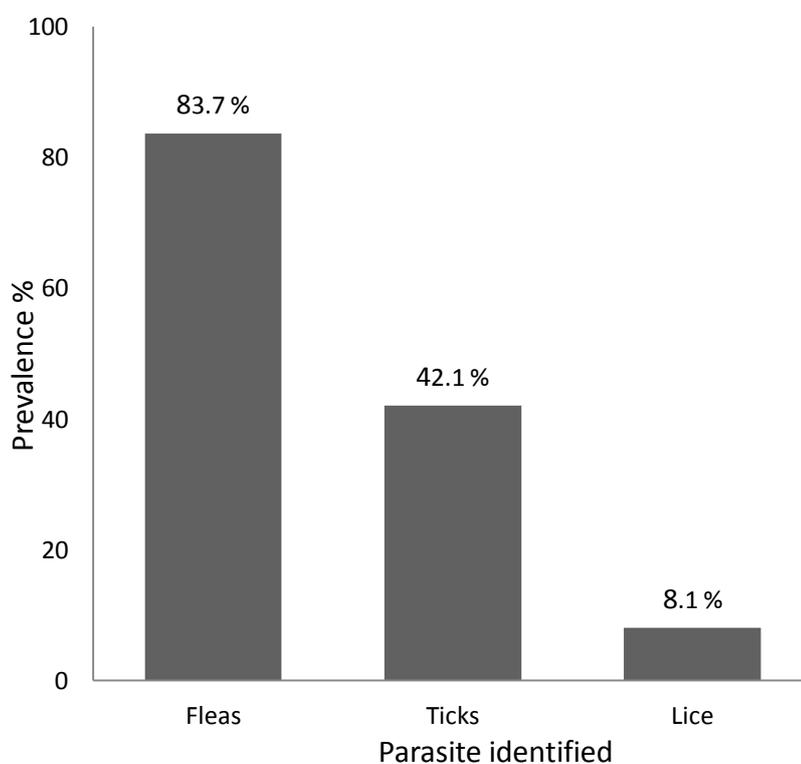


Figure 5.2: Prevalence of external parasites on 221 dogs from Samoa.

There was a significant difference in the prevalence of *R. sanguineus* between the three main areas ($p=0.013$), with significantly more dogs in Savai'i infested with ticks than on rural Upolu or urban Apia, with no difference between the latter two areas (Table 5.3).

Table 5.3: Number of positives/total tested (n), prevalence and 95% confidence intervals for *Rhipicephalus sanguineus* infestations of dogs for each area. Data from the study of 242 dogs in Samoa sampled for selected infectious diseases.

Area	Positive/n	Prevalence	Confidence Interval (95%)
Urban Apia	25/65	38.5%	26.7 – 51.4%
Rural Upolo	36/102	35.3%	26.1 – 45.4%
Savai'i	32/54	59.3%	45.0 – 72.4%

5.2 Discussion

Faecal floatation was positive in 93.1% (190/204) of samples. The vast majority of samples were positive for hookworm, with or without other parasites (Table 5.1). The overall prevalence for hookworm was extremely high, at 90.7% (185/204). Hookworm infection has rarely been documented at this high prevalence, but these results are comparable with an epidemiological study of dogs from tea growing regions of India, which demonstrated a hookworm prevalence of 93% by conventional faecal flotation methods. The dogs in that study had similarly low levels of veterinary care with only 2% de-wormed in the previous six months (Traub et al., 2002). The tropical climate in Samoa is ideal for the development and transmission of hookworm larvae, as is a large and dense roaming canine population (Traub et al., 2004). In addition, with only 5.8% (14/242) of dogs ever having received an anthelmintic treatment and only 1.7% (4/242) within the last 3 months, such a high prevalence is not surprising (Table 4.2). The species of hookworm egg was not determined in this study, however egg measurements taken on a third visit to Samoa in 2012 (Beckman et al., personal communication) suggest that the species is an *Ancylostoma* spp. which is what would be expected in a tropical climate (Prociv, 2003). A highly sensitive and species-specific PCR-RFLP test can be used to determine the *Ancylostoma* spp. directly from eggs in faeces (Traub et al., 2004), and this would be a valuable extension of this study.

An extremely high prevalence of hookworm infection in dogs could play a significant role in contributing to the incidence of HrCLM in human populations. The effect of hookworm on the Samoan or tourist population is hard to establish as there are no studies or reports in the literature of this kind. A German study investigating the most frequently diagnosed infectious diseases reported in 890 young people returning from the tropics and subtropics demonstrated cutaneous larva migrans (CLM) was the sixth most common disease diagnosed (Herbinger et al., 2012). In another French study of dematoses in patients following travel to the tropics, CLM was diagnosed in 4.8% of patients, however very few subjects in this study had visited Oceania (2.4%) (Ansart et al., 2007). Anecdotally, one of the eight students working on the 2010 sterilisation campaign with the APS developed suspected HrCLM lesions on the foot, following two weeks in Samoa. However, a brief informal survey of dermatologists in New Zealand, several of whom have worked for dermatology clinics in Samoa itself, revealed that none recall having seen HrCLM lesions in Samoans or tourists returning from Samoa (Rademaker, personal communication). Clearly further investigation is required to establish the incidence of HrCLM or other manifestations of hookworm infection in humans in Samoa and to determine the effect of such a high prevalence in the canine population. All *Ancylostoma* species are capable of producing HrCLM in humans, although it is *A. braziliense* that is most commonly implicated and produces the most severe and prolonged lesions (Prociv, 2003).

Given that canine hookworm is highly endemic among dogs in Samoa, precautions to prevent human infection should be recommended to both local and tourist populations. Current recommendations for endemic areas include wearing protective footwear when walking on the beach or contaminated soil. When lying on tropical beaches potentially frequented by dogs, areas of sand that have been washed by the tide are preferable to the dry sand, and mattresses to towels. Avoiding beaches frequented by dogs or banning animals from the beach has been shown to be very difficult (Hochedez and Caumes, 2007), however there are some dog free villages and resorts in Samoa which may pose less of a public health threat. Importantly, any suspect lesions should be investigated and treated by a medical practitioner.

Giardia was also shown to be endemic in Samoan dogs, with a prevalence of 29% (27/93) (Table 5.1). The parasite is found in dogs worldwide, and prevalence studies have varied from very low to as high as 55.2%. The higher prevalences tend to occur in studies examining shelter and kennelled dogs (Itoh et al., 2005; Upjohn et al., 2010), in younger populations, and in winter months (Fontanarrosa et al., 2006; Gates and Nolan, 2009b; Little et al., 2009). Some recent studies have shown that *Giardia* spp. cysts are becoming more commonly detected in

dogs than helminth eggs, though usually without any overt clinical signs of infection (Little et al., 2009; Palmer et al., 2008a). Dogs can harbour *Giardia* spp. infections of both host-specific and zoonotic assemblages and the zoonotic importance of infection in dogs depends on the assemblages isolated (Caccio and Ryan, 2008). Determination of the *Giardia* assemblage requires PCR on DNA extracted from the faeces (Caccio and Ryan, 2008). Further research into the assemblages carried by dogs in Samoa is needed to assess the zoonotic potential and the importance it may have to public health. Giardiasis is the most common parasitic infection affecting humans worldwide, with the majority of infections acquired by drinking contaminated water sources. Children are most at risk of contracting the infection. As with dogs, many human infections are asymptomatic, however when symptoms do occur they can range from an acute to intermittent to chronic non-bloody diarrhoea (Thompson, 1998b). The majority of Samoan dogs are roaming and free to defaecate anywhere, so there is potential for contamination of water or food sources if the assemblages are found to be zoonotic. However even the presence of a potentially zoonotic assemblage does not confirm zoonotic transmission. Ideally, a longitudinal study that tests and subtypes at multiple loci all positive *Giardia* samples from both pets and people in the same setting, would be needed to definitively determine the role of dogs in the transmission of *Giardia* to humans (Ballweber et al., 2010). Recommendations to prevent *Giardia* infection in humans include maintaining good personal hygiene and hand washing, especially when preparing food, avoiding contaminated drinking water and cleaning up and disposing of dog faeces (Anonymous, 2012c).

No *Cryptosporidium* spp. oocysts were detected from any of the 93 faecal samples tested by direct IFA coproscopy (Table 5.1). Many prevalence studies in dogs have demonstrated a worldwide distribution with prevalences ranging up to 45% (Batchelor et al., 2008; Bugg et al., 1999; Hackett and Lappin, 2003; Lallo and Bondan, 2006; Scorza and Lappin, 2012a; Shukla et al., 2006), and in the environment *Cryptosporidium* oocysts are highly resistant, persisting in wet cool conditions for six months or longer (Xiao and Fayer, 2008). It might therefore be reasonably hypothesised that *Cryptosporidium* spp. be detected in Samoan dogs. Human studies have shown the direct IFA used in this study is a more sensitive and specific test than unconcentrated faecal smears, cytologic staining methods and faecal ELISAs (Scorza and Lappin, 2012a; Weber et al., 1991), however the test has not been completely validated for use in dogs, which shed lower numbers of oocysts than humans, and therefore could result in false negative results (Scorza and Lappin, 2012a). In addition, it is recommended that faecal samples requiring storage prior to testing by direct IFA coproscopy are fixed with 10% formalin, as this does not interfere with immunodetection methods for *Cryptosporidium* spp.

(Little and Lindsay, 2012), although the evidence for the basis of this statement remains elusive. It is therefore possible that storage of the samples in this study by freezing at -80°C and storage of some samples for periods of over two years prior to testing may have affected the results of this test. This may also have affected the *Giardia* results of this study. Before reaching the conclusion that *Cryptosporidium* infection in Samoan dogs occurs at a very low prevalence level or the organism is absent, future studies should examine faecal samples in a shorter time frame and with appropriately stored faeces.

Preventative healthcare of dogs in this study was very low with only 14 dogs (5.8%) reported as ever having been de-wormed and only 4 of these (1.7%) within the last 3 months (Table 4.2). Comparing these results with a questionnaire based study by Farnworth et al. 2012, on attitudes towards dog management in Samoa there are some similarities in the populations selected; 71% of dogs in that study were male, compared to 72.7% (176/242) in the current study. The geographical distribution of samples across both studies was fairly equivalent and is shown in Table 4.1 compared with the Samoan household distribution in the 2011 census. However there were also some differences, especially relating to previous veterinary treatments; 72% of dogs had never visited a vet, compared to 95.9% (232/242) in the current study and 12% had ever been vaccinated, compared to 3.7% (9/242) in the current study. In the Farnworth study 19% of dogs were already sterilised, suggesting that these dogs had already been presented to the APS clinic or previous mobile clinic for sterilisation. The lower figures for previous veterinary care in the present study may indicate that by selecting dogs through a free mobile sterilisation clinic, as opposed to randomly, dogs are less likely to have had any prior veterinary care. These dogs may be more likely to have certain parasitoses, therefore some prevalences may be higher in this study population than compared with that of the general Samoan dog population. People who regularly de-worm and vaccinate their dogs may be more likely to take their dog to the vet to be sterilised, rather than wait until a mobile clinic visits their village; these animals would have been excluded from this study.

In Samoa, where there is only one veterinary care provider in one location (Apia, the capital city), access to antiparasiticides is difficult. Guidelines provided by the International Companion Animal Management Coalition (ICAM) (Anonymous, 2007a), on vaccination and parasite treatment as part of humane dog population control, recommend that treatments are provided in conjunction with education about responsible ownership, sterilisation and registration or identification. Mobile 'camps' or clinics can be very effective at drawing attention to the importance of preventative treatments, but there is a limit to the distance the general public will travel for such a service. This is very clear in this study, for no dogs from the

island of Savai'i, where there is no regular veterinary companion animal service, had ever seen a veterinarian or received any preventative care previously.

The range of other intestinal parasites in this study is similar to those reported in previous prevalence studies around the world (Fontanarrosa et al., 2006; Little et al., 2009; Palmer et al., 2008a). However the prevalence rates in this study were surprisingly low given the relative absence of preventative veterinary care, with *Trichuris vulpis* (6.9%, 14/204), *Dipylidium caninum* (4.4%, 9/204), *Toxocara canis* (3.4%, 7/204), *Capillaria* spp. eggs (2.0%, 4/204) and *Sarcocystis* sporocysts (0.5%, 1/204) all detected (Table 5.1). The low levels of *D. caninum* are surprising given that fleas, involved in the lifecycle, were detected on 83.7% (185/221) of dogs examined (Table 5.2). A low detection rate may be the cause of this result, as faecal flotation has poor detection sensitivity for this particular parasite (Conboy, 2009). For 38 dogs in the study, faecal samples were not collected. This was generally due to the rectum being empty, or the presence of severe watery diarrhoea making collection of an adequate sample impossible. In cases of severe diarrhoea, parasitism such as with *T. vulpis* or giardiasis may have been a cause, but results from these dogs were not able to be included in the study, which could also have falsely lowered the prevalence reported. *T. canis* was detected in 3.4% of samples, a surprisingly low prevalence given the relative lack of preventative veterinary care (Table 5.1). All seven faecal samples positive for *Toxocara* eggs were from young dogs; one aged one year, four aged six months or less. The remaining two positive samples were from dogs without a recorded age, however both dogs were small (10kg) and on the basis of no small breeds being included in the study, can be considered immature. Previous studies have reported *T. canis* infections in dogs throughout the world, with some very high prevalences recorded; a study of 269 dogs in Nigeria reported 33.8% dogs positive on faecal examination, with a prevalence of 51.4% in puppies up to 6 months (Sowemimo, 2007). A similarly high level was seen in a Chinese study, where 45.2% of dogs slaughtered in abattoirs were positive for adult worms (Dai et al., 2009).

The eggs of *T. canis* are unembryonated when shed. Optimal conditions for their development are temperatures of 25 to 30 °C, and a relative humidity of 85 to 95%. Under these conditions eggs can develop to the infective L3 stage in as little as 9 to 15 days (Schnieder et al., 2011). In Samoa, temperatures vary little with an average of 27 °C (range 23 to 30 °C) throughout the year. The rainy season is centred around January with the dry season centred around July and August, although monthly precipitation is still approximately 80-150mm in these months (Anonymous, 2012a). Climate conditions in Samoa seem ideal for the larval development of *T.*

canis. However sampling for this study was only carried out in the middle of the dry season and there is some evidence to suggest that there are seasonal variations in prevalence with higher prevalences being recorded in winter months or wet seasons (Sowemimo, 2007). Although generally *T. canis* eggs are very resistant in the environment, temperatures over 30-35°C and desiccation will kill eggs in the environment (Lloyd, 1998a), and it is possible that conditions in Samoa in fact do not allow persistence of the eggs in the environment, thereby reducing the risk of infection. The effect of any seasonal changes on *T. canis* infection in Samoan dogs could be further investigated by sampling dogs throughout the year, which may also confirm whether the prevalence of *T. canis* is truly as low as reported in this study.

Age resistance is also seen with *T. canis* infection in dogs, due to the development of immune competence and acquired immunity (Schnieder et al., 2011). A study comparing experimental infection with *T. canis* of 3 week, 3 month and one year old dogs from an ascarid naive colony, showed that almost all larvae in the 3 week old puppies had migrated through the tracheal migration route. In contrast, most larvae in the 3 month old and one year old group were discovered in granulomas in the tissue. It was therefore concluded that the likelihood of somatic migration progressively increases from the age of 3 months onwards (Greve, 1971; Schnieder et al., 2011). In the current study, no dogs under the age of 4 months were sampled as they were estimated less than 10kg. It is therefore possible that the prevalence of 3.4% is falsely low, and may in fact be higher when younger dogs are sampled. The possibility of missing *T. canis* eggs present in faecal samples examined by faecal flotation methods is unlikely. When present, *T. canis* eggs are large and obvious and usually present in large numbers (Lloyd, 1998a), therefore this is likely to be a true prevalence within the population sampled. These results do show that *T. canis* is present in the canine population in Samoa, and with most dogs free to roam and defaecate in areas populated by humans, contamination of soil with eggs from infected dogs is inevitable. In addition to infection from the environment, there is evidence to suggest that embryonated eggs on the coats of infected dogs may also be a source of human toxocariasis to in contact people (Aydenizoz-Ozkayhan et al., 2008). Although most infections in humans remain asymptomatic, the consequences of VLM and OLM caused by *T. canis* infection can be permanent and very serious, especially in young children (Rubinsky-Elefant et al., 2010). Examination of faecal samples from puppies in this youngest of age groups would be useful in determining the true importance of this zoonotic disease in Samoa, as would examination of soil in village areas for contamination with *T. canis* eggs.

There was also a very high prevalence of external parasites on dogs in this study, with 95% (210/221) of dogs having some kind of external parasitism. Fleas were the most common

parasite detected, with fleas detected on 83.7% (185/221) of dogs examined (Table 5.2). This is unsurprising given the Samoan climate and lack of flea prevention used. The owners of only 10 dogs reported to have ever used a flea treatment on their dogs, though most owners could not say what product was used or when. Lice infestations were detected on 8.1% (18/221) of dogs, all were the species *Trichodectes canis*. Fleas and lice cause direct harm to their hosts in the form of irritation, blood loss, allergy and secondary skin infection. They are also intermediate hosts for some tapeworms, such as *Dipylidium caninum* (Blagburn and Dryden, 2009), and several pathogens have been isolated from fleas, such as *Leishmania infantum* and *Borrelia burgdorferi*, though their competence as a vector has not been proven (Piesman and Happ, 1997; Quinnell and Courtenay, 2009).

Ticks were the second most common ectoparasite detected, with 42.1% (93/221) of dogs infested, and all ticks positively identified were *R. sanguineus* (Table 5.2). A small number of ticks were not identified due to the poor quality of photographic record; these may have been *R. sanguineus* or another species of tick. *R. sanguineus* is an important vector of canine diseases worldwide, with *Ehrlichia canis*, *Babesia canis*, *Anaplasma platys*, *Mycoplasma hemocanis*, *Rickettsia conorii* in Europe, Asia and Africa, and Rocky Mountain spotted fever in the USA all reported to be transmitted by the tick (Dantas-Torres, 2008; Senevira et al., 1973). Some of these are important zoonoses as well as causing disease and potentially death in the infected dog. The exposure of dogs to *E. canis* and *A. platys* was evaluated in this study (Figure 6.1) however the investigation of other tick-borne pathogens known to be transmitted elsewhere by this vector may be valuable in further evaluating canine health in Samoa.

There was a higher prevalence of tick infestations on dogs in Savai'i compared with both urban and rural Upolu (Table 5.3). However the study's use of convenience rather than random sampling makes these comparisons less reliable. Some conclusions may still be able to be drawn however, and these results could indicate that dogs living on Savai'i are more at risk of diseases carried and spread by this tick. The higher prevalence could be explained by the fact that as a separate island, access to the veterinary care provided by the APS for dog owners on Savai'i is much harder; no dogs from Savai'i were reported as having any veterinary treatment or preventative healthcare products given. However the rate of any preventative care in this study was very low for all regions and where flea treatments were reported as used, it was not with any frequency and it was not established whether these products would have been effective against ticks as well. Habitat in which the dog lives and elevation above sea level have both been shown to have an effect on the tick infestation prevalence and density, as much of the ticks development and survival depends on off-host conditions (Wells et al.,

2012). However all the villages sampled in this study were close to sea level with similar climates and there was little difference in environmental conditions and habitat between villages on Savai'i and rural Upolu. The difference in prevalence detected in this study is hard to explain, and there is the possibility, given the small sample sizes and convenience sampling, that the trends seen may just be due to chance. Any relationship would need to be clarified with a larger scale, randomised study.

Intestinal and external parasitism is clearly a problem in the canine population in Samoa, with currently very few preventative measures taken. Some of the intestinal parasitic diseases identified in this study are known zoonoses, as well as causing disease in the dogs themselves and potentially death in young puppies. A high prevalence of infestation with *R. sanguineus* is an important finding due to its role in transmission of certain vector borne diseases. Preventative measures against both intestinal and ectoparasites can be recommended for both canine health and to help reduce environmental burden in village areas and therefore reduce public health risk. Much research has been done in Europe and the USA into and recommendations for gold standard in preventative care for canine and feline parasites have been developed. Chapter 7 discusses these in further detail and poses potential recommendations for preventative care in Samoan dogs.

Chapter 6 Prevalence of selected vector-borne diseases in Samoan dogs

6.1 Results

6.1.1 Descriptive results

Of the 242 dogs enrolled on the study, 237 had blood samples taken that were analysed. The following results are from those 237 dogs (Table 6.1). There were 171 male dogs and 64 female dogs, and two dogs of unrecorded sex within the sample population. All the dogs sampled were the mixed breed “Samoan” dog type predominantly seen in Samoa. There were 12 stray dogs, with the remaining 225 dogs being owned. All owned dogs were reported to live outdoors. Age was known or estimated in 218 dogs with a range of four months to eight years, and 19 dogs were of unknown age. There were 53 dogs of less than 12 months of age, 68 dogs were one year or older but less than two years old, 61 dogs were two years or over but less than three years, and 36 dogs were three years or over. The median age was one year. Samples were obtained from all three regions with 75 dogs sampled from the urban Apia area, 109 dogs from the rural Upolu area and 53 dogs from Savai’i.

Of the 237 dogs in the sample population, only ten had ever visited the APS veterinary clinic in Apia, nine of them for vaccination, and one for treatment for an illness. The vaccine used by the APS is the core vaccine against canine distemper virus, infectious canine hepatitis and canine parvovirus. Of these ten dogs, nine were from the urban Apia area, and the tenth from a neighbouring village just outside. Fourteen dogs had ever been treated with an anthelmintic, and less than half of these received this treatment within the previous six months. No dogs from Savai’i had ever received a vaccination, worm or flea treatment.

Twenty dogs tested positive for *Anaplasma* antibodies by ELISA, giving an overall prevalence of 8.4% (95% CI=5.2-12.7). For heartworm antigen, 111/237 dogs were positive by ELISA, giving an overall prevalence of 46.8% (95% CI=40.3-53.4). There were no positive results for *B. burgdorferi*, *E. canis* or *L. infantum*, by antibody ELISA, giving a prevalence of 0.0% (95% CI=0-1.5%) for each of these diseases (Figure 6.1).

Table 6.1: Number (N) and percentage (%) of 237 dogs sampled in the study of selected infectious disease of dogs in Samoa for each variable of interest, and number and percentage (%) of samples with a positive ELISA test result for heartworm antigen and *Anaplasma* antibody for each variable.

Variable		N (%) n=237	Heartworm (%)	Anaplasma (%)
Age	<1 year	53 (22.4)	10 (18.9)	4 (7.5)
	≥1, <2 years	68 (28.7)	30 (44.1)	7 (10.3)
	≥2, <3 years	61 (25.7)	35 (57.4)	6 (9.8)
	≥3 years	36 (15.2)	24 (66.7)	3 (8.3)
	Not recorded	19 (8.0)	7 (36.8)	0 (0.0)
Sex	Male	171 (72.2)	88 (51.5)	17 (9.9)
	Female	64 (27.0)	22 (34.4)	3 (4.7)
	Unknown	2 (0.8)	1 (50.0)	0 (0)
Area	Urban Apia area	75 (31.6)	18 (24.0)	10 (13.3)
	Rural Upolu	109 (46.0)	54 (49.5)	3 (2.8)
	Savai'i	53 (22.4)	39 (73.6)	7 (13.2)

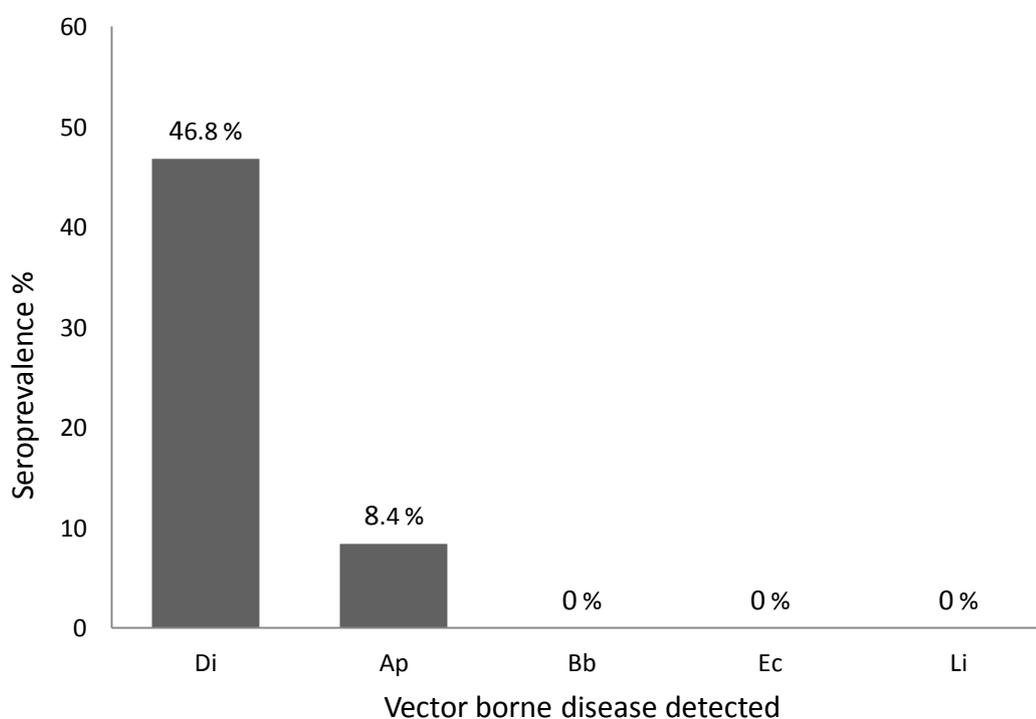


Figure 6.1: The seroprevalence of selected vector borne diseases by ELISA in 237 Samoan dogs. *Dirofilaria immitis* (Di), *Anaplasma* (Ap), *Borrelia burgdorferi* (Bb), *Ehrlichia canis* (Ec) and *Leishmania infantum* (Li).

6.1.2. Association between prevalence of canine heartworm and age and area

There was a significant association with both age and area of Samoa the dog was from, and the seroprevalence of heartworm disease. There was a statistically significant difference ($p < 0.001$) between the age categories (Table 6.2 and Figure 6.2), with older dogs having a higher seroprevalence than younger dogs. There were significant differences between all age categories except between the second and third, and the third and the fourth age categories.

Table 6.2: Number of positives/total tested (n), prevalence and 95% confidence intervals for 237 Samoan dogs with positive heartworm antigen tests within the four age categories.

Age category	Positive/n	Prevalence	Confidence Interval (95%)
<1 year	10/53	18.9%	9.4 – 32.0%
1 <2 years	30/68	44.1%	32.1 – 56.7%
2 <3 years	35/61	57.4%	44.1 – 70.0%
≥ 3 years	24/36	66.7%	49.0 – 81.4%

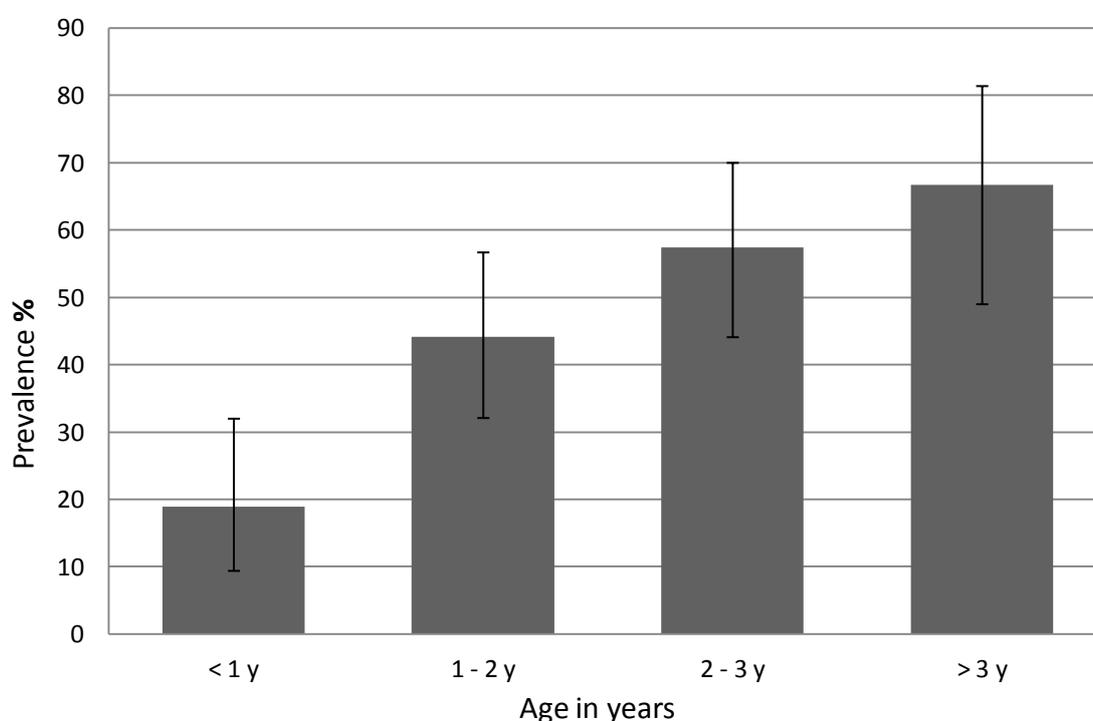


Figure 6.2: The seroprevalence of heartworm antigen in 237 Samoan dogs by age with 95% error bars. There was a significant difference between the four age groups ($p < 0.001$) using a Fisher's Exact Test, with dogs having an increasing risk of heartworm infection with age.

There was also a statistically significant difference ($p < 0.001$) between all the areas, with the urban Apia area having the lowest and Savai'i having the highest seroprevalence (Table 6.3 and Figure 6.3). Urban Apia is the main town and the base of the APS, the only veterinary care available to Samoans. Both rural Upolu and Savai'i consist mainly of small villages, predominantly coastal. Savai'i, the second island, is a more remote area with no ready access to veterinary care or medicines.

Table 6.3: Number of positives/total tested (n), prevalence and 95% confidence intervals for 237 Samoan dogs with positive heartworm antigen tests within three Samoan geographical areas.

Area	Positive/n	Prevalence	Confidence Interval (95%)
Urban Apia	18/75	24.0%	14.9 – 35.3%
Rural Upolu	54/109	49.5%	39.8 – 59.3%
Savai'i	39/53	73.6%	59.7 – 84.7%
Total	111/237	46.8%	40.3 – 53.4%

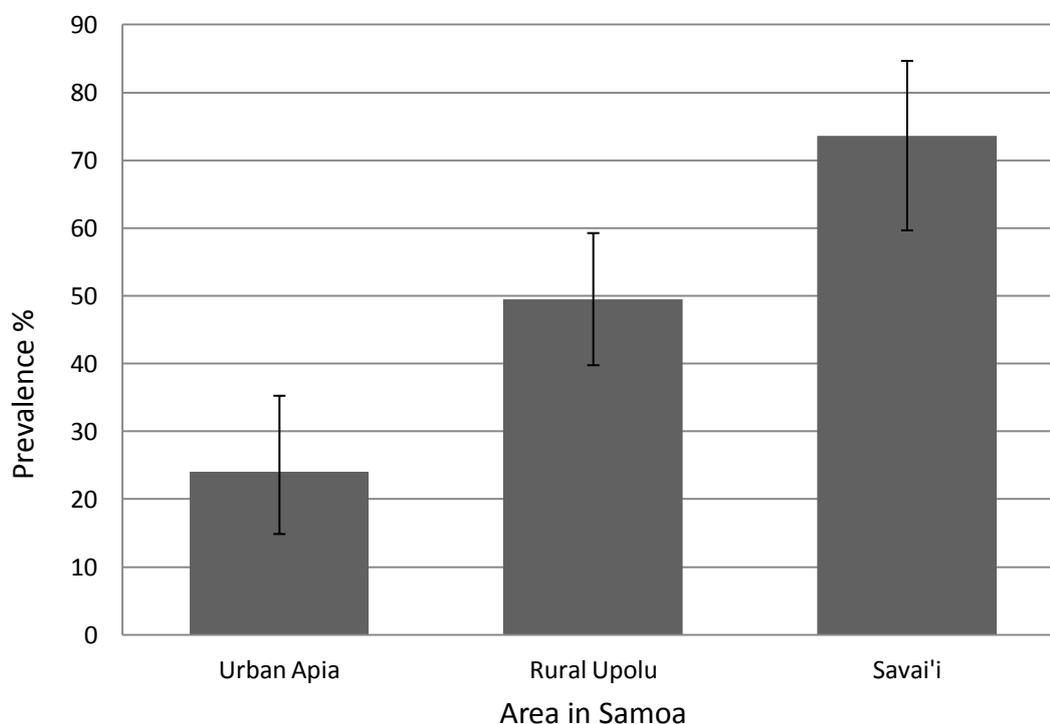


Figure 6.3: The prevalence of heartworm in 337 dogs in three main areas of Samoa with 95% error bars. There was a significant difference between the three areas ($p < 0.001$) using a Fisher's Exact Test.

On evaluation of the results, it appeared that there was a trend for dogs sampled from rural Upolu and Savai'i to be slightly older than those from the urban Apia area. An ANOVA was used to evaluate the difference in heartworm disease between the areas taking into account any effect of age, and there was still a significant difference ($p < 0.001$) (Figure 6.4).

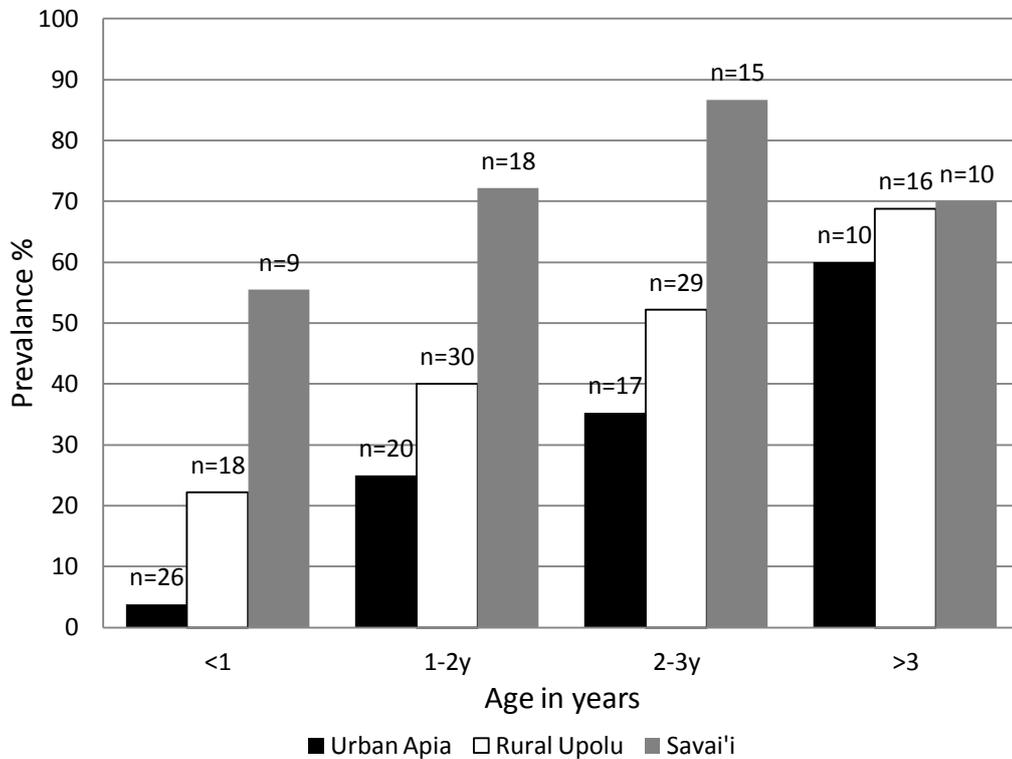


Figure 6.4: The prevalence of heartworm in 237 dogs in three main areas of Samoa, divided into age groups. There was a significant difference between the three areas ($p < 0.001$) when any effect of age was accounted for (ANOVA).

6.1.3. Association between seroprevalence of *Anaplasma* spp. and area

There was a statistically significant difference ($p=0.009$) in seroprevalence of *Anaplasma* spp. between the areas, with the urban Apia area and Savai'i having a higher seroprevalence than rural Upolu, though with no difference between the urban Apia area and Savai'i (Table 5.4).

Table 6.4: Number of positives/total tested (n), prevalence and 95% confidence intervals for 237 Samoan dogs with positive *Anaplasma* ELISA tests within three Samoan geographical areas.

	Positive/n	Prevalence	Confidence Interval (95%)
Urban Apia	10/75	13.3%	6.6 – 23.2%
Rural Upolu	3/109	2.8%	0.6 – 7.8%
Savai'i	7/53	13.2%	5.5 – 25.3%
Total	20/237	8.4%	5.2 – 12.7%

6.2 Discussion

This study is the first to provide epidemiological data on vector-borne diseases of dogs in Samoa. The overall prevalence of *D. immitis* in dogs in this study was 46.8% (111/237), a high prevalence for this parasitosis (Figure 6.1). The data from the questionnaire highlight the fact that heartworm prophylaxis in dogs in Samoa is very rare, and the climate in Samoa is ideal for both development of the microfilariae and the maintenance of a mosquito vector population. Therefore a *D. immitis* prevalence rate of this magnitude can be expected in an endemic area, with a large canine population and minimal prophylaxis. Prevalences ranging from 22.4-86% have been seen in previous studies in the Asia-Pacific region in countries with a similar climate and conditions: in a study conducted in Papua New Guinea, 86% of dogs were positive for heartworm disease on necropsy (Hamir and Onaga, 1986); in New Caledonia prevalences of 22.4-66% have been reported in the stray dog population (Watier-Grillot et al., 2011); and in Hawaii a study confirmed a prevalence of 32% in dogs by microfilarial testing (Gubler, 1966). Dogs housed outside, as were all the Samoan dogs in this study, have also been shown to be at increased risk of *D. immitis* infection, due to increased exposure to the mosquito vector (Miller and Crosbie, 2011).

There was a significant difference in the prevalence of *D. immitis* between the different age groups, with seropositivity increasing with age in the sample population (Table 6.2 and Figure 6.2). The highest prevalence rate of 66.7% (24/36) was seen in the oldest age category, dogs aged three years and over. This relationship has been seen in previous studies, with higher

prevalences recorded in dogs over three (Montoya et al., 1998) and four years old (Lefkaditis et al., 2010), and age considered an important risk factor by some authors (Montoya et al., 1998; Selby et al., 1980), attributed to prolonged exposure of dogs to mosquitoes and a lack of prophylactic measures (Montoya-Alonso et al., 2010). The age group with the lowest prevalence was dogs aged less than one year old, but for a disease with a prepatent period of seven months, a prevalence of 18.9% (10/53) is still very high for this age group (Nelson, 2012). This suggests that dogs are being exposed to infected mosquitoes from a very early age, and veterinarians must be aware that dogs as young as eight or nine months old may already be infected with *D. immitis*.

Inclusion in this study depended on presentation for sterilisation, so it could be assumed that dogs sampled may not be representative of the age of the general Samoan dog population. The median age of dogs recruited was one year old. The APS has been operating in Samoa since 1994, and in recent years has held regular free sterilisation campaigns as well as offering cost price surgery in the Apia clinic at other times. Therefore it may be assumed that older dogs are more likely to have been sterilised already, shifting the sample population age to the left. However, despite this service, sterilisation rates in Samoa still remain low, with a recent questionnaire study showing only 19% of owned dogs were sterilised (Farnworth et al., 2012). This may in part be due to the difficulty in accessing the veterinary services offered by the APS for some people in remote rural locations. Samoan dogs also receive very little preventative or veterinary care, and may generally have a short life expectancy. Taking this into consideration, the age distribution seen in this study is likely to be closer to that of the general population than might otherwise be expected. If indeed this study population is young, then the real prevalence of canine heartworm disease of dogs in Samoa is in fact likely to be even higher than our finding of 46.8%, given that risk increases with age.

There was a significant difference between the Apia urban area, where the lowest regional prevalence of 24% (18/75) was seen, and the other two more rural and beach side regions of rural Upolu and Savai'i (Table 6.3). There was no significant difference between rural Upolu's prevalence of 49.5% (54/109) and the prevalence in Savai'i of 73.6% (39/53). There is little difference in the climate between these three regions, but the increased prevalence in rural regions might be due to a higher mosquito density and therefore increased risk of mosquito bites. Access to veterinary care and products is also harder for dog owners in rural communities, and this is evident in the fact that almost all dogs that had ever received vaccines or anthelmintics were from within the Apia urban area. This, albeit mildly, increased access to veterinary medications and care might also help keep the prevalence lower in this

region, however there was no evidence than any dog enrolled on the study was receiving regular heartworm prevention. In previous studies examining prevalence between areas, any significant differences are usually attributed to differing climates and conditions (Miller and Crosbie, 2011; Montoya-Alonso et al., 2011), however as all Samoa has a similar tropical climate this cannot be used to explain these findings. Mosquito abundance depends on a number of factors including climate, habitat and availability of standing water containers. There is evidence that the preference of mosquitoes for urban, rural or coastal habitats and hotter, cooler, wetter or drier climates varies between different species (Braks et al., 2003; Reiskind and Lounibos, 2012). Without knowing what species is the vector for heartworm in dogs in Samoa, it is hard to hypothesise what habitat would be preferred.

The high prevalence of dirofilariasis in Samoa means there is a significant risk to any uninfected dog. To reduce this risk, a heartworm prevention program needs to be introduced, and both owner and veterinary awareness of the problem needs to be raised. Current recommendations from the American Heartworm Society (AHS), include year round preventative care e.g. monthly prophylaxis with an oral or topical macrolytic lactone product, and annual antigen testing in all dogs aged 8 weeks and older (Graham et al., 2012). Yet despite veterinary and owner awareness in the USA, poor owner compliance is a problem, and it is estimated that less than 50% of pet dogs in the USA receive preventative medication year round (Bowman et al., 2007; Gates and Nolan, 2010). Furthermore, only 51% of clinics actually recommend annual testing of dogs (Bowman et al., 2007). A study looking at the diagnosis and treatment of heartworm in dogs in shelters in the USA, where resources may be limited, showed that the primary reason for not adhering to the AHS guidelines for management was the costs associated with providing the care (Colby et al., 2011). These gold standard recommendations may not be practical in Samoa, where there are also financial considerations, and it should be considered in introducing a preventative program that good compliance is important for a program to be effective. New studies should be carried out in the future to evaluate the efficacy of any preventative programs introduced.

Adulticidal treatment of dogs infected with heartworm is recommended, ideally following a diagnostic work up to assess the severity of disease, however large numbers of adulticide treatments have been performed successfully with minimal diagnostic evaluation (Graham et al., 2012). A three dose protocol of melarsomine, used with doxycycline, glucocorticoids and a heartworm preventative is the recommended treatment regime (Graham et al., 2012), although again this may prove to be too expensive for many dog owners in Samoa. If adulticide treatment is unavailable or contraindicated, the long term use of ivermectin with doxycycline

has been described, and may result in a reduction of heartworm burden or even result in a cure; ivermectin has some adulticidal as well as microfilaricidal properties (Bazzocchi et al., 2008; Graham et al., 2012; Grandi et al., 2010).

D. immitis is zoonotic, and can result in the pulmonary form of human dirofilariasis. The nematode is almost always unable to develop to maturity in humans, and lesions are the result of pre-adult worms lodging in the pulmonary vasculature where they form solitary nodular pulmonary granulomas, radiographically known as coin lesions (Simon et al., 2009). Humans do not become microfilaraemic and are usually free of symptoms. However, nodular pulmonary lesions detected by radiology raise the suspicion of malignant tumour, tuberculosis, fungal infections and other serious diseases and may initiate an invasive and expensive diagnostic work up to reach a definitive diagnosis (Theis, 2005). Studies have shown that human pulmonary dirofilariasis may be under-diagnosed (Muro et al., 1999; Simon et al., 2005) and that higher seroprevalence rates in humans are present in regions where levels of canine infection are high (Montoya-Alonso et al., 2011).

The point of care ELISA (SNAP 4Dx, IDEXX Laboratories, Westbrook, ME) used in this study detects *D. immitis* antigen in circulation. These antigen tests are the recommended method for screening and diagnosis for canine heartworm disease, as per the AHS (Graham et al., 2012). The kit used has been shown to have an excellent specificity of 97%, and a good sensitivity of 84% at low heartworm burdens (<4 adult female heartworms). This sensitivity improves as heartworm burden increases (Atkins, 2003), but false negative results may occur when worm burdens are very low. False positive results are unlikely.

The presence of *Anaplasma* spp. has never before been documented in Samoa and data for countries elsewhere in the Pacific are sparse. In this study, the overall seroprevalence of *Anaplasma* spp. was 8.4% (20/237) (Figure 6.1). The point-of-care ELISA test kit (SNAP 4Dx, IDEXX Laboratories, Westbrook, ME) used detects antibodies to both *A. phagocytophilum* and *A. platys*, therefore a positive result may be due to exposure to either of these organisms (Chandrashekar et al., 2010). As a general rule, *A. platys* is the predominant *Anaplasma* spp. in the Southern Hemisphere (Abarca et al., 2007; Sanogo et al., 2003; Santos et al., 2009) and Asia-Pacific region (Brown et al., 2001; Inokuma et al., 2002; Pinyoowong et al., 2008), whereas *A. phagocytophilum* is more commonly seen in the Northern Hemisphere, where it follows the distribution of *Ixodes* spp. ticks (Diniz and Breitschwerdt, 2012; Swanson et al., 2006). No *Ixodes* spp. ticks have been reported previously in Samoa, and a concurrent study of ectoparasites on Samoan dogs in the current sample population, showed 42.1% (93/221) of

dogs were infected with ticks, and all ticks positively identified were confirmed as *Rhipicephalus sanguineus*, the vector of *A. platys* (Table 5.2). No *Ixodes* spp. ticks were detected. For these reasons it can be assumed that the *Anaplasma* species present in Samoa is most likely to be *A. platys*. To confirm this hypothesis further testing including PCR will be performed to distinguish between these two *Anaplasma* species. From a public health perspective, *A. platys* is the better of the two infections, as the organism is currently considered of low zoonotic relevance (Otranto et al., 2009b).

There was a significant difference in seroprevalence of *Anaplasma* spp. in the sample population between results in the three areas examined, with dogs in rural Upolu having a significantly lower seroprevalence (2.8%, 3/109) compared with those in urban Apia (13.3%, 10/75) and Savai'i (13.2%, 7/53) (Table 6.4). This cannot be easily explained; when compared with the difference in prevalence of *R. sanguineus* infections in dogs, the highest prevalence was seen in dogs from Savai'i (59.3%), however Apia and rural Upolu had a similar lower prevalence (38.5% and 35.3% respectively) (Table 5.3). Given the small sample sizes and lack of random sampling, there is the possibility that these trends seen may just be due to chance.

The point-of-care ELISA test kit (SNAP 4Dx, IDEXX Laboratories, Westbrook, ME) used in this study is a diagnostically accurate test for detecting exposure and seroprevalence to *Anaplasma* spp. It is both sensitive (99.1%) and specific (100%) when compared with IFA (Chandrashekar et al., 2010), and in addition is quick and easy to use without the need for any specific laboratory equipment or training. However, a positive antibody result does not always indicate current infection; antibodies may persist beyond the resolution of clinical infection (Egenvall et al., 1997; Gaunt et al., 2010), and many naturally infected dogs remain healthy with no sign of clinical illness (Beall et al., 2008).

None of the dogs tested were seropositive for *E. canis* (Figure 6.1), which is surprising given the high number of dogs in Samoa infested with its primary vector, *R. sanguineus*. In most countries the distribution of *E. canis* follows that of its vector, although some countries such as Australia appear to be free of *E. canis*, despite *R. sanguineus* being widespread throughout much of the country (Irwin, 2001). In a study into animal health in Samoa conducted in 1997 six out of ten dogs tested positive for antibodies to *E. canis* (by IFA test) (Martin, 1999). This is at odds with the findings of the current study. The *E. canis* part of point-of-care ELISA test kit (SNAP 4Dx, IDEXX Laboratories, Westbrook, ME) used in this study is calibrated to be positive at titres greater than 1:160 (Harrus et al., 2012) so at lower titres the test is less sensitive than IFA. However sensitivity and specificity are still excellent (96.2% and 100% respectively) when

compared with the IFA (Chandrashekar et al., 2010). In dogs experimentally infected with *E. canis*, serum IgG antibodies generally appear by 15 days post-infection (Waner et al., 2001). In untreated dogs, antibody titres peak at 3-5 months post infection before starting to fall again. In some dogs serum antibodies may remain elevated for as long as three years or even life in some cases (Bartsch and Greene, 1996; Harrus et al., 1998b; Perille and Matus, 1991). This provides a long window for the detection of antibodies in, often clinically healthy, infected dogs, by serology. Zero samples positive means either the organism is absent from Samoa, or the seroprevalence is very low, below 1.5%. This was an unexpected finding, and further testing using PCR or IFA would help to confirm that this is a true finding and not a problem with the test kit used or due to low antibody titres going undetected by the ELISA used in this study. It is possible in the previous study (Martin, 1999), the IFA was cross reacting with another *Ehrlichia* species. The IFA is known to cross react with *Ehrlichia chaffeensis* and *Ehrlichia ewingii* resulting in false positives, however the same may be true for the ELISA (O'Connor et al., 2006). It may also be possible that there was cross reactivity with an *Anaplasma* species, such as *A. platys*.

There were no antibodies demonstrated to either *B. burgdorferi* or *L. infantum* in any dogs in this study (Figure 6.1). This was as expected for Samoa and other Pacific regions. The global distribution of *B. burgdorferi* follows that of its *Ixodes* spp. tick vectors and in general occurs in the Northern hemisphere in temperate, cooler climate conditions (Greene et al., 2012). *Ixodes* spp. ticks have not been proven to be present in Samoa in either this study or in any previous reports. *Leishmania* tends to occur in warmer climates where a competent sand fly vector is present. Approximately 70 of 1000 known sand fly species are able to transmit leishmaniasis (Murray et al., 2005) but there is no evidence that a competent sand fly vector exists in Samoa. Most maps showing the global distribution of *Leishmania*, Southeast Asia, Australia, New Zealand and the Pacific are markedly free of the parasite (Baneth and Solano-Gallego, 2012). However recently there have been reports to suggest that *Leishmania* distribution may be wider than previously thought (Thompson and Conlan, 2011). In 2004, the first autochthonous case of leishmaniasis was reported in kangaroos in Australia, involving a novel species of the parasite (Rose et al., 2004). There have also been cases reported in Thailand and East Timor, raising the possibility of vectors in the Asia-Pacific area (Thompson and Conlan, 2011). There have been no reported cases of human or canine leishmaniasis in Samoa or the surrounding Pacific region, and this region is assumed to be free of the disease. However this may be due to a lack of surveillance studies investigating the presence of the disease in dogs, humans or

any other species. Given this and the findings of this study, it is highly likely that these two organisms are absent from Samoa, although a very low prevalence cannot be ruled out.

There were certain exclusions in this study to ensure that large blood samples were not taken from small or unwell dogs. This meant that very visibly unwell dogs were excluded, which may have been more likely to be suffering from certain parasitic or vector borne diseases, such as heartworm disease and ehrlichiosis among others. By excluding dogs which may have been exhibiting clinical signs of the diseases being tested for, prevalences reported in this study may be falsely low. In addition, the sampling period for this study was relatively limited. Any seasonal variation in diseases or their vectors would not be accounted for, although the climate in Samoa is fairly similar year round. Sample collection was also limited to villages the mobile clinic was visiting. For the most part there was a good spread across the two main islands, although it is possible by limiting sample collection to these areas hyperendemic areas of infections were inadvertently missed or included in the study.

Chapter 7 General discussion

The findings of this study are important from both a veterinary and public health standpoint. They demonstrate that intestinal and external parasitism is clearly a problem in Samoan dogs, with very few preventative measures currently taken. An extremely high hookworm prevalence of 90.7% was detected (Table 5.1). Coupled with a dog population that is allowed to roam freely throughout towns and villages, defaecating anywhere, such a high prevalence of this zoonotic disease in dogs could pose a public health threat (Hochedez and Caumes, 2007). Further investigation into the species of hookworm present in Samoan dogs, by PCR-RFLP, would provide valuable information, as would studies investigating the incidence of hookworm related disease in people. *Giardia* spp. oocysts were also detected in 29% of dogs tested (Table 5.1). Further investigation of the *Giardia* assemblage detected in the canine faecal samples, by multi-locus PCR, would help to assess the zoonotic potential of this disease in Samoa. A study assessing assemblages from humans and dogs in the same setting would be ideal in determining the role of dogs in the transmission of *Giardia* to humans (Ballweber et al., 2010). A high prevalence of the tick *R. sanguineus* (42.1%) is an important finding with regard to its potential to transmit infectious disease (Table 5.2). It is likely to be the vector of the *Anaplasma* species detected in this study. Given its presence, the investigation of other vector-borne diseases transmitted by this tick, such as *Babesia canis*, *Mycoplasma hemocanis* and *Rickettsia conorii* would be an interesting extension of this study. Another area for future research would include expanding this study to include other canine infectious diseases such as leptospirosis.

The high prevalence of *D. immitis* (46.8%) means there is a significant risk of heartworm disease to the canine population (Figure 6.1), and there is a need for veterinary awareness and owner education on the severity of this disease. Hopefully this data will encourage the use of heartworm prevention in dogs in Samoa, although the associated costs will be a consideration for many Samoan dog owners. The knowledge that an *Anaplasma* spp., likely *A. platys*, is endemic in Samoa, with a seroprevalence of 8.4% detected in this study (Figure 6.1), also provides useful information for veterinarians, and it should be considered a differential diagnosis in cases where thrombocytopenia or bleeding symptoms are observed. However, PCR is needed to determine which *Anaplasma* species is present in Samoa. Findings in the present study suggest that *E. canis*, *B. burgdorferi* and *L. infantum* are either absent or are present at a very low prevalence in dogs in Samoa (Figure 6.1). In addition, the prevalence of

canine dirofilariasis provides information for medical practitioners in order that *D. immitis* infection may be considered as a differential diagnosis for pulmonary nodules in humans. The presence of *A. platys* however is considered of little zoonotic relevance (Otranto et al., 2009b).

An important limitation of this study is the non random selection of the study population. The study design was essentially a convenience sample for a pilot study and so only animals voluntarily presented for sterilisation on the selected days were included. This could have affected the age and health status of the study population compared with the general canine population in Samoa, which could mean that the prevalences stated in this study are not representative of the general population. Any further studies should aim to sample randomly and throughout the year to minimise any seasonal bias.

Given the high levels of intestinal and external parasitism, and canine heartworm infection detected in this study, preventative healthcare measures should be recommended to dog owners in Samoa. This would not only help to improve the health status of the dogs treated, but would also help to reduce the environmental burden of parasites, reducing the risk of infection being transmitted to other dogs, or potentially to humans where zoonotic disease is concerned. Much research has been done in Europe and the USA into parasite control, and recommendations for gold standard in preventative care for canine and feline parasites have been developed (Anonymous, 2010c, 2011). For endoparasites, control of parasite eggs or larvae in the environment is essential to minimise the infection pressure to humans and animals for parasites where eggs are passed in the faeces. All cestode and nematode eggs are highly resistant in the environment and may persist in soil for months or years (Anonymous, 2010c). Appropriate disposal of faeces, on a daily basis is recommended. Fencing roaming dogs and leash laws in urban areas where density may be high is also important. Implementation of comprehensive parasite control programs is also important in preventing initial parasite environmental contamination (Anonymous, 2010c). In a country where canine heartworm disease is endemic, an endoparasite control program would include de-worming all puppies fortnightly from 2-12 weeks of age, and then monthly treatment with a heartworm preventative that also covers intestinal worms, such as a macrolytic lactone product, throughout adult life (Anonymous, 2011). In addition, control of ticks and fleas with regular and year round application of an approved product which will treat and prevent both ticks and fleas should be recommended, and dogs should be inspected regularly to check for ticks (Anonymous, 2012b).

Owner compliance to these strict recommendations has been shown to be poor, even in a relatively wealthy western country with good pet health awareness among owners (Bowman et al., 2007; Colby et al., 2011). Instigating programs such as these to all dogs in Samoa may be unrealistic, due to both cost and owner awareness of the diseases. Some pet ownership education is already provided by the APS, and cost price anthelmintics and ectoparasiticides are available through the organisation. A report on humane canine population management by ICAM suggests that the provision of free preventative treatments should be done with caution as there is a risk of de-valuing general veterinary services. Such treatments also need to be provided regularly to be effective, and so repeated access to such services should be considered (Anonymous, 2007a). Continuing this work and widespread awareness campaigns on the importance of preventative veterinary healthcare, alongside increased access to the necessary treatments would also be beneficial. The cost of this work for a not-for profit organisation such as the APS could be considerable and needs to be taken into account.

As well as preventative health care for the dogs, precautions to prevent human infection by zoonotic parasites should be recommended to both local and tourist populations. Recommendations for preventing hookworm transmission include wearing protective footwear when walking on beaches or contaminated soil and care to lie only on sand which has been washed by the tide (Hochedez and Caumes, 2007). For preventing infection with parasites transmitted by the faecal-oral route, such as *Giardia*, maintaining good personal hygiene and hand-washing, especially when preparing food and after handling animals, is particularly important (Anonymous, 2012c). Owner education to raise the profile of these diseases and their relevance to public health may also help to encourage compliance with introducing preventative care to their dogs. Through education the importance of preventative measures for zoonoses transmission such as good hygiene, removal and disposal of dog faeces promptly and regular anthelmintic treatment of dogs can be explained and subsequently implemented. Legislation to require dog owners to register their animals, to fence roaming dogs and to pick up and dispose of faeces in public areas could also be an option to help reduce the number of free roaming dogs which in turn would reduce dog fouling of public areas thereby reducing the environmental parasite burden and subsequent risk to humans. There are already some dedicated dog free villages and beaches in Samoa, and this initiative should be encouraged to help reduce the risk of zoonotic diseases, especially HrCLM and toxocariasis.

Further to the results of this study, speciation of both hookworm and *Anaplasma* spp., by PCR-RFLP and PCR respectively, would enhance our understanding of the zoonotic potential of

these diseases in Samoa, as would multi-locus PCR on *Giardia* to ascertain whether zoonotic assemblages are present in the canine population. Future studies in Samoan dogs could include widening the diseases investigated to include other canine infectious diseases such as leptospirosis and *Babesia canis*. In addition, human studies investigating the prevalence of hookworm related diseases in Samoa would help to establish the importance and relevance of the findings in this study with regard to public health. Future prevalence studies following the implementation of any of the prevention strategies detailed in this chapter would be useful to assess their efficacy.

References

- Abarca, K., Lopez, J., Perret, C., Guerrero, J., Godoy, P., Veloz, A., Valiente-Echeverria, F., Leon, U., Gutjahr, C., Azocar, T., 2007, *Anaplasma platys* in dogs, Chile. *Emerging Infectious Diseases* 13, 1392-1395.
- Acosta-Jamett, G., Cleaveland, S., Cunningham, A.A., Bronsvoort, B.M.D., 2010, Demography of domestic dogs in rural and urban areas of the Coquimbo region of Chile and implications for disease transmission. *Preventive Veterinary Medicine* 94, 272-281.
- Adler, S., Theodor, O., 1932, Investigations on mediterranean kala azar VI - Canine visceral Leishmaniasis. *Proceedings of the Royal Society of London Series B-Containing Papers of a Biological Character* 110, 402-412.
- Aguero-Rosenfeld, M.E., Donnarumma, L., Zentmaier, L., Jacob, J., Frey, M., Noto, R., Carbonaro, C.A., Wormser, G.P., 2002, Seroprevalence of antibodies that react with *Anaplasma phagocytophila*, the agent of human granulocytic ehrlichiosis, in different populations in Westchester County, New York. *Journal of Clinical Microbiology* 40, 2612-2615.
- Amusatogui, I., Tesouro, M.A., Kakoma, I., Sainz, A., 2008, Serological reactivity to *Ehrlichia canis*, *Anaplasma phagocytophilum*, *Neorickettsia risticii*, *Borrelia burgdorferi* and *Rickettsia conorii* in dogs from Northwestern Spain. *Vector-Borne and Zoonotic Diseases* 8, 797-803.
- Anderson, A., 2009, The rat and the octopus: initial human colonization and the prehistoric introduction of domestic animals to Remote Oceania. *Biol Invasions* 11, 1503-1519.
- Anderson, B.E., Dawson, J.E., Jones, D.C., Wilson, K.H., 1991, *Ehrlichia chaffeensis*, a new species associated with human ehrlichiosis. *Journal of Clinical Microbiology* 29, 2838-2842.
- Anonymous 2007a. Humane dog population management guidance: International Companion Animal Management Coalition. In www.icam-coalition.org.
- Anonymous 2007b. Samoa "Worried about the dogs...". In www.tripadvisor.co.uk.
- Anonymous 2010a. *Cryptosporidium*: Biology. In <http://www.cdc.gov/parasites/crypto/biology.html> (Centers for Disease Control and Prevention).
- Anonymous 2010b. The Dog Problem. In www.apssamoa.org (Animal Protection Society of Samoa, Apia, Samoa).
- Anonymous 2010c. Worm control in cats and dogs. In www.esccap.org, Parasites, E.S.C.C.A., ed.
- Anonymous 2011. Current Advice on Parasite Control. In www.capcvet.org (Companion Animal Parasite Council).
- Anonymous 2012a. Central Intelligence Agency: The World Factbook: Samoa. In <https://www.cia.gov/library/publications/the-world-factbook/geos/ws.html> (Washington, USA, Central Intelligence Agency).
- Anonymous 2012b. Control of ectoparasites in cats and dogs. In www.esccap.org, Parasites, E.S.C.C.A., ed.

- Anonymous 2012c. Giardia: Prevention and control. In www.cdc.gov.
- Anonymous 2012d. Hookworm- Life cycle (cutaneous larval migrans). In <http://www.dpd.cdc.gov/dpdx/HTML/Hookworm.htm> (Centres for disease control and prevention).
- Anonymous 2012e. Samoa. In www.lonelyplanet.com.
- Anonymous 2012f. Toxocariasis. In http://www.cdc.gov/parasites/toxocariasis/gen_info/index.html (Centers for Disease Control and Prevention).
- Ansart, S., Perez, L., Jaureguiberry, S., Danis, M., Bricaire, F., Caumes, E., 2007, Spectrum of dermatoses in 165 travelers returning from the tropics with skin diseases. *American Journal of Tropical Medicine and Hygiene* 76, 184-186.
- Appel, M.J.G., Allan, S., Jacobson, R.H., Lauderdale, T.L., Chang, Y.F., Shin, S.J., Thomford, J.W., Todhunter, R.J., Summers, B.A., 1993, Experimental Lyme disease in dogs produces arthritis and persistent infection. *Journal of Infectious Diseases* 167, 651-664.
- Atkins, C.E., 2003, Comparison of results of three commercial heartworm antigen test kits in dogs with low heartworm burdens. *Journal of the American Veterinary Medical Association* 222, 1221-1223.
- Atkins, C.E., 2005, Canine Heartworm Disease, In: Ettinger, S.J., Feldman, E.C. (Eds.) *Textbook of Veterinary Internal Medicine*. Elsevier Saunders, St Louis, pp. 1118-1136.
- Aydenizoz-Ozkayhan, M., Yagci, B.B., Erat, S., 2008, The investigation of *Toxocara canis* eggs in coats of different dog breeds as a potential transmission route in human toxocariasis. *Veterinary Parasitology* 152, 94-100.
- Azuma, Y., Isogai, E., Isogai, H., Kawamura, K., 1994, Canine Lyme disease- clinical and serological evaluations in 21 dogs in Japan. *Veterinary Record* 134, 369-372.
- Baker, D.C., Simpson, M., Gaunt, S.D., Corstvet, R.E., 1987, Acute *Ehrlichia platys* infection in the dog. *Veterinary Pathology* 24, 449-453.
- Bakken, J.S., Dumler, J.S., Chen, S.M., Eckman, M.R., Vanetta, L.L., Walker, D.H., 1994, Human granulocytic ehrlichiosis in the upper midwest United-Sates. A new species emerging? *Jama-Journal of the American Medical Association* 272, 212-218.
- Bakken, J.S., Dumler, S., 2008, Human granulocytic anaplasmosis. *Infectious Disease Clinics of North America* 22, 433-448.
- Bakken, J.S., Krueth, J., WilsonNordskog, C., Tilden, R.L., Asanovich, K., Dumler, J.S., 1996, Clinical and laboratory characteristics of human granulocytic ehrlichiosis. *Jama-Journal of the American Medical Association* 275, 199-205.
- Ballweber, L.R., Xiao, L.H., Bowman, D.D., Kahn, G., Cama, V.A., 2010, Giardiasis in dogs and cats: update on epidemiology and public health significance. *Trends in Parasitology* 26, 180-189.
- Baneth, G., Koutinas, A.F., Solano-Gallego, L., Bourdeau, P., Ferrer, L., 2008, Canine leishmaniosis - new concepts and insights on an expanding zoonosis: part one. *Trends in Parasitology* 24, 324-330.
- Baneth, G., Solano-Gallego, L., 2012, Leishmaniasis, In: Greene, C.E. (Ed.) *Infectious Diseases of the Dog and Cat*. Elsevier Saunders, St. Louis, pp. 734-749.
- Bartsch, R.C., Greene, R.T., 1996, Post-therapy antibody titers in dogs with ehrlichiosis: Follow-up study on 68 patients treated primarily with tetracycline and/or doxycycline. *Journal of Veterinary Internal Medicine* 10, 271-274.

- Batchelor, D.J., Tzannes, S., Graham, P.A., Wastling, J.M., Pinchbeck, G.L., German, A.J., 2008, Detection of endoparasites with zoonotic potential in dogs with gastrointestinal disease in the UK. *Transboundary and Emerging Diseases* 55, 99-104.
- Bates, P., 2008, Could exotic tick-borne diseases of dogs and cats become endemic in the UK? *GVJ - Government Veterinary Journal* 18, 21-25.
- Bazzocchi, C., Mortarino, M., Grandi, G., Kramer, L.H., Genchi, C., Bandi, C., Genchi, M., Sacchi, L., McCall, J.W., 2008, Combined ivermectin and doxycycline treatment has microfilaricidal and adulticidal activity against *Dirofilaria immitis* in experimentally infected dogs. *International Journal for Parasitology* 38, 1401-1410.
- Beall, M.J., Alleman, A.R., Breitschwerdt, E.B., Cohn, L.A., Couto, C.G., Dryden, M.W., Guptill, L.C., Iazbik, C., Kania, S.A., Lathan, P., Little, S.E., Roy, A., Sayler, K.A., Stillman, B.A., Welles, E.G., Wolfson, W., Yabsley, M.J., 2012, Seroprevalence of *Ehrlichia canis*, *Ehrlichia chaffeensis* and *Ehrlichia ewingii* in dogs in North America. *Parasites & Vectors* 5.
- Beall, M.J., Chandrashekar, R., Eberts, M.D., Cyr, K.E., Diniz, P., Mainville, C., Hegarty, B.C., Crawford, J.M., Breitschwerdt, E.B., 2008, Serological and molecular prevalence of *Borrelia burgdorferi*, *Anaplasma phagocytophilum*, and *Ehrlichia species* in dogs from Minnesota. *Vector-Borne and Zoonotic Diseases* 8, 455-464.
- Beaufils, J.P., Inokuma, H., Martin-Grel, J., Jumelle, P., Barbault-Jumelle, M., Brouqui, P., 2002, *Anaplasma platys* (*Ehrlichia platys*) infection in a dog in France: description of the case, and characterization of the agent. *Revue De Medecine Veterinaire* 153, 85-90.
- Becker, A.C., Rohen, M., Epe, C., Schnieder, T., 2012, Prevalence of endoparasites in stray and fostered dogs and cats in Northern Germany. *Parasitology Research* 111, 849-857.
- Berrahal, F., Mary, C., Roze, M., Berenger, A., Escoffier, K., Lamouroux, D., Dunan, S., 1996, Canine leishmaniasis: Identification of asymptomatic carriers by polymerase chain reaction and immunoblotting. *American Journal of Tropical Medicine and Hygiene* 55, 273-277.
- Beugnet, F., Marie, J.-L., 2009, Emerging arthropod-borne diseases of companion animals in Europe. *Veterinary Parasitology* 163, 298-305.
- Bingham, P., 2010a, Investigations of suspected exotic diseases. *Surveillance (Wellington)* 37, 22-29.
- Bingham, P., 2010b, Quarterly report of investigations of suspected exotic disease. *Surveillance (Wellington)* 37, 30-40.
- Bingham, P., 2012, Quarterly report of investigations of suspected exotic diseases. *Surveillance (Wellington)* 39, 23-26.
- Bjoersdorff, A., Bergstrom, S., Massung, R.F., Haemig, P.D., Olsen, B., 2001, *Ehrlichia*-infected ticks on migrating birds. *Emerging Infectious Diseases* 7, 877-879.
- Blagburn, B.L., Dryden, M.W., 2009, Biology, treatment, and control of flea and tick infestations. *Veterinary Clinics of North America-Small Animal Practice* 39, 1173-1198.
- Bove, C.M., Gordon, S.G., Saunders, A.B., Miller, M.W., Roland, R.M., Achen, S.E., Drourr, L.T., Boggess, M.M., 2010, Outcome of minimally invasive surgical treatment of heartworm caval syndrome in dogs: 42 cases (1999-2007). *Javma-Journal of the American Veterinary Medical Association* 236, 187-192.

- Bowman, D., Little, S.E., Lorentzen, L., Shields, J., Sullivan, M.P., Carlin, E.P., 2009, Prevalence and geographic distribution of *Dirofilaria immitis*, *Borrelia burgdorferi*, *Ehrlichia canis*, and *Anaplasma phagocytophilum* in dogs in the United States: Results of a national clinic-based serologic survey. *Veterinary Parasitology* 160, 138-148.
- Bowman, D.D., Atkins, C.E., 2009, Heartworm Biology, Treatment, and Control. *Veterinary Clinics of North America-Small Animal Practice* 39, 1127-1158.
- Bowman, D.D., Montgomery, S.P., Zajac, A.M., Eberhard, M.L., Kazacos, K.R., 2010, Hookworms of dogs and cats as agents of cutaneous larva migrans. *Trends in Parasitology* 26, 162-167.
- Bowman, D.D., Torre, C.J., Mannella, C., 2007, Survey of 11 western states for heartworm (*Dirofilari immitis*) infection, heartworm diagnostic and prevention protocols, and fecal examination protocols for gastrointestinal parasites. *Veterinary Therapeutics* 8, 293-304.
- Braks, M.A., Honorio, N.A., Lourencqo-De-Oliveira, R., Juliano, S.A., Lounibos, L.P., Reiskind, M.H., 2003, Convergent habitat segregation of *Aedes aegypti* and *Aedes albopictus* (Diptera: *Culicidae*) in southeastern Brazil and Florida. *Journal of Medical Entomology* 40, 785-794.
- Brown, G.K., Canfield, P.J., Dunstan, R.H., Roberts, T.K., Martin, A.R., Brown, C.S., Irving, R., 2006, Detection of *Anaplasma platys* and *Babesia canis vogeli* and their impact on platelet numbers in free-roaming dogs associated with remote Aboriginal communities in Australia. *Australian Veterinary Journal* 84, 321-325.
- Brown, G.K., Martin, A.R., Roberts, T.K., Aitken, R.J., 2001, Detection of *Ehrlichia platys* in dogs in Australia. *Australian Veterinary Journal* 79, 554-558.
- Brown, G.K., Martin, A.R., Roberts, T.K., Dunstan, R.H., 2005, Molecular detection of *Anaplasma platys* in lice collected from dogs in Australia. *Australian Veterinary Journal* 83, 101-102.
- Bugg, R.J., Robertson, I.D., Elliot, A.D., Thompson, R.C.A., 1999, Gastrointestinal parasites of urban dogs in Perth, Western Australia. *Veterinary Journal* 157, 295-301.
- Buret, A.G., 2005, Immunopathology of giardiasis: the role of lymphocytes in intestinal epithelial injury and malfunction. *Memorias Do Instituto Oswaldo Cruz* 100, 185-190.
- Cabral, M., O'Grady, J.E., Gomes, S., Sousa, J.C., Thompson, H., Alexander, J., 1998, The immunology of canine leishmaniosis: strong evidence for a developing disease spectrum from asymptomatic dogs. *Veterinary Parasitology* 76, 173-180.
- Caccio, S.M., Beck, R., Lalle, M., Marinculic, A., Pozio, E., 2008, Multilocus genotyping of *Giardia duodenalis* reveals striking differences between assemblages A and B. *International Journal for Parasitology* 38, 1523-1531.
- Caccio, S.M., Ryan, U., 2008, Molecular epidemiology of giardiasis. *Molecular and Biochemical Parasitology* 160, 75-80.
- Campbell, K.L., 2005, Other external parasites, In: Ettinger, S.J., Feldman, E.C. (Eds.) *Textbook of veterinary internal medicine*. Elsevier Saunders, pp. 66-70.
- Cao, W.C., Zhao, Q.M., Zhang, P.H., Dumler, J.S., Zhang, X.T., Fang, L.Q., Yang, H., 2000, Granulocytic ehrlichiae in *Ixodes persulcatus* ticks from an area in China where Lyme disease is endemic. *Journal of Clinical Microbiology* 38, 4208-4210.
- Carastro, S.M., Dugan, S.J., Paul, A.J., 1992, Intraocular dirofilariasis in a dog. *Compendium on Continuing Education for the Practicing Veterinarian* 14, 209-217.

- Carlisle, C.H., Atwell, R.B., 1984, A survey of heartworm in dogs in Australia. *Australian Veterinary Journal* 61, 356-360.
- Carrade, D.D., Foley, J.E., Borjesson, D.L., Sykes, J.E., 2009, Canine Granulocytic Anaplasmosis: A Review. *Journal of Veterinary Internal Medicine* 23, 1129-1141.
- Carroll, S.M., Grove, D.I., 1986, Experimental infection of humans with *Ancylostoma ceylanicum*- clinical, parasitological, hematological and immunological findings. *Tropical and Geographical Medicine* 38, 38-45.
- Caumes, E., Ly, F., Bricaire, F., 2002, Cutaneous larva migrans with folliculitis: report of seven cases and review of the literature. *British Journal of Dermatology* 146, 314-316.
- Cestnick, L., 1998, Lyme disease in Australia. *Australian and New Zealand Journal of Public Health* 22, 524-524.
- Chambers, E.W., McClintock, S.K., Avery, M.F., King, J.D., Bradley, M.H., Schmaedick, M.A., Lammie, P.J., Burkot, T.R., 2009, Xenomonitoring of *Wuchereria bancrofti* and *Dirofilaria immitis* infections in mosquitoes from American Samoa: trapping considerations and a comparison of polymerase chain reaction assays with dissection. *American Journal of Tropical Medicine and Hygiene* 80, 774-781.
- Chandrashekar, R., Mainville, C.A., Beall, M.J., O'Connor, T., Eberts, M.D., Alleman, A.R., Gaunt, S.D., Breitschwerdt, E.B., 2010, Performance of a commercially available in-clinic ELISA for the detection of antibodies against *Anaplasma phagocytophilum*, *Ehrlichia canis*, and *Borrelia burgdorferi* and *Dirofilaria immitis* antigen in dogs. *American Journal of Veterinary Research* 71, 1443-1450.
- Chang, A.C.H., Chang, W.L., Lin, C.T., Pan, M.J., Lee, S.C., 1996, Canine infectious cyclic thrombocytopenia found in Taiwan. *Journal of Veterinary Medical Science* 58, 473-476.
- Chen, S.M., Dumler, J.S., Bakken, J.S., Walker, D.H., 1994, Identification of a granulocytotropic *Ehrlichia* species as the etiological agent of human disease *Journal of Clinical Microbiology* 32, 589-595.
- Choi, K.S., Garyu, J., Park, J.H., Dumler, J.S., 2003, Diminished adhesion of *Anaplasma phagocytophilum*-infected neutrophils to endothelial cells is associated with reduced expression of leukocyte surface selectin. *Infection and Immunity* 71, 4586-4594.
- Ciaramella, P., Oliva, G., DeLuna, R., Gradoni, L., Ambrosio, R., Cortese, L., Scalone, A., Persechino, A., 1997, A retrospective clinical study of canine leishmaniasis in 150 dogs naturally infected by *Leishmania infantum*. *Veterinary Record* 141, 539-543.
- Colby, K.N., Levy, J.K., Dunn, K.F., Michaud, R.I., 2011, Diagnostic, treatment, and prevention protocols for canine heartworm infection in animal sheltering agencies. *Veterinary Parasitology* 176, 333-341.
- Conboy, G., 2009, Cestodes of Dogs and Cats in North America. *Veterinary Clinics of North America-Small Animal Practice* 39, 1075-1090.
- Cooley, A.J., Clemmons, R.M., Gross, T.L., 1987, Heartworm disease manifested by encephalomyelitis and myositis in a dog *Journal of the American Veterinary Medical Association* 190, 431-432.
- Cortadellas, O., del Palacio, M.J.F., Bayon, A., Albert, A., Talavera, J., 2006, Systemic hypertension in dogs with leishmaniasis: Prevalence and clinical consequences. *Journal of Veterinary Internal Medicine* 20, 941-947.

- Coutinho, M.T.Z., Bueno, L.L., Sterzik, A., Fujiwara, R.T., Botelho, J.R., De Maria, M., Genaro, O., Linardi, P.M., 2005, Participation of *Rhipicephalus sanguineus* (Acari : Ixodidae) in the epidemiology of canine visceral leishmaniasis. *Veterinary Parasitology* 128, 149-155.
- Dai, R.S., Li, Z.Y., Li, F., Liu, D.X., Liu, W., Liu, G.H., He, S.W., Tan, M.Y., Lin, R.Q., Liu, Y., Zhu, X.Q., 2009, Severe infection of adult dogs with helminths in Hunan Province, China poses significant public health concerns. *Veterinary Parasitology* 160, 348-350.
- Dambach, D.M., Smith, C.A., Lewis, R.M., VanWinkle, T.J., 1997, Morphologic, immunohistochemical, and ultrastructural characterization of a distinctive renal lesion in dogs putatively associated with *Borrelia burgdorferi* infection: 49 cases (1987-1992). *Veterinary Pathology* 34, 85-96.
- Dantas-Torres, F., 2008, The brown dog tick, *Rhipicephalus sanguineus* (Latreille, 1806) (Acari : Ixodidae): From taxonomy to control. *Veterinary Parasitology* 152, 173-185.
- Davoust, B., Bourry, O., Gomez, J., Lafay, L., Casali, F., Leroy, E., Parzy, D., 2006, Surveys on seroprevalence of canine monocytic ehrlichiosis among dogs living in the Ivory Coast and Gabon and evaluation of a quick commercial test kit dot-ELISA, In: Hechemy, K.E., Oteo, J.A., Raoult, D.A., Silverman, D.J., Blanco, J.R. (Eds.) *Century of Rickettsiology: Emerging, Reemerging Rickettsioses, Molecular Diagnostics, and Emerging Veterinary Rickettsioses*. pp. 464-469.
- Day, M.J., 2011, One health: the importance of companion animal vector-borne diseases. *Parasites & Vectors* 4.
- de la Fuente, J., Torina, A., Naranjo, V., Nicosia, S., Alongi, A., La Mantia, F., Kocan, K.M., 2006, Molecular characterization of *Anaplasma platys* strains from dogs in Sicily, Italy. *BMC veterinary research* 2, 24.
- Derraik, J.G.B., Slaney, D., 2005, Container aperture size and nutrient preferences of mosquitoes (Diptera : Culicidae) in the Auckland region, New Zealand. *Journal of Vector Ecology* 30, 73-82.
- Desjeux, P., 2004, Leishmaniasis: current situation and new perspectives. *Comparative Immunology Microbiology and Infectious Diseases* 27, 305-318.
- Diba, V.C., Whitty, C.J.M., Green, T., 2004, Cutaneous larva migrans acquired in Britain. *Clinical and Experimental Dermatology* 29, 555-556.
- Diniz, P.P., Breitschwerdt, E.B., 2012, *Anaplasma phagocytophilum* infection (Canine Granulocytic Anaplasmosis), In: Greene, C.E. (Ed.) *Infectious Diseases of the Dog and Cat*. Elsevier Saunders, St. Louis, pp. 244-254.
- Donatien, A., Lestoquard, F., 1937, State of the present knowledge concerning rickettsiosis of animals. *Archives de l'Institut Pasteur d'Algérie Institut Pasteur d'Algérie*, 142-187.
- Doudier, B., Olano, J., Parola, P., Brouqui, P., 2010, Factors contributing to emergence of *Ehrlichia* and *Anaplasma* spp. as human pathogens. *Veterinary Parasitology* 167, 149-154.
- Dryden, M.W., Payne, P.A., Smith, V., 2006, Accurate diagnosis of *Giardia* spp and proper fecal examination procedures. *Veterinary Therapeutics* 7, 4-14.
- Dumler, J.S., Barbet, A.F., Bekker, C.P.J., Dasch, G.A., Palmer, G.H., Ray, S.C., Rikihisa, Y., Rurangirwa, F.R., 2001, Reorganization of genera in the families Rickettsiaceae and Anaplasmataceae in the order Rickettsiales: unification of some species of *Ehrlichia* with *Anaplasma*, *Cowdria* with *Ehrlichia* and *Ehrlichia* with *Neorickettsia*, descriptions of six new species combinations and designation of *Ehrlichia equi* and 'HGE agent' as

- subjective synonyms of *Ehrlichia phagocytophila*. International Journal of Systematic and Evolutionary Microbiology 51, 2145-2165.
- Dumler, J.S., Choi, K.S., Garcia-Garcia, J.C., Barat, N.S., Scorpio, D.G., Garyu, J.W., Grab, D.J., Bakken, J.S., 2005, Human granulocytic anaplasmosis and *Anaplasma phagocytophilum*. Emerging Infectious Diseases 11, 1828-1834.
- Eddlestone, S.M., Gaunt, S.D., Neer, T.M., Boudreaux, C.M., Gill, A., Haschke, E., Corstvet, R.E., 2007, PCR detection of *Anaplasma platys* in blood and tissue of dogs during acute phase of experimental infection. Experimental Parasitology 115, 205-210.
- Egenvall, A., Bjoersdorff, A., Lilliehook, I., Engvall, E.O., Karlstam, E., Artursson, K., Hedhammar, A., Gunnarsson, A., 1998, Early manifestations of granulocytic ehrlichiosis in dogs inoculated experimentally with a Swedish *Ehrlichia* species isolate. Veterinary Record 143, 412-417.
- Egenvall, A., Lilliehook, I., Bjoersdorff, A., Engvall, E.O., Karlstam, E., Artursson, K., Heldtander, M., Gunnarsson, A., 2000, Detection of granulocytic *Ehrlichia* species DNA by PCR in persistently infected dogs. Veterinary Record 146, 186-190.
- Egenvall, A.E., Hedhammar, A.A., Bjoersdorff, A.I., 1997, Clinical features and serology of 14 dogs affected by granulocytic ehrlichiosis in Sweden. Veterinary Record 140, 222-226.
- Fairley, R., Heath, A.C.G., 1997, Exotic ticks intercepted in New Zealand since 1980. Surveillance (Wellington) 24, 21-22.
- Farnworth, M.J., Blaszak, K.A., Hiby, E.F., Waran, N.K., 2012, Incidence of dog bites and public attitudes towards dog care and management in Samoa. Animal Welfare 21, 477-486.
- Feldmeier, H., Schuster, A., 2012, Mini review: hookworm-related cutaneous larva migrans. European Journal of Clinical Microbiology & Infectious Diseases 31, 915-918.
- Fernandez-Bellon, H., Solano-Gallego, L., Bardagi, M., Alberola, J., Ramis, A., Ferrer, L., 2006, Immune response to *Leishmania infantum* in healthy horses in Spain. Veterinary Parasitology 135, 181-185.
- Ferroglio, E., Centaro, E., Mignone, W., Trisciuglio, A., 2007, Evaluation of an ELISA rapid device for the serological diagnosis of *Leishmania infantum* infection in dog as compared with immunofluorescence assay and Western blot. Veterinary Parasitology 144, 162-166.
- Font, A., Mascort, J., Altimira, J., Closa, J.M., Vilafranca, M., 2004, Acute paraplegia associated with vasculitis in a dog with leishmaniasis. Journal of Small Animal Practice 45, 199-201.
- Fontanarrosa, M.F., Vezzani, D., Basabe, J., Eiras, D.F., 2006, An epidemiological study of gastrointestinal parasites of dogs from Southern Greater Buenos Aires (Argentina): Age, gender, breed, mixed infections, and seasonal and spatial patterns. Veterinary Parasitology 136, 283-295.
- French, T.W., Harvey, J.W., 1983, Serological diagnosis of infectious cyclic thrombocytopenia in dogs using an indirect fluorescent antibody test. American Journal of Veterinary Research 44, 2407-2411.
- Gallego, E., Alvarado, M., Wasserman, M., 2007, Identification and expression of the protein ubiquitination system in *Giardia intestinalis*. Parasitology Research 101, 1-7.
- Gates, M.C., Nolan, T.J., 2009a, Comparison of Passive Fecal Flotation Run by Veterinary Students to Zinc-Sulfate Centrifugation Flotation Run in a Diagnostic Parasitology Laboratory. Journal of Parasitology 95, 1213-1214.

- Gates, M.C., Nolan, T.J., 2009b, Endoparasite prevalence and recurrence across different age groups of dogs and cats. *Veterinary Parasitology* 166, 153-158.
- Gates, M.C., Nolan, T.J., 2010, Factors influencing heartworm, flea, and tick preventative use in patients presenting to a veterinary teaching hospital. *Preventive Veterinary Medicine* 93, 193-200.
- Gaunt, S.D., Beall, M.J., Stillman, B.A., Lorentzen, L., Diniz, P., Chandrashekar, R., Breitschwerdt, E.B., 2010, Experimental infection and co-infection of dogs with *Anaplasma platys* and *Ehrlichia canis*: hematologic, serologic and molecular findings. *Parasites & Vectors* 3.
- Gavvani, A.S.M., Mohite, H., Edrissian, G.H., Mohebali, M., Davies, C.R., 2002, Domestic dog ownership in Iran is a risk factor for human infection with *Leishmania infantum*. *American Journal of Tropical Medicine and Hygiene* 67, 511-515.
- Genchi, C., Kramer, L.H., Prieto, G., 2001, Epidemiology of Canine and Feline *Dirofilariasis*: A Global View, In: Simon, F., Genchi, C. (Eds.) *Heartworm Infections in Humans and Animals*. Ediciones Universidad Salamanca, Barcelona, pp. 121-129.
- Genchi, C., Kramer, L.H., Rivasi, F., 2011a, *Dirofilaria* Infections in Europe. *Vector-Borne and Zoonotic Diseases* 11, 1307-1317.
- Genchi, C., Mortarino, M., Rinaldi, L., Cringoli, G., Traldi, G., Genchi, M., 2011b, Changing climate and changing vector-borne disease distribution: The example of *Dirofilaria* in Europe. *Veterinary Parasitology* 176, 295-299.
- Genchi, C., Rinaldi, L., Mortarino, M., Genchi, M., Cringoli, G., 2009, Climate and *Dirofilaria* infection in Europe. *Veterinary Parasitology* 163, 286-292.
- Georges, K., Ezeokoli, C.D., Newaj-Fyzul, A., Campbell, M., Mootoo, N., Mutani, A., Sparagano, O.A.E., 2008, The Application of PCR and Reverse Line Blot Hybridization to Detect Arthropod-borne Hemopathogens of Dogs and Cats in Trinidad, In: Sparagano, O.A.E., Maillard, J.C., Figueroa, J.V. (Eds.) *Animal Biodiversity and Emerging Diseases: Prediction and Prevention*. pp. 196-199.
- Georgi, J.R., Lejambre, L.F., Ractliff, L.H., 1969, *Ancylostoma caninum* burden in relationship to erythrocyte loss in dogs. *Journal of Parasitology* 55, 1205-1211.
- Germani, Y., Morillon, M., Begaud, E., Dubourdieu, H., Costa, R., Thevenon, J., 1994, Two year study of endemic enteric pathogens associated with acute diarrhea in New Caledonia. *Journal of Clinical Microbiology* 32, 1532-1536.
- Gioia, G., Lecova, L., Genchi, M., Ferri, E., Genchi, C., Mortarino, M., 2010, Highly sensitive multiplex PCR for simultaneous detection and discrimination of *Dirofilaria immitis* and *Dirofilaria repens* in canine peripheral blood. *Veterinary Parasitology* 172, 160-163.
- Goh, S., Reacher, M., Casemore, D.P., Verlander, N.Q., Chalmers, R., Knowles, M., Williams, J., Osborn, K., Richards, S., 2004, Sporadic cryptosporidiosis, North Cumbria, England, 1996-2000. *Emerging Infectious Diseases* 10, 1007-1015.
- Gomes, Y.M., Cavalcanti, M.P., Lira, R.A., Abath, F.G.C., Alves, L.C., 2008, Diagnosis of canine visceral leishmaniasis: Biotechnological advances. *Veterinary Journal* 175, 45-52.
- Goodman, J.L., Nelson, C.M., Klein, M.B., Hayes, S.F., Weston, B.W., 1999, Leukocyte infection by the granulocytic ehrlichiosis agent is linked to expression of a selectin ligand. *Journal of Clinical Investigation* 103, 407-412.
- Gookin, J.L., Nordone, S.K., Argenzio, R.A., 2002, Host responses to *Cryptosporidium* infection. *Journal of Veterinary Internal Medicine* 16, 12-21.

- Gordon, W.S., Brownlee, A., Wilson, D.R., Macleod, J., 1932, " Tick-borne Fever " (A hitherto undescribed Disease of Sheep). *Journal of Comparative Pathology* 45, 301-307 pp.
- Graham, W., Rubin, S.B., Boeckh, A., Buzhardt, L.F., Jones, S., Miller, M., Payne, P.A., Rehn, C., Smith-Blachmore, M., Stannard, R., Nelson, C.T., Atkins, C.E., Carithers, D., McCall, J. 2012. Current Canine Guidelines for the Diagnosis, Prevention, and Management of Heartworm (*Dirofilaria immitis*) Infection in Dogs
- In <http://www.heartwormsociety.org/veterinary-resources/canine-guidelines.html#4> (American Heartworm Society).
- Gramiccia, M., Gradoni, L., 2005, The current status of zoonotic leishmaniasis and approaches to disease control. *International Journal for Parasitology* 35, 1169-1180.
- Grandi, G., Quintavalla, C., Mavropoulou, A., Genchi, M., Gnudi, G., Bertoni, G., Kramer, L., 2010, A combination of doxycycline and ivermectin is adulticidal in dogs with naturally acquired heartworm disease (*Dirofilaria immitis*). *Veterinary Parasitology* 169, 347-351.
- Greene, C.E., Straubinger, R.K., Levy, S.A., 2012, Borreliosis, In: Greene, C.E. (Ed.) *Infectious diseases of the dog and cat*. pp. 447-465.
- Greig, B., Asanovich, K.M., Armstrong, P.J., Dumler, J.S., 1996, Geographic, clinical, serologic, and molecular evidence of granulocytic ehrlichiosis, a likely zoonotic disease, in Minnesota and Wisconsin dogs. *Journal of Clinical Microbiology* 34, 44-48.
- Greve, J.H., 1971, Age resistance to *Toxocara canis* in ascarid free dogs. *American Journal of Veterinary Research* 32, 1185-&.
- Groves, M.G., Dennis, G.L., Amyx, H.L., Huxsoll, D.L., 1975, Transmission of *Ehrlichia canis* to dogs by ticks (*Rhipicephalus sanguineus*). *American Journal of Veterinary Research* 36, 937-940.
- Gubler, D.J., 1966, A comparative study on the distribution, incidence and periodicity of the canine filarial worms *Dirofilaria immitis* Leidy and *Dipetalonema reconditum* Grassi in Hawaii. *Journal of Medical Entomology* 3, 159-167.
- Gutierrez, C.N., Martinez, M., Sanchez, E., De Vera, M., Rojas, M., Ruiz, J., Triana-Alonso, F.J., 2008, Cultivation and molecular identification of *Ehrlichia canis* and *Ehrlichia chaffeensis* from a naturally co-infected dog in Venezuela. *Veterinary Clinical Pathology* 37, 258-265.
- Hackett, T., Lappin, M.R., 2003, Prevalence of enteric pathogens in dogs of north-central Colorado. *Journal of the American Animal Hospital Association* 39, 52-56.
- Hamel, D., Rohrig, E., Pfister, K., 2011, Canine vector-borne disease in travelled dogs in Germany- A retrospective evaluation of laboratory data from the years 2004-2008. *Veterinary Parasitology* 181, 31-36.
- Hamer, S.A., Tsao, J.I., Walker, E.D., Mansfield, L.S., Foster, E.S., Hickling, G.J., 2009, Use of tick surveys and serosurveys to evaluate pet dogs as a sentinel species for emerging Lyme disease. *American Journal of Veterinary Research* 70, 49-56.
- Hamir, A.H., Onaga, I., 1986, Canine spirocercosis and dirofilariasis infection in Papua New Guinea. *Australian Veterinary Journal* 63, 98-99.
- Harrus, S., Aroch, I., Lavy, E., Bark, H., 1997, Clinical manifestations of infectious canine cyclic thrombocytopenia. *Veterinary Record* 141, 247-250.

- Harrus, S., Day, M.J., Waner, T., Bark, H., 2001, Presence of immune-complexes, and absence of antinuclear antibodies, in sera of dogs naturally and experimentally infected with *Ehrlichia canis*. *Veterinary Microbiology* 83, 343-349.
- Harrus, S., Ofri, R., Aizenberg, I., Waner, T., 1998a, Acute blindness associated with monoclonal gammopathy induced by *Ehrlichia canis* infection. *Veterinary Parasitology* 78, 155-160.
- Harrus, S., Waner, T., Aizenberg, I., Foley, J.E., Poland, A.M., Bark, H., 1998b, Amplification of ehrlichial DNA from dogs 34 months after infection with *Ehrlichia canis*. *Journal of Clinical Microbiology* 36, 73-76.
- Harrus, S., Waner, T., Bark, H., Jongejan, F., Cornelissen, A., 1999, Recent advances in determining the pathogenesis of canine monocytic ehrlichiosis. *Journal of Clinical Microbiology* 37, 2745-2749.
- Harrus, S., Waner, T., Friedmann-Morvinski, D., Fishman, Z., Bark, H., Harmelin, A., 2003, Down-regulation of MHC class II receptors of DH82 cells, following infection with *Ehrlichia canis*. *Veterinary Immunology and Immunopathology* 96, 239-243.
- Harrus, S., Waner, T., Neer, T.M., 2012, *Ehrlichia canis* infection, In: Greene, C.E. (Ed.) *Infectious diseases of the dog and cat*. Elsevier Saunders, St Louis, pp. 227-238.
- Harvey, J.W., 2012, *Anaplasma platys* infection (thrombocytotropic anaplasmosis), In: Greene, C.E. (Ed.) *Infectious Diseases of the Dog and Cat*. Elsevier Saunders, St. Louis, pp. 256-259.
- Harvey, J.W., Simpson, C.F., Gaskin, J.M., 1978, Cyclic thrombocytopenia induced by a rickettsia-like agent in dogs. *Journal of Infectious Diseases* 137, 182-188.
- Herbinger, K.H., Drerup, L., Alberer, M., Nothdurft, H.D., von Sonnenburg, F., Loscher, T., 2012, Spectrum of imported infectious diseases among children and adolescents returning from the tropics and subtropics. *Journal of Travel Medicine* 19, 150-157.
- Hill, F., 1999, Infectious and parasitic diseases of dogs in New Zealand. *Surveillance (Wellington)* 26, 3-5.
- Hirsch, M., Pantchev, N., 2008, Occurrence of the travel diseases leishmaniasis, ehrlichiosis, babesiosis and dirofilariosis in dogs living in Germany. *Kleintierpraxis* 53, 154-160.
- Hochedez, P., Caumes, E., 2007, Hookworm-related cutaneous larva migrans. *Journal of Travel Medicine* 14, 326-333.
- Hoskins, J.D., Breitschwerdt, E.B., Gaunt, S.D., French, T.W., Burgdorfer, W., 1988, Antibodies to *Ehrlichia canis*, *Ehrlichia platys* and spotted-fever group rickettsiae in Louisiana dogs. *Journal of Veterinary Internal Medicine* 2, 55-59.
- Hovius, J.W., Schuijt, T.J., de Groot, K.A., Roelofs, J., Oei, G.A., Marquart, J.A., de Beer, R., Veer, C.V., van der Poll, T., Ramamoorthi, N., Fikrig, E., van Dam, A.P., 2008, Preferential protection of *Borrelia burgdorferi sensu stricto* by a Salp15 homologue in *Ixodes ricinus* saliva. *Journal of Infectious Diseases* 198, 1189-1197.
- Hutton, T.A., Goldstein, R.E., Njaa, B.L., Atwater, D.Z., Chang, Y.F., Simpson, K.W., 2008, Search for *Borrelia burgdorferi* in kidneys of dogs with suspected "Lyme nephritis". *Journal of Veterinary Internal Medicine* 22, 860-865.
- Huxsoll, D.L., 1976, Canine ehrlichiosis (tropical canine pancytopenia)- review. *Veterinary Parasitology* 2, 49-60.
- IDEXX 2012. SNAP 4Dx Plus Test. In http://www.idexx.com/view/xhtml/en_us/smallanimal/inhouse/snap/4dx.jsf (IDEXX Laboratories).

- Inokuma, H., Fujii, K., Matsumoto, K., Okuda, M., Nakagome, K., Kosugi, R., Hirakawa, M., Onishi, T., 2002, Demonstration of *Anaplasma (Ehrlichia) platys* inclusions in peripheral blood platelets of a dog in Japan. *Veterinary Parasitology* 110, 145-152.
- Irwin, P.J., 2001, The first report of canine ehrlichiosis in Australia. *Australian Veterinary Journal* 79, 552-553.
- Itoh, N., Kanai, K., Hori, Y., Hoshi, F., Higuchi, S., 2009, Prevalence of *Giardia intestinalis* and other zoonotic intestinal parasites in private household dogs of the Hachinohe area in Aomori prefecture, Japan in 1997, 2002 and 2007. *Journal of Veterinary Science* 10, 305-308.
- Itoh, N., Muraoka, N., Saeki, H., Aoki, M., Itagaki, T., 2005, Prevalence of *Giardia intestinalis* infection in dogs of breeding kennels in Japan. *Journal of Veterinary Medical Science* 67, 717-718.
- Jaderlund, K.H., Egenvall, A., Bergstrom, K., Hedhammar, A., 2007, Seroprevalence of *Borrelia burgdorferi sensu lato* and *Anaplasma phagocytophilum* in dogs with neurological signs. *Veterinary Record* 160, 825-831.
- Jaenson, T.G.T., Talleklint, L., 1992, Incompetence of roe deer as reservoirs of the Lyme borreliosis spirochete. *Journal of Medical Entomology* 29, 813-817.
- Jameson, L.J., Medlock, J.M., 2011, Tick Surveillance in Great Britain. *Vector-Borne and Zoonotic Diseases* 11, 403-412.
- Jiang, J., Jiang, B., Yu, J., Zhang, W., Gao, H., Zhan, L., Sun, Y., Zhang, X., Zhang, P., Liu, W., Wu, X., Xu, R., Cao, W., 2011, *Anaplasma phagocytophilum* infection in ticks, China-Russia border. *Emerging Infectious Diseases* 17, 932-934.
- Joffe, D., Van Niekerk, D., Gagne, F., Gilleard, J., Kutz, S., Lobingier, R., 2011, The prevalence of intestinal parasites in dogs and cats in Calgary, Alberta. *Canadian Veterinary Journal- Revue Veterinaire Canadienne* 52, 1323-1328.
- Kartashev, V., Batashova, I., Kartashov, S., Ermakov, A., Mironova, A., Kuleshova, Y., Ilyasov, B., Kolodiy, I., Klyuchnikov, A., Ryabikina, E., Babicheva, M., Levchenko, Y., Pavlova, R., Pantchev, N., Morchon, R., Simon, F., 2011, Canine and human dirofilariosis in the Rostov region (Southern Russia). *Veterinary Medicine International* 2011, 1-5.
- Katavolos, P., Armstrong, P.M., Dawson, J.E., Telford, S.R., III, 1998, Duration of tick attachment required for transmission of granulocytic ehrlichiosis. *Journal of Infectious Diseases* 177, 1422-1425.
- Keirans, J.E., Hutcheson, H.J., Durden, L.A., Klompen, J.S.H., 1996, *Ixodes (Ixodes) scapularis* (Acari: Ixodidae): Redescription of all active stages, distribution, hosts, geographical variation, and medical and veterinary importance. *Journal of Medical Entomology* 33, 297-318.
- Kelly, P.J., Eoghain, G.N., Raoult, D., 2004, Antibodies reactive with *Bartonella henselae* and *Ehrlichia canis* in dogs from the communal lands of Zimbabwe. *Journal of the South African Veterinary Association-Tydskrif Van Die Suid-Afrikaanse Veterinere Vereniging* 75, 116-120.
- Killick-Kendrick, R., 1999, The biology and control of phlebotomine sand flies. *Clinics in Dermatology* 17, 279-289.
- Killick-Kendrick, R., Killick-Kendrick, M., Pinelli, E., Del Real, G., Molina, R., Vitutia, M.M., Canavate, M.C., Nieto, J., 1994, A laboratory model of canine leishmaniasis: the

- inoculation of dogs with *Leishmania infantum* promastigotes from midguts of experimentally infected phlebotomine sandflies. *Parasite (Paris, France)* 1, 311-318.
- Kim, C.M., Yi, Y.H., Yu, D.H., Lee, M.J., Cho, M.R., Desai, A.R., Shringi, S., Klein, T.A., Kim, H.C., Song, J.W., Back, L.J., Chong, S.T., O'Guinn, M.L., Lee, J.S., Lee, I.Y., Park, J.H., Foley, J., Chae, J.S., 2006, Tick-borne-borne rickettsial pathogens in ticks and small mammals in Korea. *Applied and Environmental Microbiology* 72, 5766-5776.
- Kittelberger, R., 2012, Difference in performance of an *Ehrlichia canis* immunofluorescence antibody test in two dog populations. *Surveillance (Wellington)* 39, 12-13.
- Klempner, M.S., Hu, L.T., Evans, J., Schmid, C.H., Johnson, G.M., Trevino, R.P., Norton, D., Levy, L., Wall, D., McCall, J., Kosinski, M., Weinstein, A., 2001, Two controlled trials of antibiotic treatment in patients with persistent symptoms and a history of Lyme disease. *New England Journal of Medicine* 345, 85-92.
- Klose, C., Mravak, S., Geb, M., Bienzle, U., Meyer, C.G., 1996, Autochthonous cutaneous larva migrans in Germany. *Tropical Medicine & International Health* 1, 503-504.
- Kohn, B., Galke, D., Beelitz, P., Pfister, K., 2008, Clinical features of canine granulocytic anaplasmosis in 18 naturally infected dogs. *Journal of Veterinary Internal Medicine* 22, 1289-1295.
- Kommenou, A.A., Mylonakis, M.E., Kouti, V., Tendoma, L., Leontides, L., Skountzou, E., Dessiris, A., Koutinas, A.F., Ofri, R., 2007, Ocular manifestations of natural canine monocytic ehrlichiosis (*Ehrlichia canis*): a retrospective study of 90 cases. *Veterinary Ophthalmology* 10, 137-142.
- Kontos, V.I., Papadopoulos, O., French, T.W., 1991, Natural and experimental infections with a Greek strain of *Ehrlichia platys*. *Veterinary Clinical Pathology* 20, 101-105.
- Korenberg, E.I., Nefedova, V.V., Romanenko, V.N., Gorelova, N.B., 2010, The Tick *Ixodes pavlovskyi* as a Host of *Spirochetes* Pathogenic for Humans and Its Possible Role in the Epizootiology and Epidemiology of Borrelioses. *Vector-Borne and Zoonotic Diseases* 10, 453-458.
- Koutinas, A.F., Polizopoulou, Z.S., Saridomichelakis, M.N., Argyriadis, D., Fytianou, A., Plevraki, K.G., 1999, Clinical considerations on canine visceral leishmaniasis in Greece: A retrospective study of 158 cases (1989-1996). *Journal of the American Animal Hospital Association* 35, 376-383.
- Kuenzle, S., von Budingen, H.C., Meier, M., Harrer, M.D., Urich, E., Becher, B., Goebels, N., 2007, Pathogen specificity and autoimmunity are distinct features of antigen-driven immune responses in neuroborreliosis. *Infection and Immunity* 75, 3842-3847.
- Kurtenbach, K., Sewell, H.S., Ogden, N.H., Randolph, S.E., Nuttall, P.A., 1998, Serum complement sensitivity as a key factor in Lyme disease ecology. *Infection and Immunity* 66, 1248-1251.
- Labarthe, N., Guerrero, J., 2005, Epidemiology of heartworm: What is happening in South America and Mexico? *Veterinary Parasitology* 133, 149-156.
- Lallo, M.A., Bondan, E.F., 2006, Prevalence of *Cryptosporidium* sp in institutionalized dogs in the city of Sao Paulo, Brazil. *Revista De Saude Publica* 40, 120-125.
- Landmann, J.K., Procvic, P., 2003, Experimental human infection with the dog hookworm, *Ancylostoma caninum*. *Medical Journal of Australia* 178, 69-71.
- Lasek-Nesselquist, E., Welch, D.M., Sogin, M.L., 2010, The identification of a new *Giardia duodenalis* assemblage in marine vertebrates and a preliminary analysis of G.

- duodenalis population biology in marine systems. *International Journal for Parasitology* 40, 1063-1074.
- Lebbad, M., Mattsson, J.G., Christensson, B., Ljungstrom, B., Backhans, A., Andersson, J.O., Svard, S.G., 2010, From mouse to moose: Multi locus genotyping of *Giardia* isolates from various animal species. *Veterinary Parasitology* 168, 231-239.
- Lee, A.C.Y., Montgomery, S.P., Theis, J.H., Blagburn, B.L., Eberhard, M.L., 2010a, Public health issues concerning the widespread distribution of canine heartworm disease. *Trends in Parasitology* 26, 168-173.
- Lee, A.C.Y., Schantz, P.M., Kazacos, K.R., Montgomery, S.P., Bowman, D.D., 2010b, Epidemiologic and zoonotic aspects of ascarid infections in dogs and cats. *Trends in Parasitology* 26, 155-161.
- Lefkaditis, M., Koukeri, S., Cozma, V., 2010, An endemic area of *Dirofilaria immitis* seropositive dogs at the eastern foothills of Mt Olympus, Northern Greece. *Helminthologia* 47, 3-7.
- Levy, S.A., Magnarelli, L.A., 1992, Relationship between development of antibodies to *Borrelia burgdorferi* in dogs and the subsequent development of limb joint borreliosis. *Journal of the American Veterinary Medical Association* 200, 344-347.
- Lewis, G.E., Ristic, M., Smith, R.D., Lincoln, T., Stephenson, E.H., 1977, Brown dog tick *Rhipicephalus sanguineus* and dog as experimental hosts of *Ehrlichia canis*. *American Journal of Veterinary Research* 38, 1953-1955.
- Li, J., Zhang, P., Wang, P.Y., Alsarakibi, M., Zhu, H.B., Liu, Y.J., Meng, X.L., Li, J.P., Guo, J.C., Li, G.Q., 2012, Genotype identification and prevalence of *Giardia duodenalis* in pet dogs of Guangzhou, Southern China. *Veterinary Parasitology* 188, 368-371.
- Liang, F.T., Jacobson, R.H., Straubinger, R.K., Grooters, A., Philipp, M.T., 2000, Characterization of a *Borrelia burgdorferi* VlsE invariable region useful in canine Lyme disease serodiagnosis by enzyme-linked immunosorbent assay. *Journal of Clinical Microbiology* 38, 4160-4166.
- Lilliehook, I., Egenvall, A., Tvedten, H.W., 1998, Hematopathology in dogs experimentally infected with a Swedish granulocytic *Ehrlichia* species. *Veterinary Clinical Pathology* 27, 116-122.
- Lim, S., Irwin, P.J., Lee, S., Oh, M., Ahn, K., Myung, B., Shin, S., 2010, Comparison of selected canine vector-borne diseases between urban animal shelter and rural hunting dogs in Korea. *Parasites & Vectors* 3.
- Lissman, B.A., Bosler, E.M., Camay, H., Ormiston, B.G., Benach, J.L., 1984, Spirochete associated arthritis (Lyme disease) in a dog. *Journal of the American Veterinary Medical Association* 185, 219-220.
- Little, M.D., Halsey, N.A., Cline, B.L., Katz, S.P., 1983, *Ancylostoma* larva in a muscle fiber of man following cutaneous larva migrans. *American Journal of Tropical Medicine and Hygiene* 32, 1285-1288.
- Little, S.E., Johnson, E.M., Lewis, D., Jaklitsch, R.P., Payton, M.E., Blagburn, B.L., Bowman, D.D., Moroff, S., Tams, T., Rich, L., Aucoin, D., 2009, Prevalence of intestinal parasites in pet dogs in the United States. *Veterinary Parasitology* 166, 144-152.
- Little, S.E., Lindsay, D.S., 2012, Laboratory diagnosis of protozoal infections, In: Greene, C.E. (Ed.) *Infectious diseases of the dog and cat*. pp. 711-717.

- Littman, M.P., Goldstein, R.E., Labato, M.A., Lappin, M.R., Moore, G.E., 2006, ACVIM small animal consensus statement on Lyme disease in dogs: Diagnosis, treatment, and prevention. *Journal of Veterinary Internal Medicine* 20, 422-434.
- Liu, S., Yuan, C., Cui, Y.F., Li, B.X., Wu, L.J., Liu, Y., 2012, Investigation of *Borrelia* spp. in ticks (Acari: Ixodidae) at the border crossings between China and Russia in Heilongjiang Province, China. *Asian Pacific Journal of Tropical Medicine* 5, 459-464.
- Lloyd, D., 1998a, Toxocarosis, In: Palmer, S.R., Soulsby, E.J.L., Simpson, D.I.H. (Eds.) *Zoonoses*. Oxford University Press, pp. 841-854.
- Lloyd, S., 1998b, Other cestode infections: Hymenolepiosis, diphyllbothriosis, coenurosis and other adult and larval cestodes, In: Palmer, S.R., Soulsby, E.J.L., Simpson, D.I.H. (Eds.) *Zoonoses*. Oxford University Press, pp. 651-663.
- Lopez, J., Castillo, A., Munoz, M., Hildebrandt, S., 1999, *Ehrlichia canis* in Chile; preliminary report. *Archivos De Medicina Veterinaria* 31, 211-214.
- Lucio-Forster, A., Liotta, J.L., Yaros, J.P., Briggs, K.R., Mohammed, H.O., Bowman, D.D., 2012, Morphological differentiation of eggs of *Ancylostoma caninum*, *Ancylostoma tubaeforme*, and *Ancylostoma braziliense* from dogs and cats in the United States. *Journal of Parasitology* 98, 1041-1044.
- M'Ghirbi, Y., Ghorbel, A., Amouri, M., Nebaoui, A., Haddad, S., Bouattour, A., 2009, Clinical, serological, and molecular evidence of ehrlichiosis and anaplasmosis in dogs in Tunisia. *Parasitology Research* 104, 767-774.
- Machado da Silva, A.V., de Souza Candido, C.D., Pereira, D.d.P., Brazil, R.P., Araujo Carreira, J.C., 2008, The first record of American visceral leishmaniasis in domestic cats from Rio de Janeiro, Brazil. *Acta Tropica* 105, 92-94.
- Macleod, J., Gordon, W.S., 1933, Studies in tick-borne fever of sheep I. Transmission by the tick *Ixodes ricinus*, with a description of the disease produced. *Parasitology* 25, 273-283 pp.
- Magnarelli, L.A., Anderson, J.F., Schreier, A.B., Ficke, C.M., 1987, Clinical and serologic studies of canine borreliosis. *Journal of the American Veterinary Medical Association* 191, 1089-1094.
- Mahdy, M.A.K., Lim, Y.A.L., Ngui, R., Fatimah, M.R.S., Choy, S.H., Yap, N.J., Al-Mekhlafi, H.M., Ibrahim, J., Surin, J., 2012, Prevalence and zoonotic potential of canine hookworms in Malaysia. *Parasites & Vectors* 5.
- Maretzki, C.H., Fisher, D.J., Greene, C.E., 1994, Granulocytic ehrlichiosis and meningitis in a dog. *Journal of the American Veterinary Medical Association* 205, 1554-1556.
- Martin-Sanchez, J., Acedo, C., Munoz-Perez, M., Pesson, B., Marchal, O., Morillas-Marquez, F., 2007, Infection by *Leishmania infantum* in cats: Epidemiological study in Spain. *Veterinary Parasitology* 145, 267-273.
- Martin, M.E., Caspersen, K., Dumler, J.S., 2001, Immunopathology and ehrlichial propagation are regulated by interferon-gamma and interleukin-10 in a murine model of human granulocytic ehrlichiosis. *Am J Pathol.* 158, 1881-1888.
- Martin, T. 1999. The animal health status of Samoa, Technical report, Secretariat of the Pacific Community.
- Martins, C.A.P., Guerrant, R.L., 1995, *Cryptosporidium* and cryptosporidiosis. *Parasitology Today* 11, 434-436.

- Masona, R.J., Lee, J.M., Curran, J.M., Moss, A., Van Der Heide, B., Daniels, P.W., 2001, Serological survey for *Ehrlichia canis* in urban dogs from the major population centres of northern Australia. *Australian Veterinary Journal* 79, 559-562.
- Mavromatis, K., Doyle, C.K., Lykidis, A., Ivanova, N., Francino, M.P., Chain, P., Shin, M., Malfatti, S., Larimer, F., Copeland, A., Detter, J.C., Land, M., Richardson, P.M., Yu, X.J., Walker, D.H., McBride, J.W., Kyrpides, N.C., 2006, The genome of the obligately intracellular bacterium *Ehrlichia canis* reveals themes of complex membrane structure and immune evasion strategies. *Journal of Bacteriology* 188, 4015-4023.
- McBride, J.W., Corstvet, R.E., Gaunt, S.D., Chinsangaram, J., Akita, G.Y., Osburn, B.I., 1996, PCR detection of acute *Ehrlichia canis* infection in dogs. *Journal of Veterinary Diagnostic Investigation* 8, 441-447.
- McKenna, P.B., 2009, An updated checklist of helminth and protozoan parasites of terrestrial mammals in New Zealand. *N. Z. J. Zool.* 36, 89-113.
- McSparran, K.D., 1994, Canine dirofilariasis - the case for the introduction of import controls on Australian dogs entering New Zealand. *Surveillance (Wellington)* 21, 17-21.
- Meinkoth, J.H., Hoover, J.P., Cowell, R.L., Tyler, R.D., Link, J., 1989, Ehrlichiosis in a dog with seizures and nonregenerative anemia. *Journal of the American Veterinary Medical Association* 195, 1754-1755.
- Meriem-Hind, B.M., Mohamed, M., 2009, Prevalence of canine *Dirofilaria immitis* infection in the city of Algiers, Algeria. *African Journal of Agricultural Research* 4, 1097-1100.
- Michel, G., Pomares, C., Ferrua, B., Marty, P., 2011, Importance of worldwide asymptomatic carriers of *Leishmania infantum* (*L. chagasi*) in human. *Acta Tropica* 119, 69-75.
- Midlej, V., Benchimol, M., 2009, *Giardia lamblia* behavior during encystment: how morphological changes in shape occur. *Parasitol Int.* 58, 72-80. Epub 2008 Dec 2003.
- Miller, L.L., Crosbie, P.R., 2011, Canine heartworm (*Dirofilaria immitis*) in Fresno and Madera Counties, California: Prevalence differences between foothill and valley habitats. *Veterinary Parasitology* 175, 84-91.
- Miller, T.A., 1966a, Blood loss during hookworm infection determined by erythrocyte labelling with radioactive 51-chromium. 1. Infection of dogs with normal and with X-irradiated *Ancylostoma caninum*. *Journal of Parasitology* 52, 844-855.
- Miller, T.A., 1966b, Blood loss during hookworm infection, determined by erythrocyte labelling with radioactive 51-chromium. 2. Pathogenesis of *Ancylostoma braziliense* infection in dogs and cats. *Journal of Parasitology* 52, 856-860.
- Mircean, V., Gyorke, A., Cozma, V., 2012, Prevalence and risk factors of *Giardia duodenalis* in dogs from Romania. *Veterinary Parasitology* 184, 325-329.
- Miro, G., Cardoso, L., Pennisi, M.G., Oliva, G., Baneth, G., 2008, Canine leishmaniosis - new concepts and insights on an expanding zoonosis: part two. *Trends in Parasitology* 24, 371-377.
- Miyoshi, T., Tsubouchi, H., Iwasaki, A., Shiraishi, T., Nabeshima, K., Shirakusa, T., 2006, Human pulmonary dirofilariasis: A case report and review of the recent Japanese literature. *Respirology* 11, 343-347.
- Montoya-Alonso, J.A., Carreton, E., Corbera, J.A., Juste, M.C., Mellado, I., Morchon, R., Simon, F., 2011, Current prevalence of *Dirofilaria immitis* in dogs, cats and humans from the island of Gran Canaria, Spain. *Veterinary Parasitology* 176, 291-294.

- Montoya-Alonso, J.A., Carreton, E., Juste, M.C., Mellado, I., Morchon, R., Simon, F., 2010, Epidemiological survey of canine heartworm disease on the island of Gran Canaria (Canary Islands - Spain) between 2000 and 2008. *Vet Parasitol* 173, 165-168.
- Montoya, J.A., Morales, M., Ferrer, O., Molina, J.M., Corbera, J.A., 1998, The prevalence of *Dirofilaria immitis* in Gran Canaria, Canary Islands, Spain (1994-1996). *Veterinary Parasitology* 75, 221-226.
- Moraes-Silva, E., Antunes, F.R., Rodrigues, M.S., Julido, F.D., Dias-Lima, A.G., Lemos-de-Sousa, V., de Alcantara, A.C., Reis, E.A.G., Nakatani, M., Badaro, R., Reis, M.G., Pontes-de-Carvalho, L., Franke, C.R., 2006, Domestic swine in a visceral leishmaniasis endemic area produce antibodies against multiple *Leishmania infantum* antigens but apparently resist to L-infantum infection. *Acta Tropica* 98, 176-182.
- Motoi, Y., Satoh, H., Inokuma, H., Kiyuuna, T., Muramatsu, Y., Ueno, H., Morita, C., 2001, First detection of *Ehrlichia platys* in dogs and ticks in Okinawa, Japan. *Microbiology and Immunology* 45, 89-91.
- Muro, A., Genchi, C., Cordero, M., Simon, F., 1999, Human dirofilariasis in the European union. *Parasitology Today* 15, 386-389.
- Murray, H.W., Berman, J.D., Davies, C.R., Saravia, N.G., 2005, Advances in leishmaniasis. *Lancet* 366, 1561-1577.
- Mylonakis, M.E., Koutinas, A.F., Billinis, C., Leontides, L.S., Kontos, V., Papadopoulos, O., Rallis, T., Fytianou, A., 2003, Evaluation of cytology in the diagnosis of acute canine monocytic ehrlichiosis (*Ehrlichia canis*): a comparison between five methods. *Veterinary Microbiology* 91, 197-204.
- Nadelman, R.B., Nowakowski, J., Fish, D., Falco, R.C., Freeman, K., McKenna, D., Welch, P., Marcus, R., Aguero-Rosenfeld, M.E., Dennis, D.T., Wormser, G.P., Tick Bite Study, G., 2001, Prophylaxis with single-dose doxycycline for the prevention of lyme disease after an ixodes scapularis tick bite. *New England Journal of Medicine* 345, 79-84.
- Nadelman, R.B., Wormser, G.P., 1998, Lyme borreliosis. *Lancet* 352, 557-565.
- Ndip, L.M., Ndip, R.N., Ndiva, V.E., Awuh, J.A., Walker, D.H., McBride, J.W., 2007, Ehrlichia species in Rhipicephalus sanguineus ticks in Cameroon. *Vector-Borne and Zoonotic Diseases* 7, 221-227.
- Neer, T.M., Breitschwerdt, E.B., Greene, R.T., Lappin, M.R., 2002, Consensus statement on ehrlichial disease of small animals from the infectious disease study group of the ACVIM. *Journal of Veterinary Internal Medicine* 16, 309-315.
- Nelson, C.T., 2012, Heartworm Disease, In: Greene, C.E. (Ed.) *Infectious Diseases of the Cat and Dog*. Elsevier Saunders, St. Louis, pp. 865-877.
- Nyindo, M., Huxsoll, D.L., Ristic, M., Kakoma, I., Brown, J.L., Carson, C.A., Stephenson, E.H., 1980, Cell-mediated and humoral immune responses of German shepherd dogs and beagles to experimental infection with *Ehrlichia canis*. *American Journal of Veterinary Research* 41, 250-254.
- O'Connor, T.P., Hanscom, J.L., Hegarty, B.C., Groat, R.G., Breitschwerdt, E.B., 2006, Comparison of an indirect immunofluorescence assay, western blot analysis, and a commercially available ELISA for detection of *Ehrlichia canis* antibodies in canine sera. *American Journal of Veterinary Research* 67, 206-210.

- Ogden, N.H., Bigras-Poulin, M., O'Callaghan, C.J., Barker, I.K., Kurtenbach, K., Lindsay, L.R., Charron, D.F., 2007, Vector seasonality, host infection dynamics and fitness of pathogens transmitted by the tick *Ixodes scapularis*. *Parasitology* 134, 209-227.
- Ohnishi, J., Piesman, J., de Silva, A.M., 2001, Antigenic and genetic heterogeneity of *Borrelia burgdorferi* populations transmitted by ticks. *Proceedings of the National Academy of Sciences of the United States of America* 98, 670-675.
- Okewole, E.A., Oduye, O.O., 2000, Experimental studies on hookworm infection. I. Clinical observations following *Ancylostoma caninum* infections in dogs. *Tropical Veterinarian* 18, 150-157.
- Oliva, G., Scalone, A., Manzillo, V.F., Gramiccia, M., Pagano, A., Di Muccio, T., Gradoni, L., 2006, Incidence and time course of *Leishmania infantum* infections examined by parasitological, serologic, and nested-PCR techniques in a cohort of naive dogs exposed to three consecutive transmission seasons. *Journal of Clinical Microbiology* 44, 1318-1322.
- Otranto, D., Capelli, G., Genchi, C., 2009a, Changing distribution patterns of canine vector borne diseases in Italy: leishmaniosis vs. dirofilariosis. *Parasites & Vectors* 2, S2.
- Otranto, D., Dantas-Torres, F., Breitschwerdt, E.B., 2009b, Managing canine vector-borne diseases of zoonotic concern: part one. *Trends in Parasitology* 25, 157-163.
- Overgaauw, P.A.M., van Zutphen, L., Hoek, D., Yaya, F.O., Roelfsema, J., Pinelli, E., van Knapen, F., Kortbeek, L.M., 2009, Zoonotic parasites in fecal samples and fur from dogs and cats in The Netherlands. *Veterinary Parasitology* 163, 115-122.
- Owen, I.L., 2005, Parasitic zoonoses in Papua New Guinea. *Journal of Helminthology* 79, 1-14.
- Palmer, C.S., Thompson, R.C.A., Traub, R.J., Rees, R., Robertson, I.D., 2008a, National study of the gastrointestinal parasites of dogs and cats in Australia. *Veterinary Parasitology* 151, 181-190.
- Palmer, C.S., Traub, R.J., Robertson, I.D., Devlin, G., Rees, R., Thompson, R.C.A., 2008b, Determining the zoonotic significance of *Giardia* and *Cryptosporidium* in Australian dogs and cats. *Veterinary Parasitology* 154, 142-147.
- Palmer, C.S., Traub, R.J., Robertson, I.D., Hobbs, R.P., Elliot, A., While, L., Rees, R., Thompson, R.C.A., 2007, The veterinary and public health significance of hookworm in dogs and cats in Australia and the status of *A. ceylanicum*. *Veterinary Parasitology* 145, 304-313.
- Pampiglione, S., Rivasi, F., Gustinelli, A., 2009, *Dirofilaria immitis* human cases in the Old World, attributed to *Dirofilaria immitis* : a critical analysis. *Histopathology* 54, 192-204.
- Pantchev, N., Norden, N., Lorentzen, L., Rossi, M., Rossi, U., Brand, B., Dyachenko, V., 2009, Current surveys on the prevalence and distribution of *Dirofilaria* spp. in dogs in Germany. *Parasitology Research* 105, 63-74.
- Papini, R., Gorini, G., Spaziani, A., Cardini, G., 2005, Survey on giardiasis in shelter dog populations. *Veterinary Parasitology* 128, 333-339.
- Parola, P., Cornet, J.P., Sanogo, Y.O., Miller, R.S., Thien, H.V., Gonzalez, J.P., Raoult, D., Telford, S.R., Wongsrichanalai, C., 2003, Detection of *Ehrlichia* spp., *Anaplasma* spp., *Rickettsia* spp., and other eubacteria in ticks from the Thai-Myanmar border and Vietnam. *Journal of Clinical Microbiology* 41, 1600-1608.
- Payne, P.A., Artzer, M., 2009, The Biology and Control of *Giardia* spp and *Trichostrongylus axei*. *Veterinary Clinics of North America-Small Animal Practice* 39, 993-1007.

- Perez, M., Rikihisa, Y., Wen, B.H., 1996, *Ehrlichia canis*-like agent isolated from a man in Venezuela: Antigenic and genetic characterization. *Journal of Clinical Microbiology* 34, 2133-2139.
- Perille, A.L., Matus, R.E., 1991, Canine ehrlichiosis in 6 dogs with persistently increased antibody titres. *Journal of Veterinary Internal Medicine* 5, 195-198.
- Petersen, C.A., 2009, Leishmaniasis, an Emerging Disease Found in Companion Animals in the United States. *Topics in Companion Animal Medicine* 24, 182-188.
- Piesman, J., Happ, C.M., 1997, Ability of the Lyme disease spirochete *Borrelia burgdorferi* to infect rodents and three species of human-biting ticks (blacklegged tick, American dog tick, lone star tick) (Acari: Ixodidae). *Journal of Medical Entomology* 34, 451-456.
- Pinyowong, D., Jittapalapong, S., Suksawat, F., Stich, R.W., Thamchaipenet, A., 2008, Molecular characterization of Thai *Ehrlichia canis* and *Anaplasma platys* strains detected in dogs. *Infection, Genetics and Evolution* 8, 433-438.
- Poitout, F.M., Shinozaki, J.K., Stockwell, P.J., Holland, C.J., Shukla, S.K., 2005, Genetic variants of *Anaplasma phagocytophilum* infecting dogs in western Washington State. *Journal of Clinical Microbiology* 43, 796-801.
- Popov, V.L., Han, V.C., Chen, S.M., Dumler, J.S., Feng, H.M., Andreadis, T.G., Tesh, R.B., Walker, D.H., 1998, Ultrastructural differentiation of the genogroups in the genus *Ehrlichia*. *Journal of Medical Microbiology* 47, 235-251.
- Prociv, P., 2003, Zoonotic hookworm infections (Ancylostomosis), In: Palmer, S.R., Soulsby, E.J.L., Simpson, D.I.H. (Eds.) *Zoonoses*. Oxford medical publications, pp. 803-822.
- Pusterla, N., Pusterla, J.B., Deplazes, P., Wolfensberger, C., Muller, W., Horauf, A., Reusch, C., Lutz, H., 1998, Seroprevalence of *Ehrlichia canis* and of canine granulocytic Ehrlichia infection in dogs in Switzerland. *Journal of Clinical Microbiology* 36, 3460-3462.
- Quinnell, R.J., Courtenay, O., 2009, Transmission, reservoir hosts and control of zoonotic visceral leishmaniasis. *Parasitology* 136, 1915-1934.
- Ramos, R., Ramos, C., Araujo, F., Oliveira, R., Souza, I., Pimentel, D., Galindo, M., Santana, M., Rosas, E., Faustino, M., Alves, L., 2010, Molecular survey and genetic characterization of tick-borne pathogens in dogs in metropolitan Recife (north-eastern Brazil). *Parasitology Research* 107, 1115-1120.
- Ravnik, U., Tozon, N., Strasek, K., Zupanc, T.A., 2009, Clinical and haematological features in *Anaplasma phagocytophilum* seropositive dogs. *Clinical Microbiology and Infection* 15, 39-40.
- Reiskind, M.H., Lounibos, L.P., 2012, Spatial and temporal patterns of abundance of *Aedes aegypti* L. (*Stegomyia aegypti*) and *Aedes albopictus* (Skuse) [*Stegomyia albopictus* (Skuse)] in southern Florida. *Med Vet Entomol* 27, 12000.
- Remy, V., Hansmann, Y., De Martino, S., Christmann, D., Brouqui, P., 2003, Human anaplasmosis presenting as atypical pneumonitis in France. *Clinical Infectious Diseases* 37, 846-848.
- Reupena, M.S. 2012a. Annual International Migration Report 2011 (Apia, Samoa, Samoa Bureau of Statistics).
- Reupena, M.S. 2012b. Population and Housing Census 2011 (Apia, Samoa, Samoa Bureau of Statistics).
- Rikihisa, Y., 2006, *Ehrlichia* subversion of host innate responses. *Current Opinion in Microbiology* 9, 95-101.

- Rishniw, M., Liotta, J., Bellosa, M., Bowman, D., Simpson, K.W., 2010, Comparison of 4 Giardia Diagnostic Tests in Diagnosis of Naturally Acquired Canine Chronic Subclinical Giardiasis. *Journal of Veterinary Internal Medicine* 24, 293-297.
- Rose, K., Curtis, J., Baldwin, T., Mathis, A., Kumar, B., Sakthianandeswaren, A., Spurck, T., Choy, J.L., Handman, E., 2004, Cutaneous leishmaniasis in red kangaroos: isolation and characterisation of the causative organisms. *International Journal for Parasitology* 34, 655-664.
- Rubinsky-Elefant, G., Hirata, C.E., Yamamoto, J.H., Ferreira, M.U., 2010, Human toxocariasis: diagnosis, worldwide seroprevalences and clinical expression of the systemic and ocular forms. *Annals of Tropical Medicine and Parasitology* 104, 3-23.
- Rupprecht, C.E., Shlim, D.R., 2012, Infectious Disease Related to Travel: Rabies, In: Brunette, G.W. (Ed.) *CDC Health Information for International Travel 2012*. Oxford University Press, Oxford, pp. 273-278.
- Russell, R.C., Doggett, S.L., Munro, R., Ellis, J., Avery, D., Hunt, C., Dickeson, D., 1994, Lyme disease- a search for the causative agent in ticks in south-eastern Australia. *Epidemiology and Infection* 112, 375-384.
- Sainz, A., Amusatogui, I., Tesouro, M.A., 1999, Ehrlichia platys infection and disease in dogs in Spain. *Journal of Veterinary Diagnostic Investigation* 11, 382-384.
- Samarawickrema, W.A., Kimura, E., Sones, F., Paulson, G.S., Cummings, R.F., 1992, Natural infections of *Dirofilaria immitis* in *Aedes (Stegomyia) polynesiensis* and *Aedes (Finlaya) samoanus* and their implication in human health in Samoa. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 86, 187-188.
- Sanogo, Y., Davoust, B., Inokuma, H., Camicas, J.L., Parola, P., Brouqui, P., 2003, First evidence of *Anaplasma platys* in *Rhipicephalus sanguineus* (Acari : Ixodida) collected from dogs in Africa. *Onderstepoort Journal of Veterinary Research* 70, 205-212.
- Santos, F., Coppede, J.S., Pereira, A.L.A., Oliveira, L.P., Roberto, P.G., Benedetti, R.B.R., Zucoloto, L.B., Lucas, F., Sobreira, L., Marins, M., 2009, Molecular evaluation of the incidence of *Ehrlichia canis*, *Anaplasma platys* and *Babesia* spp. in dogs from Ribeirao Preto, Brazil. *Veterinary Journal* 179, 145-148.
- Sarih, M., Jouda, F., Gern, L., Postic, D., 2003, First isolation of *Borrelia burgdorferi* sensu lato from *Ixodes ricinus* ticks in Morocco. *Vector-Borne and Zoonotic Diseases* 3, 133-139.
- Schantz, P.M., 1994, Of worms, dogs and human hosts- continuing challenges for veterinarians in prevention of human disease. *Journal of the American Veterinary Medical Association* 204, 1023-1028.
- Schnieder, T., Laabs, E.M., Welz, C., 2011, Larval development of *Toxocara canis* in dogs. *Veterinary Parasitology* 175, 193-206.
- Scorza, A.V., Duncan, C., Miles, L., Lappin, M.R., 2011, Prevalence of selected zoonotic and vector-borne agents in dogs and cats in Costa Rica. *Veterinary Parasitology* 183, 178-183.
- Scorza, V., Lappin, M.R., 2012a, Cryptosporidiosis and Cyclosporiasis, In: Greene, C.E. (Ed.) *Infectious Diseases of the Dog and Cat*. Elsevier Saunders, St Louis, pp. 840-851.
- Scorza, V., Lappin, M.R., 2012b, Giardiasis, In: Greene, C.E. (Ed.) *Infectious Diseases of the Dog and Cat*. Elsevier Saunders, St. Louis, pp. 785-792.
- Seaman, R.L., Kania, S.A., Hegarty, B.C., Legendre, A.M., Breitschwerdt, E.B., 2004, Comparison of results for serologic testing and a polymerase chain reaction assay to determine the

- prevalence of stray dogs in eastern Tennessee seropositive to *Ehrlichia canis*. American Journal of Veterinary Research 65, 1200-1203.
- Selby, L.A., Corwin, R.M., Hayes, H.M., 1980, Risk factors associated with canine heartworm infection. Journal of the American Veterinary Medical Association 176, 33-35.
- Senevira, P., Weerasin, N., Ariyadas, S., 1973, Transmission of *Haemobartonella canis* by dog tick, *Rhipicephalus sanguineus*. Research in Veterinary Science 14, 112-114.
- Shires, P.K., Turnwald, G.H., Qualls, C.W., King, G.K., 1982, Epidural dirofilariasis causing paraparesis in a dog. Journal of the American Veterinary Medical Association 180, 1340-1343.
- Shukla, R., Giraldo, P., Kraliz, A., Finnigan, M., Sanchez, A.L., 2006, Cryptosporidium spp. and other zoonotic enteric parasites in a sample of domestic dogs and cats in the Niagara region of Ontario. Canadian Veterinary Journal-Revue Veterinaire Canadienne 47, 1179-1184.
- Silva, F., Monobe, M.M., Lopes, R.S., Araujo, J.P., 2012, Molecular characterization of Giardia duodenalis in dogs from Brazil. Parasitology Research 110, 325-334.
- Simon, F., Lopez-Belmonte, J., Marcos-Atxutegi, C., Morchon, R., Martin-Pacho, J.R., 2005, What is happening outside North America regarding human dirofilariasis? Veterinary Parasitology 133, 181-189.
- Simon, F., Morchon, R., Gonzalez-Miguel, J., Marcos-Atxutegi, C., Siles-Lucas, M., 2009, What is new about animal and human dirofilariasis? Trends in Parasitology 25, 404-409.
- Simpson, R.M., Gaunt, S.D., Hair, J.A., Kocan, K.M., Henk, W.G., Casey, H.W., 1991, Evaluation of *Rhipicephalus sanguineus* as a potential biologic vector of *Ehrlichia platys*. American Journal of Veterinary Research 52, 1537-1541.
- Solano-Gallego, L., Koutinas, A., Miro, G., Cardoso, L., Pennisi, M.G., Ferrer, L., Bourdeau, P., Oliva, G., Baneth, G., 2009, Directions for the diagnosis, clinical staging, treatment and prevention of canine leishmaniosis. Veterinary Parasitology 165, 1-18.
- Solano-Gallego, L., Morell, P., Arboix, M., Alberola, J., Ferrer, L., 2001, Prevalence of Leishmania infantum infection in dogs living in an area of canine leishmaniosis endemicity using PCR on several tissues and serology. Journal of Clinical Microbiology 39, 560-563.
- Sood, S.K., Salzman, M.B., Johnson, B.J.B., Happ, C.M., Feig, K., Carmody, L., Rubin, L.G., Hilton, E., Piesman, J., 1997, Duration of tick attachment as a predictor of the risk of Lyme disease in an area in which Lyme disease is endemic. Journal of Infectious Diseases 175, 996-999.
- Sowemimo, O.A., 2007, Prevalence and intensity of Toxocara canis (Werner, 1782) in dogs and its potential public health significance in Ile-Ife, Nigeria. Journal of Helminthology 81, 433-438.
- Sparagano, O.A.E., de Vos, A.P., Paoletti, B., Camma, C., de Santis, P., Otranto, D., Giangaspero, A., 2003, Molecular detection of Anaplasma platys in dogs using polymerase chain reaction and reverse line blot hybridization. Journal of Veterinary Diagnostic Investigation 15, 527-534.
- Stone, M., 2005, Quarterly report of investigations of suspected exotic diseases. Surveillance (Wellington) 32, 20-23.
- Stone, W.M., Girardeau, M., 1968, Transmammary passage of Ancylostoma caninum larvae in dogs. Journal of Parasitology 54, 426-429.

- Straubinger, R.K., Straubinger, A.F., Harter, L., Jacobson, R.H., Chang, Y.F., Summers, B.A., Erb, H.N., Appel, M.J.G., 1997, *Borrelia burgdorferi* migrates into joint capsules and causes an up-regulation of interleukin-8 in synovial membranes of dogs experimentally infected with ticks. *Infection and Immunity* 65, 1273-1285.
- Straubinger, R.K., Straubinger, A.F., Summers, B.A., Jacobson, R.H., 2000, Status of *Borrelia burgdorferi* infection after antibiotic treatment and the effects of corticosteroids: An experimental study. *Journal of Infectious Diseases* 181, 1069-1081.
- Suksawat, J., Hegarty, B.C., Breitschwerdt, E.B., 2000, Seroprevalence of *Ehrlichia canis*, *Ehrlichia equi*, and *Ehrlichia risticii* in sick dogs from North Carolina and Virginia. *Journal of Veterinary Internal Medicine* 14, 50-55.
- Suksawat, J., Pitulle, C., Arraga-Alvarado, C., Madrigal, K., Hancock, S.I., Breitschwerdt, E.B., 2001a, Coinfection with three Ehrlichia species in dogs from Thailand and Venezuela with emphasis on consideration of 16S ribosomal DNA secondary structure. *Journal of Clinical Microbiology* 39, 90-93.
- Suksawat, J., Yu, X.J., Hancock, S.I., Hegarty, B.C., Nilkumhang, P., Breitschwerdt, E.B., 2001b, Serologic and molecular evidence of coinfection with multiple vector-borne pathogens in dogs from Thailand. *Journal of Veterinary Internal Medicine* 15, 453-462.
- Swanson, S.J., Neitzel, D., Reed, K.D., Belongia, E.A., 2006, Coinfections acquired from *Ixodes* ticks. *Clinical Microbiology Reviews* 19, 708-727.
- Tangtrongsup, S., Scorza, V., 2010, Update on the Diagnosis and Management of *Giardia* spp Infections in Dogs and Cats. *Topics in Companion Animal Medicine* 25, 155-162.
- Telford, S.R., III, Dawson, J.E., Katavolos, P., Warner, C.K., Kolbert, C.P., Persing, D.H., 1996, Perpetuation of the agent of human granulocytic ehrlichiosis in a deer tick-rodent cycle. *Proceedings of the National Academy of Sciences of the United States of America* 93, 6209-6214.
- Theis, J.H., 2005, Public health aspects of dirofilariasis in the United States. *Veterinary Parasitology* 133, 157-180.
- Thompson, J., 1998a, Blood parasites of animals in New Zealand. *Surveillance (Wellington)* 25, 6-8.
- Thompson, R.C.A., 1998b, *Giardia* infections, In: Palmer, S.R., Soulsby, E.J.L., Simpson, D.I.H. (Eds.) *Zoonoses*. Oxford University Press, pp. 545-561.
- Thompson, R.C.A., Conlan, J.V., 2011, Emerging issues and parasite zoonoses in the SE Asian and Australasian region. *Veterinary Parasitology* 181, 69-73.
- Thompson, R.C.A., Palmer, C.S., O'Handley, R., 2008, The public health and clinical significance of *Giardia* and *Cryptosporidium* in domestic animals. *Veterinary Journal* 177, 18-25.
- Tonks, M.C., Brown, T.J., Ionas, G., 1991, GIARDIA INFECTION OF CATS AND DOGS IN NEW-ZEALAND. *N. Z. Vet. J.* 39, 33-34.
- Traub, R.J., Hobbs, R.P., Adams, P.J., Behnke, J.M., Harris, P.D., Thompson, R.C.A., 2007, A case of mistaken identity - reappraisal of the species of canid and felid hookworms (*Ancylostoma*) present in Australia and India. *Parasitology* 134, 113-119.
- Traub, R.J., Inpankaew, T., Reid, S.A., Sutthikornchai, C., Sukthana, Y., Robertson, I.D., Thompson, R.C.A., 2009, Transmission cycles of *Giardia duodenalis* in dogs and humans in Temple communities in Bangkok-A critical evaluation of its prevalence using three diagnostic tests in the field in the absence of a gold standard. *Acta Tropica* 111, 125-132.

- Traub, R.J., Inpankaew, T., Sutthikornchai, C., Sukthana, Y., Thompson, R.C.A., 2008, PCR-based coprodiagnostic tools reveal dogs as reservoirs of zoonotic ancylostomiasis caused by *Ancylostoma ceylanicum* in temple communities in Bangkok. *Veterinary Parasitology* 155, 67-73.
- Traub, R.J., Robertson, I.D., Irwin, P., Mencke, N., Thompson, R.C.A., 2002, The role of dogs in transmission of gastrointestinal parasites in a remote tea-growing community in northeastern India. *American Journal of Tropical Medicine and Hygiene* 67, 539-545.
- Traub, R.J., Robertson, I.D., Irwin, P., Mencke, N., Thompson, R.C.A., 2004, Application of a species-specific PCR-RFLP to identify *Ancylostoma* eggs directly from canine faeces. *Veterinary Parasitology* 123, 245-255.
- Traub, R.J., Robertson, I.D., Irwin, P.J., Mencke, N., Thompson, R., 2005, Canine gastrointestinal parasitic zoonoses in India. *Trends in Parasitology* 21, 42-48.
- Traversa, D., 2011, Are we paying too much attention to cardiopulmonary nematodes and neglecting old-fashioned worms like *Trichuris vulpis*? *Parasites & Vectors* 4.
- Troeger, H., Epple, H.J., Schneider, T., Wahnschaffe, U., Ullrich, R., Burchard, G.D., Jelinek, T., Zeitz, M., Fromm, M., Schulzke, J.D., 2007, Effect of chronic *Giardia lamblia* infection on epithelial transport and barrier function in human duodenum. *Gut* 56, 328-335.
- Trotta, M., Fogliazza, A., Furlanello, T., Solano-Gallego, L., 2009, A molecular and serological study of exposure to tick-borne pathogens in sick dogs from Italy. *Clinical Microbiology and Infection* 15, 62-63.
- Tzipory, N., Crawford, P.C., Levy, J.K., 2010, Prevalence of *Dirofilaria immitis*, *Ehrlichia canis*, and *Borrelia burgdorferi* in pet dogs, racing greyhounds, and shelter dogs in Florida. *Veterinary Parasitology* 171, 136-139.
- Upjohn, M., Cobb, C., Monger, J., Geurden, T., Claerebout, E., Fox, M., 2010, Prevalence, molecular typing and risk factor analysis for *Giardia duodenalis* infections in dogs in a central London rescue shelter. *Veterinary Parasitology* 172, 341-346.
- Vargas-Hernandez, G., Andre, M.R., Faria, J.L.M., Munhoz, T.D., Hernandez-Rodriguez, M., Machado, R.Z., Tinucci-Costa, M., 2012, Molecular and serological detection of *Ehrlichia canis* and *Babesia vogeli* in dogs in Colombia. *Veterinary Parasitology* 186, 254-260.
- Verocai, G.G., Tavares, P.V., Ribeiro, F.D., Correia, T.R., Scott, F.B., 2010, Effects of Disinfectants on *Toxocara canis* Embryogenesis and Larval Establishment in Mice Tissues. *Zoonoses and Public Health* 57, E213-E216.
- Verster, A., Cilliers, W.J., Schroeder, H., 1991, A case of heartworm (*Dirofilaria immitis*) in an imported dog and a report of the occurrence of canine microfilariae in the Republic of South Africa. *Journal of the South African Veterinary Association* 62, 33-34.
- Vinasco, J., Li, O., Alvarado, A., Diaz, D., Hoyos, L., Tabachi, L., Sirigireddy, K., Ferguson, C., Moro, M.H., 2007, Molecular evidence of a new strain of *Ehrlichia canis* from South America. *Journal of Clinical Microbiology* 45, 2716-2719.
- Waner, T., Harrus, S., Bark, H., Bogin, E., Avidar, Y., Keysary, A., 1997, Characterization of the subclinical phase of canine ehrlichiosis in experimentally infected beagle dogs. *Veterinary Parasitology* 69, 307-317.
- Waner, T., Harrus, S., Jongejan, F., Bark, H., Keysary, A., Cornelissen, A., 2001, Significance of serological testing for ehrlichial diseases in dogs with special emphasis on the

- diagnosis of canine monocytic ehrlichiosis caused by *Ehrlichia canis*. *Veterinary Parasitology* 95, 1-15.
- Washabau, R.J., Holt, D.E., 2005, Diseases of the large intestine, In: Ettinger, S.J., Feldman, E.C. (Eds.) *Textbook of Veterinary Internal Medicine*. Elsevier Saunders, pp. 1378-1408.
- Watier-Grillot, S., Marie, J.L., Cabre, O., Davoust, B., 2011, Survey of canine *Dirofilaria immitis* infection in New Caledonia. *Veterinary Medicine International* 2011, 1-5.
- Weber, R., Bryan, R.T., Bishop, H.S., Wahlquist, S.P., Sullivan, J.J., Juranek, D.D., 1991, Threshold of detection of *Cryptosporidium* oocysts in human stool specimens-evidence for low sensitivity of current diagnostic methods. *Journal of Clinical Microbiology* 29, 1323-1327.
- Webster, K.A., Smith, H.V., Giles, M., Dawson, L., Robertson, L.J., 1996, Detection of *Cryptosporidium parvum* oocysts in faeces: Comparison of conventional coproscopical methods and the polymerase chain reaction. *Veterinary Parasitology* 61, 5-13.
- Wells, K., Beaucournu, J.C., Durden, L.A., Petney, T.N., Lakim, M.B., O'Hara, R.B., 2012, Ectoparasite infestation patterns of domestic dogs in suburban and rural areas in Borneo. *Parasitology Research* 111, 909-919.
- Woody, B.J., Hoskins, J.D., 1991, Ehrlichial diseases of dogs. *Veterinary Clinics of North America-Small Animal Practice* 21, 75-98.
- Xia, Z., Yu, D., Mao, J., Zhang, Z., Yu, J., 2012, The occurrence of *Dirofilaria immitis*, *Borrelia burgdorferi*, *Ehrlichia canis* and *Anaplasma phagocytophium* in dogs in China. *Journal of Helminthology* 86, 185-189.
- Xiao, L.H., Cama, V.A., Cabrera, L., Ortega, Y., Pearson, J., Gilman, R.H., 2007, Possible transmission of *Cryptosporidium canis* among children and a dog in a household. *Journal of Clinical Microbiology* 45, 2014-2016.
- Xiao, L.H., Fayer, R., 2008, Molecular characterisation of species and genotypes of *Cryptosporidium* and *Giardia* and assessment of zoonotic transmission. *International Journal for Parasitology* 38, 1239-1255.
- Xu, Q.L., McShan, K., Liang, F.T., 2008, Essential protective role attributed to the surface lipoproteins of *Borrelia burgdorferi* against innate defences. *Molecular Microbiology* 69, 15-29.
- Yoshiie, K., Kim, H., Mott, J., Rikihisa, Y., 2000, Intracellular infection by the human granulocytic ehrlichiosis agent inhibits human neutrophil apoptosis. *Infection and Immunity* 68, 1125-1133.
- Yutuc, L.M., 1954, The incidence and pre-patent period of *Ancylostoma caninum* and *Toxocara canis* in prenatally infected puppies. *Journal of Parasitology* 40, 18-19.
- Zajak, A.M., Conboy, G.A., 2012, Faecal examination for the diagnosis of parasitism: Faecal exam procedures, In: *Veterinary Clinical Parasitology*. Wiley-Blackwell, pp. 4-12.

Appendix 1: Questionnaire completed by the owner of the dog

Samoa canine study questionnaire 2011

Sample number: _____

Area from: _____

Dog details:

Dog name: _____ **Colour:** _____

Breed: Samoan mix Other breed _____

Approx weight: _____

Age: _____ known estimated

Sex: Male Female

Lifestyle: Pet Stray Indoors Outdoors

Ever been seen by a vet before Yes No

If yes, what for? _____

Ever vaccinated Yes No Unsure

If yes, when? _____

Vaccine type: core (DHP) Lepto Unsure

Ever treated for worms, mites or fleas Yes No Unsure

If yes, when? _____

With what product? _____