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Prevalence of selected infectious diseases in Samoan dogs

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CONTENTS

Contents	5
Abstract.....	7
Sammanfattning (Abstract in Swedish).....	8
Introduction.....	9
Background.....	9
Parasites	10
Hookworms.....	10
<i>Dirofilaria immitis</i>	10
<i>Toxocara canis</i>	11
<i>Trichuris vulpis</i>	12
<i>Dipylidium caninum</i>	12
Vector borne diseases	13
<i>Anaplasma phagocytophilum</i>	13
<i>Anaplasma platys</i>	13
<i>Ehrlichia canis</i>	14
<i>Borrelia burgdorferi</i>	14
<i>Leishmania infantum</i>	15
Prevalence of canine diseases in Samoa	15
Aims.....	16
Material and Methods	17
Selection of dogs.....	17
Sample collection.....	17
Sample analysis	17
Faecal analysis	17
Serum analysis	17
Statistical analysis.....	18
Results.....	19
Questionnaire results	19
Serum samples	19
Antibodies against <i>Anaplasma phagocytophilum</i> in serum.....	19
<i>Dirofilaria immitis</i> antigen in serum	19
<i>Ehrlichia canis</i> , <i>Borrelia burgdorferi</i> and <i>Leishmania infantum</i>	20
Faecal floats	20
Hookworm eggs in faecal floats	20
<i>Trichuris vulpis</i> eggs in faecal floats	20

<i>Toxocara canis</i> eggs in faecal floats.....	21
Other findings in faeces	21
Skin examination	21
Discussion.....	23
Animal health and public health	24
Acknowledgements.....	26
References.....	27

ABSTRACT

The aim of this study was to investigate the prevalence of selected infectious diseases of dogs in Samoa. Samples were collected from 192 dogs coming from 21 different villages on the two islands Upolu and Savai'i in August 2011. The dogs sampled were all undergoing anaesthesia for desexing. In total 187 serum samples and 159 faecal samples were analysed, and 181 dogs' furs examined for ectoparasites. Microscopy of the faecal samples, using a faecal float method, was performed in Samoa, whereas the serum samples were analysed in New Zealand using a commercial ELISA (enzyme-linked immunosorbent assay) test. The sampled population had a prevalence of 9.6% for *Anaplasma* spp, 43.9% for *Dirofilaria immitis*, 97.5% for hookworm, 6.9% for *Trichuris vulpis* and 4.4% for *Toxocara canis*. None of the 187 serum samples were positive for *Ehrlichia canis*, *Borrelia burgdorferi* or *Leishmania infantum*. There was a high prevalence of ectoparasites, with fleas found on 79.7%, lice on 9.1% and ticks on 42.8% of the examined dogs.

SAMMANFATTNING (ABSTRACT IN SWEDISH)

Målet med denna studie var att undersöka prevalensen av utvalda infektiösa sjukdomar hos hundar på Samoa. Prover togs från 192 hundar från 21 olika byar på de två öarna Upolu och Savai'i under augusti 2011. Hundarna som provtogs var samtliga under generell anestesi för kastrering eller sterilisering. Totalt 187 serumprover och 159 träckprover analyserades. Pälsen på 181 hundar undersöktes för ektoparasiter. Mikroskopering av träckproverna, efter fekal flotationsmetod, utfördes på Samoa. Serumproverna analyserades i Nya Zeeland med ett kommersiellt ELISA-test (enzyme-linked immunosorbent assay). Den provtagna populationen hade en prevalens på 9.6% för *Anaplasma spp*, 43.9% för *Dirofilaria immitis*, 97.5% för hakmask, 6.9% för *Trichuris vulpis* samt 4.4% för *Toxocara canis*. Ingen av de 187 serumprovna var positiva för *Ehrlichia canis*, *Borrelia burgdorferi* eller *Leishmania infantum*. Prevalensen för ektoparasiter var hög, med loppor på 79.7%, löss på 9.1% och fästingar på 42.8% av de undersökta hundarna.

INTRODUCTION

Background

Samoa is a small South Pacific nation consisting of two main islands, Upolu and Savai'i, and several much smaller islands. The total area of the country is 2,831 km². The climate is tropical. The only city is the capital Apia which according to the last census from 2006 (Samoa Bureau of Statistics, 2008) had 21% of the total population of 180,741. The rest of the population lives in villages, most of them coastal.



Fig. 1. Map over Samoa (© Wikimedia Commons, images available under a Creative Commons Attribution-Noncommercial license).

Although there are no official data on the number of dogs (*Canis familiaris*) in Samoa, the consensus is that it is vastly overpopulated, with free roaming dogs seen as malevolent to tourism, and also a problem for the locals constantly under risk of being attacked.

The only veterinary clinic for companion animals in Samoa is run by the Animal Protection Society (APS), a non-profit, non-governmental organisation founded in 1994 with the aim of improving the health and welfare of cats and dogs in Samoa. They are open for regular veterinary care on normal business hours as well as offering emergency care at any time, all at cost price. They also run free education on animal welfare in schools and to the public. One of their important missions is desexing of cats and dogs as a mean of keeping the population down in a humane way. For this purpose the APS regularly do visits to villages so that dog owners in remote areas also can get a chance to have their dogs or cats desexed.

Dogs in Samoa live a different life than that of Swedish dogs. They are never kept on a leash, and even though most dogs technically are owned by someone they are free to roam the streets as they wish during the day and often night. Since the dogs are free roaming, they often get hit by cars, and it is not rare to see lame dogs with old fractures. This also seems to be a common cause of death, and the dog population is young. Dogs are fed food scraps, often bone or bread.

Puppies are adored but as soon as they get older they are no longer as appreciated. Adult dogs are mostly kept as guard dogs, and may be treated badly so as to make them more aggressive.

Some Samoans attempt to keep the dog population under control by killing newborn puppies, especially female ones. As a result there are more male dogs than female

dogs in Samoa. Adult dogs are at risk of being maliciously poisoned by neighbours, attacked by machetes or having stones thrown at them, which Samoans see as self-defence. This has led to a vicious cycle where dogs become aggressive because of the abuse they have to endure. Once aggressive, Samoans are more prone to use violence against them. The APS aim to break this vicious cycle with humane education programs in schools and the wider community.

The high population of dogs, and their close contact with people, make them a possible threat to public health since they may pose as reservoirs for agents causing human disease.

Parasites

Hookworms

Hookworms are nematodes of the superfamily *Ancylostomatoidea*. Hookworms seen in dogs include *Ancylostoma caninum*, *A. braziliense*, *A. ceylanicum*, and *Uncinaria stenocephala*. It is not possible to morphologically differentiate eggs from different *Ancylostoma* species. *U. stenocephala* can be morphologically differentiated from *Ancylostoma* spp by measuring the size (Bowman et al, 2010). *A. caninum* has previously been reported in Samoa (Martin, 1999).

Lifecycle and disease in dogs

Hookworm eggs are passed in faeces, and under the right conditions they hatch and develop to third stage larvae. Dogs can be infected by ingesting these larvae, or percutaneously, where third-stage larvae migrate through the skin (Bowman et al, 2010). Transmammary infection also occurs with *A. caninum*. The disease caused in dogs varies with the hookworm species involved. *A. caninum* attaches to the intestinal mucosa and removes blood from arterioles, causing an acute or chronic haemorrhagic anaemia, particularly severe in young pups infected through the transmammary route. *A. braziliense*, *A. ceylanicum* and *U. stenocephala* in dogs usually only cause mild digestive upsets, diarrhoea and sometimes low-grade anaemia and hypoalbuminemia. They may also cause dermatological reactions when migrating through the skin, particularly dogs already sensitised may suffer from pedal dermatitis (Taylor et al 2007).

Zoonotic potential

The percutaneous route is the most common in humans infected by canine hookworms. In humans these cutaneous larva migrans are often self-limiting but larval migration to other sites has been seen, such as to the lungs causing eosinophilic pneumonitis, to muscle tissue causing localised myositis, to the eye causing neuroretinitis, and to the bowels causing eosinophilic enteritis. A few cases with animal hookworms developing to adults have been reported (Bowman et al, 2010).

Dirofilaria immitis

Dirofilaria immitis, the canine heartworm, is a nematode of the superfamily *Filarioidea*, prevalent in warm-temperate and tropical zones, including Australia. The final hosts are canids, occasionally felids and rarely humans (Taylor et al, 2007). *D. immitis* has previously been reported in Samoa (Martin, 1999).

Lifecycle and disease in dogs

The host gets infected by *D. immitis* when an infected mosquito (*Aedes*, *Culex*, *Anopheles*, *Mansonia*) take a bloodmeal (Lee et al, 2010). Third-stage larvae enter the bite wound and develop to adults in the final host. The adults reside in the heart and adjacent blood vessels, causing the disease associated with *D. immitis*. Due to obstruction of the normal blood stream, chronic congestive right-sided heart failure may occur, and endocarditis in the heart valves and pulmonary endarteritis may be caused by an immune response to parasite excretion (Taylor et al, 2007). The prevalence of *D. immitis* has been shown to increase with age, and in one study no dogs under the age of twelve months were positive (Vezzani, 2011).

Zoonotic potential

Humans can sometimes be infected, and the larvae tend to follow the same pathway as in the canine host. However, humans are dead-end hosts to *D. immitis*, meaning they cannot proliferate in human tissue. The infection in people is asymptomatic most of the time, but symptoms may occur including cough, chest pain, fever, haemoptysis and pleural effusion. Another problem with human infection is that the dying adult larvae in pulmonary tissue cause granulomas that are hard to differentiate from more serious disease, such as lung cancer, without the use of invasive diagnostic tests (Lee et al, 2010). In a study of *D. immitis* prevalence in Gran Canaria it was found that the prevalence of *D. immitis* in humans in different areas, according to different climate, was related to the prevalence of dogs in the same area (Montoya-Alonso et al. 2011).

Toxocara canis

Toxocara canis is a nematode whose predilection site is the small intestine. *T. canis* has previously been reported in Samoa (Martin, 1999).

Lifecycle and disease in dogs

There are several routes of infection, such as ingesting embryonated eggs present in the environment, by eating paratenic hosts (a host not needed for the development of the parasite), as well as a transplacental and transmammary route. Some of these routes include a pulmonary migration (Taylor et al, 2007).

In adult dogs the larvae usually stay developmentally arrested in somatic tissues, but in the pregnant bitch they usually resume development and infect their litter transplacentally (Rubinsky-Elefant et al, 2010). Once a bitch is infected she may harbour enough larvae for all of her subsequent litters to be transplacentally infected (Taylor et al, 2007).

Pulmonary migration in mild-moderate infections is often subclinical, with little damage to the tissues. In heavy infections however, it can lead to pneumonia, sometimes accompanied by pulmonary oedema, and in worst case death. Adult worms in the intestine cause little reaction from mild-moderate infections, but in heavy infections they cause a mucoid enteritis and may partially or completely occlude the gut. They may also cause a potbelly, with failure to thrive, and sometimes vomiting or diarrhoea (Taylor et al, 2007).

Once infection is manifest in an area it is difficult to eliminate. Female worms are able to produce an extreme amount of eggs passed in the faeces every day, and eggs can survive in the environment for several years. Also, larvae in somatic tissue are not susceptible to most anthelmintics making treatment complicated (Taylor et al, 2007).

Zoonotic potential

Human toxocariasis, caused by *T. canis*, is one of the most common zoonotic infections worldwide. Humans, usually children, get infected by accidentally ingesting embryonated eggs present in the soil or in sandpits on playgrounds. These eggs come from littering dogs. People can also get infected by eating raw meat of paratenic hosts, a common route of infection in East Asia. *T. canis* cannot develop into adults in humans, and even though the infection is most commonly asymptomatic, it may also lead to severe organ damage, all depending on the infection load, route of migration and the host's immune response (Rubinsky-Elefant et al, 2010).

There are two severe syndromes recognised in man: Visceral larva migrans (VLT) and ocular larva migrans (OLM). In VLT, larvae travel in the blood stream and migrate through vital organs causing damage, the liver being the visceral organ most commonly affected. In OLM the disease is localised to the eyes and optic nerves, and the inflammation may be sight-threatening (Rubinsky-Elefant et al, 2010).

Trichuris vulpis

Trichuris vulpis, also called whipworm, is a nematode that infects dogs, foxes and cats (Taylor et al, 2007). *T. vulpis* has previously been reported in Samoa (Martin, 1999).

Lifecycle and disease in dogs

Dogs get infected by *T. vulpis* by ingesting infectious eggs present in the environment. Most infections are mild and subclinical, only heavy infections cause disease. Dogs then get a haemorrhagic colitis and/or diphteritic inflammation of the caecal mucosa, sometimes with anaemia and weight loss. Eggs may survive and stay infectious for several years in the environment (Taylor et al, 2007). *T. vulpis* is more common in older dogs (Traversa, 2011).

Zoonotic potential

The zoonotic potential of *T. vulpis* is being debated (Traversa, 2011).

Dipylidium caninum

The cestode *Dipylidium caninum* is the most common tapeworm of the domestic dog (Taylor et al, 2007). It has previously been reported in Samoa (Martin, 1999).

Lifecycle and disease in dogs

Dogs get infected when they get bitten by infected lice (*Trichodectes canis*) or fleas (*Ctenocephalides* spp). Its predilection site is small intestine, and although dogs may be irritated by active segments crawling around their anus, adult *D. caninum* is non-pathogenic (Taylor et al, 2007).

Zoonotic potential

D. caninum rarely infects man (Taylor et al, 2007).

Vector borne diseases

Anaplasma phagocytophilum

Anaplasma phagocytophilum is an intracellular bacterium of the order *Rickettsiales*. It is the causative agent of canine and human granulocytic anaplasmosis. It is found in many areas of the Northern hemisphere, such as North America, Europe, Tunisia and Asia, but neither molecular evidence nor culture cases have been documented in the Southern hemisphere, although a bacterium closely related to *A. phagocytophilum* was recently detected in South Africa (Carrade et al, 2009). *A. phagocytophilum* has not officially been reported in Samoa, however in a pilot study from 2010 a small number of dogs tested positive using the commercial ELISA (enzyme-linked immunosorbent assay) test kits IDEXX SNAP® 4Dx® Test (Acke et al personal communication, 2011).

Lifecycle and disease in dogs

A. phagocytophilum is transmitted to mammals through different *Ixodes* ticks (Ettinger & Feldman, 2010). *A. phagocytophilum* infects and forms morulae, a cluster of bacteria, within circulating granulocytes, preferably neutrophils (Taylor et al, 2007). Infection with *A. phagocytophilum* in dogs may be subclinical, and the most common clinical signs seen are non-specific such as fever, depression and inappetence. Trombocytopenia is seen in 80% of cases, and lymphopenia, eosinopenia and a mild nonregenerative anaemia can also be seen. Infection seems to be self-limiting in dogs (Ettinger & Feldman, 2010).

Zoonotic potential

By bringing infected ticks into contact with humans, dogs may be an indirect source of infection. Similarly to the disease in dogs, the most common clinical signs in people with human granulocytic anaplasmosis are fever, malaise, myalgia and headache, and the most common abnormal laboratory finding is thrombocytopenia, with leukopenia also often being seen. (Dumler et al, 2007) Life-threatening complications due to secondary infections are sometimes seen, even though death is rare (Ettinger & Feldman, 2010).

Anaplasma platys

Anaplasma platys, the cause of canine thrombocytic anaplasmosis, is phylogenetically closely related to *A. phagocytophilum* (Carrade et al, 2009). It has been reported worldwide (Ettinger & Feldman, 2010), but has not been officially reported in Samoa.

Lifecycle and disease in dogs

The vector of *A. platys* is thought to be the worldwide distributed brown dog tick (*Rhipicephalus sanguineus*) (Inokuma et al, 2000). *A. platys* is an obligate intracellular bacterium that infects and form morulae within platelets, causing cyclic thrombocytopenia, likely due to immune-mediated mechanisms. The severity of the

disease varies depending on geographic location, from subclinical to more serious disease with fever, splenomegaly, and haemorrhages (Ettinger & Feldman, 2010).

Zoonotic potential

A. platys has not been confirmed to infect humans (Greene, 2006).

Ehrlichia canis

Ehrlichia canis is grouped in the same family as the *Anaplasma* spp, Anaplasmataceae. It is an intracellular bacterium infecting and forming morulae in circulating monocytes, causing the disease canine monocytic ehrlichiosis. Its geographical distribution is worldwide, in tropical and temperate areas, it is however not present in Australia despite the presence of suitable vectors (Greene, 2006). In 1997 antibodies against *E. canis* were detected in 6 of 10 Samoan dogs tested serologically using indirect fluorescent antibody test (IFAT) (Martin, 1999).

Lifecycle and disease in dogs

This disease is divided in an acute and a chronic phase, even though they can be difficult to distinguish from one another. The clinical signs of the acute phase are non-specific such as fever, depression, inappetence and weight loss. Neurological signs may occur as a result of meningeal inflammation or haemorrhage. Lymphadenopathy and splenomegaly may be seen, as well as thrombocytopenia and sometimes mild leukopenia and anaemia. Dogs may recover from the acute phase without treatment, or they may remain subclinically infected for months to years, with bacteria evading the host immune system through antigenic variation. The clinical signs of chronic ehrlichiosis are also non-specific and can be mild to life-threatening, pancytopenia in the severe form (Ettinger & Feldman, 2010).

Zoonotic potential

Human monocytic ehrlichiosis is caused by *E. chaffeensis*, a bacteria closely related to *E. canis*. In a Venezuelan study DNA from *E. canis* was detected in 6 of 20 human patients with clinical signs of human monocytic ehrlichiosis, suggesting that *E. canis* can cause clinical infection in humans (Perez et al, 2006).

Borrelia burgdorferi

Borrelia burgdorferi is the spirochete causing Lyme borreliosis, and has been reported from North America, Europe and Asia (Greene, 2006). It has not been reported in Samoa.

Lifecycle and disease in dogs

B. burgdorferi is transmitted to mammals by a variety of *Ixodes* ticks, which need to be attached for at least 50 hours for transmission to occur. Clinical signs of Lyme borreliosis in dogs include fever, shifting leg lameness, lymphadenomegaly and polyarthritis. A mild focal meningitis, encephalitis and perineuritis has been seen in experimental infections, but no neurological signs were observed (Greene, 2006).

Zoonotic potential

Signs of Lyme borreliosis in people include dermatitis, arthritis, myocarditis and meningoencephalitis, often with neurological signs. Direct horizontal transmission from dogs to humans is unlikely, and in general having a pet dog should not be a risk factor for humans since *Ixodes* ticks do not survive long indoors, and fed ticks do not reattach until after they have moulted (Greene, 2006).

Leishmania infantum

The protozoan genus *Leishmania* includes several species that cause disease in dogs and people, *Leishmania infantum* being the main cause of the canine visceral leishmaniasis. Another form of disease that *L. infantum* causes is cutaneous leishmaniasis, although both forms are often seen at the same time. *L. infantum* is distributed worldwide (Greene, 2006). It has not been reported in Samoa.

Lifecycle and disease in dogs

L. infantum is transmitted by a sandfly and replicates inside macrophages before disseminating throughout the body. It primarily travels to the hemolymphatic system organs and remote dermal areas, creating a generalised infection (Greene, 2006).

Most dogs infected by *L. infantum* will not develop disease. Either they will eliminate the infection or remain infected subclinically and capable to transmit the infection to sandflies (Greene, 2006). Clinical signs of canine visceral leishmaniasis may take months to years to develop (Taylor et al, 2007). It is a chronic systemic disease and the signs are highly variable, often beginning with slight but progressive dullness and exercise intolerance. The most common findings on physical examination include lymphadenomegaly, skin involvement and cachexia (Greene, 2006).

Zoonotic potential

L. infantum is an important zoonosis in endemic countries, such as those of the Mediterranean Basin. Although humans too get the infection from sandflies, dogs in these countries act as a reservoir. Direct infection from dogs to humans is unlikely, but infection may be transmitted through contaminated needles and blood transfusion. Human visceral leishmaniasis is a severe disease that may cause death if left untreated. People affected by the disease typically include young children, adults infected with HIV or people receiving cytostatic or immunosuppressive drugs (Greene, 2006).

Prevalence of canine diseases in Samoa

Very few studies on canine disease prevalence in Samoa have been performed. It is of significance not only to the dogs, but also from a public health perspective to know what diseases are common in Samoa, since many diseases can transmit from dogs to humans, either directly or via a vector where dogs may represent a reservoir.

In 2010 a not yet published pilot study was carried out, sampling 50 dogs for selected infectious diseases. A high prevalence of hookworm (65%), *Dirofilaria immitis* (58%), fleas (90%) and brown dog tick (32.5%) was detected, and also evidence of *Dipylidium caninum*, *Trichuris vulpis*, *Capillaria* spp, *Anaplasma* spp, and lice. None

of the 50 serum samples were positive for *Leishmania infantum*, *Ehrlichia canis* or *Borrelia burgdorferi* (Acke et al, personal communication 2011).

Aims

The aim of this study was to investigate the prevalence of selected infectious diseases of dogs in Samoa.

The study was performed in Samoa and New Zealand with supervisors from Massey University and the Hopkirk Institute, Palmerston North, New Zealand and SLU (Swedish University of Agricultural Sciences), Uppsala, Sweden.

MATERIAL AND METHODS

Selection of dogs

Samples were collected during four weeks in August 2011. At this time the APS in Samoa was running a free desexing campaign where they visited seven villages on Upolu, two villages on Savai'i, and on two occasions the APS set up mobile free desexing clinics outside the Samoan Tourist Authority in Apia. The APS clinic in Avele was also open for free desexing on several occasions. Dogs sampled were undergoing anaesthesia for desexing and were estimated to weigh at least 10 kg. The owner, unless the dog was a stray, also had to give his/her consent and fill out a questionnaire with some basic information about the dog. This included age (estimated or known), lifestyle of the dog and if the dog previously had seen a veterinarian, been vaccinated, dewormed or defleaed. For stray dogs the age was estimated by the APS staff, and it was assumed the dog had never been vaccinated or dewormed.

Sample collection

While under anaesthesia, 10 ml of blood was collected from the jugular vein using a 20 gauge needle and a 10 ml syringe, and then put into a serum tube and an EDTA tube for future use. The tubes were then immediately placed in a chilled container and refrigerated within eight hours. The serum tubes were left overnight in a refrigerator for the blood to clot. The following morning the clot was removed and the serum was stored in a freezer pending analysis.

A faecal sample was collected from the rectum using a latex glove during general anaesthesia. Faecal samples were stored in a chilled container immediately and refrigerated within eight hours, pending analysis.

The coat was checked for fleas, lice and ticks.

Sample analysis

Faecal analysis

Faecal floats were performed within four days of the sample being taken. Half a teaspoon of faeces was mixed with a saturated sodium nitrate solution and mashed through a small sieve, the faeces solution was then poured into a small bottle. The bottle was filled to the rim with more saturated sodium nitrate solution and a cover slip was put on top of it, and left for 15 minutes for any parasite eggs to float up. The cover slip was then put onto a slide and put under the microscope. The whole of the slide was looked at in 4x magnification. The eggs seen on the slide were identified, and when an egg was found where identification could not be made, a photo was taken down the microscope and later given to the parasitology unit at Massey University. No egg count was performed.

Serum analysis

The analyses of the serum samples were performed in New Zealand, within 8 weeks of the first sample being taken. The frozen serum tubes were defrosted and centrifuged at 3.1 rpm for 10 minutes. The serum was then used for the commercial

ELISA (enzyme-linked immunosorbent assay) kits SNAP® 4Dx® Test (IDEXX Laboratories) and SNAP® Leishmania Test (IDEXX Laboratories), following the manufacturer's instructions. The 4Dx kit detects *Dirofilaria immitis* antigen (sensitivity 99.2%, specificity 100%) and antibodies against *Anaplasma* spp (sensitivity 99.1%, specificity 100%), *Borrelia burgdorferi* (sensitivity 98.8%, specificity 100%) and *Ehrlichia canis* (sensitivity 96.2%, specificity 100%). Dogs exposed to *Anaplasma platys* will also turn up positive for *Anaplasma phagocytophilum* on the kit, due to a serological cross-reaction (Chandrashekar et al, 2010). The Leishmania kit detects antibodies against *Leishmania infantum*, with a sensitivity of 91.1-93.4% and a specificity of 98.3-99.2% (Ferroglio, 2007).

Statistical analysis

The prevalence of the different agents was calculated for the total sampled population as well as for three separate geographical groups:

- Savai'i: Rural, coastal villages.
- Apia Urban Area (AUA): A geographic region with 21% of the human population, being the area with the highest density of people.
- The rest of Upolu: Rural, coastal villages.

The villages included in the groups Savai'i and rural Upolu were all strictly coastal villages, whereas some of the villages included in AUA were slightly more inland.

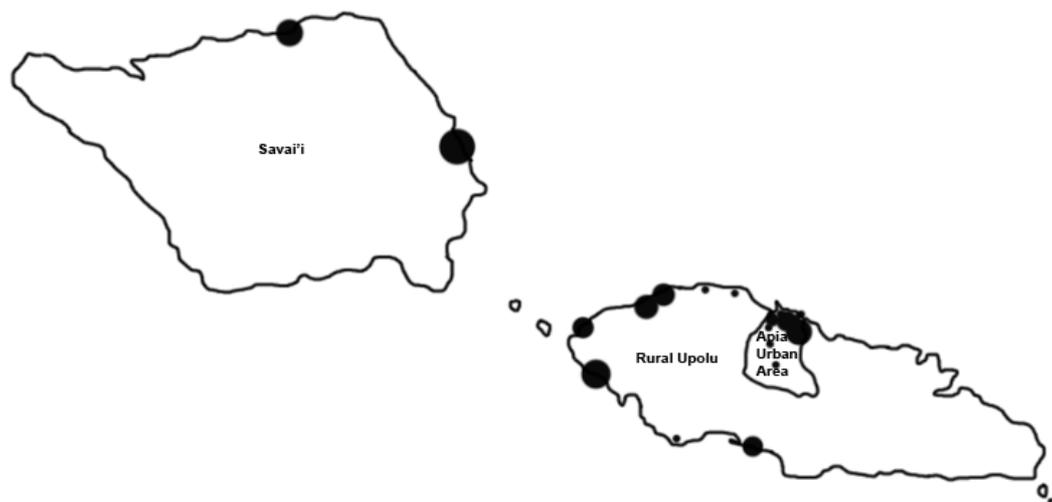


Fig. 2. Map of Samoa showing the three geographical areas that the samples were divided into. Dots represent samples, size of dot proportional to how many samples were from that village.

Fisher's exact test was used to examine the difference in prevalence between these geographical groups. This test was chosen because the numbers of positive samples in several cases were too low for a chi-square test to be suitable. A cut off value of 0.05 was used for p to be significant. A binominal exact confidence interval was calculated for all prevalence values. The computer software R was used for all calculations.

RESULTS

Most dogs presented for desexing matched the criteria of weighing at least 10 kg. Dogs weighing less than 10 kg were all puppies. A few dogs that weighed at least 10 kg were not sampled as they were either deemed unfit, or because there was no time to sample them.

Of the 192 dogs sampled, 185 were presented by an owner, and 7 were stray dogs either caught by local villagers or by the APS.

Of the dogs sampled 133 were males and 57 were females.

Questionnaire results

Of the 185 dogs with an owner to answer questions about the dog's previous health status, six stated that the dog had seen a veterinarian previously in its life. Five of these were for puppy check-up, vaccination and deworming, and one was because the dog was not eating well. These firstly mentioned five dogs were the only dogs that, according to the owners, had been vaccinated. They were usually not sure which vaccine the dog had been given, however the vaccine normally stocked by the APS is DHP (Distemper, Hepatitis, Parvovirus). Another two owners stated that they had dewormed their dogs when they were young.

All dogs were stated to be outdoors and free roaming.

For 172 dogs the age was either known or estimated. The ages ranged from four months to eight years, with 150 dogs less than three years old.

Serum samples

Antibodies against Anaplasma phagocytophilum in serum

There was a statistically significant difference ($p=0.004$) in prevalence of *Anaplasma* spp between the three geographical areas. There was no statistical significance between AUA and Savai'i ($p=1$), but there was between AUA and rural Upolu ($p=0.004$), and rural Upolu and Savai'i ($p=0.015$).

Table 1. Prevalence of antibodies against Anaplasma phagocytophilum in serum samples

	No. of positives	Prevalence	Confidence interval (95% confidence level)
Apia Urban Area (n=62)	10	16.1%	8.0-27.7%
Rural Upolu (n=84)	2	2.4%	0.3-8.3%
Savai'i (n=41)	6	14.6%	5.6-29.2%
Total (n=187)	18	9.6%	5.8-14.8%

Dirofilaria immitis antigen in serum

There was a statistically significant difference ($p<0.001$) in prevalence of *D. immitis* between the three areas. The difference was highest between AUA and Savai'i

($p < 0.001$), but still significant between AUA and rural Upolu ($p = 0.002$) and rural Upolu and Savai'i ($p = 0.007$)

Table 2. Prevalence of *Dirofilaria immitis* antigen in serum samples

	No. of positives	Prevalence	Confidence interval (95% confidence level)
Apia Urban Area (n=62)	13	21.0%	11.7-33.2%
Rural Upolu (n=84)	39	46.4%	35.5-57.6%
Savai'i (n=41)	30	73.2%	57.1-85.8%
Total (n=187)	82	43.9%	36.6-51.3%

Ehrlichia canis*, *Borrelia burgdorferi* and *Leishmania infantum

Of the 187 serum samples, none were positive for *E. canis*, *B. burgdorferi* or *L. infantum*. This gives a confidence interval of 0-2.0% with a 95% confidence level.

Faecal floats

Hookworm eggs in faecal floats

Almost all dogs had hookworm eggs, and there was no statistically significant difference between the three areas ($p = 0.555$). Differentiation between *Uncinaria stenocephala* and *Ancylostoma* spp could not be performed.

Table 3. Prevalence of hookworm eggs in faecal floats

	No. of positives	Prevalence	Confidence interval (95% confidence level)
Apia Urban Area (n=54)	53	98.1%	90.1-100.0%
Rural Upolu (n=68)	65	95.6%	87.6-99.0%
Savai'i (n=37)	37	100.0%	90.5-100.0%
Total (n=159)	155	97.5%	93.7-99.3%

***Trichuris vulpis* eggs in faecal floats**

There was a statistically significant difference ($p = 0.029$) in the finding of *Trichuris* eggs between the three areas. This difference was between AUA and rural Upolu ($p = 0.021$), with no significant difference between AUA and Savai'i ($p = 0.520$) or rural Upolu and Savai'i ($p = 0.124$)

Table 4. Prevalence of *Trichuris vulpis* eggs in faecal floats

	No. of positives	Prevalence	Confidence interval (95% confidence level)
Apia Urban Area (n=54)	7	13.0%	5.4-24.9%
Rural Upolu (n=68)	1	1.5%	0.04-7.9%
Savai'i (n=37)	3	8.1%	1.7-21.9%
Total (n=159)	11	6.9%	3.5-12.0%

***Toxocara canis* eggs in faecal floats**

There was no statistically significant difference in the finding of *T. canis* eggs between the three areas ($p=1$). All of the positive samples were from young dogs; 1 had been estimated to be 1 years old, 4 were estimated or known to be 6 months or less, and two did not have an age specified, however they weighed 10 kg meaning they were most likely less than 6 months old since no small breeds were present in the study.

Table 5. Prevalence of *Toxocara canis* eggs in faecal floats

	No. of positives	Prevalence	Confidence interval (95% confidence level)
Apia Urban Area (n=54)	3	3.7%	0.5-12.7%
Rural Upolu (n=68)	2	4.4%	0.9-12.4%
Savai'i (n=37)	2	5.4%	0.7-18.2%
Total (n=159)	7	4.4%	1.8-8.9%

Other findings in faeces

In three dogs mobile tapeworm fragments (proglottids) were seen when the faecal sample was collected. In two faecal floats *Capillaria* spp eggs were seen. In three faecal samples a structure resembling coccidial oocysts, and in one sample a sarcocystis sporocyst were found when looking at 40x magnification. In one dog a mite egg was found in the faeces.

Skin examination

Table 6. Prevalence of ectoparasites in 181 examined dogs

	No. of dogs	Prevalence	Confidence interval (95% confidence level)
Fleas	149	79.7%	73.2-85.2%
Lice	17	9.1%	5.4-14.2%
Ticks	80	42.8%	35.6-50.2%

The species of the lice and fleas were not identified. The ticks were mostly the brown dog tick (*Rhipicephalus sanguineus*). About ten ticks could not be identified.

DISCUSSION

Almost all samples were positive for hookworm. Samoa is a country with tropical climate, a perfect environment for hookworm eggs to develop into infectious stages. Only a very few owners stated that they had ever used deworming medicine for their dogs. The canine population density is high, further leading to a high infectious disease pressure. Although not surprising, it is definitely disconcerting. If practically all dog faeces in Samoa contain hookworm, infectious stages of hookworm are expected to be present in the soil anywhere that dogs have been. In Samoa this is practically everywhere. It is not uncommon to see Samoans and tourist walk around with bare feet, putting them at risk of cutaneous larva migrans.

Almost half of the samples were positive for *D. immitis*. Considering that the sampled population was young, and that prevalence for *D. immitis* has been shown to increase with age (Vezzani et al, 2011), the true prevalence in the population of Samoan dogs may be higher. There was a significant difference between all the three areas, where Savai'i had the highest numbers and AUA had the lowest. Since no preventative measures are being taken in Samoa, one explanation for this difference may be that the degree of exposure to its vector, mosquitoes, would play a big role in exposure to *D. immitis*. There are however no recent studies on the whether the mosquito population differs between these areas.

Ectoparasites were also a very common finding, which in part might be explained by the low use of prophylactic measures. There should be a certain number of false negatives since the chance of finding an ectoparasite may vary with the time available for skin examination during this study, meaning the true number could be even higher. A high ectoparasite load may lead to anaemia, but even a low load is an animal welfare problem since it often causes itching and may transmit disease.

Approximately one tenth of the serum samples tested positive for antibodies against *Anaplasma phagocytophilum*. However, the vector of *A. phagocytophilum* is *Ixodes* ticks (Carrade et al, 2009), and no *Ixodes* ticks were for certain found on the examined dogs. Some ticks detected on the dogs could not be identified, and therefore may or may not have been of an *Ixodes* species. All ticks identified were the brown dog tick (*Rhipicephalus sanguineus*). This tick has not been reported to transmit *A. phagocytophilum*, but is instead the suspected vector of *A. platys* (Inokuma et al, 2000). Therefore it is likely that the dogs were in fact carrying antibodies against *A. platys*, known to serologically cross-react with *A. phagocytophilum* and therefore test positive in the kits used in this study. Further analysing of positive samples using a polymerase chain reaction (PCR) method to identify the correct species would be useful; especially since neither *A. phagocytophilum* nor *A. platys* has been reported in Samoa before. According to the statistical analyses, rural Upolu had a significantly lower prevalence compared to AUA and Savai'i.

Eggs from *Toxocara canis* were found in seven faecal samples (4.4%), all from young dogs. This might seem like a low number, but since *T. canis* stay arrested in the tissues of adult dogs and is therefore not passed in faeces (Rubinsky-Elefant et al, 2000), the true prevalence of infected dogs is most likely much higher. Although a bitch close to parturition may pass eggs in faeces, it is more commonly puppies that

do (Rubinsky-Elefant et al, 2000). Puppies are also the dogs that live the closest to Samoans, being more accepted as a pet and often carried around and admired by Samoan children. *T. canis* may cause severe disease in humans when accidentally ingested. Children, who have a lesser degree of hygiene, are more commonly affected than adults (Rubinsky-Elefant et al, 2000). Therefore it is of interest from a public health perspective to treat puppies with anthelmintics, in addition to the obvious reason that *T. canis* also cause disease in infected puppies. Since eggs of *T. canis* may survive in the environment for several years it would be interesting to take soil samples to see to what extent *T. canis* is present in the environment.

The vector for *Ehrlichia canis*, the brown dog tick, is as mentioned before present on Samoa. In a study from 1999, 6 out of 10 dogs tested serologically using IFAT (indirect fluorescent antibody test) tested positive for antibodies against *E. canis* (Martin, 1999). However, in the test performed in our study none of the 187 serum samples tested positive. It would be interesting to further investigate whether the positive samples from 1999 could have been the result of a cross-reaction. Another possibility would be that if *E. canis* is present in Samoa it is of a strain that do not turn up positive on the ELISA kits used in this study. In Australia the brown dog tick is present but *E. canis* is not (Greene, 2006), meaning it is possible that *E. canis* is actually not present in Samoa.

Eggs resembling *Capillaria* spp were found in two samples. *Capillaria* spp has not before been officially documented in dogs on Samoa; it would therefore have been interesting to confirm the diagnosis by PCR.

Trichuris vulpis and tapeworm fragments were also detected. *T. vulpis* and *Dipylidium caninum* have been reported in Samoa before (Martin, 1999). Both of these parasites are of low pathogenicity in dogs and are not considered to be zoonotic.

Since a convenience sampling design was used, and the total canine population in Samoa is unknown, the numbers presented is not a true prevalence for the entire canine population of Samoa. However, the results should still be indicative of the true prevalence. There might be a selection bias, especially in regards to the age. Since dogs weighing less than 10 kg were not sampled, this means that the youngest puppies were not sampled. Older dogs might not have been presented for desexing in the same extent as younger dogs, although the author got the impression that most dog owners seemed to bring all of their dogs regardless of age. Some dogs presented for desexing were deemed unfit for surgery and therefore were not sampled; these dogs might have been infected with one or several of the diseases tested for in this study.

Animal health and public health

Dogs in Samoa carry several agents that may cause disease in humans. The high prevalence of these diseases in dogs, combined with the high number of dogs present on Samoa, means that the risk for human disease may be considerable. It is therefore of value not only for animal welfare, but also for public health, to prevent parasite infection in dogs and to keep the number of dogs down.

Regular deworming in combination with tick prophylaxis of dogs would be of value to keep the disease pressure down. Due to economic reasons it might however be hard to persuade the majority of Samoans to pay for expensive tick collars or spot-on

treatments and to deworm their dogs on a regular basis, if at all. Therefore education on how to prevent transmission from dogs to humans is essential. This is done today by the APS. Furthermore, an initiative to make locals safely remove and destroy all dog faeces found in the environment might be a cheap but efficient way to reduce the transmission of those parasites with a direct life cycle, if this were to be practiced extensively. Tourists coming to Samoa also need to be aware of the risks and how to avoid getting infected.

The author's recommendations for anyone visiting or living on Samoa include:

- Avoid being bitten by mosquitoes, which may transmit *D. immitis* (along with other diseases present on Samoa).
- Avoid walking bare feet, including on beaches, to lower the risk for larva migrans caused by hookworms.
- Hands should be washed thoroughly after any handling of dogs and before any meal. It is a Samoan tradition to eat with your hands, which probably increases the risk of accidentally ingesting infectious stages of several zoonotic parasites. The use of cutlery might be preferred from this point of view.

Further studies on what impact canine zoonotic diseases have on public health in Samoa would be valuable.



Fig. 3. Free roaming intact male dog on beach.

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