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General health conditions in the dog population of Lilongwe

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Generell häslostatus hos hundpopulationen i Lilongwe

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ABSTRACT

In many cases, disease in the animal population is regarded as only an animal health issue, but may often also constitute a risk to public health. This applies especially in developing countries where people often are living under conditions of extreme poverty and then often in close proximity to their animals. It is often cheaper to prevent diseases within the animal population than it is to treat disease in the human population. However, to prevent diseases in a population it is important to know which infectious diseases are circulating. The aim of this study was to determine the prevalence of selected infectious diseases in the dog population in Lilongwe, Malawi, by testing a sample population of 100 dogs. Samples were collected from 40 free roaming dogs and 40 dogs kept chained outdoors, all participating in vaccination and spaying campaigns. In addition, samples were collected from 20 dogs visiting the Lilongwe Society for Protection and Care of Animals veterinary clinic as clients. In total 100 blood samples and 40 faecal samples were analysed and 100 dogs went through a general physical exam including getting their coats checked for ectoparasites. The blood samples were analysed using commercial snap tests and the faecal samples by faecal floatation in saturated NaCl. The sampled population had a prevalence of 12% for *Anaplasma* spp, 22% for *Ehrlichia canis*, 4% for *Dirofilaria immitis* and 1% for *Leishmania* spp. No dogs were positive for antibodies against *Borrelia burgdorferi*. Eggs from *Ancylostoma* spp were found in 80% of the collected faecal samples. *Trichuris vulpis, Toxocara canis* and *Toxoscaris leonine* were not as common with a prevalence of 2.5%, 7.5% and 12.5% respectively. There was a high prevalence of ectoparasites, especially fleas which were present on 97.5% of the sampled community dogs and also lice that were present on 25.0%. Ticks were not as common with a prevalence of 11.3%. Ectoparasites were not as common on the client dogs, assumingly because of regular treatment with different spot-on medication and lesser contact with other dogs and infected environment.

SAMMANFATTNING (ABSTRACT IN SWEDISH)

I många fall anses sjukdomar i djurpopulationen vara enbart ett djurvälfärdsproblem men de kan även utgöra en risk för humanhälsan. Detta gäller framför allt i utvecklingsländer där människor lever i utbredd fattigdom och djuren ofta hålls i närmiljön. Det är ofta billigare att förebygga sjukdom i djurpopulationen än vad det är att behandla sjukdom i den humana populationen. För att förebygga sjukdom i en population är det dock viktigt att veta vilka infektiösa sjukdomar som cirkulerar. Målet med denna studie var att bestämma prevalensen av utvalda infektiösa sjukdomar hos hundpopulationen i Lilongwe, Malawi. Detta gjordes genom att en grupp på 100 hundar provtogs. Prover samlades in från 80 hundar som deltog i vaccinations eller kastrationskampanjen samt 20 hundar som besökte Lilongwe Society for Protection and Care of Animals-kliniken som patienter. Av kampanjhundarna hölls 40 frigående och 40 fastkedjade utomhus. Totalt samlades 100 blodprover och 40 avföringsprov in och analyserades. Samtliga 100 hundar genomgick först en generell hälsokontroll som inkluderade att deras päls undersöktes för närvaro av ektoparasiter. Blodproverna analyserades med hjälp av kommersiella snap-tester och avföringsproverna genom faecal flotation i mättad NaCl. I den provtagna populationen var prevalensen 12% för *Anaplasma* spp, 22% för *Ehrlichia canis*, 4% för *Dirofilaria immitis* och 1% för *Leishmania* spp. Inga hundar var positiva för antikroppar mot *Borrelia burgdorferi*. Ägg från *Ancylostoma* spp påvisades i 80% av de insamlade avföringsproverna. *Trichuris vulpis, Toxocara canis* och *Toxoscaris leonine* var inte lika vanliga med prevalenser på 2,5%, 7,5% och 12,5%. Prevalensen för ektoparasiter var hög, speciellt för loppor vilka 97,5% av kampanjhundarna hade. Löss var närvarande hos 25% av kampanjhundarna men fästingar var inte lika vanliga med en prevalens på enbart 11,3%. Ektoparasiter var inte lika vanligt förekommande hos hundarna som kom till kliniken, troligen tack vare regelbunden lusbehandling med spot on preparat och mindre kontakt med andra hundar och infekterad miljö.

Contents

INTRODUCTION

According to the concept of "One Health" improving the health of animals also affects the health of the human population. In many cases, disease in the animal population also constitutes a risk to the public health (Lavallen et al., 2011; Schurer et al., 2013; Elwing, 2013), especially in developing countries, such as Malawi, where people often are living in extreme conditions of poverty (Acosta-Jamett et al., 2010, Schurer et al., 2013).

Malawi is one of the world's least-developed and most densely populated countries. Besides high infant mortality and a high adult prevalence rate of HIV/AIDS there is also a very high risk of several other major infectious diseases such as typhoid fever, hepatitis, malaria, plaque etc (www.landguiden.se, 2014). Because of these major issues, zoonotic diseases, even rabies, are not considered a major priority.

Free roaming dogs can be the source of zoonotic diseases spreading to humans (Slater, 2001). However, with the exception of rabies, there have been very few studies done on which infectious diseases are present in Malawi's dog population, although there is a high number of free roaming dogs (Boone, 2014). There was a survey done by Fitzsimmons, published 1967, which explored what parasites were present within the dog population of Southern Malawi. Other than this article though, there has not been any studies published.

The overall purpose of this study was to determine if a number of selected diseases were present in the dog population in Lilongwe, the capital of Malawi, and if so to what extent. Rabies was not one of the diseases included in this study, mainly because a post mortem examination of the test subject is needed for a positive diagnosis (Ettinger & Feldman, 2010).

THE OBJECTIVES OF THE STUDY

The objectives of this study were to:

- Investigate the general condition of the dog population in Lilongwe by doing physical examinations and basic laboratory tests.
- Determine the prevalence of selected infectious diseases within the dog population.
- Find out if there were differences in disease prevalence between different types of dog populations.

LITERATURE REVIEW

Background

Malawi

The Republic of Malawi, is a developing country in southeast Africa. It is one of the world's least-developed and most densely populated countries with around 85% of the population living in rural areas. Agriculture is the most important source of income. The majority of the population are under 20 years old and the life expectancy is 53 years. Official languages are Chichewa and English and a majority of the population are Christians although old traditions of animism still remain (www.landguiden.se, 2014).

Besides high infant mortality and a high adult prevalence rate of HIV/AIDS there is also a very high risk of several other major infectious diseases such as rabies (www.landguiden.se, 2014). However, because of problems with HIV/AIDS, diarrhoea, malaria, hepatitis, typhoid fever, schistosomiasis and plague, rabies and other zoonotic infections in the dog population are not a major priority (www.landguiden.se, 2014; Knobel et al., 2005).

Lilongwe

Lilongwe is the largest city in Malawi and also the capital. It has a rapidly growing human population which in 2012 reached 780 000 people. The city is divided into 57 areas and consists of a diverse matrix of unplanned low-income housing, planned housing, agricultural zones, rural zones, commercial zones and industrial zones (Boone, 2014).

In September 2013, the Humane Society International (HSI) conducted a survey estimating the size of the street dog population in Lilongwe. Based on this survey, the HSI estimated that there are approximately 36 500 street dogs in Lilongwe. A street dog was in this study defined as a unconfined outdoor dog, regardless of ownership status (i.e. included both stray dogs with no owner and free roaming dogs that had owners). Furthermore the survey estimated an owned dog population in Lilongwe of 65 000, this category included both dogs that were kept as pets indoors or in a yard and guard dogs that were kept chained outdoors or free roaming. Therefore, many of the owned dogs also fall into the street dog category, how many is hard to tell. Findings during this survey suggest that the annual dog mortality rates are high and that the population turnover is rapid, making it difficult to achieve target rabies immunization rates. It was therefore concluded that a comprehensive control program that emphasize sterilization and better animal care is necessary to achieve the goal of largely eliminating dog-mediated rabies transmission (Boone, 2014).

One health

According to the concept of "One Health" improving the health of animals also affects the health of the human population. It is important to consider all aspects of health care to attain optimal health for people, animals and the environment. In many cases, disease in the animal population is regarded as only an animal health issue, but may often also constitute a risk to public health (Lavallen et al., 2011; Schurer et al., 2013; Elwing, 2013). This applies especially in developing countries where people often are living in extreme conditions of poverty (Acosta-Jamett et al., 2010, Schurer et al., 2013). Roaming stray dogs pose a threat to society if they are ignored and not cared for in a proper way. It is often cheaper to prevent diseases within the animal population than it is to treat the disease in the human population. All of the factors mentioned above emphasise the importance of knowing what kind of diseases the animals are carrying, not only in order to prevent them from spreading within that animal's population, but also to prevent the zoonotic diseases to cause sickness also in the human population.

The Lilongwe Society for the Protection and Care of animals (LSPCA)

The LSPCA was originally conceived by individuals concerned about the state of animal welfare in the country, but since 2008 have had the Ministry of Agriculture and Food security's full support. With the support of the Royal Society for the Prevention of Cruelty to Animals International (RSPCA) the LSPCA strive to address issues such as animal abuse, neglect and the rising number of feral dogs. They also try to raise the knowledge in the population about animal welfare.

Managing the dog population in Lilongwe is important to achieve the goals listed above (Reece et al., 2013). Through various activities, such as rabies vaccination, spaying and neutering of dogs and opening community veterinary clinics, the LSPCA aspires to control the disease situation within the city's dog population. Besides the spaying and neutering of owned dogs, the LSPCA also gathers neglected stray dogs from the street and vaccinate and spay or neuter them before putting them up for adoption or releasing them back to the streets. The main goal is to reduce the risks with unwanted animals on the streets and thus increase the life quality of the dogs that already live there. By doing so, the risk of zoonotic diseases spreading to the human population will also be reduced.

The Lilongwe Society for the Protection and Care of animals also run humane education in public schools with the goal of promoting compassion for animals and install a sense of responsibility within the children towards the animals in their care (Lunney et al., 2011). Primary education is not compulsory in Malawi but in 1994, the government established a free primary education for all children and the attendance rates are improving for each year and through these programs the LSPCA hope to reach out to a large number of people.

Parasites

While some parasites only infect one host species others are generalists and thus able to cross species barriers. Some parasites, such as *Echinococcus granulosus*, *Ancylostoma* spp, *Toxocara canis*, even cross between animals and people and are then classified as zoonotic. These parasites may cause significant public health problems, both in the human and animal population (Martínez-Moreno et al., 2007; Schurer et al., 2013). The parasites listed below are those that were suspected to be present in the Lilongwe dog population and whose presence could be determined through available resources during this time period.

Ectoparasites

Fleas

Fleas, of the order Siphonaptera, are small, wingless parasites who feed blood of mammals and birds worldwide. Many species are ubiquitous and move easily between hosts. Part of the life cycle is off the host, making them hard to control. The fact that the flea is ubiquitous and hard to control makes it important both from a veterinary and human medical stand point (Taylor et al., 2007).

There are 15 to 16 different families of fleas, but only two families contain species of veterinary importance; Ceratophyllidae and Pulicidae. Dog fleas, *Ctenocephalides canis*, is of the Pulicidae family but since fleas are ubiquitous dogs may also be infected by a range of other species, including *Ctenocephalides felis* (Taylor et al., 2007).

A flea infection may cause inflammation, pruritus, anaemia and in some cases cutaneous hypersensitivity reactions in the dog. Fleas may also act as vectors for a range of viruses, bacteria and protozoa. The pathogen transmission is enhanced by the fact that fleas do not just bite a specific host but that they will feed on any available animal, including humans. Fleas also act as an intermediate host for the nematode *Acanthocheolonema reconditum* and the common tapeworm of cats and dogs, *Dipylidium caninum* (Soulsby, 1982; Taylor et al., 2007), both of which can infect humans (Soulsby, 1982).

Lice

Lice, of the order Phthiraptera, are small, wingless parasitic insects who feed on blood, epidermal tissue debris, feathers/fur or sebaceous secretion from mammals and birds worldwide. All species live their entire lives on their host (they are so called permanent obligate ectoparasites) and are highly host specific. Unlike fleas, lice are not vectors for bacteria or viruses but they may, like fleas, act as intermediate hosts for the common tape worm of cats and dogs, *Dipylidium caninum*. Despite this the veterinary interest lies in the direct damage that the lice can cause to their host (Taylor et al., 2007).

There is approximately 3500 species of lice. The order is divided into four sub-orders with *Anoplura* representing the (blood) sucking lice and *Amblycera* and *Ischnocera* the (fur/feather) chewing lice. Examples of species that may infest dogs are *Linognatus setosus* of the sucking sub-order and *Heterodoxus spiniger* and *Trichodectes canis* of the chewing sub-order (Taylor et al., 2007).

The effect of a louse infestation on a dog depends on the density of lice. In the case of a light infection there may not be any clinical signs. In cases where the louse infection is heavy however, clinical signs such as pruritus, alopecia, excoriation, lethargy and loss of body weight can be seen. If the lice are of the sucking sub-order the host may also become anaemic. Infections which are heavy enough to cause clinical signs are known as pediculosis and are mainly associated with young animals, old animals in poor health or animals kept in unhygienic conditions (Taylor et al., 2007).

Ticks

Ticks, sub-order Ixodida, class *Arachnida* are ectoparasites and obligate blood-feeders whom feed on vertebrates all over the world. Ticks are divided into two families; *Ixodidae* and *Argasidae*. *Ixodidae* is thought to be of greater importance and are known as hard ticks where as *Argasidae* are soft ticks. What makes ticks important out of a human and veterinary medical standpoint is that they act as vectors for a number of bacteria, viruses and protozoa (Taylor et al., 2007).

The number of hosts that one individual tick will bite varies between one and three depending on the species. Dogs are host to a number of species of ticks. *Rhipicephalus sanguineus*, also known as the brown dog tick, is of special importance because of the pathogens for which it acts as a vector. It is responsible for the transmission of *Babesia canis* and *Ehrlichia canis* and can also cause tick paralysis in the dog (Taylor et al., 2007).

Odd tick bites on the dog will only cause irritation, inflammation and in some cases cutaneous hypersensitivity reactions. In large numbers though, tick bites can cause anaemia and production losses. The salivary production in certain tick species, such as the brown dog tick, can also cause toxicosis and paralysis (Taylor et al., 2007).

Endoparasites

Dirofilaria immitis

The nematode *Dirofilaria immitis*, also known as canine heartworm, superfamily *Filariodea*, is present in warm-temperate and tropical zones throughout the world. Parasitic larvae are transmitted to the host through the bite of a female mosquito of the genera *Aedes, Anopheles* or *Culex*. There are a number of host species including felines and in rare cases also humans, but the host species most susceptible to infection is dogs (Taylor et al., 2007). Prevalence in the dog population increases significantly with age and it is very unusual for dogs below the age of 1 year to be infected (Taylor et al., 2007; Vezzani et al., 2011).

Once the larvae has infected the dog it begins to mature and starts making its way to the pulmonary arteries and the right ventricle of the heart (Wilmington, 2010). The adult female then releases microfilariae that can survive for several months in the bloodstream. When a mosquito bites the infectious host the microfilariae, which are ingested with the blood, continue to mature in the mosquito before infecting the next host that the mosquito bites (Taylor et al., 2007).

Mature heartworms create a blockage in the cardiac blood vessels and cause an inflammatory reaction. This reaction leads to a weakening of the heart muscle, decreasing the cardiac output and may eventually lead to complications such as liver and kidney failure. Clinical signs of infection in the dog are all associated to right sided heart failure and include coughing, exercise intolerance, abnormal lung sounds, dyspnoea, hepatomegaly, syncope, ascites and abnormal heart sounds (Taylor et al., 2007; Wilmington, 2010). Clinical signs are however not present in the early stages making testing and preventative actions critical (Wilmington, 2010).

As mentioned above *D. immitis* has the potential to infect the human although it happens rarely (Lee et al., 2010; Tyler et al., 2007). Tests have shown a relation between prevalence of infection in the dog and human population (Montoya-Alonso et al., 2011). The parasite does not proliferate in human tissue, making humans a dead end host. Most commonly the infection is asymptomatic in humans but may cause fever, chest pains and respiratory symptoms (Lee et al., 2010). Worth noting is that the benign pulmonary nodules that arise in human infections are often confused with pulmonary carcinomas (Simón et al., 2009).

Hookworm

Hookworms are nematodes within the superfamily Ancylostomatoidea. Among the hookworms who claim dogs as hosts are *Ancylostoma caninum* and *A. braziliense,* both of which are distributed in tropical regions making them possible infectious agents in Malawi (Taylor et al., 2007). It is however impossible to morphologically distinguish these two species eggs from one another (Bowman et al., 2010).

The lifecycle of hookworms is direct and given optimal conditions, the eggs can develop and hatch within 5 days of passing with the dogs faeces. The larvae then infect a new host by oral and/or transcutaneous transmission. *A. caninum* can also pass transmammary to the puppies. In the case of an oral infection the larvae may either pass directly to the intestine or burrow through the buccal mucosa and then migrate via the bloodstream to the lungs where it matures in the bronchi and trachea before being coughed up and swallowed. If the infection is transcutaneous the larvae will penetrate a blood vessel and migrate via the bloodstream to the lungs and undergo the same pulmonary migration as described for the oral infection. Once in the intestine the mature hookworm will attach itself to the intestine mucosa and ingest blood from the arterioles while producing eggs that pass with the host's faeces. An infected dog may pass more than a million eggs daily for several weeks (Taylor et al., 2007). One major contributing factor to the success of *A. caninum* in tropical conditions is thought to be its ability to evade unfavourable conditions by migrating to the host's skeletal muscles and there entering into a state of hypobiosis. There it remains dormant until either the host is put through stress, at which point the larvae migrates to the intestine, or the bitch gives birth, and the larvae migrates to the milk and infects the puppies directly (Taylor et al., 2007; Traub et al., 2014).

Clinical signs are more severe in young dogs, usually under one year of age, because their marrow is not able to compensate for the blood loss as well as the adult dogs (Taylor et al., 2007). In puppies, clinical signs such as severe haemorrhagic diarrhoea, anaemia and hypoproteinaemia may be seen in the acute phase (Areekul et al., 1975; Miller, 1968; Taylor et al., 2007). Infections in adult dogs tend to be of a chronic nature resulting in microcytic hypochromic anaemia (Carrol and Grove, 1984; Taylor et al., 2007). Other signs of infection are respiratory embarrassment and skin reactions such as moist eczema and ulceration at the sites of cutaneous penetration (Taylor et al., 2007).

Some species of hookworm can occasionally infect humans transcutaneously causing a temporary pruritic popular rash commonly known as “ground itch” (Maplestone, 1933). With most species the larvae does not reach full maturity, making humans a final host with larvae migrating aimlessly within the epidermis (Bowman et al., 2007). *A. caninum* may still enter the human intestine and cause eosinophilic enteritis (Taylor et al., 2007).

Trichuris vulpis

Trichuris vulpis, also known as whipworm, is a nematode within the superfamily *Trichuroidea*. The parasite infects dogs, cats or foxes (Taylor et al., 2007) all over the world (Traversa, 2011).

Infectious eggs are present in the environment and can under optimal circumstances stay viable for several years (Taylor et al., 2007; Traversa, 2011). Unless exposed to desiccation or sunlight for long periods of time, these conditions will not affect the eggs viability (Travesa, 2011). Infection occurs after oral ingestion with eggs hatching and penetrating the glands of the distal intestines. After moulting in the glands the adult worms emerge and lie in the mucosa, their anterior ends used as anchors as they ingest blood from the host and release eggs that are passed with the faeces (Taylor et al., 2007).

Most infections in dogs are mild, with no clinical signs. Only in cases of massive infection can watery and sometimes bloody diarrhoea be seen, caused by haemorrhagic colitis and/or diphteritic inflammation of the caecal mucosa. Occasionally the diarrhoea is accompanied by anaemia and weight loss. Massive infections are more common in older dogs (Taylor et al., 2007).

The zoonotic potential of *Trichuris vulpis* is still being debated; it is occasionally incriminated as a cause of zoonosis but it has not as of yet been substantiated (Traversa, 2011).

Toxocara canis

Toxocara canis is a nematode within the superfamily *Ascaridoidea*. The parasite infects dogs and foxes worldwide (Taylor et al., 2007).

There are four possible modes of infection: peroral infection by ingestion of infectious eggs or an infected paratenic host, transplacental infection or transmammary infection. The peroral infection is followed by either a hepatic-tracheal migration or by somatic migration resulting in hypobiosis in tissues such as liver, lungs, CNS, heart, skeletal muscle or the abdominal wall (Taylor et al., 2007). Once infection manifests in the population it is difficult to eliminate; infected hosts can pass extremely high amounts of eggs with their faeces and the eggs are very persistent and can survive in the environment for several years. Somatic larvae are not susceptible to treatment with anthelmintics making treatment problematic (Taylor et al., 2007).

In mild to moderate infections of dogs the clinical signs are, if any, mild with a tucked up or dilated abdomen, occasional vomiting (sometimes containing intact worms) and diarrhoea. In the case of heavy infection respiratory symptoms such as coughing, tachypnea and frothy nasal discharge are seen. Some puppies die because of the pulmonary oedema. Nervous convulsions have also been attributed to toxocariosis but this has not yet been substantiated (Taylor et al., 2007).

Human toxocariasis is one of the most common zoonotic infections (Macpherson, 2013; Rubinsky-elefant et al., 2010). The infectious agents responsible are *Toxocara canis* or, to a lesser extent, *T. mystax* (Chong *et al*., 2014; Macpherson, 2013; Overgaauw et al., 2013; Rubinsky-elefant et al., 2010). *T. mystax*, formerly known as *T. cati*, is the equivalent to T. canis but with cats as the definitive host. Infection is oral, with the larvae hatching in the small intestine before penetrating the intestinal wall and, via the bloodstream, migrating to the lungs, liver, muscles, eyes and central nervous system (Rubinsky-elefant et al., 2010). Common clinical signs are fever, stomach pain and respiratory symptoms similar to those caused by asthma (Bogtish et al., 2005). Children are more commonly affected since their hygiene is not as developed as adults and the risk of accidentally ingesting infectious eggs therefore is greater (Rubinsky-elefant et al., 2010).

Toxoscaris leonina

Toxoscaris leonina, formerly known as *Toxoscaris limbata*, is of the same class and superfamily as *Toxocara* spp. but is, though common, of less significance since its parasitic phase is non-migratory and also because it is not a known zoonotic species. Pathological effects are rarely seen on the dog and clinical signs are mostly limited to unthriftiness, distended abdomen and diarrhoea (Taylor et al., 2007).

Vector borne diseases

Anaplasma spp.

Canine anaplasmosis is the result of infection by the bacterium *Anaplasma phagocytophilum* or the closely related *Anaplasma platys*, both of the rickettsiales-order. Although infection by *A. phagocytophilum* is generally more severe than that of *A. platys,* both forms of the disease are of veterinary importance. *A. phagocytophilum* is the causative agent of both canine and humane granulocytic anaplasmosis and *A. platys* of canine thrombocytopenia (Ettinger & Feldman, 2010).

Both species of *Anaplasma* are obligate intracellular bacteria and use ticks of the *Ixodes* family as vectors in their transfer to mammals (Ettinger & Feldman, 2010; Taylor et al., 2007). *A. phagocytophilum* uses the common deer tick as its vector, in Europe *Ixodes ricinus* and in North America *Ixodes scapularis* and *pacificus*, and is therefore found only in the northern hemisphere (Ettinger & Feldman, 2010). *A. platys* uses the more wide spread brown dog tick, *Rhipicephalus sanguineus*, as a vector and has therefore got a worldwide distribution (Inokuma et al., 2000; Sanogo, 2003). Multiple reports have however been made of a bacterium closely related to *A. phagocytophilum* in the dog population of South Africa (Carrade et al., 2009; Inokuma et al., 2005).

The bacterium enters the dermis when the tick bites the host and then spreads via the blood and/or the lymph. *A. phagocytophilum* infects and forms morulae within the host's granulocytes, predominantly the neutrophils but also the eosinophils of the peripheral blood whereas *A. platys* mainly infects platelets. Infection may cause haematological abnormalities such as leucopenia, neutropenia and most consistently thrombocytopenia (present in 80% of dog cases). Also a mild non regenerative anaemia can be seen (Ettinger & Feldman, 2010; Taylor et al., 2007).

Infection of dogs can cause a wide range of clinical and pathological abnormalities. It is not uncommon for the infection to be subclinical but if there are clinical signs it is most commonly in the form of a non-specific acute fever accompanied with lethargy, inappetence/anorexia, arthritis-like stiffness and occasionally splenomegaly. Less commonly seen are signs such as diarrhoea, lameness and nervous symptoms in the form of seizures and neck pain. The infection may manifest systemically; clinical signs may then also include shock, haemorrhage and multi organ failure (Ettinger & Feldman, 2010; Taylor et al., 2007).

There is no risk of an infected dog transmitting the disease to a human directly. However, the same tick that infected a dog can also transmit the disease to a human. Therefore dogs may act as indirect sources of infection by bringing infected ticks into contact with humans (Dumler et al., 2007). *A. platys* has never been documented to infect humans (Greene, 2006). *A. phagocytophilum* however may cause human granulocytic anaplasmosis (Ettinger & Feldman, 2010; Dumler et al., 2007) with symptoms such as fever, malaise, muscle pain, headache (Dumler et al., 2007) and in rare cases death due to secondary infections (Ettinger & Feldman, 2010).

Borrelia burgdorferi

Borrelia burgdorferi, a bacterium of the spirochete phylum, is the causing agent of Lymes disease, also known as borreliosis. The disease is a zoonotic, vector borne disease transmitted by deer ticks of the Ixodes genus (Ettinger & Feldman, 2010). Because the ticks used as vectors are confined to the northern hemisphere, so also is the disease (Greene, 2006).

B. burgdorferi enters the dermis after the tick has been attached for at least 50 hours (Greene, 2006). It then spreads via the blood and/or the lymph. Because *B. borgdorferi* uses the same species of ticks as *A. phagocytophilum*, mixed infections are common (Taylor et al., 2007).

Clinical manifestations of the disease are highly varied from no clinical signs to fever, shifting leg lameness, polyarthritis and lymphadenomegaly (Ettinger & Feldman, 2010; Greene, 2006). The massive systemic lymf node enlargement is especially observed in regional lymph nodes near the site of the tick bite (Steere, 1989; Summers et al., 2005). *B.*

burgdorferi also causes mild meningitis in dogs, but usually without clinical symptoms (Greene, 2006).

As with *A. phagocytophilum* there is no risk of direct horizontal transmission between dogs and humans. As mentioned above the same tick that infected a dog can also transmit the disease to a human, making dogs potential indirect sources of infection (Dumler et al., 2007). Symptoms seen in humans include dermatitis, myocarditis, arthritis and meningoencephalitis. Unlike the meningitis in dogs the meningoencephalitis in humans often causes neurological symptoms (Greene, 2006).

Ehrlichia canis

Canine monocytic ehrlichiosis, also known as tick fever, is caused by infection with the gram-negative bacterium *Ehrlichia canis* of the same family as *Anaplasma* spp. It is an obligate intracellular organism, primarily infecting circulating monocytes. Erlichiosis is transmitted to dogs via the brown dog tick, *Rhipicephalus sanguineus* (Hoskins, 2000; Taylor et al., 2007). Because the brown dog tick has a wide distribution so does *E. canis.* The tick does however have a low tolerance for cold making *E. canis* common in both tropical and subtropical regions but not in the colder parts of the world (Dantas-Torres, 2008; Ristic, 1993; Taylor et al., 2007).

Following the tick bite, the bacteria migrate through the bloodstream and lymphatics to the spleen and liver where they infect macrophages. The infected macrophages then spread the infection to other organs (Taylor et al., 2007).

E. canis infections in dogs can be fatal in both its acute and chronic form. The infection alters the blood's ability to clot, putting a strain on the bone marrow. Over time the disease may cause the bone marrow to fail, resulting in a pancytopenia. The severity may vary but the clinical signs seen are discharge from eyes and nose, depression and loss of appetite, enlarged lymph nodes, spleen and liver, muscle and joint pain, lameness, bruising, nose bleeds and severe anaemia (Hoskins, 2000). Neurological signs may also occur secondary to meningitis, meningoencephalitis or haemorrhage (Ettinger & Feldman, 2010; Taylor et al., 2007). The infection can also be subclinical with clinical signs limited to blood changes only (Taylor et al., 2007), it is therefore important to note that dogs may seem healthy even when infected with *E. canis.*

Human ehrlichiosis is a description of an infection caused by one of five separate agents in the family Anaplasmaceae. Even though caused by different underlying pathogens, all forms of human echrlichiosis share many clinical signs and haematological changes. Symptoms include fever, headache, muscle pain and malaise combined with laboratory findings such as thrombocytopenia and leukopenia. The agents that infect humans include *Anaplasma phagocytophilum, Ehrlichia chaffeensis, E. ewingii, E. canis* and *Neorickettsia sennetsu*. *E. chaffeensis* and *E. ewingii* cause human monocytic ehrlichiosis (HME) and human ewingii ehrlichiosis respectively. These two, together with *A. phagocytophilum*, are the most common causes of human ehrlichioses. It is however only these three agents that have been sufficiently investigated, making an evaluation of the severity and frequency of the other infectious agents in humans difficult (Dumler *et al.*, 2007). It is possible to use enzyme linked immunosorbant assays, ELISA, to diagnose an *Ehrlichia* spp infection, but since there is a risk of cross-reactivity (Hegarty, 2009) the most common method used today is polymerase chain reaction, PCR (Beall *et al*., 2012). When analysing 20 human patients with clinical signs of HME in Venezuela, it was found that 6 out of 20 (30%) were positive for *E. canis* on PCR suggesting that also *E. canis* can cause human ehrlichiosis similar to that caused by *E. ewingii* (Perez et al., 2006).

Leishmania spp

The protozoan genus *Leishmania* is currently comprised of some 30 species, of which around 20 cause disease in the human population (Ashford, 2000). There is a number of *Leishmania* species that may cause canine leishmaniosis, *L. tropica, L. infantum* and *L. braziliensis* being the more common agents. All members of the genus infect the macrophages of dogs, humans and a variety of wild animals (Taylor et al., 2007). *Leishmania* spp have almost a worldwide distribution, with *L. tropica* being present in southern Africa (Taylor et al., 2007).

The protozoa use the blood-sucking sandflies, *Phlebotomus* spp, as both vectors and intermediate hosts in the Old world (in the New world they have other species as intermediate hosts). If the vector also acts as an intermediate host, the protozoa undergoes a morphological transformation after ingestion and multiplies while in the gut. They then migrate to the proboscis and become inoculated into the new host when the fly feeds. The protozoa then infect macrophages and replicates inside them before disseminating throughout the body, primarily to the hemolymphatic system (Taylor et al., 2007).

L. tropica causes cutaneous leishmaniosis in the dog with shallow skin ulcers developing at the site of the insect bite, often on the eyelids (Taylor et al., 2007). *L. infatum* can also cause cutaneous leishmaniosis but may also cause visceral leishmaniosis with lesions in internal organs (Taylor et al., 2007). It may take years before an infected dog develops clinical signs such as exercise intolerance, depilation of hair around the eyes followed by generalised alopecia and eczema, intermittent fever, anaemia, cachexia and generalised lymphadenopathy. The disease is often chronic with low mortality but may also manifest in an acute, rapidly fatal form. In some cases the active lesions persist and lead to a chronic enlargement of the spleen, liver and lymph nodes and persistent cutaneous lesions (Greene, 2006; Taylor et al., 2007). The visceral form is more common but often the two forms are seen together (Greene, 2006; Taylor et al., 2007).

Leishmaniasis in humans may take the form of cutaneous leishmaniasis (oriental sore), visceral leishmaniasis (kal azar), mucocutaneous leishmaniasis, post-kala azar dermal leishmaniasis or diffuse cutaneous leishmaniasis (Ashford, 2000). All human infections are either actively zoonotic or have been acquired by recent host transfer from a zoonotic source (Ashford, 2000). People typically affected by the disease are young children, HIV-positive adults or patients receiving cytostatic or immunosuppressive drugs (Ashford, 2000; Greene, 2006).

MATERIAL AND METHODS

Selection of dogs

Dogs from two populations were included in the study:

- Dogs from different areas of Lilongwe who participated in the vaccine or spaying campaign run by the LSPCA. The rabies campaign runs every autumn and this year (2014) included approximately 16 000 dogs. The spaying campaign runs irregularly but the aim is for the clinic to go out twice a week and spay a varying number of dogs (average of 11 dogs per day). The dogs in this category were free roaming or kept chained outdoors. In total 80 dogs from this category were tested for a selection of infectious diseases, 40 from each lifestyle group (free roaming versus chained).
- Dogs who visited the clinic as paying customers during the time for this study. The dogs in this category were kept indoors and/or in fenced off areas. 20 dogs from this category were tested for the same infectious diseases as the campaign dogs.

The areas included in the study were initially picked at random but this was later modified to include how the dogs in the area were kept. The first two areas, by chance, included a majority of chained dogs. This resulted in 40 samples from chained dogs and only 7 from free roaming dogs. This is not representative of how dogs are kept in Lilongwe, where a majority of dogs are kept free roaming. Areas elected by the staff at the LSPCA for their large number of free roaming dogs was later used so that both dogs kept free roaming and dogs kept chained would be equally represented with 40 samples each. All campaign areas included in the study were semi urban while the client dogs as a rule lived in the more urban parts of Lilongwe.

Questionnaire

All dogs sampled had owners. The owners to the dogs that participated in the campaigns were asked a series of questions, often translated into Chichewa (the most commonly language spoken in Lilongwe), while the owner to the client dogs were handed a questionnaire in English before leaving the dog at the clinic (see appendix).

The questions asked were:

- How old is the dog?
- How do you keep the dog? Free roaming, chained, fenced in area, indoors, other?
- Have you ever visited the veterinarian with your dog before? If yes, why?
- Have you ever vaccinated your dog before? If yes, against what and how often?
- Have you ever dewormed your dog? If yes, how often?
- Have you ever de-flead your dog? If yes, how often and what method? Shampoo, dip, injection, spot on, other?

Sample collection

The selected dogs first went through a physical examination which included evaluation of the dogs weight and palpation of the lymph nodes. During this examination the coat was also checked for fleas, lice and ticks.

Blood samples were collected from all selected dogs. An aliquot of 5 ml blood was collected from the *vena cephalica* using a 30 gauge needle and an EDTA tube. Blood was then either immediately transferred to snap tests (see below) using a pipette or held cool until analysis (within 24 hours).

Faecal samples were collected from the 40 dogs that were possible to sample, the rest were either too stressed or did not have enough faeces in their rectum to collect a sample. Primarily faeces was collected from the rectum of dogs participating in the vaccine campaign but also from dogs under anaesthesia for spaying/neutering during one of the spaying campaigns. Nitrile gloves were used during the collecting of the samples and the faeces was then put in a 10 ml plastic transport tube for analysis within 24 hours. If the dog was awake, as the dogs from the vaccine campaign were, oil was used as a lubricant.

Sample analysis

Ocular analysis

The coat of the dogs was visually examined for the presence of ectoparasites such as lice, fleas and ticks. Special care was taken while examining the skin area between the toes and in the ears since this is the predilection site for ticks.

Serological analysis

When deciding which agents to test for in this study, factors such as climate, environment and available tests were considered. The blood was analysed for circulating antibodies directed against *Anaplasma* spp. (*A. phagocytophilum* and *A. platys*), *Borrelia burgdorferi, Dirofilaria immitis, Ehrlichia canis* and *Leishmania infantum*. Two commercial ELISA (Enzyme-Linked ImmunoSorbent Assay) tests were used for these analyses; Idexx SNAP® 4Dx® Plus (*Anaplasma* spp. Se: 90.3%, Sp: 94.3%; *Borrelia burgdorferi* Se: 94.1%, 96.2%; *Dirofilaria immitis* Se: 99.0%, Sp: 99.3%; *Ehrlichia canis* Se: 97.1%, Sp: 95.3%) and BVT Speed Leish K (*Leishmania infantum* Se: 98.0%, Sp: 100.0%).

Faecal analysis

The faecal analysis was done using a flotation method. A small amount of fresh faeces, approximately a teaspoon (5ml), was placed in a 10 ml transport tube before adding clean tap water and mixing the solution until it became homogenous. The solution was then sieved through a 150 µm mesh into a test tube and centrifuged until the eggs were sedimented. The fluid was then replaced with saturated NaCl. At first the test tube was only filled half way up, then after mixing the solution with a pipette, more was added so that the liquid reached the brim of the test tube. A coverslip was then placed on top of the liquid's surface and left standing for 15 minutes. The coverslip was removed and placed on a slide and examined under a microscope with 100x and 400x magnification. Findings were then compared to picture references in literature (Taylor *et al*., 2007). At examination the genus of the eggs which were present was recorded. However, no egg count was performed.

Statistical analysis

When analysing the results from the study the prevalences of the different agents was calculated both for the total study population and for the different populations of dogs, i.e. campaign, free roaming, chained and clinic clients.

Fishers exact test was used when comparing prevalence's in different populations of dogs due to a number of values being lower than 5. The cut off value used for significance of the *p*-value was 0.05. A binominal exact confidence interval with a 95% confidence level was calculated for all prevalence values using the Clopper-Pearson (exact) method (www.danielsoper.com, 2014).

RESULTS

In total, 80 blood and 40 faecal samples were collected during the rabies and spaying campaigns. Out of these 50% were free roaming and 50% were kept chained outdoors. The samples were collected from five different areas of Lilongwe, all semi urban areas, representative of Lilongwe. Depending on which area the samples were collected from the dog population was either primarily free roaming or chained. In two areas all dogs were kept free roaming while in the areas with primarily chained dogs there was also the odd free roaming dog.

In total, 20 blood samples were collected from dogs visiting the LSPCA clinic in Lilongwe for spaying, general check-ups, vaccination or treatment. These dogs were all kept indoors and/or within a yard except one whom was kept free roaming.

Questionnaire results

The dogs from the campaigns were 0.5 to 13 years old and had an average age of 3.8 years (median 3 years). The client dogs were between 0.6 and 13 years old with an average age of 4.1 years (median 3.5 years).

Of the campaign dog's owners, 41% (33/80) answered that they had visited a veterinarian at some point. Out of these, 3 answered that the reason for the visit was for a general check-up and one for a cough, the rest answered that the reason for the visit was for vaccination against rabies. The corresponding number for the client dogs was 85% (17/20). They answered that they had visited a veterinarian previously for deworming, annual vaccinations against rabies and parvo virus and general check-ups. Also, one dog had been brought to the clinic at the time of the sample collection for anaemia, two for wounds, one for a broken bone and one for fever.

In total 70% (56/80) of the campaign dogs had been vaccinated before. Of the vaccinated dogs all were vaccinated against rabies but only one was also vaccinated against parvo virus. A higher percentage of the client dogs were vaccinated, with 95% (19/20) vaccinated against rabies and 80% (16/20) also vaccinated against parvo virus.

Of the campaign dogs only 31% (25/80) had ever been dewormed while 80% (16/20) of the client dogs got dewormed once every 6-12 months.

Out of the campaign dogs 55% got de-flead once every 1-3 months. In some areas most dogs got de-flead regularly, while in other areas almost none. In the areas where people de-flead their dogs it was by communal dipping of all the village's dogs in antiparasitic liquid once every 1-2 months (amitraz). Of the client dogs owners 90% (18/20) answered that they de-flead their dog once a month. Most of these answered that they use antiparasitic shampoo (pyrethrin). Only one answered that they used spot on (fipronil). None of the owners, irrespective of population, answered that they used tick collars or other prophylaxis against ectoparasites.

Physical examination

General health status

Of the dogs examined in this study all but two were without clinical signs of systemic disease. One of the chained campaign dogs and one of the client dogs were anaemic and depressed. The campaign dog was also severely underweight.

Among the adult females 10% showed signs of ongoing lactation.

A number of the client dogs were moderately overweight (35%) while some of the campaign dogs were underweight (15%).

Many dogs had open wounds on their scalps and outer ears. This was generally attested to flies laying eggs in the skin (so called fly strike). Usually these wounds were full of flies also during examination.

Fleas

Ocular examination of the dogs' coats showed a high prevalence of fleas. Of the examined dogs 85% had fleas. There was a significant difference between the prevalence in the campaign and the client population where almost all campaign dogs were positive (97.5%) but only 35% of the client dogs ($p<0.05$). There was not, however, a significant difference between the different groups within the campaign ($p=1.00$). The species of the fleas were not identified.

Table 1. Prevalence of fleas in 100 examined dogs

	No. of positives	Prevalence	Confidence interval (95% confidence level)
Campaign (n=80)	78	97.5%	88.9 – 99.9%
Free roaming (n=40)	39	97.5%	86.8 – 99.9%
Chained (n=40)	39	97.5%	86.6 – 99.9%
Client (n=20)	7	35%	15.4 – 59.2%
Total (n=100)	85	85%	78.0 – 92.0%

Lice

Ocular examination of the dogs' coats showed a considerably lower prevalence of lice compared with fleas. Only 22% of the examined dogs had fleas. There was no significant difference in prevalence between the different populations and groups ($p=0.228$ between the campaign and client dogs and $p=0.196$ between the free roaming and chained dogs). The species of the lice were not identified.

Table 2. Prevalence of lice in 100 examined dogs

	No. of positives	Prevalence	Confidence interval (95% confidence level)
Campaign (n=80)	20	25%	16.0 – 35.9%
Free roaming (n=40)	13	32.5%	18.6 – 49.1%

Chained (n=40)	7	17.5%	7.3 – 32.8%
Client (n=20)	2	10%	1.2 – 31.7%
Total (n=100)	22	22%	14.3 – 31.4%

Ticks

Ticks were only present on the campaign dogs with 9 dogs out of 80 being positive when examined in the ears and between the toes. Of these 9 dogs, 8 were free roaming and 7 were positive for the tick borne disease *E. canis*. None of the client dogs had any ticks at the time of examination. All ticks found were brown dog ticks (*Rhipicephalus sanguineus*). There was no statistically significant difference in prevalence between the campaign- and client population (p=0.198). There was however a statistically significant difference in prevalence between the free roaming- and the chained group (p=0.029).

Table 3. Prevalence of ticks in 100 examined dogs

	No. of positives	Prevalence	Confidence interval (95% confidence level)
Campaign (n=80)	9	11.3%	5.3 – 20.3%
Free roaming (n=40)	8	20%	9.1 – 35.6%
Chained (n=40)	1	2.5%	0.1 – 13.2%
Client (n=20)	0	0%	0.0 – 16.8%
Total (n=100)	9	9%	4.2 – 16.4%

Serology

Anaplasma spp

Of the dogs included in this study, 12 were positive for antibodies against *Anaplasma* spp. Out of these, 8 were free roaming dogs participating in one of the campaigns. There was however no statistically significant difference between the prevalence in the free roaming group and the chained group (p=0.192). Nor was there between the campaign- and client population (p=0.451). Four out of the 12 dogs with antibodies against *Anaplasma* spp also had ticks during examination.

Table 4. Prevalence of antibodies against Anaplasma in 100 blood samples

	No. of positives	Prevalence	Confidence interval (95% confidence level)
Campaign (n=80)	11	13.8%	7.1 – 23.3%
Free roaming (n=40)	8	20%	9.1 – 35.6%
Chained (n=40)	3	7.5%	1.6 – 20.4%
Client (n=20)	1	5%	0,1 – 24.9%
Total (n=100)	12	12%	6.4 – 20.0%

Borrelia burgdorferi

Of the 100 serum samples analysed, none were positive for *Borrelia burgdorferi*.

Ehrlichia canis

Out of the vector borne infectious agents analysed, *E. canis* was the most common. Out of the 100 dogs tested 22 were positive for antibodies against the disease. There was no statistically significant difference in prevalence between the campaign and the client population (*p*=0.451). Out of the client dogs only one was positive for antibodies against *E. canis*, this dog was also the only free roaming dog within this group. There was a significant difference in prevalence between the free roaming and chained group within the campaign population (*p*=0.041). Seven out of 22 dogs positive for antibodies against *E. canis* had ticks during examination.

Table 5. Prevalence of antibodies against Ehrlichia canis in 100 blood samples

	No. of positives	Prevalence	Confidence interval (95% confidence level)
Campaign (n=80)	21	26.3%	14.7 – 40.7%
Free roaming (n=40)	15	37.5%	22.7 – 54.2%
Chained (n=40)	6	15%	5.7 – 29.8%
Client (n=20)	1	5%	0.1 – 24.9%
Total (n=100)	22	22%	14.3 – 31.4%

Dirofilaria immitis

Out of the 100 blood samples analysed, only 4 were positive for antibodies against heartworm. All positives were collected from free roaming campaign dogs. Two of the dogs were elderly (8-10 years old), one was middle aged but with unknown exact age and one was only 9 months old.

Table 6. Prevalence of antibodies against Dirofilaria immitis in 100 blood samples

	No. of positives	Prevalence	Confidence interval (95% confidence level)
Campaign (n=80)	4	5%	1.4 – 12.3%
Free roaming (n=40)	4	10%	2.8 – 23.7%
Chained (n=40)	0	0%	0.00 – 8.8%
Client (n=20)	0	0%	0.00 – 16.8%
Total (n=100)	4	4%	1.1 – 9.9%

Leishmania spp

Only one dog in this study was positive for antibodies against *Leishmania* spp. This dog was a one year old free roaming dog participating in the rabies campaign. The dog had never been seen by a veterinarian and had never been vaccinated, dewormed or de-flead.

Table 7. Prevalence of antibodies against Leishmania spp *in 100 blood samples*

	No. of positives	Prevalence	Confidence interval (95% confidence level)
Campaign (n=80)	1	1.3%	0.0 – 6.8%
Free roaming (n=40)	1	2.5%	0.1 – 13.2%
Chained (n=40)	0	0%	0.0 – 8.8%
Client (n=20)	0	0%	0.0 – 16.8%

Total (n=100)	1	1%	0.00 – 5.4%

Faecal samples

Ocular examination of faeces

Three faecal samples collected during the campaigns had live nematode larvae present in the sample when collected. The larvae were white, 5mm in length and 2mm wide. The species was not identified from the larvae, however all three faecal samples were also positive for hookworm eggs.

Endoparasites identified through faecal floats

Hookworm eggs (*Ancylostoma* spp) were present in the majority of the faecal samples collected (80.0%).

Other genus identified through faecal floats were *Trichuris* spp, *Toxocara* spp and *Toxoscaris* spp. Considering which species use dogs as hosts these eggs are thought to be from the species *Trichuris vulpis, Toxocara canis* and *Toxoscaris leonine* (Table 8). There were also some findings that were not identified: what looked like tapeworm eggs and fragments of proglottids (mobile tapeworm fragments) was seen in several (6/40) of the samples.

Table 8. Prevalence of endoparasites in 40 examined dogs

	No. of positives	Prevalence	Confidence interval (95% confidence level)
Ancylostoma spp	32	80.0%	64.4 – 90.9%
Trichuris vulpis	1	2.5%	0.0 – 13.2%
Toxocara canis	3	7.5%	1.6 – 20.1%
Toxoscaris leonina	5	12.5%	4.2 – 26.8%

DISCUSSION

Study design

Even though the prevalence of a disease often was higher within the campaign population compared to the client population, there was often no statistical significance in the finding. To detect significant differences in prevalence, the size of the test population would have to be larger. Especially, the client population tested would have to be larger, preferably the same size as the two test groups within the campaign population. Due to the restricted budget and also a limited supply of client dogs, this was however not possible at the time of the study.

Because of lacking experience in parasitology, some species of endoparasite eggs were not identified during the examination of the faecal floats. There were some findings that could have been tapeworm or fragments of proglottides which were never identified.

Questionnaire

When asked if they had taken their dog to see a veterinarian 41% of the campaign dog owners answered yes. Out of these all but three answered that the reason was for rabies vaccination. This is probably a misunderstanding since most of the dog's previous vaccination shots were given during earlier year's campaigns. There are usually no veterinarians present during these vaccinations; hence these dogs have most likely not been to see a veterinarian before.

Most client owners answered that their dogs were vaccinated against rabies and parvo virus but since the clinic only keeps the vaccines for rabies and DHPPi (Distemper, Hepatitis, Parvo and Para-Influenza) in stock, it is likely that the dogs were also vaccinated against all diseases included in the DHPPi vaccine.

General health conditions

The dogs sampled carried several infectious agents that may cause disease and issues of welfare within the dog population.

During the brief general examination done in connection to the sample collection, many of the campaign dogs were underweight. This could be a consequence of underfeeding but could also indicate chronical illness. The average age was also low (3.8 – 4.1 years) indicating a quick turnover within the population. Considering the amount of infectious diseases circulating in the population, it is likely that chronical illness contributes to the low age average.

Prevalences

Ectoparasites were very common within the Lilongwe dog population with fleas being present in practically every dog's coat examined. A high prevalence of fleas within the dog population has been reported in several studies conducted in developing countries (Colombo, 2011; Kumsa, 2011; Sjölander, 2012; Wells, 2012) and was therefore also expected in Lilongwe. The high prevalence within Lilongwe can be explained by both the high density of dogs within the city but also by the fact that many are free roaming and therefore infect a larger area and also each other in a larger extent than if they were kept in a restricted area. The use of prophylaxis against ectoparasites is also close to non-existent in the areas included in this study. Even in the areas where the dogs were dipped regularly, fleas were very common since the environment never got sanitised. The lower number of lice-infested dogs may be explained by the fact that lice are more difficult to find during a brief ocular examination, making the number of false negatives high. Most likely the prevalence of lice is higher than shown in this study. The number of dogs with ticks present on their body was surprisingly low, however the high prevalence of tick borne diseases indicate that it is not uncommon for dogs in Lilongwe to be bitten by the brown dog tick. During more thorough examinations on dogs under anaesthesia (not included in the sample population), ticks were found more frequently. The reason why there was a significant difference in prevalence between the campaign and client dogs is probably that the client dogs more often get regularly de-flead, which also has effect on other ectoparasites such as ticks. They were also not allowed to roam freely, making the risk for direct contact with an infected dog or environment less likely. A high load of ectoparasites may lead to issues of animal welfare because of the constant itching but the main reason they are of veterinary and medical importance is because of the risk of the parasites spreading infectious diseases (Taylor et al., 2007).

Of the population sampled, 4% were positive for antibodies against *D. immitis.* Considering that the average age of the sampled population, both campaign and client, was young, and the prevalence for *D. immitis* increases with age (Vezzani et al., 2011), the true prevalence could be higher. However, the study population was picked at random without any consideration to age, meaning that this low average age may very well be representative of the population as a whole, making the prevalence of 4% a representative one. None of the client dogs were positive for heartworm and even though there was no statistical significance to this it might indicate that the risk of mosquito bites, and therefore infection with *D. immitis*, decreases when dogs are kept indoors during the mosquito's active periods, dusk and dawn. A larger number of client dogs would have to be sampled to be sure about this interpretation.

Of the dogs sampled in this study, 12% were positive for antibodies against *Anaplasma* spp. It is not possible, through the EDTA-test used in this study, to determine if it is *A. phagocytophilum* or *A. platys* that the positive dogs have been exposed to. However, no *Ixodes* ticks were present on any of the dogs examined, and these ticks are the vector for *A. phagocytophilum* (Ettinger & Feldman, 2010; Taylor et al., 2007). Neither were any of the dogs sampled positive for *Borrelia burgdorferi*, a bacterium which also uses *Ixodes* ticks as a vector (Ettinger & Feldman, 2010; Taylor et al., 2007), further indicating the absence of *Ixodes* ticks. The vector for *A. platys*, the brown dog tick (Inokuma *et* al., 2000; Sanogo, 2003), on the other hand was present on 9% of the dogs and 22% of the sampled population was positive for *E. canis*, which also uses the brown dog tick as a vector. Considering this information, it is most likely that the antibodies detected were against *A. platys* and not *A. phagocytophilum*. Since *A. platys* has never been recorded to infect humans (Greene, 2006) it would be positive in a human medicine perspective if this is the case. To make a certain diagnosis, further analysis using a polymerase chain reaction (PCR) method of the positive samples would be necessary. This would be especially interesting since it is possible that the bacterium closely related to *A. phagocytophilum* (Carrade et al., 2009; Inokuma et al., 2005) that has been reported in South Africa may also be present in Malawi. However, no such studies have, at this time, been done.

Ehrlichia canis was, in this study, the bacteria with the highest prevalence. The prevalence was especially high within the free roaming population in which the prevalence was as high as 37.5%. In comparison a study conducted in the south and central regions of the United States showed a prevalence of only 0.8% (Beal, 2012). The free roaming population in Lilongwe was also the group that had a 9% prevalence of the brown dog tick, a vector of *E. canis* (Hoskins, 2000; Taylor et al., 2007), indicating that the group was exposed to tick infested environments to a greater extent than the other groups and therefore endured a greater disease pressure. The dogs included in the American study were pets visiting clinics across the region and were most likely kept in environments similar to those of the clinic dogs in this study. The only client dog in this study which was positive for antibodies against *E. canis* was also the only free roaming dog within this group, further strengthening this theory. Besides from a lower disease pressure the client dogs (both in Malawi and in the USA) are also probably kept tick free in a greater extent since their owners keep them indoors and are therefore more prone to remove ticks from the coat and treat them with prophylactic drugs against ectoparasites.

Only one dog (1%) sampled in this study was positive for antibodies against *Leishmania* spp. This is not a high number but since it is a zoonotic disease of importance (Ashford, 2000; Greene, 2006) it is worth noting.

Almost all faecal samples analysed in this study were positive for *Ancylostoma* spp (80%). This result is similar to that reported in north-west Ethiopia by Abere et al. (2013) but significantly higher than reported in other areas of Ethiopia by Eleni et al. (2011) and Yacob et al. (Yacob et al., 2007). Abere et al. (2013) reported a 78.89% prevalence of *A. caninum* while the latter two only reported prevalences of 32 and 4.6% respectively. A study from Brazil (Heukelbach et al., 2012) also reported a lower prevalence of hookworm (19.4%). The difference in prevalence of hookworm between areas or countries is probably due to differences in climate, veterinary facilities and public awareness of the parasite; why it should be treated and how (Abere et al., 2013). Malawi has a tropical climate so it is not surprising that the parasite thrives here. Even though direct sunlight will kill the eggs the conditions are good if the faeces lies in the shade, especially during the rainy season when the environment is moist. Because the parasite also has alternative routes, such as trans placental or trans mammary migration (Taylor et al., 2007; Traub et al., 2014) even the dry summer is not a problem for the parasite. Many owners deworm their dogs but because not everybody does this and because nobody picks up their dogs faeces, the risk for reinfection is high. Because the canine density is high in the semi urban areas so also is the disease pressure. This also constitutes a health risk to the human population since they live in so close proximity to the dogs. It is not common praxis for adults to walk barefoot in Lilongwe, but children very often do and the people spend a lot of their time outdoors preparing food, washing etc. The children also play in the soil. All contact with possibly infected soil puts the humans at risk of cutaneous larva migrans (Bowman et al., 2007; Maplestone, 1933).

Eggs from *Toxocara canis* were also found in three faecal samples (7,5%) during this study. At first glance this seems like a low number (compared with 39.8% reported by Abere et al., 2013) but since *T. canis* goes into hypobiosis in adult dogs (Rubinsky-elefant et al., 2010; Taylor et al., 2007), and it was primarily adult dogs that were included in this study, the true prevalence of infected dogs was most likely higher. The main source of eggs being passed, is puppies, and they were excluded in this study. Puppies under 6 months of age were however included in the study by Abere et al. (2013) and it was also in this age group that the prevalence of *T. canis* was significantly higher. This could explain the difference in prevalence between the two studies. As mentioned above climate, veterinary facilities and public awareness also affects the prevalence of this type of parasitic infection. However since *T. canis* eggs are extremely resilient and can survive in the environment for several years (Taylor et al., 2007), even a low number of dogs passing a large number of eggs in their faeces is of importance. The part of the human population that is at most risk of infection is children (Rubinsky-elefant et al., 2010). This is most likely a result of them more frequently handling the puppies. Children also play in the dirt and generally have lower hygiene standards than adults, further increasing the risk of them getting infected. It is therefore important, from a veterinary and public health perspective, to deworm puppies.

Trichuris vulpis and *Toxoscaris leonine* were also diagnosed. Both of these parasites are however of low importance seen both from a veterinary and medical view point since they are of low pathogenicity and are not proven to be zoonotic (Taylor et al., 2007; Traversa, 2011).

Differences in prevalence between the different dog populations

The prevalence of infectious diseases was often higher within the campaign population compared to the client population. Most likely this is a direct effect of both better prophylaxis against vectors within the client population and lower disease pressure because of less contact with infected dogs and the environment. The infectious diseases included in this study are either transferred directly or via biological vectors in the environment. The client dogs were de-flead more regularly than the campaign dogs and also usually slept indoors, protecting them to some extent from mosquitos, lice, fleas and ticks. They also do not meet as many other dogs or move in infected areas to the same extent as the campaign dogs, making the risk of contracting an infectious agent directly from other dogs or indirectly through the environment less likely. In a study from 2014 (Petterson, 2014) the author reflects on if it would be a good idea to try to influence the people in the community to keep their dogs confined instead of letting them roam free. The author concludes that this would indeed reduce the spreading of diseases, but it would also reduce the animals' welfare since many households are not surrounded by fences resulting in that the dogs would be chained at all times since walking one’s dog is not the norm in Lilongwe.

Future measures to promote better health status

The following measures are potentially useful in the future to lessen the prevalence of infectious diseases:

- Education of the general public in zoonotic risks and prophylaxis
- Parasitic prophylaxis
- Vaccination
- Spaying/neutering
- Better hygiene

The most important step to promote a better health status within the dog population would be to educate the general public about the risks of zoonotic diseases and how they spread. The public in Lilongwe do not have much money to spare so if they are to spend money on prophylactic measures such as tick collars, spot on or deworming agents, they need motivation as to why this is necessary.

At the moment it is not public knowledge that diseases may spread from animals to humans. In a study conducted in Lilongwe during the same time period as this study, children between the ages of 9 and 15 years that had participated in animal welfare lessons were asked if a human could catch diseases from an animal and 69% of 162 children answered that this is not possible (Gyllenhammar, 2015). Education on risks for the public health and how to prevent transmission is essential. A small thing such as instructing the public to safely remove and dispose of dog faeces found in the environment would be an economical and efficient way to lessen the disease pressure. Also avoiding walking with bare feet would lessen the risk of larvae migrants through the skin (Sjölander, 2012).

The LSPCA already runs a successful vaccination campaign once a year, this year vaccinating over 16 000 dogs against rabies virus (Mr. E. Mitembo, pers. comm., nov 2014). It is however also important to control the size and lessen the turnover of the population to keep disease pressure down. This is achieved by spaying and castrating adult dogs (Boone, 2014) in campaigns such as those also run by the LSPCA. Several studies have shown that by combining vaccination and castration programs in this way the prevalence of rabies can be reduced significantly (Reece et al., 2013, WHO 2014).

The public should also be educated about the health risks of handling food without proper hand hygiene. As it is today many only wash their hands with water from a bowl, if at all. It is tradition in Malawi to eat with your bare hands, as a matter of fact it is a must while eating the Malawi staple food nsima, which must be kneaded and rolled before eating. If the hands are not cleaned properly before handling food there is a major risk of accidently ingesting infectious stages of a number of zoonotic parasites while eating. Since food such as nsima needs to be kneaded before ingestion cutlery will probably not be an option, hence better hand hygiene is of utmost importance.

Conclusion

The dog population in Lilongwe, Malawi, carry several agents that may not only cause disease and welfare issues within the dog population but also pose a health risk within the human population because of their zoonotic potential. The relatively high prevalence of parasites and vector borne diseases combined with the high number of dogs in Lilongwe makes the disease pressure considerable. There are a number of diseases present in Malawi that are, for humans, more lethal than those listed in this study; rabies, HIV/ AIDS etc. However, many zoonotic diseases can be prevented with relatively small measures such as public education and prophylactic treatment of the animals. These small measures could be enough to, not only improve the animal's welfare, but also have a positive effect on the public health and should therefore not be forgotten. It is also important to consider that by improving the dogs' health, their lifespan will also become longer, slowing down the population turnover. As mentioned earlier, slowing down the population turnover would improve the results from the rabies vaccination campaign and both animals and humans alike would suffer a lesser risk of contracting rabies.

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APPENDIX

Questionnaire:

1. How old is your dog (estimated or known)?
   ....
2. How do you keep your dog? Indoors, goes for walks with owner, free roaming?
   ....
   ....
3. Have you taken your dog to the veterinarian before? Yes/No
   If yes: why?
   ....
   ....
4. Is your dog vaccinated? Yes/No
   If yes: how often do you vaccinate and against what?
   ....
5. Is your dog dewormed? Yes/No
   If yes: how often do you deworm?
   ....
6. Is your dog de-flead? Yes/No
   If yes: how often do you deflea?
   ....

THANK YOU!