The potential health impact of ivermectin mass drug administration on swine for malaria control in Mozambique

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Academic Abstract

Background: Both endo- and ectoparasites pose a great challenge to the health of pigs worldwide, placing a significant burden on low-resource countries where veterinary care is minimal. As part of a larger clinical trial assessing the use of ivermectin (IVM) mass drug administration to humans and pigs for the control of malaria vectors in the Mopeia district in Mozambique, a longitudinal study to assess the impact of IVM administration on pig health was performed.

Methods: Beginning in March 2022, IVM was administered to pigs in the intervention area once a month for three consecutive months. Seventy pigs from the treatment group and 70 pigs from the control group were randomly selected for inclusion in the study. Fecal samples were collected monthly for three months and analyzed for the presence of strongyle eggs, strongyle eggs in the larval stage (strongyles – larval) and *Ascaris suum* using the modified McMaster test. Fecal samples were also collected two weeks after each dose of IVM was given to pigs in the treatment group for determination of a fecal egg reduction count. Juvenile pigs were measured twice a month for the first 3 months of the study, then once monthly for another three months. Visual exam for ectoparasites was performed on all pigs for the presence of ticks, lice or scabies at the same time points.

Results: Overall, 55% [95% CI: 48-62%] of pigs were positive for *Ascaris suum*, 95.2% [95% CI: 91-98%] were positive for strongyle eggs, and 72.5% [95% CI: 65.5-79%] were positive for strongyle – larval. A significant difference in the ivermectin treatment group was only seen one month after the second dose of ivermectin was administered: pigs in the treatment group had a 23.6% lower prevalence of strongyles ($p = 0.003$) and 18% lower prevalence of strongyles – larval ($p = 0.03$). Pigs in the treatment group also had lower EPG for *Ascaris suum* (diff = 102 EPG; $p = 0.006$), strongyles (diff = 642 EPG; $p < 0.001$), and strongyles - larval (diff = 217 EPG; $p < 0.001$).

Analysis of covariance regression found no significant difference($P>0.05$) in average daily weight gain (ADG) between the treatment and control groups.

Conclusion: IVM delivered once monthly for three months has a small impact on pig health. To counteract the multiple health challenges pigs face in these settings, different dosing schedules along with education on husbandry issues related to nutrition and sanitation should be investigated in order to maximize impact on pig health.

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General Audience Abstract

A study was conducted in rural Mozambique in the district of Mopeia to determine the effectiveness of ivermectin against common parasites of swine when administered to pigs. The study began in March of 2022, at the beginning of rainy season, and ivermectin was given to pigs once a month for three months. Pigs were visited twice a month for the first three months, and then once a month for another three months. At various time points, fecal samples were collected, pigs were examined for evidence of ectoparasites (ticks, lice and scabies infestation), and young pigs were measured to determine their rate of growth. Fecal samples were analyzed for the presence of common internal parasites (endoparasites) affecting pigs in the area. The burden of endo and ectoparasites was estimated before any ivermectin was administered, and then compared in treated and untreated pigs over the course of the study. Similarly, the effect of ivermectin on growth rates in young animals was determined. The results of this study found that there was a high burden of common endoparasites in pigs in the Mopeia district, which was only minimally affected by the use of ivermectin delivered once a month. Among the treated pigs, a fecal egg count reduction test suggests that these parasites are potentially resistant to ivermectin, although other issues may be responsible for these results. The burden of ectoparasites was generally low (<10%), with ivermectin only significantly reducing the prevalence of ticks. Young animals that received ivermectin had a 15% increase in their growth rate, but this was not statistically significant. In conclusion, the use of ivermectin once a month for three months in pigs, as part of a malaria intervention, has some minimal positive health effects on treated pigs. Given the poor management practices, poor nutrition and lack of veterinary care in these pigs, it is likely that to have a greater impact on pig health, ivermectin will need to be delivered under a different dosing schedule and alongside owner education on pig management practices.

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Dedication

I dedicate this work to my dear Father and Mother who have been very encouraging and supportive to me before and after I started this degree program. Their prayers have paid off.

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CHAPTER 1: LITERATURE REVIEW

This review will discuss the important gastrointestinal nematodes (GIN) and ectoparasites of veterinary importance that are known to affect health and productivity of pigs, but are also known to be effectively treated by ivermectin. Resistance to ivermectin in GINs will also be discussed.

1.1 Overview of Gastrointestinal nematodes in pigs

Gastrointestinal nematodes are parasitic worms that reside in the stomach and intestine of a vertebrate host animal. They have a broad distribution throughout the world and throughout livestock populations. There are various gastrointestinal parasites that affect pig health and productivity in African countries, as in other parts of the world. These parasites are known to enter the host animal via the fecal-oral route. Once inside the host animal, these parasites compete for nutrients with the host leading to reduced animal productivity, weight loss, diseases and rarely death. The effects caused by these pathogens not only affect the animal but also affect the farmers who depend on the animal as a source of food and income through selling of the animal or its by-products[1,2].

Pigs can be parasitized by different groups of internal parasites such as nematodes, trematodes as well as cestodes[3]. Below is the discussion of several important species of veterinary importance that are known to affect pig health and productivity worldwide, and are also known to be effectively treated by ivermectin. These include Ascarids (*Ascaris suum)* and strongyles (*Strongyloides ransomi, Hyostrongylus rubidus*, *Metastrongylus sp.* as well as *Oesophagostomum spp.*). We expect that these parasites will be significantly impacted by ivermectin administration in our study area.

1.2 Epidemiology and life cycle of gastrointestinal nematodes in pigs

Generally, the life cycle of GIN consists of two phases; the first phase takes place within the host animal while the other phase takes place in the environment and include the developmental stages of the free-

living parasite in the environment. This life cycle can either be direct with only one host or indirect with two hosts.[4].

In the direct life cycle, the cycle begins when eggs are released from an infected animal into the environment. The eggs then develop into the first stage larvae (L1). This larva is environmentally protected by the released manure in which it grows. The larva feeds on bacteria after which it develops through successive molts from the first stage larvae (L1) to the second stage larvae (L2) and finally to the infective third stage larvae (L3). Depending on the parasite species, this process can take one to two weeks if temperature and humidity are optimal. The larval stage is aquatic and non-feeding but is able to go wherever a film of moisture exists, be it underground, on vegetation or on the surface of the soil. L3 maintain the cuticle (a protective exoskeleton) of L2 making themselves more resistant to harsh environmental conditions and allowing their survival for many months up to more than a year on pasture[3]. An animal host will be infected when it ingests food or drinks water containing the L3 larvae. After being ingested by the host the L3 larvae exsheath and develops further in the mucosa of the intestine depending on the host species. The parasite then molts twice and adults emerges on the mucosal surface around three weeks after infection. Males and females will mate and the female hatches eggs which are then released with fecal material onto the terrestrial environment from which the cycle repeats itself[5].

GINs with an indirect life cycle involve an invertebrate as an intermediate host. In this kind of cycle, after the eggs are passed by an infected animal in the feces, an intermediate host must ingest them for development to an infective L3 stage to occur. The animal becomes infected when it ingests an intermediate host containing this infective L3 larval[3].

1.3 Major GINS of importance in swine

1.3.1 *Oesophagostomum spp.*

Oesophagostomum spp are among the common GIN found mostly in adult pigs. These parasites have a direct life cycle and the eggs are typical of Strongyle-type eggs and are passed in the animal feces from which they hatch and develop into an infective L3 in about a week. Once ingested, the infective larvae then penetrate and develop in the mucosal lining of the large intestine causing small nodules. A large percent of these infective larvae return to the lumen or cavity of the intestine in about one week, from which they transition to adults and start laying eggs in about a month. The larvae are known to cause pea-sized nodules in the large intestine, which are believed to interfere with intestinal absorption. These nodules generally disappear within five weeks although since most pigs will be reinfected, the nodules may persist. Adults however, cause minor damage to the host. When the animal is severely infected with this parasite it loses weight significantly and experiences what is called the "thin sow syndrome" during lactation[3,6].

1.3.2 *Hyostrongylus rubidus*

Hyostrongylus rubidus is the most common and most pathogenic stomach worm in pigs, and has some features in common with *Oesophagostomum spp*. The eggs of this parasite are typical of strongyle eggs and are therefore morphologically similar and hard to distinguish from other strongyle eggs. This nematode also has a direct life cycle, with pigs becoming infected by ingesting the free-living infective L3. The infective larva then enter the stomach mucosa and produce nodules from which it emerges in about 14 days, develop into adults in about 3 weeks after infection. Severe infection with larvae or adults can be fatal to young animals and can lead to anemia and stomach inflammation which may be associated with loss of appetite and diarrhoea[3,6].

1.3.3 *Metastrongylus spp.*

Metastrongylus spp are the lungworms and they have an indirect life cycle requiring an intermediate earthworm host for the larval development. Pigs become infected after consuming an earthworm containing the infective L3 larvae. After ingestion, the larvae are freed from the earthworm upon digestion and then enter the lymphatics and migrate to the right part of the heart and lungs where they mature in the airspace. Heavy infections lead to clinical disease which is caused by tiny hemorrhages caused by migrating larvae or when adults may obstruct the airways. Pigs infected with this parasite are predisposed to respiratory infection due to influenza, mycoplasma, or other bacterial pathogens[3,6].

1.3.4 *Strongyloides ransomi*

Strongyloides ransomi is the threadworm of swine and has a unique life cycle with free-living generations comprising adult males and females, and parasitic partheno- genetic females (capable of reproduction without males) in the small intestine. Thin-shelled eggs are passed in the feces, hatch, and develop to the infective L3 larval on the ground. As with other nematodes, pigs get infected through the infective L3 larvae which is contracted orally or percutaneously. Sow to neonatal piglet transmission occurs through colostrum.

After being ingested the L3 larvae migrate to the lungs through the circulatory system up the bronchial tree and then swallowed. A large number of larvae migrate to the mammary glands where they become dormant but later activated in the lactating sow and infect the nursing piglets through the colostrum. Those L3 larvae migrating to the intestine are parthenogenetic and develop to adults and then begin to lay eggs about seven days post infection. They normally cause little tissue damage. Severe infection can cause diarrhea in piglets during the first two weeks. Severe infection can also cause mortality as high as 75%. The surviving animals may become stunted and with very poor feed-conversion rates[3,6].

1.3.5 *Ascaris suum*

Ascaris suum is the large roundworm of pigs. It has a prepatent period of 6-8 weeks and high prevalence rate in pigs worldwide and has a zoonotic ability to cause ascariasis to humans[7–9]. Adults are primarily found in the small intestine although they may also be found in the stomach or bile duct. The female produces as many as one million thick and round shelled eggs per day that are passed into feces. *Ascaris* eggs are thick and resistant, which not only protects them from adverse environmental temperatures, but it also aids in easy identification[6]. Swine ascariasis is known to interfere with pig health and productivity leading to liver condemnation, impairment of feed conversion rate and reduced growth rate all of which results in economic losses[10]

Like with most gastrointestinal nematodes, the life cycle of *Ascaris suum* is direct, involving only a vertebrate host animal such as a pig. However, there are some important differences to note. The host is infected when it eats food or drinks water contaminated with the embryonated eggs containing the infective L3 larvae covered by L2 cuticle. The ingested eggs then hatch in the small and large intestine, penetrate the intestinal wall, and then the larvae migrate to the liver where they shed the L2 cuticle and passes through the bloodstream to the lungs. In the lungs, they penetrate the alveolar space and move to the pharynx where they are swallowed and returned to the small intestine where they molt again to the L4 stage. The larvae then mature and reach sexual maturity in the small intestine. Although the adult worms may reside in the intestines for nearly a year, the majority of worms are expelled by the sixth month of infection. Unembryonated eggs are expelled outside the animal body through defecation but once in the environment they can remain viable for up to 15 years. The larvae in the eggs will undergo two molts during embryonation. Once in the environment they can then infect another or the same pig via the fecal-oral route and continue their life cycle[3,8].

The migration in the host liver causes local lesions which may lead to liver condemnation at slaughter. The presence of the larvae in the lungs may cause transient pneumonia, with clinical symptoms if the number of larvae is significant. The presence of immature and adult worms in the small intestine may

cause diarrhea if worm loads are too high. Other effects include slower weight gain and poor feed conversion rates[3].

1.4 Diagnostics

Diagnosis of parasite infection in livestock may be challenging because one method of diagnosis alone may not be entirely reliable and therefore it is recommended to use a combination of methods for more accurate diagnosis. Nematode infections in pigs can be diagnosed through regular monitoring of signs of anemia and combining this with examination of fecal samples by performing a fecal egg count(FEC) [3,11]. When pigs experience diarrhea, weight loss and anemia, this can be an indication that the animal is parasitized. Generally, piglets will develop severe symptoms and even die from intestinal parasitism. This is because they have not yet developed immunity against these parasites and are therefore much more vulnerable as compared to adult pigs which are already immunologically strong. Adult pigs are not severely affected and they rarely show any clinical symptoms[3].

1.4.1 *Fecal egg count*

Fecal analysis is the most common method of diagnosing nematode infections in pigs. This involves collecting fresh fecal samples, examining the samples for nematode eggs and performing a FEC. There are a variety of techniques that can be used to perform a FEC, all using freshly collected fecal samples. GIN eggs can easily be identified because of their unique appearances. Although several methods exist for FEC, the Modified McMaster Test is the most commonly used and an efficient method of counting parasite eggs in pigs, and other livestock[3,12]. The procedure employs a floatation technique that isolate eggs of endoparasites from fecal detritus by using their weight. The egg counting chamber keeps the eggs afloat ready for counting. [11]. The method makes use of specific microscope slide containing two chambers from which the eggs are counted and the results are reported as eggs per gram of feces because

a known volume of floatation solution and a known weight of feces is used to make a suspension which is placed in McMaster two counting chambers and parasite eggs are counted from there. The results of fecal examination provide the type and intensity of infection in an animal and this can guide farmers during treatment.

1.5 Control and treatment of gastrointestinal nematodes

The purpose of controlling GIN infection is to minimize the detrimental effects of parasites to the animals, which improves their health and productivity. It is estimated that 20% of any livestock group will have about 80% of the parasites[13]. By identifying and treating this 20%, the number of parasite larvae in the environment should be lowered and the chances of selecting for anthelmintic resistance could be reduced. Treatment is often based on the use of anthelmintics, which are anti-parasitic drugs used to treat GINs, trematodes as well as cestodes. The most commonly used anthelmintics for the treatment and control of GIN in pigs are macrocyclic lactones, probenzimidazoles and levamisole[3,14]. Others include tetrahydropyrimindines-imidazothiazoles, aminoacetonitrile derivatives (AAD) and spiroindoles. These drugs have compounds that can act against a broad range of nematodes, although some can also act against arthropod ectoparasites[15]. To be approved and used, anthelmintics must demonstrate high levels of safety and effectiveness against all stages of the parasites, must be economical to farmers, available in convenient formulations and compatible with other commonly used compounds[16].

Avermectins which include drugs such as eprinomectin, ivermectin, doramectin and moxidectin are in the class of macrocyclic lactones, and are considered to have high potency against all parasitic life stages of nematodes[17]. This class of drug is also considered to have a persistent efficacy for several weeks after administration and is normally administered to the animal as an injection, although topical and oral formulations are also approved. [3,14]

Although the use of anthelmintics is an important control tool, their use alone will not be effective. GIN control should incorporate other approaches such as good livestock management and sanitation. Management practices such as housing the animal in a confined area on slatted floors or concrete, allows for proper sanitation. Sanitation through cleaning to remove parasite eggs from the environment plus disinfecting pens between use is vital. Disinfection may involve cleaning the pigs by washing them properly at regular intervals. Both management and sanitation is intended to interfere with the parasite life cycle and prevent the infection and reduce transmission[18,19].

1.6 Ectoparasites in pigs

There are a variety of ectoparasites in pigs but two major ones are known to affect the health and productivity of pigs. These are the mange mites (*Sarcoptes scabiei* var. *suis)* and lice (*Haematopinus suis)*.

1.6.1 *Haematopinus suis*

The infestation of pigs with lice causes irritation and discomfort leading to wounds, poor performance, significant hair loss and increased risk of secondary infection all of which significantly affects pig productivity. The entire life cycle of *Haematopinus suis* is spent on the pig and the parasite cannot survive off the host for more than a few days. Adult gravid females lay eggs (nits) and glue them to the base of the hair shaft. The eggs incubate for about 10 to 14 days and then hatch into nymphs which feeds on host blood. The nymphs undergo three molts before they transition to adults which also feed on host blood. The entire life cycle completes in about three weeks.

1.6.2 *Sarcoptes scabiei var. suis*

Another important ectoparasite of swine is *Sarcoptes scabiei* var. *suis*, which is one of the most common external parasites of pigs. Infected animals flap their ears, experiences stress and pruritus (itchy skin) making it waste a lot of time rubbing and scratching itself against the crates or the walls. The excessive rubbing damages the skin which then exposes the animal to secondary bacterial infections. By spending

a lot of time rubbing and scratching itself, the animal is unable to eat properly and this leads to weight loss. It also causes decreased fertility as well as decreased feed conversion ratios making the animal weak and less productive[22].

The life cycle of *Sarcoptes scabiei* occurs entirely on the host animal and it begins when females dig tunnels in the outer epidermal layers where they lay eggs after they have mated. Mating occurs in small depressions in the epidermis. After the eggs have been laid, they hatch as six-legged larvae which molt to eight-legged nymphs which then molt to adults. This life cycle is completed in about 10-15 days. Although the parasite has no capacity to reproduce outside the host, they however may survive outside the pig for up to 12 days at a temperature around 7 °C to18 °C and relative humidity around 65 - 75%[22,23].

There are a variety of compounds such as doramectin and ivermectin that are available to treat these two ectoparasites in pigs[3,24]. Similar to GINs, cleaning and disinfecting housing areas and washing the animal is helpful, as is avoiding overcrowding to minimize contacts between animals, since the primary means of lice infestation is through direct contact.

1.7 Avermectins and avermectin resistance

Avermectins are anthelmintic macrocyclic lactones derived from the *Streptomycetaceae* family of actinobacteria. In this class, four drugs which are ivermectin, doramectin, eprinomectin and moxidectin have been approved for veterinary use for the control of parasites (both roundworms and arthropods). Ivermectin was the first of the macrocyclic lactones to be discovered.

After a prolonged use of avermectins in veterinary medicine, resistance to avermectins is now widespread. Resistance to one of the avermectins in the macrocyclic lactone class typically means resistance to all macrocyclic lactones. Drug resistance occurs when a susceptible population manifests a decreased response to treatment and is complete when the maximum dose of the drug that can be

tolerated by the host no longer has any effect. In other words, resistance is the ability of parasites to survive doses of drugs that would normally kill parasites of the same species and stage[12,14,25,26]. This trait is inherited by offspring from parent parasites, and therefore will be available in the next generation. These resistant alleles are initially rare in the population or arise as rare mutations but as selection continues their population increases as does the proportion of resistant parasites[3,14,25].

Macrocyclic lactones act and bind selectively, with high affinity for glutamate-gated chloride channels causing the irreversible opening of these channels leading to the paralysis of the parasite neuromusculature including the pharynx which ultimately prevent the parasite from food intake and therefore parasite death. Additionally, ivermectin affects γ-aminobutyric acid (GABA) chloride ion channels present in the invertebrate peripheral nervous system and in the vertebrate central nervous system[20]. Molecular mechanisms of nematode resistance to ivermectin in nematode parasites is unclear but some studies have shown that P-glycoprotein may play a role in nematode resistance to ivermectin and other macrocyclic lactones[28]

The main drivers of drug resistance in any population including nematode populations is high-treatment frequency, under-dosing and the use of the same anthelmintic class over several years[3,7,12,14,25,27]. Reports about anthelmintic resistance in pigs are very limited. A few studies report finding *Oesophagostomum spp.* resistant to levamisole, pyrantel and benzimidazoles[12,26,27], as well as some reports showing that ivermectin efficacy has been reduced for *Oesophagostomum spp.* in pigs[12,31]. Some studies from Nigeria have shown that *Oesophagostomum* spp. are susceptible to ivermectin treatment while another study conducted in Kenya showed that ivermectin was less effective against the same species[30,32]. All of the existing studies on the use of ivermectin for the control of internal parasites in pigs shows that ivermectin is 100% effective against *Ascaris suum*. There are no studies of ivermectin resistance in other strongylids such as *Metastrongylus* spp, *Hyostrongylus rubidus* or *Strongyloides ransomi.*

Ivermectin is known for its ability in controlling not only endoparasites but also ectoparasites. Some studies have shown that ivermectin is effective against lice, ticks and mange mites such as *Sarcoptes scabiei var. suis* that are known to impair the health livestock animals across the world[33]. To the best of my knowledge, there are currently no reports of resistance to ivermectin in pig ectoparasites and therefore this drug is expected to be more than 95% efficacious against most swine ectoparasites such as lice, ticks and mites.

1.7.1 *Monitoring of avermectin resistance*

One of major methods of detection of anthelmintic resistance is the use of fecal egg count reduction test (FECRT). In this technique the populations of gastrointestinal nematodes are considered susceptible when drug efficacy exceeds 90% (reduction in FERCT). If drug efficacy is below 90% then the population is described as resistant. This method and other methods of monitoring anthelmintic resistance such as egg hatch test, larval development assay, and the use of molecular techniques for anthelmintic resistance monitoring have been described in detail by Morutse et al[3,12,25].

CHAPTER 2: THE POTENTIAL HEALTH IMPACT OF IVERMECTIN MASS DRUG ADMINISTRATION ON SWINE FOR MALARIA CONTROL IN MOZAMBIQUE

2.1. Introduction

Ivermectin is a commonly used anthelmintic that has been used for over 40 years in veterinary medicine for the control of both endo- and ectoparasites. It was first identified in the 1970s by a researcher at the Kitasato Institute in Tokyo, in partnership with the US-based company Merck, Sharp and Dohme, and was the first endectocidal[i] drug ever discovered. Ivermectin is actually a derivative of the compound avermectin, which is naturally produced by *Streptomyces avermitilis,* a soil bacteria only found in Japan[34,35]

In addition to its long history of use in animals, ivermectin has been used in humans for the control of onchocerciasis and lymphatic filariasis, two parasitic neglected tropical diseases that for years have caused serious morbidity and disability to communities in resource-poor countries. Onchocerciasis causes itching, scars, depigmentation, skin thickening, visual impairment and total blindness all of which affects community productivity[36,37]. Similarly, lymphatic filariasis, which is a public health problem in over 70 countries globally, is also associated with serious morbidity and disability resulting from lymphoedema and hydrocoele[38–40]. The discovery of ivermectin and its application in mass drug administration programs in the affected communities has played a major role in combating these two debilitating diseases[39,41,42].

In the past decade, there has been a growing interest in the use of ivermectin for the control of malaria, a life-threating disease of public health importance caused by protozoan parasite of the genus *Plasmodium*, which is transmitted to people through the bites of infected *Anopheles* mosquitoes[43]. Malaria is known to cause more than 200 million cases and about half a million deaths annually. Children under the age of five, pregnant women and other immunocompromised individuals in low-

[i] **An endectocide** is a drug that is effective against both endo- and ectoparasites.

income countries are the most severely affected, with the world health organization (WHO) African region carrying disproportionately large percent of malaria cases and deaths[44,45].

There are about 537 Anopheline mosquitoes but only about 70-80 are known to transmit malaria worldwide[46,47]. Anopheles mosquitoes known to be the dominant malaria vectors include *Anopheles gambiae*, *Anopheles arabiensis* and *Anopheles funestus.* These species are known to carry several plasmodium parasites such as *Plasmodium falciparum* and *Plasmodium vivax* which are the two most common malaria parasites[44,45]. Some of these malaria vectors are known to be zoophilic[ii] while some are known to be anthropophilic[iii]. Zoophilic mosquitoes are known to contribute to residual malaria transmission[iv].

For decades now, insecticide treated nets (ITNs) and indoor residual spraying (IRS) have been the major tools to fight malaria worldwide. Even though these tools have significantly lowered malaria incidence and deaths, they are now facing some setbacks. These setbacks include behavioral avoidance mechanisms and development of insecticide resistance by *Anopheles* mosquitoes which threaten malaria control efforts[48]. Some anopheline mosquitoes have shifted their biting behavior by becoming crepuscular and hence biting when people are outside houses and unprotected, while others have shifted their biting behavior by opting to become partly zoophilic and only bite humans opportunistically when the human host is available and unprotected[49]. The ITNs and IRS only target the endophagic[v] and endophilic[vi] mosquitoes but are unable to target exophagic[vii] , zoophagic and exophilic[viii] mosquitoes. It is the exophagic, zoophagic and exophilic mosquitoes that contribute to maintenance of residual transmission of malaria, and they are the ones that must also be dealt with if we are to address the problem of residual malaria transmission[49,50].

[ii] **Zoophilic** mosquitoes are those that prefer animal blood over human blood
[iii] **Anthropophilic** mosquitoes prefer biting humans as sources of their blood meal
[iv] **Residual malaria transmission** is any ongoing transmission despite the widespread use of conventional malaria control tools such as insecticide treated nets (ITNs) and indoor residual spray (IRS)
[v] **Endophagic** mosquitoes prefer getting blood sources from inside houses
[vi] **Endophilic** mosquitoes prefer resting indoors after blood feeding
[vii] **Exophagic** mosquitoes prefer feeding outside houses
[viii] **Exophilic** mosquitoes prefer resting outdoors after getting their blood meal

Because of the behavioral shift and resistance to conventional insecticides shown by malaria vectors, a novel control is needed. Since residual malaria transmission is partly maintained by zoophagic mosquitoes, treating livestock with ivermectin is considered a potential option to address this problem[50]. Giving ivermectin to livestock and humans together is expected to reduce residual malaria transmission to an even greater extent by effectively 'poisoning' the two blood sources. Once ivermectin is given to either humans or animals, it exerts toxic effects to any host-seeking mosquitoes that feed on a treated host regardless of its feeding, resting, or host preference by reducing its survival time[51,52]. By delivering ivermectin as a mass drug administration, it is expected to drive the *Anopheles* vector population down and hence reduce malaria transmission and the number of clinical cases. [50,53]

2.2 Broad One Health Endectocide-based Malaria Intervention in Africa (BOHEMIA)

The BOHEMIA project implemented a clinical trial for ivermectin mass drug administration (iMDA) in the Mopeia district in Mozambique in 2022, focusing on the use of ivermectin in humans and pigs. The study was designed to determine the safety (in humans) and efficacy of iMDA, and has generated data needed to support a policy recommendation by the WHO for the use of iMDA as a new vector control tool in malaria endemic regions. To supplement the data needed for a policy recommendation, an economic analysis of the intervention was included as a component of the study. Economic analyses of public health interventions are essential to inform and ensure efficient use of resources prior to widespread implementation, and are valuable for helping governments and intergovernmental organizations prioritize interventions relative to their impact[54]

The inclusion of pigs in iMDA for malaria control makes the economic analysis of this potential intervention different from traditional assessments of other malaria control interventions. Although pigs essentially serve as a treated blood source, the animals may also benefit from the effect of ivermectin as an anti-parasitic. Ivermectin is commonly used in swine to treat gastrointestinal helminths such as *Oesophagostomum* spp. and *Metastrongylus* spp. and ectoparasites such as *Sarcoptes scabiei* var. *suis*, which has the potential to improve livestock productivity[55]. The BOHEMIA clinical trial in

Mozambique offers an opportunity to measure the potential impact of iMDA on pig health and estimate subsequent economic gains in malaria-endemic areas. This information can be incorporated into economic analyses, highlighting the value of a One Health approach to measuring costs and benefits of iMDA strategies for malaria control.

The rationale for selecting pigs as our target animals is that they are the most abundant type of livestock kept by Mozambican farmers in the Mopeia district and they are also sources of blood meals for malaria vectors in the area. Although most of these pigs are free roaming, they do not go very far from human habitations[56]. There are some cattle kept by some farmers, but their numbers are very low. Cattle were treated as part of the clinical trial, but the impact of iMDA on their health and household economics was not captured as part of this study, due to their low numbers.

Pig production in Mozambique contributes significantly to the improvement of individual farmer's income, as well as the country's GDP. Farmers generate income through selling the whole animal or its by-products. This farming activity also contributes to the production of manure which is essential for agricultural crop production[57]. Like in many other parts of Africa, there are two major animal husbandry systems in Mozambique. The first is the semi-intensive animal production system and the second, which is most common and the one found in Mopeia, is the extensive production system in which households own small numbers of pigs that are housed in pens at night, but allowed to roam free during the day[56,57].

Although ivermectin has been used in veterinary medicine for decades to control endo- and ectoparasites in livestock, what is not known is how its use at a population level (i.e., as part of iMDA) might have health and economic impacts beyond the individual animal or herd, leading to broader community benefits. On the other hand, there is also a concern for growing evidence of resistance in gastrointestinal helminths to ivermectin and other drugs in its class due to its common use throughout the world. In order to include costs and benefits of using iMDA in pigs for malaria control, a better understanding of the direct health and productivity impacts ivermectin has on common parasites of swine is needed.

2.3 Study Objectives

Objective 1: Identify the prevalence of common endo- and ectoparasites of swine in Mopeia.

Objective 2: Quantify ivermectin effectiveness in the reduction of common endo- and ectoparasites of swine.

Objective 3: Determine if there is any evidence for pre-existing resistance in gastrointestinal helminths.

Objective 4: Associate changes in endo- and ectoparasite burden with changes in growth rates in juvenile pigs.

2.4 Materials and Methods

2.4.1 *Study site*

This study was conducted in the Mopeia district (Figure 1), which is located in the Zambezia province in Mozambique. The district has a population of 180,000 people with a land surface of 7,668 km^2 and a population density of 11.7 inhabitants per square kilometers[58]. The district receives an average annual rainfall ranging from 500 to 800 mm between November and March, while the average annual temperature is 26.5ºC. Agriculture is the main economic activity and, after poultry, the most predominant livestock in the area are pigs. Pigs are managed in extensive production systems characterized by small herds that are allowed to roam free during the day and scavenge for food[59,60]. Based on a 2020 census of the district, 8% of households owned pigs, with an average of 3 to 4 pigs per household. There are little to no veterinary services available in the area, therefore most pigs are poorly nourished and prone to various forms of parasite infections and other diseases[56]

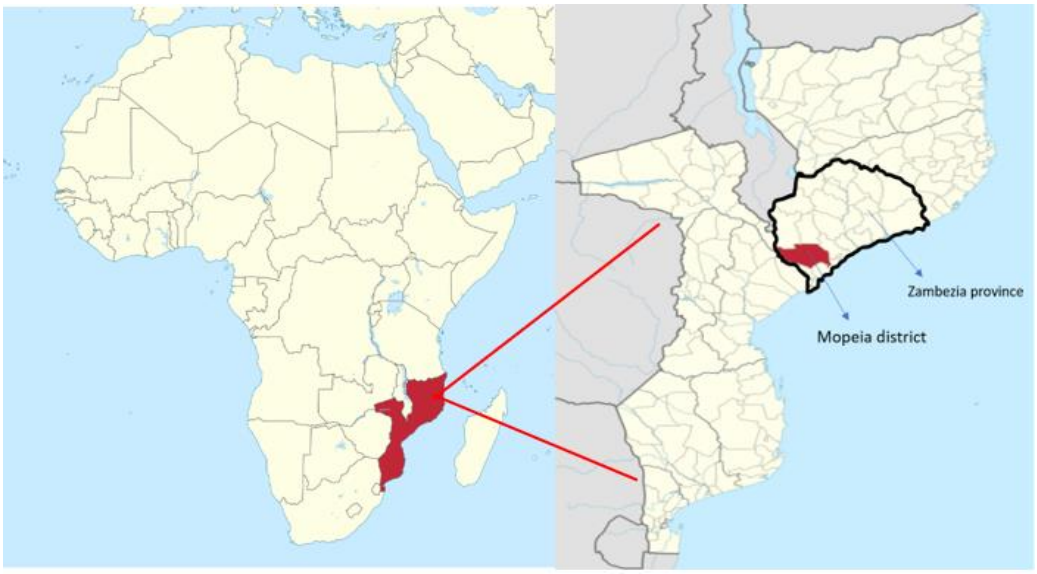


Figure 1. Map showing Mozambique and Mopeia district. [Source: https://commons.wikimedia.org]

2.4.2 *Study Design*

This study used data collected as part of the larger BOHEMIA clinical trial that was conducted in the Mopeia district from March 2022 to September 2022. The primary study was a cluster randomized, controlled trial with three parallel arms:

Humans and livestock (H+L): ivermectin was delivered to humans and livestock
Humans only (H): ivermectin was delivered to humans only
Control (C): albendazole was delivered to humans, as a control

Table 1 provides a summary of the total number of households, households with pigs, and total pigs for each trial arm, as determined by the 2020 census of the district. Delivery of ivermectin or albendazole occurred once a month for three consecutive months beginning at the start of the rainy season in the month of March. Pigs in the H+L arm were injected subcutaneously with 1% injectable solution dosed at 300mcg/kg, once a month for three months, concurrent with delivery of ivermectin to humans.

Table 1. Number of households (HH) by ownership of pigs– all clusters from census data

Arm	Total HH	HH w/o pigs	HH with pigs	Total pigs
1(H+L)	2468	2297	156	560
2(H)	2603	2397	194	718
3(C)	2511	2344	149	691

2.4.3 *Household and livestock selection*

A total of 90 pig-owning households from the H+L arm and 90 households from the H only arm were selected, for a total of 189 selected households (Figure 2). The households were selected, along with households that did not own pigs, to provide longitudinal survey data on household economics, which was part of the larger BOHEMIA study on the cost-effectiveness of iMDA.

From the selected pig-owning households, 35 juvenile pigs (age 6 to 20 weeks) and 35 adult pigs (age > 40 weeks), were randomly selected, for a total of 70 pigs per arm. Pigs selected from the H+L arm represented ivermectin treated animals, while pigs selected from the H arm were used as the control (i.e., untreated animals). In order to ensure broad sampling of pigs across the various clusters in each arm, the number of pigs selected per household was limited to two juvenile and two adult pigs, and no more than four pig-owning households were enrolled per cluster.

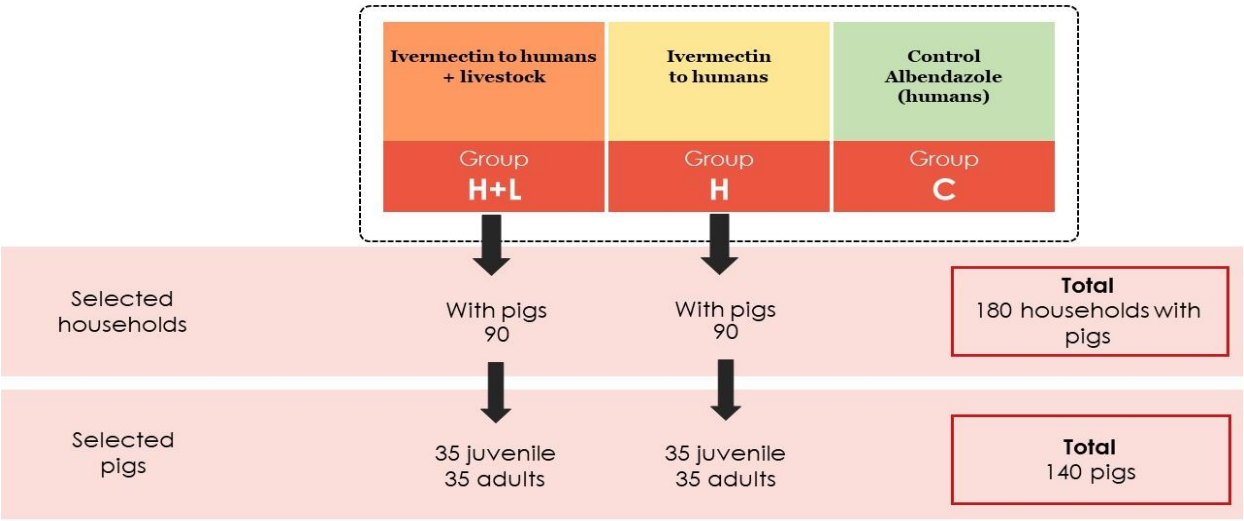


Figure 2. Household and pig selection in the study area

2.4.4 *Sample size determination*

The objectives of this study that focus on determining the effectiveness of ivermectin on the reduction of common endoparasites of swine and the effect on average daily weight gain provided the basis for sample size determination.

The sample size calculation for detecting a difference in two means is:

$n = (Z_{a/2} + Z\beta)^2 * 2 * \sigma^2 / d^2$

where:

$Z_{a/2}$ = critical value of the Normal distribution at $\alpha/2$

$Z\beta$ = critical value of the Normal distribution at β

σ = standard deviation of population mean

d = difference to detect

2.4.5 *Average daily weight gain*

Sample size was calculated to detect a 15% difference in average daily weight gain (d) in juvenile pigs, equal to 0.02 kg/day (0.044 lb/day), between treated and untreated animals, where alpha = 0.05 and beta = 0.2.

Assumptions:

- Swine have an average daily weight gain of 0.13 kg/day (σ 0.025), or 0.29 lb/day (σ 0.055)[61]

Sample size calculated:

- Per arm: 26 juvenile pigs + an additional 9 due to concerns for loss to follow up. A total of 35 juvenile per arm, 70 juvenile pigs in total, will be selected for heart girth and length measurements.

2.4.6 *Fecal egg counts (FEC)*

Assuming ivermectin is effective in the study population, I anticipate a 90% difference between the average eggs/gram (AEG) found in treated and untreated cattle and pigs. Given this significant anticipated difference, I calculate our sample size based on estimating the mean EPG per arm in a simple random sample (H and H+L), where alpha = 0.05 and beta = 0.2.

Assumptions:

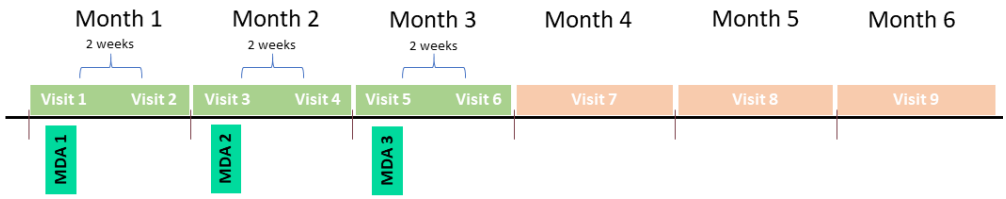
- swine untreated: AEG = 300 eggs/gram (σ 20 AEG)
- pigs will be stratified by age (juvenile vs. adult)

Sample size calculated:

- 35 juvenile swine and 35 adult swine per arm (H and H+L). A total of 70 per arm, or 140 animals total
- Juvenile animals selected for average daily weight gain measurements will be selected for use in the FEC

2.4.7 *Timeline for data collection activities*

Data collection for the study took place over six months, beginning in month 1, which correlated with the start of the clinical trial and first dose of ivermectin given to pigs in the H+L arm. Households with selected pigs were visited twice a month in the first three months, then monthly for months 4, 5, and 6 (Figure 3). To identify enrolled pigs, photos were taken and long-lasting marker was applied and reapplied at every visit.



PIG SAMPLING									
Fecal sample collection (control)	X		X		X				X
Fecal sample collection (treated)	X	X	X	X	X	X			X
Juvenile body measurements	X	X	X	X	X	X	X	X	X
Visual inspections	X	X	X	x	X	X	X	X	X

Figure 3. Summary of data collection activities over the six-month study.

Fresh fecal samples were collected from the rectum of pigs (Appendix A) on a monthly basis for the first 3 months of the study, and then again at 6 months. For those in the treated group, fecal collection was done immediately prior to or within 1 hour of the administration of ivermectin. For the 70 pigs selected from the treated group, additional fecal samples were collected 2 weeks after ivermectin administration in months 1-3 to assess ivermectin efficacy using the fecal egg reduction count test (FERCT) as described by Torgesson et al[62].

All selected pigs were visually inspected for evidence of external parasites (Appendix B), including the presence and number of ticks, presence of lice, and evidence of a scabies infection, twice a month in months 1-3, and then monthly in months 4-6.

Heart girth and body length (Figure 4) were measured on the 70 selected juvenile pigs (4 to 20 weeks of age) twice a month in months 1-3, and then monthly in months 4-6 of the study.

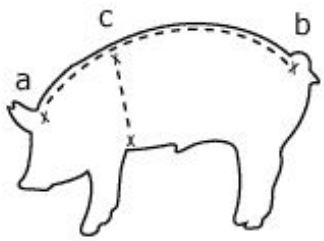


Figure 4. Visualization of heart girth (circumference c) and length (a to b) measurements

2.5 Data analysis

All the following statistical data analysis was performed using R Statistical software version 4.2.1.

2.5.1 *Fecal sample analysis*

A fecal egg count (FEC) was performed for each fecal sample collected using the McMaster's FEC test[3], following the approved study protocol (Appendix C). This test is used to estimate the number of helminth eggs per gram (EPG) of feces, which is an indicator of intestinal helminth burden. This test requires observation and quantification of parasite eggs under a compound microscope. For strongyles, this method does not allow identification of eggs to the species level, therefore I categorized Strongyle eggs as "strongyles", most likely representing nematodes such as *Hyostrongylus rubidus*, *Oesophagostomum* spp. and *Strongyloides ransomi* among others; or "strongyles – laval", most likely representing *Metastrongylus* spp. that pass in feces as larvated eggs. Eggs of *Ascaris suum* are easy to identify and were counted separately.

The percent prevalence of each of the three categories of endoparasites (i.e., strongyles, strongyles – larval, and *A. suum*) were estimated for the entire pig population studied, as well as comparing juveniles and adults, using data from visit 1. Prevalence was estimated and compared between pigs in the treatment and control groups using data from visits 1, 3, and 5. Visit 1 occurred prior to treatment with ivermectin, visit 3 occurred one month after the first dose of ivermectin, and visit 5 occurred one month after the second dose of ivermectin. For comparing prevalence of selected endo-parasites I used the Chi-squared test ($\alpha = 0.05$).

The average EPG was also estimated for all pigs using visit 1 data, and estimated and compared between treatment and control groups at visits 1, 3, and 5. A two-sample t-test for the mean of independent groups were used when measuring difference in mean EPGs between control and treatment groups ($\alpha = 0.05$). As part of the analysis, I log transformed the EPG variable before running the statistical test because I observed non-normal distribution of EPG. I also checked for equal variance before analysis.

For pigs in the treated group, I conducted the fecal egg reduction count test (FERCT) in months 1-3. FERCT is calculated as:

$$\frac{100\ x\ (1 - (2 - weeks\ post - deworming\ FEC))}{Pre - deworming\ FEC}$$

A FERCT of 90-95% means the parasite treatment is effective; < 90% indicates resistance.

2.5.2 *Analysis of findings on visual inspection*

For ticks, the following predilection sites were examined for the presence of ticks: (i) ears; (ii) inguinal area (iii) rectum and (iv) axilla. For each site, number of ticks were categorised based on number of ticks found; 1-5, 6-20 and > 20. Lice were recorded as present or absent. Signs commonly associated with a scabies infection were noted as present or absent, and included excessive ear shaking, thick black debris in ears, intense itching of the body, red papules covering body, thickened and crusted skin and hair loss.

The percent prevalence of pigs with visible ticks, lice, and evidence of mites was determined at visit 1. I compared prevalence of pigs with visible ticks, lice or evidence of mites between treatment and control groups using visual inspection data taken at visits 1-6. Chi-square test was used for comparison of ectoparasite prevalence between control and treatment group (α=0.05)

2.5.3 *Average daily gain (ADG)*

Growth rates in juvenile pigs were determined using weights estimated from heart girth and length measurements over the six-month study period. Weights were estimated at each time point (visits 1-9) using Schaeffer's formula[63]:

Estimated weight (pounds) = [heart girth (inches) x heart girth (inches) x length (inches)] / 400

This was measured as the average daily gain which is the average amount of weight an animal gains each day during the trial period[12]. ANCOVA test was used to compare ADG between control and treated groups.

2.6 RESULTS

A total of 140 pigs were enrolled in visit 1 of the study; 35 adults and 35 juveniles in the treatment group (N=70) and 35 adults and 35 juveniles in the control group (N=70). At the end of the study there were total 128 pigs remaining, 65 in the treatment group and 63 in the control group.

2.6.1 *Burden of endo- and ectoparasites in swine*

Overall, Fifty-five percent [95% CI: 48-62%] of pigs were positive for *Ascaris suum*, 95.2% [95% CI: 91-98%] were positive for Strongyle eggs, and 72.5% [95% CI: 65.5-79%] were positive for Strongyle – larval eggs (Figure 5). There was no statistically significant difference in endoparasite prevalence between juvenile and adult pigs for any of the three parasite categories at visit 1 (Appendix D: Supplementary tables). The overall mean EPG for *Ascaris suum* was 179.3 [95% CI: 147.3-211.3 CI] while Strongyle eggs and Strongyle eggs-larval stage had an overall mean EPG of 637.2 [95% CI: 505.5-768.95 CI] and 307.7 [95% CI: 224.2-391.1] respectively. There was no significant difference ($p>0.05$) in mean EPG between juveniles and adult pigs for any of the three categories of endoparasites (Figure 6).

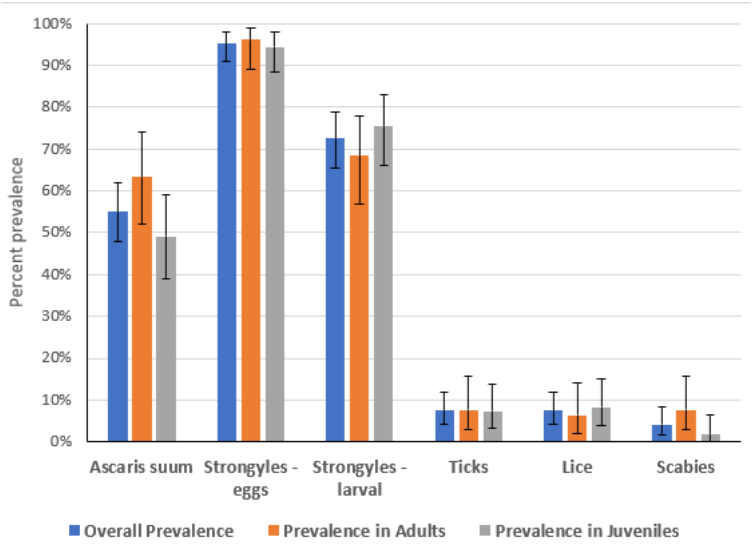


Figure 5. Baseline prevalence of selected endo- and ectoparasites

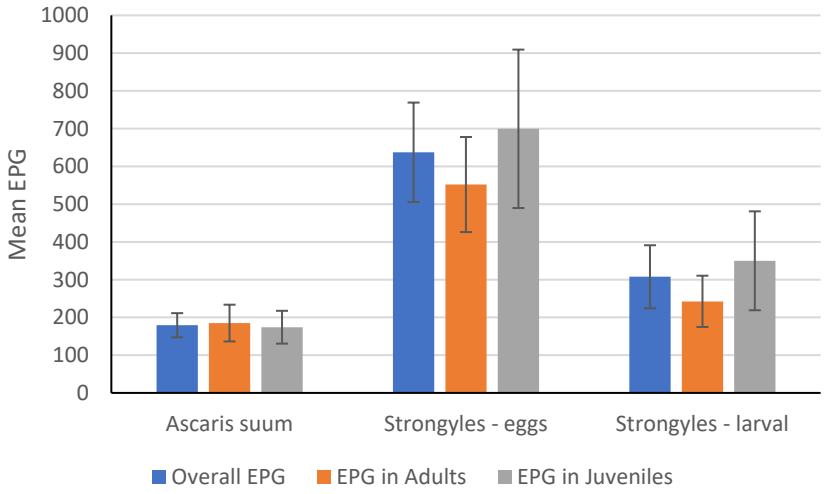


Figure 6. Baseline average eggs per gram (EPG) for selected endoparasites

Ectoparasite infestation was generally low (Figure 5). I found that 7.4% (C.I: 4.1%-12%) of pigs were positive for ticks while those positive for lice were 7.4% (C.I: 4.1%-12%) and those positive for scabies were 4.2% (C.I: 1.8%-8.2%). Like endoparasite prevalence, there was no significant difference in prevalence of any ectoparasite between juveniles and adult pigs (Appendix D: Supplementary table, Table B). When investigating co-infestation for ectoparasites, I found no co-infestation of ticks and

scabies in any pigs. Overall, co-infestation with ticks and lice, and lice and scabies were both 0.53% (95% C.I: 0.01%-2.9%) (Appendix D: Supplementary table, Table C).

2.6.2 *Ivermectin effectiveness in the reduction of common endo- and ectoparasites of swine.*

All prevalence and EPG values for visits 1, 3, and 5 can be found in Appendix D, Table A. At visit 1 (prior to ivermectin treatment) and visit 3 (one month after first dose of ivermectin), there was no difference between the treatment or control groups in prevalence (Figure 7) or average EPG (Figure 8) for any of the three endoparasite groups. However, at visit 5 (one month after the second dose of ivermectin), pigs in the treatment group had a 23.6% lower prevalence of strongyles ($p = 0.003$) and 18% lower prevalence of strongyles – larval ($p = 0.03$) as compared to controls. There was no significant difference in prevalence for *A. suum* ($p = 0.8$).

At visit 5 a significant difference in average EPGs was similarly observed between the control and treatment groups. Pigs in the treatment group had lower EPG of *Ascaris suum* (diff = 102; $p = 0.006$) for strongyles (diff = 642 EPG; $p < 0.001$), and for strongyles – larval (diff = 218 EPG; $p < 0.001$), as compared to controls.

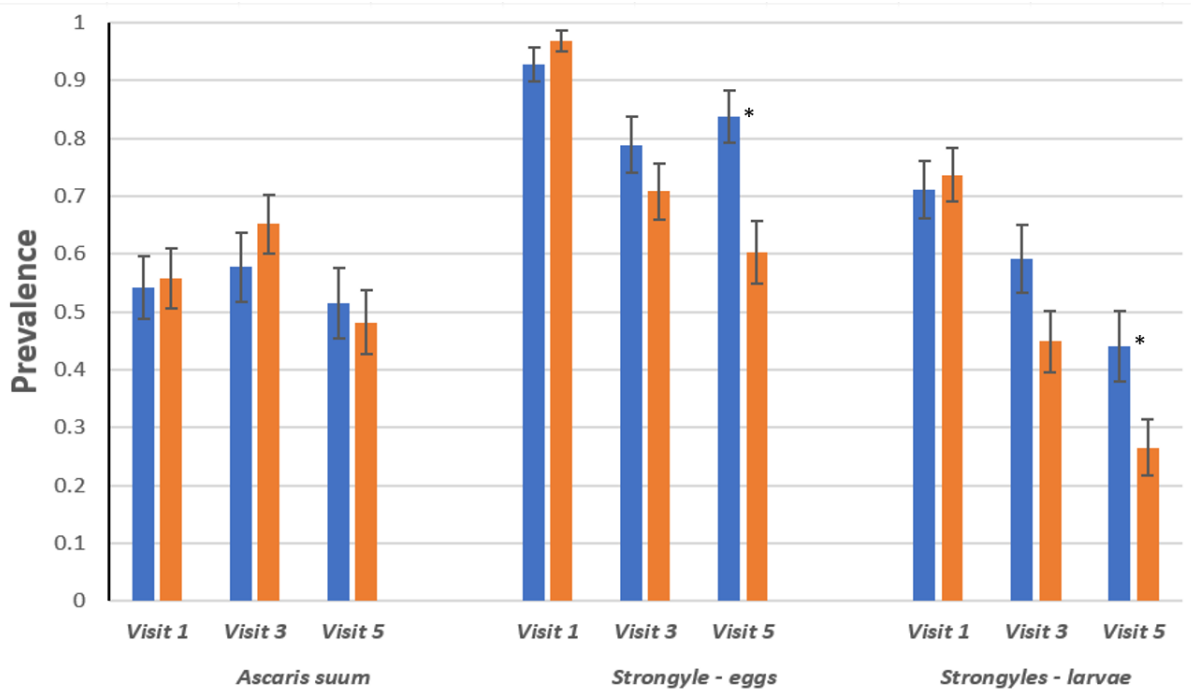


Figure 7. Prevalence of selected endoparasites by visit. * (p<0.05)

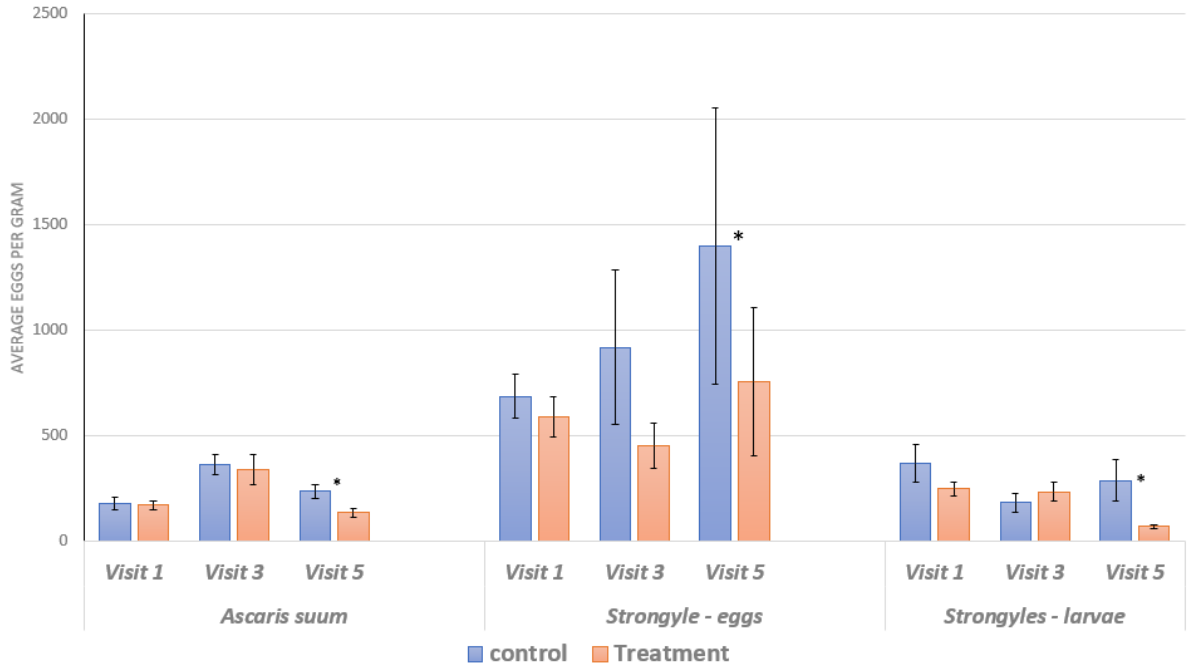


Figure 8. Average eggs per gram of feces for selected parasites by visit. * (p<0.05)

At visit 1 and visit 2, the prevalence of ticks, lice and scabies infestation was very low with no significant difference between control and treatment. At visits 3 through 6 a significant difference was

observed between control and treatment groups for ticks, but no statistically significant difference was found for lice or scabies (Figure 9). More information on ectoparasite prevalence by visit can be found in the supplementary tables of Appendix D (Table B).

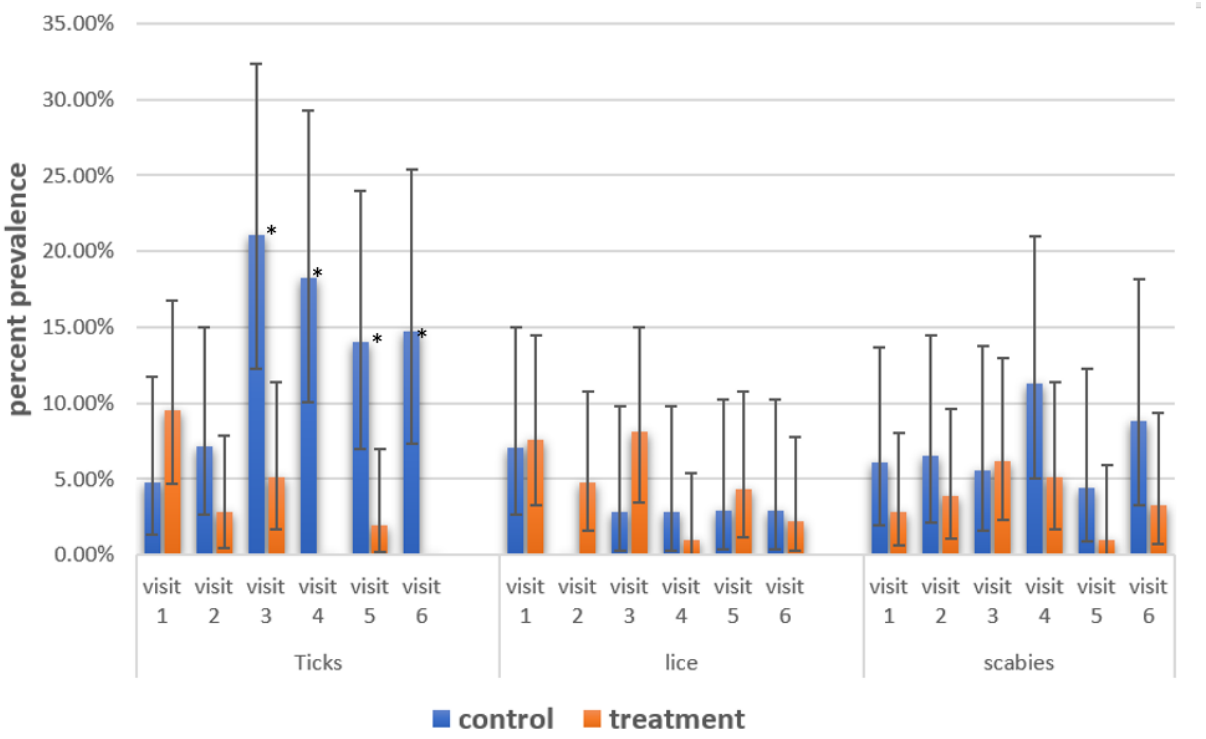


*Figure 9. Prevalence of selected ectoparasites by visit. * ($p<0.05$)*

2.6.3 ***Resistance to ivermectin in endoparasites***

Table 2 provides results of the FERCT, performed in month 1 (visits 1 and 2), month 2 (visits 3 and 4), and month 3 (visits 5 and 6) of the study. All FERCT results are all well below the 90% reduction that is expected when the drug delivered is effective against the parasite. For example, in month one, the FERCT for strongyle eggs was 51%, and 53% for strongyle eggs-larval. The FERCT for *A. suum* was negative 52%, suggesting no effect of ivermectin at all.

Table 2. FECRT of selected endoparasites from all sampled pigs

PARASITE									
	Visit 1	visit 2	FECRT	Visit 3	Visit 4	FECRT	Visit 5	Visit 6	FECRT
Ascaris suum	100	152	-52.4	245	98.5	59.8	75.5	63.89	15.4
strongyle eggs	583.8	285	51.21	307.1	61	80.13	431.5	581.7	-34.8
strongyle eggs-larval	185.2	88	52.5	104.55	21	79.91	23.9	15	37.24

Given these results, we reviewed the timing between fecal sample collection for pre and post ivermectin treatment, which was intended to be 2 weeks (14 days). Due to challenging field conditions, fecal samples were not collected after every 14 days as designed. Some fecal samples were collected 16 to 20 days after ivermectin treatment. A second analysis was performed eliminating animals with post treatment samples collected outside of a 13-15-day window, however this did not change the results in a meaningful way. All FERCT values were still well below the expected 90% reduction (Table 3).

Table 3. FECRT of pigs sampled after 13,14 and 15 days after ivermectin treatment

PARASITE	*Mean EPG in treatment*								
	visit1	visit2	FECRT	visit3	visit4	FECRT	visit5	visit6	FECRT
Ascaris suum	101.7	183	-79.94	212.7	86.6	59.29	69.86	64.4	7.82
strongyle eggs	527.1	344	34.73	262	67.6	74.2	150.7	642	-325.94
strongyle eggs-larval	168.6	94	44.25	102.8	23.9	76.75	20.54	15	26.97

2.6.4 ***Average daily weight gain***

A total of eleven pigs were removed from the analysis for comparing ADG between treatment and control groups. The exclusion of these pigs was based on finding biologically implausible values, such as a negative ADG, or weights that were 50% lower on subsequent visits (e.g., first weight of 10 pounds,

then two weeks later, a weight of 5 pounds). The assumption is that these pigs were incorrectly measured, or the incorrect pig was measured.

The mean ADG for the control group was 0.162 lbs/day (σ = 0.083), whereas the treatment group was 0.207 lbs/day (σ = 0.131). Simple regression analysis (Figure 9) found no difference in ADG between the treatment and control groups (β = 021, se = 0.025, p = 0.418). Results of the ANCOVA regression (Table 4), which adjusted for starting weight found a 0.021 pound (se = 0.025) increase in ADG for the treatment group, but this was also not significant (p = 0.418).

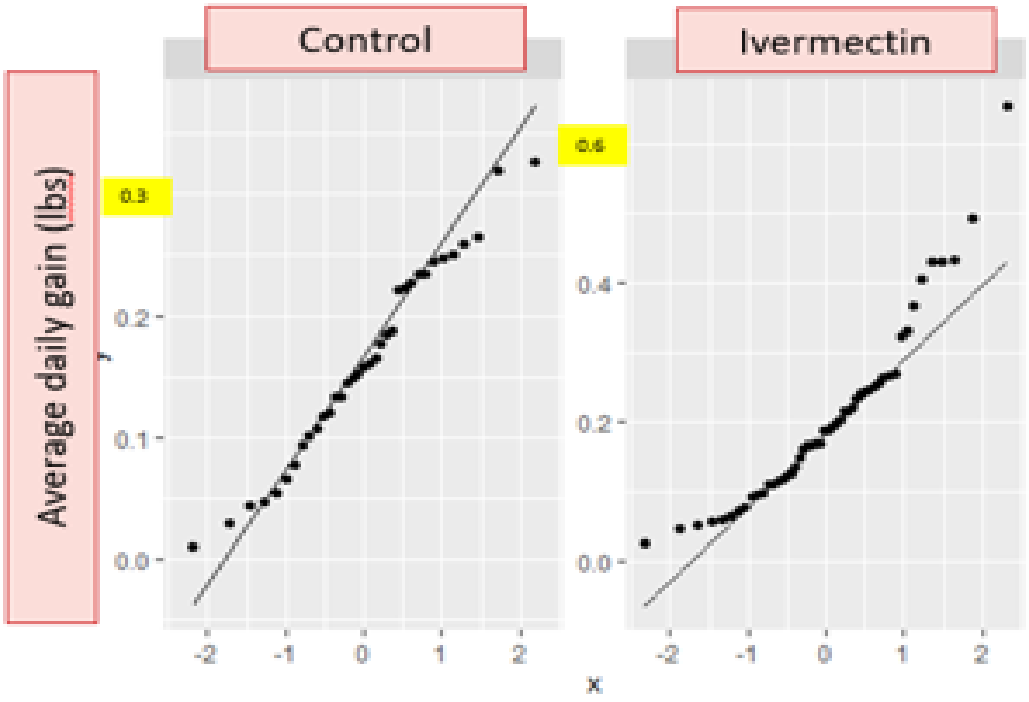


Figure 10. Growth rates (Average daily weight gain)

Table 4. ANCOVA-comparing treatment groups after adjusting for staring weight

	β	***SE***	***P***
Intercept	0.009	0.028	<0.001
Starting weight	0.005	0.002	0.003
ADG (ivm)	0.021	0.025	**0.418**

2.7 Discussion

Our results indicate that endoparasite prevalence and intensity of infection is high in pigs in the Mopeia district. Greater than 50% of pigs were positive for *A. suum* eggs, over 90% were positive for strongyle eggs, and around 70% were positive for strongyle eggs in the larval stage. The average EPG was similar to that found by other studies in pigs in Sub-Saharan Africa such as those observed by Kouam et al[64,65]. I expected that juvenile pigs would have a higher prevalence of infection and higher average EPGs, however no significant difference was found. This is contrary to our expectation and contrary to what the literature suggests, as young animals normally have weaker immunity against parasites as compared to adult animals which I would expect to have relatively lower parasite burden due to their strong acquired immunity resulting from repeated infections[66]. One possible explanation for this observation is that, according to responses from household questionnaires (data unpublished), most pigs in the area do not receive any nutritional supplementation or veterinary care, and are therefore burdened by poor nutrition and ill health making both juveniles and adults equally susceptible to parasitic infection. Another possible explanation is that the environment may be heavily contaminated with parasite eggs due to the type of production system in the area. The extensive production system is characterized by

free roaming pigs and/or makeshift housing with poor environmental hygiene, which are major risk factors for infection for both juveniles and adult animals.[51]

The impact of three monthly ivermectin treatments on selected endoparasites was minimal, with significant differences between treatment and control groups only identified in visit 5, or one month after the second dose of ivermectin. It is also important to note that the differences in EPG seem to occur because the average EPG in the control group went up, rather than average EPG in the treatment group going down. Potential explanations for this include the fact that iMDA was started at the beginning of the rainy season and during the rainy season parasite burden is expected to intensify which it does in controls.

Also, one month after the first dose of ivermectin administration we again see no significant difference in either prevalence or in mean EPGs between control and treatment groups although there were nearly twice as many *Strongyle* eggs in the control group as compared to the treatment group but this difference was not statistically significant. This non-difference can be explained by the fact that in veterinary practice, treatment is recommended every 14 days, but this was not the case in this study because this study was a sub-study of a larger clinical trial, which was aimed at malaria control by administering ivermectin to people and swine once a month. Due to this, there were higher chances that after 14 days the animals would be in the same risk of parasite infection as it was before ivermectin administration. This is because 14 days is beyond the ivermectin drug elimination half-life in pigs which is known to be 6-10 days meaning that by day 14 the drug was already cleared from the body and hence reinfection was possible especially because the environment was highly contaminated due to the type of production system conducted in the study site. [52]

The results of the FERCT in all three months when iMDA was delivered suggest that the selected endoparasite groups were not affected by the ivermectin dose administered. This is contrary to what I would expect since the dose used was the approved labeled dose, based on weight, and ivermectin is labeled for the treatment of the endoparasites examined in this study. Current literature shows that ivermectin has over 95% effectiveness in treating strongyles and *A. suum* in pigs in Sub-Saharan

Africa[67,68]. Although resistance to ivermectin and other anthelmintics has been reported across the world[32,69,70], this typically happens where they are routinely given to the animals or where it is used off-label. However, based on household questionnaires (data unpublished), only 3% of pig-owning households in the study area reported deworming any of their pigs in the year prior to the start of the study. It is therefore unlikely that the endoparasites in this area are actually resistant to ivermectin, but rather other potential factors were responsible. For example, parasite misidentification, sampling of the wrong pigs, or underdosing of ivermectin by study field staff.

Similar to endoparasites, the impact of three monthly ivermectin treatments on selected ectoparasites was minimal. This is not surprising as the overall prevalence was generally low (< 10%) and the study was not powered to detect a difference in ectoparasite burden. Interestingly, the only significant difference identified was the reduction of ticks in the treatment group. Although ivermectin is labeled for treatment of lice (*Haematopinus suis*) and mange mites (*Sarcoptes scabiei* var. *suis*), it is not labeled for the treatment or prevention of ticks in pigs. However, some studies such as the one conducted in Tanzania have shown that ivermectin is actually effective against ticks in pigs. [71]

Given the minimal impact on prevalence and EPG, it is not surprising that I also did not identify a significant difference in ADG between treatment and control groups. However, it is important to note that I based my sample size on the assumption of an ADG = 0.286 lbs/day, with a standard deviation of 0.025 and the ability to detect a 15% difference between the groups. I did not anticipate that the ADGs of pigs in Mopeia would be so low (0.162 for control pigs, 0.207 for treatment pigs) and have such large standard deviations. Although the difference between the groups is roughly 15%, I suspect our sample size was too small to determine a significant impact on weight gain.

Although ivermectin has a broad spectrum of activity against a variety of ectoparasites and endoparasites, its ecological impact needs to be considered when implementing ivermectin mass drug administration programs. Some studies have indicated that ivermectin that is excreted via animal feces has toxic effects on dung microbiota that are responsible for degrading animal feces in the environment. Several ecologically important microbiota such as diptera and coleopterans as well as earthworms are

known to be negatively affected by ivermectin contained in the animal feces and therefore increasing and frequent use of ivermectin in livestock is discouraged[72].

2.8 Limitations

This study was part of a larger study investigating the effects of iMDA in humans, and humans and pigs as a novel vector control tool for malaria. Therefore, the study described here was not designed to study the effect of ivermectin delivery for pig health, but rather to capture any secondary health and productivity effects that might come from the delivery of ivermectin in pigs to achieve a human health outcome. This is an important difference. Typically, due to the life cycle of the parasites, ivermectin would be dosed two times, 14 days apart to effectively treat most endo- and ectoparasite infections. Environmental decontamination and sanitation is recommended to prevent reinfection but this is not a common practice in the area, and was not a part of the iMDA intervention, therefore this may have contributed to the poor performance of ivermectin against the selected parasites[73,74]. Potentially, challenges with animal identification, lack of skilled veterinary technicians in the area and inadequate training on parasite identification may have contributed to data quality issues and consequently to the results I have observed. Parasite identification was completed within three days of sample collection, but a there was not a veterinarian or parasitologist with the knowledge to monitor and ensure that identification was done correctly, therefore this may have affected data quality and results. There is also the potential that our animal team might have administered a wrong dose (underdose) to pigs hence masking the true efficacy of ivermectin.

2.9 Concluding Remarks

Our results suggest that ivermectin delivered once a month for three months as part of a malaria intervention trial is not highly effective against endo- and ectoparasites of pigs in Mopeia. I am unable to rule out if this is due to the fact that ivermectin was not provided in a typical manner (after every 14 days) in combination with poor sanitation and a heavily contaminated environment, or if parasite resistance was present, as the results of the FERCT would suggest. Resistance has been detected across the world and in some African countries such as Kenya which is not very far from Mozambique,[32]

therefore there is a possibility that these parasites are also resistant to ivermectin even though I have evidence of minimal previous exposures to anthelmintics. Due to significantly low numbers of ectoparasites observed, I also cannot make safe conclusions on the efficacy of ivermectin against ectoparasites.

Given the fact that over 95% of pigs in Mopeia have no history of vaccination or deworming, monthly administration of ivermectin for 3 months may not have the benefit we would expect to see with different dose schedule or under a different management system and therefore this is likely to have contributed to the type of results I have obtained. Despite the results that I see, the animal team reported that owners were pleased with the results of ivermectin treatment to the extent that the control group was requesting that their pigs also be treated so they could experience similar benefits as the treatment group. This implies that ivermectin treatment produced some visible positive results although I was not able to capture much of it from the data I have.

Interestingly, the animal health team that was responsible for collecting data reported stated that owners whose pigs received ivermectin reported that they looked healthier and grew faster. Future analyses with data collected from this study will investigate how ivermectin treatment may have affected pig sales, reproduction, slaughter and income generation to the household.

What seems certain is that iMDA in pigs for public health purposes alone is not likely to be highly effective for pig health in such resource poor settings where environments are highly contaminated, the population isn't aware of basic management practices, and veterinary care is poor. If iMDA in pigs is successful in reducing malaria, a true One Health approach going forward would be to deliver ivermectin to pigs alongside with the proper environmental sanitation programs and other pig management programs including nutrition and other veterinary care. Reducing parasite burdens in these animals isn't only an animal health and economic issue. The fact that 50% of pigs in this area are infected with *Ascaris suum* is also a human health risk. *Ascaris suum* is known to have zoonotic

capacity and is transmitted to humans who come in contact with animal feces or contaminated crops such as vegetables[75,76] .

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APPENDIX A. Fecal sample collection from pigs

1 OBJECTIVES

To train BOHEMIA's animal health fieldworkers on how to collect fecal samples from pigs.

2 DEFINITIONS

Term	Definition
CISM	Centro de investigaçao en Saúde de Manhiça
BOHEMIA	Broad One Health Endectocide- based Malaria Intervention in Africa
H+L arm	Set of clusters/households in which ivermectin is to be administered to both humans and livestock.
H arm	Set of clusters/households in which ivermectin is to be administered only to humans.
Animal health fieldworker	Fieldworker responsible for animal sampling in the context of the Health and Economics study.

3 APPLICABLE TO

Applicable to all animal health fieldworkers taking part in the Health Economics study of the BOHEMIA project, for them to be able to correctly collect, identify and store fecal samples prior to analysis.

4 RESPONSIBILITIES

It is the responsibility of the animal health lead and field coordinators to train the animal health fieldworkers in the procedures described below and coordinate their work. It is the responsibility of the animal health fieldworkers to follow the procedures described below and collect fecal samples as explained by researchers and coordinators during the training.

5 RELATED SOPs

- How to conduct a visit for animal sampling
- Livestock selection for data collection
- External examination.
- Biometrics.
- Fecal sample analysis
- Restraining livestock for ivermectin administration or tick counting

6 MATERIALS AND EQUIPMENT

Exam gloves, pens, labels for sample identification, marker, lubricant, cooler with ice packs.

7 PROCEDURES

For samples collected from pigs in the H+L arm during visits 1, 3, and 5, samples must be collected at the time of ivermectin administration or within 1 hour after ivermectin is administered.

Sample collection:

1. Wear your exam gloves. Apply small amount of lubricant to the index and middle fingers of your dominant hand
2. Fix the pig by holding its tail with the other hand.
3. Insert index and middle fingers into the rectum of the animal, one finger at a time. There is no need to go very deep. Spread your fingers to allow air into the rectum – this can stimulate defecation.
    - NOTE: If the pig is small (6-8 weeks of age) you may only be able to insert one finger, or just your pinky finger. Gentle movement of the finger should stimulate defecation.
4. Remove approximately 2 to 4 grams of feces.
5. If sampling from the rectum is not possible then collect a sample immediately after it has been naturally deposited by the animal. Take care only to collect fecal material that has not been in contact with the ground or other surface.
6. Peel the glove off your hand keeping the feces encased within it.
7. Squeeze as much air as possible out of the glove. Twist the wrist portion and tie off the glove before placing the label

Sample identification:

8. The label should include the household ID, animal ID, date of collection, and visit number.
    - Visit number options: 1st visit, 1st follow-up, 2nd visit, 2nd follow-up, 3rd visit, 3rd follow-up, 6th visit.
    - NOTE: on visits 4 and 5, no fecal samples are collected
    - NOTE: fecal samples are only collected in the follow-up visits for pigs that received ivermectin as part of the BOHEMIA study.
9. Place the label around the tied portion of the glove, making sure it sticks to itself.

Sample storage:

10. Place the sample in the cooler with ice packs immediately after collection.
11. At the end of the day, all samples should be stored in a refrigerator.
12. Every three days, samples should be taken to the central point identified for sample analysis.

8 REFERENCES

Study protocol: Economic assessment of ivermectin as a vector control tool

APPENDIX B. Study specific procedure; external examination

1 OBJECTIVES

To train BOHEMIA's animal health fieldworkers on how to perform an external examination of pigs according to protocol.

2 DEFINITIONS

Term	Definition
CISM	Centro de investigaçao en Saúde de Manhiça
BOHEMIA	Broad One Health Endectocide- based Malaria Intervention in Africa
H+L arm	Set of clusters/households in which ivermectin is to be administered to both humans and livestock.
H arm	Set of clusters/households in which ivermectin is to be administered only to humans.
Animal health fieldworker	Fieldworker responsible for animal sampling in the context of the Health and Economics study.
Biometric data	Measurements of an animal's body length and circumference

3 APPLICABLE TO

Applicable to all animal health fieldworkers taking part in the Health Economics study of BOHEMIA project, for them to be able to correctly examine pigs according to protocol.

4 RESPONSIBILITIES

It is the responsibility of the animal health lead and field coordinators to train the animal health fieldworkers in the procedures described below and coordinate their work. It is the responsibility of the animal health fieldworkers to follow the procedures described below and examine pigs as explained by the animal health lead and coordinators during the training.

5 RELATED SOPs

- How to conduct a visit for animal sampling.
- Livestock selection for data collection.
- Fecal sample collection from pigs.
- Biometrics.
- Livestock restraint.

6 MATERIALS AND EQUIPMENT

Tablet, exam gloves, materials for animal restriction.

7 PROCEDURES

From all BOHEMIA fieldworkers, 18 will be trained for animal sampling duty. These animal health fieldworkers will be responsible for collecting biometric and biological data from pigs in households recruited for the Health Economics study where an informed consent has been signed, both among H+L and H arm clusters. See SSP - Livestock selection for data collection.

All pigs that are selected for inclusion in the study will undergo external examination at every visit (total 9 visits). Once the animal is restrained (See SSP for livestock restraint), a visual examination for ticks, lice and signs of mite infestation is performed and results are documented in the data collection sheet (Annex 1 of SSP *Livestock selection for data collection*). A visual aid is provided (Annex 2) to help animal health fieldworkers differentiate between ticks and lice, and to identify physical signs of infestation with mites, which are not visible to the naked eye.

Ticks:

Ticks come in colors such as brown or black. They have a teardrop shaped body, small compact head, 8 legs and no antenna. They may appear flattened (if before a blood meal) or large and engorged, if during or after a blood meal. Ticks crawl slowly or are attached firmly to the pig's skin while feeding.

1. Look for ticks in the following locations: 1) behind or inside right ear; 2) behind or inside left ear; 3) in right and left axilla areas; 4) in right and left inguinal areas; 5) under the tail and around the rectum
2. For each area where ticks are found, note the number of ticks observed as: 1-5, 6-20, >20

Lice:

Lice are greyish brown and roughly 0.6 cm in length. They have a soft body, oblong head, six legs and 2 antennae. Lice do not swell with a blood meal and move fast through the hairs. They often leave small white sticky eggs on the pig's hair. They often cause severe itching in infested animals.

1. Note if any lice are present on the animal. Answer as: yes or no

Mites:

Mites are external parasites that are not visible to the naked eye. Mites cause intense itching in pigs, which can lead to hair loss, secondary skin infections, and reddening of the skin.

2. Note if any of the following signs are present in the pig: Excessive ear shaking, thick black debris in ears (not mud, must be thick and black), intense itching, red papules covering the body, thickened or crusted skin (often around tail base, face/mouth, ears, between the legs), or hair loss.

8 REFERENCES

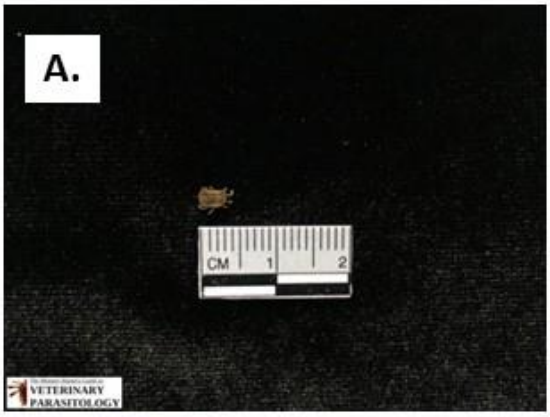
Study protocol: Economic assessment of ivermectin as a vector control tool.

9 ANNEXES

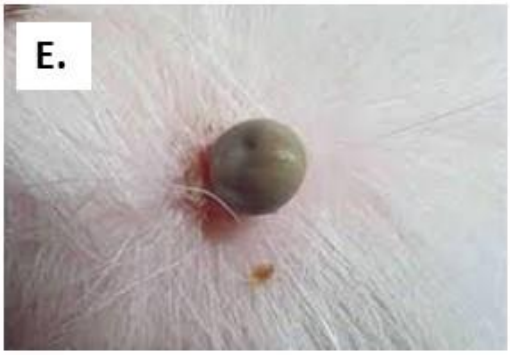
Annex name	Responsible Person	Reference
1. Visual inspection aid for ticks, lice and signs of mites		

Visual Inspection Aid

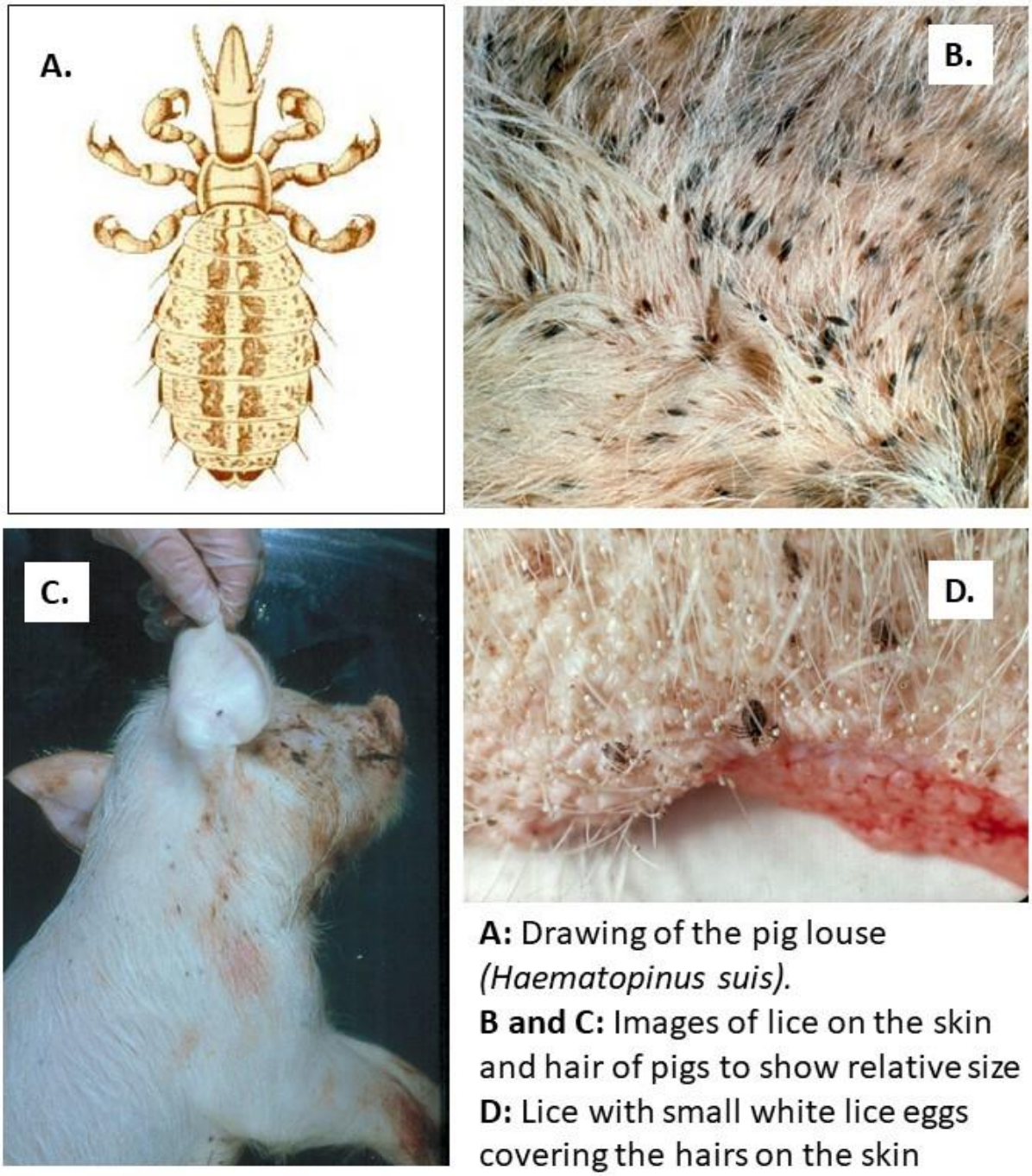
Ticks



A-B: Soft ticks (*Ornithodoros* spp.) only feed for very short periods of time (30 minutes) so they are rarely seen on their pig hosts.
C-E: Hard ticks are attached to the pigs skin and may appear flat (**C** and **D**) when they first attach, or engorged (**E**) when they have been attached for several days.

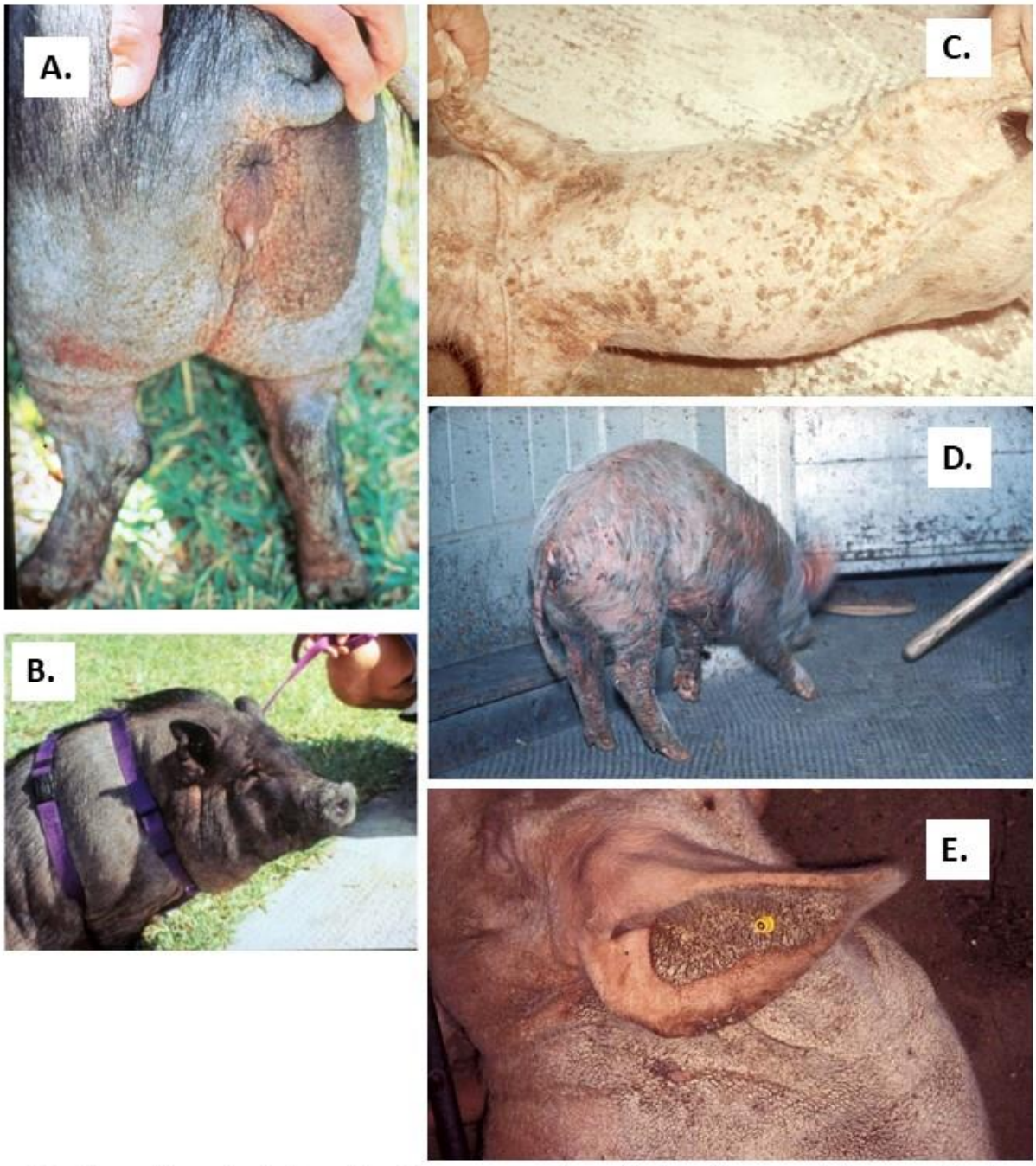


Lice



A: Drawing of the pig louse *(Haematopinus suis).*
B and C: Images of lice on the skin and hair of pigs to show relative size
D: Lice with small white lice eggs covering the hairs on the skin

Signs of mite infestation



A-E: Pigs with a mite infestation. You can see the red raised bumps with areas of red and thickened skin and hair loss (Images **A-D**). Pigs may only be affected on some parts of their bodies (**A, B, E**), or may have generalized lesions (**C** and **D**). Mites are NOT visible to the naked eye.

APPENDIX C. Fecal sample analysis

1 OBJECTIVES

To train BOHEMIA's animal health fieldworkers on how to analyze fecal samples from pigs.

2 DEFINITIONS

Term	Definition
CISM	Centro de investigaçao en Saúde de Manhiça
BOHEMIA	Broad One Health Endectocide- based Malaria Intervention in Africa
Animal health fieldworker	Fieldworker responsible for animal sampling in the context of the Health and Economics study.

3 APPLICABLE TO

Applicable to animal health fieldworkers taking part in the Health Economics study of the BOHEMIA project, and selected to perform the fecal analysis for all samples collected from pigs during the trial.

4 RESPONSIBILITIES

It is the responsibility of the animal health lead and field coordinators to train the animal health fieldworkers in the procedures described below and to coordinate their work. It is the responsibility of the animal health fieldworkers to follow the procedures described below as explained by the animal health lead and coordinators during the training.

5 RELATED SOPs

- Fecal sample collection from pigs

6 MATERIALS AND EQUIPMENT

Exam gloves, permanent marker, digital scale, 2 plastic cups, fecal flotation solution, plastic dispenser bottle (500 ml), 50 ml measuring cylinder, tongue depressor or spoon for mixing, gauze 4 x 4 pads, 2-chamber McMaster slide, plastic transfer pipette, compound microscope with 10x, 40x and 100x objectives, timer, tally counter, microfiber cloth.

To make fecal flotation solution: table sugar, table salt, digital scale, 2 large plastic measuring cups, 3-L Erlenmeyer flask, hot plate, magnetic stir bar, water, flask with screw cap (for storage)

7 PROCEDURES

Two animal health fieldworkers will be selected to perform the fecal sample analyses for the study. They will stay in a central location, where they will receive fecal samples from the field every 1-3

days. Additional animal health fieldworkers may be trained on fecal sample analysis to provide additional support as needed.

Preparation of fecal flotation solution:

1. Weigh out 1125 grams of table sugar in a plastic container - make sure to tare the scale after placing the container and before adding sugar
2. Weigh out 748 grams of salt in a plastic container - make sure to tare the scale after placing the container and before adding sugar
3. Add exactly 2 L water in a 3L-Erlenmeyer flask and put it on a stirring hot plate. Add a magnetic stir bar to the flask and turn on the heat to the hot plate
4. Place a funnel into the top of the flask and slowly pour the sugar into the water
5. Then pour the salt into the flask
6. Add water to complete 3000 ml on the Erlenmeyer flask, then measure another 125 ml of water and add that to the flask. There should be a total of 3125 ml of water in the flask
7. Allow the sugar and salt completely dissolve and then remove the flask from the plate and let the solution come down to room temperature
8. Transfer the solution into a clean container with secure lid
    - NOTE: The solution is good for one year at room temperature
9. At the time of analysis, pour some of the solution into the 500 ml plastic dispenser bottle - refill as needed.

McMaster slide preparation:

1. Wear exam gloves.
2. Label two cups with household and animal ID.
3. Tare one labeled cup on scale.
4. If feces are pelleted, crush the pellets in the glove and knead the feces in glove to mix. Cut off fingertip of glove containing feces to access fecal material, making sure to leave label intact.
5. Weight out 2 grams of feces into the cup on the scale.
6. Dispense 28 ml flotation solution from the plastic dispenser bottle into the cup, mix well and **let soak for approximately 5 minutes (set timer)**.
    - NOTE: Once you are confident in the procedure you can weigh out multiple samples, add flotation solution and mix until 5-6 samples are set up.
7. After five minutes, mix again. Place the 4 x 4 gauze on top of the second cup (don't stretch fabric tight across the cup). Pour the mixture of feces and flotation solution through, pressing fluid through with the tongue depressor.
8. **Immediately**, fill both chambers of the McMaster slide using a transfer pipette collecting fluid from the top of the solution. If large bubbles are present, empty the slide and refill. Even if a large bubble is not actually under the grid, the slide should be refilled. Fill the entire chamber, not just the area under the grid.
9. Set slide aside for **5 minutes (set timer)** to allow parasite eggs to float to the surface. Read slides **within 20 minutes** of filling the slide. If slides are left too long, fluid evaporates and salt crystals form.

Counting the McMaster chambers:

10. Place McMaster slide onto the microscope stage.
11. Bring the grid lines on the McMaster slide into focus using the low power (4X) objective and the coarse adjust knob. Turn to the 10X objective and refocus grid lines using the fine adjust knob.
12. Count all eggs inside of the grid areas of both chambers using the 10X objective. Include eggs on the grid line if greater than ½ of the egg is inside the grid.
13. Always start at the same point on the McMaster slide (for example, top left or bottom right). That way, you won't lose track of whether you have counted only one or both chambers.

14. With the help of the Pig Parasite Identification document (annex 1), count each of the following categories of parasite eggs, individually in both chambers:
    - Category 1: Eggs of *Ascaris suum*
    - Category 2: Multicellular strongyle eggs
    - Category 3: Strongyle eggs, larval stage

Data Entry (annex 2):

15. Look at the labels on the glove and enter the following information: Household ID, Pig ID, date collected and visit number [1st visit, 1st follow-up, 2nd visit, 2nd follow-up, 3rd visit, 3rd follow-up, 6th visit – note, on visits 4 and 5, no fecal samples are collected].
16. For each of the categories listed above, enter the total egg count for chambers 1 and 2, separately

Cleaning McMaster slides:

17. After entering results, the McMaster chamber should be washed under a stream of water, shaken to remove most of the water and then dried with a microfiber cloth on the outside and inside of the chambers.

8 REFERENCES

Study protocol: Economic assessment of ivermectin as a vector control tool.

TABLE A. Impact of ivermectin on endoparasites of swine, when delivered once a month for three months

	Parasite	Control	Treatment	p-value	Control	Treatment	p-value
		% (95% C.I)	% (95% C.I)		**EPG** (95% C.I)	**EPG** (95% C.I)	
Baseline	*Ascaris suum*	55.% (43.5%,65.7%)	55.% (45.2%,65%)	1	178.89 (122-235)	171(133.7-209.7)	0.61
	strongyles - eggs	93% (85%,97%)	97% (92%,99%)	0.3	688.3(477.7-898.9)	588.58(398.6-778.5)	0.3
	strongyles - larval	71% (60.5%,80.8%)	73% (63.8%,81.5%)	0.82	368.8(189.95-547.3)	248.5(188.3-308.8)	0.06
Visit 3: 4 weeks	*Ascaris suum*	57.7% (45%,69%)	65% (56.5%,75.8%)	0.4	363.4(266.7, 460)	339.6(195-484)	0.15
	strongyles - eggs	78.9% (67.6%,87.7%)	70.7% (60.7%,79%)	0.32	920.52(186.8,1654)	452(240-664)	0.12
	strongyles - larval	59.2% (46.8%,70.7%)	45.5% (35.4%,55.8%)	0.1	183.3(92.4,274.3)	235(144-325)	0.9
Visit 5: 8 weeks	*Ascaris suum*	51.5% (39%,64%)	48.2% (39%,64%)	0.8	238.57(172.2,305)	136(93-178)	0.006
	strongyles - eggs	83.8% (72%,92%)	60.2% (49%,71%)	0.003	1399.12(88.86,2709)	757(53-1460)	0.0009
	strongyles - larval	44% (32%,56.7%)	26% (17 %,37%)	0.027	288.33(85,491.7)	70.5(49-91)	0.0006

TABLE B. Impact of ivermectin on ectoparasites of swine, when delivered once a month for three months

		Control	*Treatment*	*p-value*
Baseline		%	%	
	Ticks	4.76% (1.3%,11.7%)	9.5% (4.7%,16.8%)	0.34
	Lice	7.1% (2.7%,15%)	7.6% (3.3%,14.5%)	1
	Scabies	6.1% (2%,13.7%)	2.86% (0.6%,8%)	0.47
Visit 2: 2 weeks				
	Ticks	7.2% (2.7%,15%)	2.8% (0.5%,7.9%)	0.28
	Lice	0%	4.8% (1.6%,10.8%)	0.13
	Scabies	6.5% (2.1%,14.5%)	3.9% (1.1%,9.6%)	0.65
Visit 3: 4 weeks				
	Ticks	21.1% (12.3%,32.4%)	5.1% (1.7%,11.4%)	0.003
	Lice	2.8% (0.3%,9.8%)	8.1% (3.5%,15%)	0.26
	Scabies	5.6% (1.6%,13.8%)	6.2% (2.3%,13%)	1
Visit 4: 6 weeks				
	Ticks	18.3% (10.1%,29.3%)	0%	0.000032
	Lice	2.8% (0.3%,9.8%)	1% (0%,5.4%)	0.76
	Scabies	11.3% (5%,21%)	5.1% (1.7%,11.4%)	0.23
Visit 5: 8 weeks				
	Ticks	14.% (7%,24%)	2% (0.2%,7%)	0.0061
	Lice	2.9% (0.35%, 10.2%)	4.3% (1.2%,10.8%)	0.9
	Scabies	4.4% (0.9%,12.3%)	1% (0%,5.9%)	0.4
Visit 6: 10 weeks				
	Ticks	14.7% (7.3%,25.4%)	0%	0.0005
	Lice	2.9% (0.4%,10.2%)	2.2% (0.3%,7.8%)	1
	Scabies	8.8% (3.3%,18.2%)	3.3% (0.7%,9.4%)	0.26

Table C. Baseline prevalence of ectoparasites in juveniles and adults

		overall	Adults	Juveniles
Ectoparasites		% (95% CI)	% (95% CI)	% (95% CI)
	Ticks	7.4% (4.1%,12%)	7.6% (2.8%,15.8%)	7.3% (3.2%,13.8%)
	Lice	7.4% (4.1%,12%)	6.3% (2.1%,14.2%)	8.2% (3.8%,15%)
	Scabies	4.2% (1.8%,8.2%)	7.6% (2.8%, 15.8%)	1.8% (0.2%,6.4%)
	Ticks and lice	0.53% (0.01%,2.9%)	0%	0.91% (0.02%,5%)
	Ticks and scabies	0%	0%	0%
	Lice and scabies	0.53% (0.01%,2.9%)	0%	0.91% (0.02%,5%)