CHARACTERISTICS OF PRODUCTION SYSTEM, EPIDEMIOLOGY AND CONTROL OF PIG PARASITES IN BUSIA DISTRICT, KENYA

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DECLARATION

This thesis is my original work and has not been presented for a degree in any other University

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This thesis is dedicated to my wife Anne and my children Kevin and Tiffany

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LIST OF ABBREVIATIONS AND ACRONYMS

χ^2	chi square statistic
μl	microlitre
Ag-ELISA	Antigen Enzyme Linked Immunosorbent Assay
AIDS	Acquired immunodeficiency Disease syndrome
AWMA	Adult worm mortality assay
ANOVA	one-way analysis of variance
ASF	African Swine Fever
bw	body weight
CI	Confidence interval
DPI	Days post infection
DPT	Days post treatment
DVO	District Veterinary Officer
DWG	daily weight gain
ED50	Effective dose 50
EHA	Egg hatch assay
EM	Electron Microscopy
EPG	Eggs Per Gram
FAO	Food and Agricultural Organization
FCC	Farmers Choice Company
Fig.	Figure
FITCA	Farming In Tsetse Controlled Areas
FTC	Farmers Training Centre

g	Grams
GDP	Gross Domestic Product
GIT	Gastrointestinal tract
GoK	Government of Kenya
GPS	Geographical Positioning System
HIV	Human Immunodeficiency Virus
hrs	Hours
KARI	Kenya Agricultural Research Institute
Kg	Kilograms
Km	Kilometer
LD50	Lethal Dose 50
LDA	Larval Development Assay
LMA	Larval Mortality Assay
mg	Milligrams
Min	Minutes
ml	Millilitre
MLFD	Ministry of Livestock and Fisheries Development
mm	Millimetres
NGOs	Non-Governmental Organization
°c	Degrees Celsius
OD	Odds ratios
OPG	Oocysts Per Gram
PBS	Phosphate Buffered Saline

PCV	Packed Cell Volume
PPM	Parts Per Million
r	Pearson correlation
rpm	Revolution Per Minute
SE	standard error
spp	species
TEM	Transmission Electron Microscope
TRC	Trypanosomiasis Research Institute
USD	United States Dollar

ABSTRACT

A study was carried out in Busia District to characterize the pig production system, determine the prevalence and intensity of pig parasites, identify the risk factors associated with occurrence of these parasites and determine the efficacy of pawpaw products and neem oil against *Oesophagostomum* spp and *Haematopinus suis* infections in pigs. In order to achieve these objectives, socio-economic studies, slaughter slab and farm surveys and efficacy trials were carried out.

For the socio-economic survey, questionnaire data on farm characteristics was collected from 182 farmers selected from the six divisions of Busia District. The mean number of pigs per farm was 3.6, mainly (178/182, 98%) for income generation. The production systems were mainly farrow to weaner (22/182, 12%) and weaner to finisher (65/182, 36%). The main production constraints as perceived by farmers included pig diseases (148/182, 81%) and high cost or lack of feed (148/182, 81%). The slaughter slab survey involved a questionnaire survey on 16 butchers and 6 slaughter-slabs in the main urban centres in the District. The average net income per annum for each butcher was Ksh 62,688 (897 USD), while the average profit per slaughtered pig was Ksh. 268 (3.8 USD). The main constraints to butchery business were conflicts with regulatory authorities (16/16, 100%) and lack of slaughter pigs (15/16, 94%).

The study on prevalence and intensity of parasites was carried out at both slaughter slabs (37 pigs) and farms (135 farms, 306 pigs) level in Busia District. The prevalences of nematodes shed by pigs at both the slaughter slab and farm level were:

Oesophagostomum spp (100% (37/37), 75% (230/306)), *Strongyloides ransomi* (16% (6/37), 37% (113/306)), *Ascaris suum* (19% (7/37), 18% (55/306)), *Trichuris suis* (3% (1/37), 7% (21/306)), *Metastrongylus* spp (54% (20/37), 11% (34/306)) and *Physocephalus sexalatus* (24% (9/37), 3% (9/306)). The gastrointestinal protozoan parasites in both the slaughtered and farm sampled pigs respectively included: coccidia spp (85% (32/37), 33% (101/306)), *Balantidium coli* (89% (33/37), 64% (196/306)), *Tritrichomonas suis* (89% (33/37), 42% (129/306)) and *Entamoeba* spp (100% (37/37), 87% (266/306)). The ectoparasites observed on slaughtered and farm pigs respectively were *Sarcoptes scabiei* (63% (21/33), 64 (195/306)), *Haematopinus suis* (85% (28/33), 96% (294/306)), *Ixodid* ticks (40% (13/33), 30% (92/306)). The prevalence of cysticercosis was 4% (11/284).

There was a positive correlation ($p<0.05$) between the amount of rainfall in a given Division of sampling and prevalence of all the nematodes except *S. ransomi*. The prevalence of nematodes was associated with age, being highest in adults (*Oesophagostomum* spp), growers and finishers (*A. suum, T. suis*) and piglets (*S. ransomi* and *P. sexalatus, Metastrongylus* spp). There was negative correlation between the amount of rainfall in the Division of pig origin and prevalence of coccidia, *Tritrichomonas suis*, and *Entamoeba* spp, but a positive correlation with prevalence of *B. coli*. The prevalence of *H. suis* was significantly ($p<0.05$) associated with amount of rainfall (negative correlation), class of pigs (highest in finishers) and lack of provision of housing. The prevalence of mange was negatively associated with amount of rainfall

(negative correlation) and class (highest in sows) of pigs. Lack of latrines was the only significant ($p<0.05$) factor associated with the prevalence of porcine cysticercosis.

The efficacy trials evaluated the effectiveness of pawpaw and neem products on *Oesophagostomum* spp and *H. suis* infections in pigs using *in vitro* and *in vivo* tests. Papain and papaya latex were the most effective herbal products against *Oesophagostomum* spp. In the *in vivo* tests, levamisole, pawpaw latex, neem, papain, pawpaw powder caused faecal egg count reductions of 84.6%, 57.1%, 56%, 43.2%, 27.1%, respectively. At *in vitro* level, neem oil caused 100% mortalities in concentrations above 12.5% while amitraz at concentration of 5mg/ml was able to kill 100% lice in 24 hours. At *in vivo* level, lice counts on pigs treated with neem oil and amitraz declined significantly ($p<0.0001$) up to 28 days post treatment.

In conclusion, the serious production constraints reported in this study can be addressed through education of farmers/traders using affordable extension services and improvement of the pig markets. The parasites reported in pigs for the first time in Kenya included: *Metastrongylus* spp, *G. urosulubatus*, *B. coli*, *Tt. suis*, *Entamoeba* spp, ixodid ticks and *T. penetrans*. The results of this study demonstrate that pawpaw and neem oil products have significant efficacy against *Oesophagostomum* spp and *H. suis*. Further studies on the pawpaw and neem oil products including elucidation of the possible toxic effects on the animals, dosage formulation and effectiveness of the products in integrated control programmes for pig parasites are recommended.

CHAPTER ONE: GENERAL INTRODUCTION

1.1 Introduction and objectives

Livestock are kept by majority of Kenyan communities and the livestock industry contributes 10% of the National Gross Domestic Product (GDP) and employs 50% of the agricultural work force (GoK, 2002a). However, only 25% of the land in Kenya is suitable for arable farming and the current demand for livestock products is high (GoK, 2002a; FAO, 2005). With the declining grazing land in Kenya due to high human population growth, farmers have been encouraged to keep livestock which require minimal space such as pigs. In recent years, the population of pigs in Kenya has grown steadily, rising from 77,000 in 1980 to 415,200 in 2005 (FAO, 2005). Majority of pigs in the country are hybrids of Large White and Land Race breeds and are mainly kept by small-scale farmers in high potential areas of the country (Kagira, 2001; Wabacha, 2001). Crossbred pigs are also found in Western Kenya and in slums in urban areas.

In the past, studies on livestock health and productivity in Kenya have focused mainly on ruminants, with very few studies focussing on pigs. This is in spite of the fact that sustainable interventions in pig farming will require a thorough understanding of existing management and husbandry practices as well as constraints associated with production. The studies in pigs have mainly been undertaken in the high agricultural potential areas in Central Province of Kenya (Kagira, 2001; Wabacha, 2001; Ng'ang'a *et al.*, 2007). However, very little is known about the socio-economic characteristics of the pig farmers and pig trade in Western Kenya.

Previous studies on parasites of pigs kept in free range conditions has mainly focussed on cysticercosis (Githigia *et al.*, 2005; Mutua *et al.*, 2007) with minimal attention to other parasites. The gastrointestinal nematodes of economic importance in pigs kept in confinement in the country include *Ascaris suum*, *Oesophagostomum* spp., *Trichuris suis* and *Strongyloides ransomi* (Kagira, 2001; Wabacha, 2001). It would be expected that a more diverse spectrum of nematodes occurs in pigs kept under extensive production system due to increased transmission of parasites under this system (Roepstorff and Nansen, 1994). The helminth parasites can cause losses due to sub-clinical helminthosis, acute illness and death, premature slaughter and rejection of some parts of the carcass at meat inspection (Stewart and Hale, 1988).

Sarcoptes scabiei var. suis and *Haematopinus suis* are the main ectoparasites of pigs and are of high economic importance in the pig industry and can reduce the production efficiency in pigs (Davies, 1995). Indeed, sarcoptic mange is a leading production constraint in pigs kept intensively in Central Province, Kenya (Wabacha, 2001). On the other hand, *Haematopinus suis* (hog louse) causes pruritus and have moderate effect on productivity of pigs (Damriyasa *et al.*, 2004). In spite of known epidemiology for mange and louse infestations in the intensive system of farming, little is known about the occurrence of the two conditions in pigs kept in traditional extensive production system in Kenya.

The existence of pigs under free-range conditions exposes them to a variety of vector-borne diseases including trypanosomosis, eperythrozoonosis and babesiosis (Permin *et* *al.*, 1999). Trypanosomosis in pigs is caused by trypanosomes, which are mainly transmitted by the *Glossina* spp (tsetse flies). The numbers of reported cases of pig trypanosomosis in Kenya are few, possibly due to minimal surveillance (Angus, 1996). In recent years, the occurrence of *Trypanosoma brucei* in pigs in Busia District, Kenya has raised concern that the pigs could be acting as reservoir hosts for the human infective trypanosomes (Ng'ayo *et al.*, 2005).

Apart from affecting the productivity of pigs, a number of pig parasites are of zoonotic importance and these include *Taenia solium*, *Ascaris suum*, *Cryptosporidium, Giardia* and *Trichinella spiralis* (Olson and Guselle, 2000). The disease burden and risk factors associated with transmission of these parasites have not been well documented in Kenya owing to lack of awareness and scarce resources to conduct epidemiological surveys.

Intensive housing systems, broad-spectrum anthelmintics and acaricides are used widely in the pig industry in Kenya and provide an opportunity for reducing the prevalence and intensity of parasites in pigs (Kagira, 2001). However, pigs kept in free-range systems require different control strategies owing to the high risk of exposure, diversity and seasonal incidence of the parasites (Roepstorff and Nansen, 1994). There is no recorded information on the current parasite control strategies in free-range pigs in Kenya. With the emergence of drug resistance and rising costs of anthelmintics (Kagira *et al.*, 2003), the use of ethnoveterinary products for control of parasites has gained importance (Githiori, 2004). Although the use of ethnoveterinary products is common in Western Kenya (Okitoi *et al.*, 2007b), the use of these alternative remedies in control of pig parasites has not been scientifically evaluated.

1.2 Objectives

1.2.1 Overall objective

The overall objective of the study was to characterize the pig production systems and determine the epidemiology of internal and external parasites in pigs kept under extensive production system in Busia District, Kenya and the efficacy of existing dewormers and alternative remedies with a view of recommending suitable intervention strategies.

1.2.2 Specific objectives

1. To describe the farm-level characteristics, pig production constraints and pig trade in the study area
2. To determine the prevalence and intensity of ecto-parasites, endo-parasites and hemo-parasites in pigs at farm and slaughter-slab levels in the study area
3. To determine the effect of host, management, and environmental factors on occurrence of the parasites in pigs in the study area
4. To determine the occurrence of risk factors for transmission of zoonotic parasites, with emphasis on *T. solium* in the study area
5. To determine the efficacy of pawpaw products and neem oil against *Oesophagostomum* spp and *H. suis* infections in pigs

1.3 Justification of the study

With the rapid increase in the human population leading to higher demand for food, it is becoming increasingly important to maximise agricultural production through improved management practices and control of diseases that limit production. Pig farming, with annual growth rate of 9.3% and contributing 79% of total meat exported (FAO, 2005), has emerged as an important livestock enterprise in Kenya as it requires less acreage and has high potential for high economic gain. In western Kenya, farmers keep the pigs in free-range systems, which expose the animals to a wide variety of productivity-limiting parasites. Such pigs may also become a public health risk due to the emergence of zoonoses. With the emergence of drug resistance (Conder and Campbell, 1995; Kagira, 2001) and rising costs of anthelmintics, the use of ethnoveterinary products for control of pig parasites has gained importance. Information on the characteristics of the pig production system and production constraints, prevalence and intensity of the parasites and effectiveness of drugs and ethnoveterinary products used is essential in formulating surveillance and control programme for the parasites. This study aimed at providing this information for pigs kept under the extensive system of production in Busia District.

CHAPTER TWO: LITERATURE REVIEW

2.1 Pig production in the world

World pig population is estimated to be 923 million, of which 552 million are found in Asia, 72 million in North America, 194 million in Europe, 81 million in Latin America, and 18 million in Africa (FAO, 2002). Pork contributes 44% of the meat protein consumed in the world, making it the most popular meat (FAO, 2002). In Europe and North America, large scale commercial pig farming is predominant and is characterized by keeping of improved breeds and under intensive conditions. Commercial small-scale and large scale production systems are also found in Asia and Africa, but most are on the decline due to the high cost involved (Lekule and Kyvsgaard, 2003). The intensive production constitutes 20% of pigs in developing countries and is economically viable in countries with shortage of land to grow feeds and in large cities because of availability of industrial by-products (Lekule and Kyvsgaard, 2003).

Free range and tethering systems are common in most developing countries and contributes 80% of the pigs kept in East Africa, 75% in Zimbabwe, 70% in Botswana and 65% in West Africa (Lekule and Kyvsgaard, 2003; Nsoso *et al.*, 2006). Although most of the pigs in developing countries are crosses of exotic breeds, indigenous breeds are also common. Economic studies have shown that the traditional production system in Africa is wasteful and unprofitable due to poor feed conversion, high mortality rates, low reproductive rates and poor final products. However, experiences from Africa show that intensive pig farming is stagnant with farmers favouring the free range system (Lekule and Kyvsgaard, 2003; Nsoso *et al.*, 2006). This is mainly due to low fixed cost in the traditional pig production where the pigs are rarely provided with housing, commercial feeds and parasite control is minimal (Lekule and Kyvsgaard, 2003).

2.2 Pig production and its constraints in Kenya

The exotic pigs introduced in Kenya by the white settlers in 1920s were mainly the Landrace, Large White and Hampshire breeds. The Upland Bacon Factory established in 1946 influenced the performance of the pig industry during the colonial and post independence era and the closure of the factory in 1985 due to lack of markets led to a decline in pig production in the country. However, the entry of Farmers Choice Company Kenya (FCC) Limited, in processing of pork and pork products in addition to other local butcheries has boosted pig production by small holder farmers especially in Central and Nairobi Provinces (Wahome *et al.*, 1992). FCC enhances the production of pigs through provision of breeding stock to farmers, delivery of extension services and facilitates delivery of pig to the processing plants. Thus in recent years, the population of pigs in Kenya has grown steadily, rising from 77,000 in 1980 to 415, 200 pigs in 2005 (GoK, 2002a; FAO, 2005). The annual growth rate increased from 5.6% in 1980 to 9.3% in 1990s. Further, it has been shown that pig meat in Kenya accounted for 79% of total meat exported in 2002; earning the country a total of 1.1 million USD (FAO, 2005).

Apart from the exotic breeds of pigs which are reared in Central, Nairobi and Rift Valley provinces, a substantial number of crossbred and suspected indigenous pigs are found in the Nyanza and Western Province of the country. These pigs are kept in free-range conditions where they graze and scavenge for feed (Githigia *et al.,* 2002; Mutua *et al.*, 2007). The constraints in the free ranging pig production system have however not been characterized. On the contrary, a number of studies on pig health and productivity have been conducted in the high potential areas and have indicated the main constraints to pig production as diseases, lack of quality feeds, lack of breeding animals, poor extension services and poor marketing (Gichohi *et al.,* 1988; Munyua *et al.,* 1991; Wabacha, 2001). Due to the climatic and management factors, high parasite burdens are common in most pig farms in Kenya (Wabacha, 2001; Kagira *et al*., 2003). The farmers in most cases have limited knowledge as regards management and control of parasite infections (Kagira *et al*., 2003).

2.3. Helminths of domestic pigs

Helminths that are of economic importance in pigs kept intensively in Kenya include *Ascaris suum, Oesophagostomum* spp., *Trichuris suis* and *Strongyloides ransomi* (Langat, 1999; Kagira, 2001; Wabacha, 2001). Other helminths which have been found in free range pigs elsewhere include *Hyostrongylus rubidus*, *Metastrongylus* spp, *Stephanurus dentatus*, *Ascarops strongylina*, *Globocephalus* spp., *Physiocephalus sexalatus* and *Trichnella* spp (Murrell, 1986).

2.3.1 Major nematodes: life cycles and pathogenesis

Oesophagostomum (nodular worm) is the most prevalent nematode in pigs kept under intensive system in Kenya (Kagira *et al*., 2001) and occurs as two species (*O. dentatum* and *O. quadrispinulatum*) which have a direct life cycle. Both the eggs and free-living larvae are sensitive to desiccation, but the third stage larvae (L_3) are resistant and may survive in humid environment for approximately one year, thus exposing outdoor kept pigs to continuous infection. Pigs experimentally infected with *O. dentatum* require more feed per unit weight gain than the controls (Roepstorff and Nansen, 1994), implying that the parasite significantly affects the productivity of pigs.

Globally, *A. suum* is one of the most prevalent nematode parasites of pigs occurring in both the extensive and intensive production systems (Roepstorff and Nansen, 1994). The few studies done in Kenya have shown that *A. suum* is the second most prevalent nematode in pigs kept indoors (Kagira, 2001). *Ascaris suum* eggs have a thick and resistant shell, which protects them against adverse conditions such as desiccation and allows them to remain viable for up to 10 years in the environment. Pigs raised in extensive conditions have been shown to have high prevalence of *A. suum* (Ajayi *et al.*, 1988) possibly because of continuous exposure. *Ascaris suum* is associated with decreased daily weight gain and condemnation of infected livers during meat inspection (Hale *et al.*, 1985).

Trichuris suis has also been reported in indoor pigs in Kenya (Kagira, 2001). The eggs with the infective larvae are very resistant to environmental conditions and may remain infective for years (Soulsby, 1982). Pigs reared outdoors have been shown to have high prevalence of *T. suis* due to continuous infection (Carstensen *et al.*, 2002) and heavy infections cause inflammation of the bowel wall leading to unthriftness, weakness, anaemia and emaciation.

Strongyloides ransomi (threadworm of swine) has a unique direct life cycle that includes free-living generations of adult females and males, plus parasitic parthenogenetic females in the small intestines. Pigs may be infected orally or percutaneously by larval stage 3 (L_3) and in addition, neonatal infection may take place by colostral transmission of larvae. Infected pigs have anorexia, reduced growth rate, vomiting and intestinal haemorrhages (Roepstorff and Nansen, 1994).

Three species of *Metastrongylus* (*M. elongatus*, *M. pudendotectus* and *M. salmi*), lungworms often co-exist in pigs and have an indirect life cycle with earthworms as true intermediate hosts. The thick shelled, resistant eggs embryonate in the environment, but do not hatch until they are ingested by an earthworm in which further development takes place. Pigs become infected by ingesting earthworms containing the L_3. In the pig, the larvae have systemic migration and cause serious lung pathology. Clinically, metastrongylosis is characterised by coughing, difficult breathing, loss of appetite and retarded growth rate (Soulsby, 1982).

2.3.2 Diagnosis and control of pig nematodes

The gastrointestinal nematodes are mainly diagnosed by coproscopy, using morphological features of the eggs as described by various authors (e.g. Soulsby, 1982; MAFF, 1986). Pathological lesions caused by various worms and the morphology of the adult parasites can be used in diagnosis at post-mortem. Nematode infections in swine are largely sub clinical and therefore farmers are not motivated to change the hygiene practices radically in order to control the infections. Instead, routine anthelmintic treatments appeal to farmers for reasons of convenience. The effect of each treatment may be transitory when pigs are re-infected continuously (Roepstorff and Nansen, 1994). A number of narrow and broad-spectrum anthelmintics are available for treatment of pigs. The most commonly used anthelmintics are piperazine, levamisoles, benzimidazoles and ivermectin (Conder and Campbell, 1995; Kagira, 2001).

There are two different principles of treatment, namely; treatment of pigs at specific times i.e., strategic treatment adjusted to age and reproduction cycle, or treatment of all pigs after specific period. In strategic treatment, sows are treated 1-2 weeks before farrowing; this is often followed by a move to clean farrowing units. To reduce the latter transmission of infection, piglets may be treated at weaning and once or twice during the fattening period. The second method requires that all pigs in the herds be treated at specific intervals based not only on parasite population but also for labour saving and technical reasons. The latter is the most common method amongst pig farmers in central Kenya where farmers treat animals after every 2-3 months (Kagira, 2001, Wabacha, 2001).

2.3.3 *Taenia solium*

Taenia solium, the pork tapeworm, is emerging as a significant problem affecting the smallholder pig production and posing a serious public health risk to human population in most countries in Africa, South America and Asia. Approximately 50 million people worldwide carry adult *T. solium*, 20 million are infected with cysticerci and 50,000 deaths occur every year due to neurocycticercosis (Borneo and Garcia, 2001). Two recent preliminary studies in Western Kenya, showed that 6-14% of the pig examined by ante-mortem lingual examination were infected with *T. solium* cysts (Githigia *et al.*, 2002; Mutua *et al.*, 2007) while Busia Hospitals records indicate a taeniosis prevalence of between 4 and 10% in human patients in a division where free-range pigs are kept (GoK, 2001)

The *T. solium* taeniosis/cycticercosis complex is associated with poor sanitation and hygiene, poor pig husbandry, lack of proper meat inspection and disease control measures. In developing countries, pigs can be considered to function as natural sources of sanitation, as they clean the villages of faecal material and garbage (Copado *et al.*, 2004). The life cycle of *Taenia solium* includes the pig as the normal intermediate host, harbouring the larval cysticerci. Infections in pigs arise from ingestion of eggs through scavenging of feeds contaminated with eggs of *T. solium*. Humans act as both the definitive and intermediate hosts. The development of human neurocysticercosis, arising from lodging of cysts in the brain and spinal cord causes a variety of neurological symptoms including headache, epileptic seizures, blindness, and death (Nash, 2003). The impact of the human disease is enormous and arises from hospitalisation costs and social stigmatisation (Nash, 2003).

The method most commonly employed in the diagnoses of pig cysticercosis is the post-mortem inspection of the carcasses in abattoirs. In developing countries like Kenya, the method can be hampered by lack of meat inspectors, home slaughter and presence of informal slaughterhouses in some communities (Langat, 1999). Under optimal conditions, the method has a sensitivity of 10.6% (Boa *et al.*, 1995). Lingual examination method has been used before in Kenya (Githigia *et al.*, 2005, Mutua *et al.*, 2007) but its limitations include: low sensitivity (21%), requirement of expertise and only heavily infected pigs can be detected (Sciutto *et al.*, 1998). Immunodiagnostic tools for diagnosis of pig and human cysticercosis, which have high (>80%) sensitivity and specificity have been developed in recent past (Dorny *et al.*, 2004) and have contributed to better understanding of the epidemiology and impact of the disease in endemic areas. A commonly used immunodiagnostic tests is the antigen ELISA which has a sensitivity and specificity of 85% and 97%, respectively (Nguekam *et al.*, 2003). In humans, control of the disease can be achieved through treatment of tapeworm carriers and health education, and this has been tried in Latin America and South Africa (Mafojane *et al.*, 2003, Borneo and Garcia, 2001). In pigs, the methods, which have been successfully used, include restraint and housing of pigs, cestocidal treatment, and vaccination (Mafojane *et al.*, 2003).

2.4 Protozoan infections in pigs

2.4.1 Trypanosomes

Trypanosomosis in pigs is caused by *Trypanosoma simiae*, *T. congolense* and *T. brucei* (Stephen, 1986). These trypanosomes are mainly transmitted by the tsetse flies (*Glossina* spp). Compared to *T. brucei* and *T. congolense, T. simiae* is very virulent and infections occur in outbreaks and cause a rapid fatal disease (Stephen, 1986). Unlike in the ruminants, most *T. congolense* and *T. brucei* infections cause very little pathogenic effect in pigs. However, some strains may cause a disease with noticeable pathogenic effects (Stephen, 1986). The occurrence of concurrent helminthosis in pigs is thought to increase the pathogenicity of the trypanosomes (Stephen, 1986). However, such interactions have not been investigated to date.

Compared to other animals, the numbers of reported cases of trypanosomosis in pigs in Kenya are few. Although this may indicate that pigs are less affected by trypanosomes, a closer look at the records indicates that most of trypanosomosis surveillance studies have focused on cattle and small ruminants with little attention being given to pigs. In limited studies undertaken by Angus (1996) and Ngayo *et al.*, (2005) the prevalence of trypanosomosis in pigs in Busia District ranged from 2-5%. One pig was observed to be infected with human infective *T.b. rhodesiense* (Ng'ayo *et al.*, 2005). To estimate the sleeping sickness risk in this focus there is a need to elucidate the epidemiological significance of the pig reservoir.

2.4.2 Gastrointestinal protozoa

The main gastrointestinal protozoan parasites of pigs include coccidia, *Giardia* spp and *Balantidium coli.* Coccidiosis is an important cause of gastrointestinal disturbances in piglets and is a differential diagnosis for gastrointestinal nematodosis especially *S. ransomi* infections (Kagira, 2001). *Isospora suis* predominates in young piglets and is highly immunogenic while *Eimeria* spp. being the most common in older pigs particularly those having access to outdoor runs (Roepstorff *et al.*, 1998). Although climatic conditions of areas where pig are reared in Kenya favour the sporulation and survival of coccidial oocysts throughout the year, information on the prevalence of coccidia in pigs is scanty.

Giardiasis is caused by protozoan parasite *Giardia duodenalis,* which is common in domestic animals and human. The prevalence of giardiasis in humans is 2-7% in Europe and North America, but can be as high as 40% in developing countries (Olson and Giselle, 2000). *Giardia* spp is predominantly transmitted through fecal-oral routes usually between humans but waterborne and food-borne zoonotic transmission has been reported (Olson and Guselle, 2000). Zoonotic transmission is associated with poor animal husbandry, where animal faeces contaminate water and food for human consumption. *Giardia* spp prevalence of up to 70% has been reported in pig farms in parts of the world (Roepstorff *et al.*, 1998). However, little is known about the Kenyan situation, which the current study investigated.

Balantidium coli is the largest protozoan and the only ciliate parasite of humans. Although only a few epidemiological studies have been conducted worldwide, high prevalences of up to 29% have been reported (Barnish and Ashford, 1989). Among the domestic animals, pigs have a particularly high rate of infection (20-100%) and are thus regarded as the main reservoir of this parasite (Solaymani-Mohammad and Petri Jr, 2006). The parasite is a commensal organism in the pigs and is common in pigs kept in poor hygienic conditions. However, in humans living in close proximity with pigs, chronic and acute infections can occur sporadically and in epidemics (Solaymani-Mohammad and Petri Jr, 2006).

2.5 Sarcoptic mange and hog lice

Sarcoptes scabiei var. suis, the causative agent of sarcoptic mange, is the most important ectoparasite of pigs throughout the world (Soulsby, 1982; Davies, 1995). The infestation is spread chiefly by direct contact between hosts. Animals in poor condition appear to be most susceptible although conditions especially overcrowding, in which mange occurs often go hand in hand with poor feeding and management. Many infestations have little or no effect on weight gain and feed efficiency (Davies, 1995), but in some, loss of condition, production and vitality may be severe, and the appearance of affected animals may be aesthetically displeasing. In addition, the economic conditions associated with the infestation results from the costs of acaricides used to control mange (Davies, 1995). The diagnosis of the disease is mainly based on the presence of clinical signs and observation of mites in the skin scrapings and earwax. Mange is a leading production constraint in pigs kept intensively in Kenya and control is mainly through use of sprays such as amitraz (Wabacha, 2001). The use of ivermectin to control both the gastrointestinal nematodes and mange has also gained importance in the country although the prohibitive cost limits its use to high potential areas and farmers affluence (Wabacha, 2001; Kagira, 2001). Farmers keeping pigs indoors are also known to opt for other non-conventional products for control of mange (Wabacha, 2001). The use of such products has not been documented in farms keeping outdoor pigs.

Hog louse (*Haematopinus suis*) is a common ecto-parasite of pigs all over the world (Davis and Williams, 1986). The parasite is host specific and affects pigs of any age or condition. However, pigs kept outdoors and those in poor body condition are more susceptible (Nsoso *et al*., 2006). The blood sucking activity of the hog louse results in irritation and discomfort of the pigs. Such pigs have been shown to have extensive hair loss and wounds, have reduced performance and are more susceptible to other diseases (Davis and Williams, 1986; Nsoso *et al*., 2006). The hog louse has also been implicated in the transmission of diseases such as swine pox, eperythrozoonosis and African Swine Fever (Permin *et al*., 1999). In Africa, only a few studies have reported the occurrence of *H. suis*. In Ghana and Botswana, the prevalence of *H. suis* in free-range pigs was reported as 38% and 100%, respectively (Permin *et al*., 1999; Nsoso *et al*., 2006). However, no study on either the prevalence or control of *H. suis* has been undertaken in Kenya.

2.6 Use of plant extracts in control of parasites

The use of drugs has several limitations including the emergence of resistance, consumer concerns and high costs (Conder and Campbell, 1995). Consequently, livestock farmers are encouraged to adopt alternative or novel parasite control methods, plant-based anthelmintics being chief amongst them (Githiori, 2004). These methods can be used to cure infections or manage parasite infections (i.e., assist in maintaining infections below the economic threshold) within a livestock production system. The use of ethno-veterinary preparations as anthelmintics has been documented in different parts of the world, including Kenya (Githiori, 2004). However, all the studies undertaken to date in Kenya have targeted helminths found in ruminants and laboratory animals, with none addressing the parasites of pigs. This is in spite of the fact that pig farmers are known to use several preparations in control of the parasites, which have not been scientifically evaluated. Some of the plants grown or present in Busia District, and with known anthelmintics properties include pawpaw and neem tree (Okitoi *et al.*, 2007b). The pawpaw latex has been shown to be effective against some nematodes of laboratory animals and *A. suum* in pigs (Satrija *et al.*, 1994; 1995). In India, a study by Satrija *et al.*, (1995) showed that papaya latex given at a dosage of 4 and 8 mg/Kg was able to reduce faecal egg counts by 99% and the number of adult worms by 80 and 100%, respectively. This shows that paw paw products should be further investigated as possible anthelmintics for control of pig nematodes.

The use of neem extracts has gained importance in research towards the control of external and internal parasites of livestock. In Kenya, the plant has been promoted by different institutions and non-government organizations as a drug for many diseases and thus most households have planted the herbal plant (Okitoi *et al.*, 2007b). Neem, the popular name for *Azadirachta indica*, belongs to the Meliaceae family and is reported to have several medicinal properties, including use as an anti-inflammatory, antipyretic, analgesic, immuno-stimulant, hypoglycemic, anti-fungal and antibacterial (Mitchell *et al.*, 1997). Neem extracts also demonstrate activity against insects and parasites of both plants and animals. The activity of *A. indica* on animal parasites was observed in *Bovicola ovis* (Heath *et al.*, 1995), *Hyalomma anatolicum excavatum* (Abdel-Shafy and Zayed, 2002) and biting louse *Damalinia limbata* (Habluetzel *et al.*, 2007). In the study by Habluetzel *et al.*, (2007), an application of Neem Azal®, reduced the louse burden in goats by up to 96% with the activity lasting for a period of 18 weeks. With the observed excellent activity of neem on *D. limbata*, it would be important to examine whether this product has similar efficacy on other lice including the hog louse.

Studies on the efficacy of various plant products have been tried at both the *in vitro* and *in vivo* level. The *in vitro* tests are more versatile than *in vivo* ones because they require less amount of drug, time, are simple and economical. For example, worms from few pigs are sufficient to test many drugs and concentrations and no previous toxicity data are required. However, the interpretation of the *in vitro* results is always hampered by the fact that host conditions in the gut might change the efficacy of the drug against a target parasite.

CHAPTER THREE: CHARACTERISTICS OF PIG PRODUCTION SYSTEM AND TRADE IN BUSIA DISTRICT, KENYA

3.1 INTRODUCTION

Pigs in Kenya are produced under a variety of production systems ranging from intensive commercial pig farms in Central Kenya to free range traditional system in Western Kenya. The smallholder intensive system in Central Province has been well characterized by Gichohi *et al.*, (1988), Munyua *et al.*, (1991), Kagira, (2001) and Wabacha, (2001). Majority of pigs in the country are either produced by these farmers or by large commercial piggeries owned by pig processing companies in Kenya. Improved breeds bred under confinement characterize the commercial pig production system in these areas.

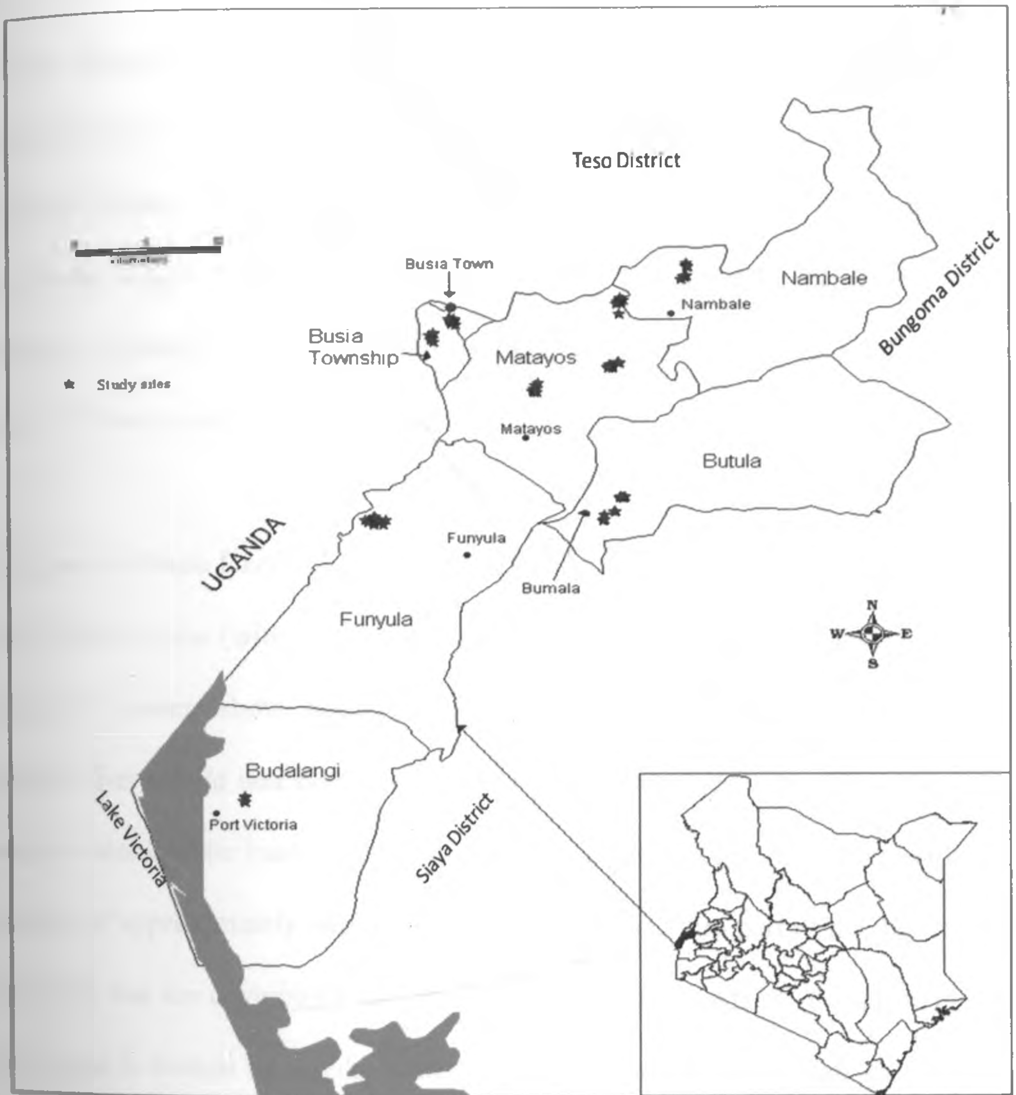
The free-range traditional system of rearing pigs in Western Kenya has however, not been well characterized. In other developing countries, this system is characterized by high mortality rate; low off take, low reproductive rates, absence or minimal health care, lack of supplementary feeding and lack of proper housing (Nsoso *et al.*, 2000, Hide, 2003, Deca *et al.*, 2007). In spite of this, studies in Africa have shown that given a choice, most farmers opt for the traditional pig farming rather than intensive farming due to the high input costs associated with the latter (Verhulst, 1976). In Busia District, where traditional village pig production is common, the level of human development index is low and is characterized by amongst others high prevalence of poverty and HIV (GoK, 2002b; UNDP, 2008). Most families in the district are subsistence resource poor farmers who have adopted small-scale pig farming to improve their living standards (GoK, 2002b). Sustainable interventions under this type of farming will require a thorough understanding of pig management and husbandry practices as well as constraints associated with production. The only markets for these pigs are the local butchers who are located either at village level or in the regional urban centers. In spite of its importance in the local economy, pig trade in developing countries has not been well characterized. In an effort to understand and improve pig production in this area, the current study was formulated to characterize the socio-economics of pig production system and the main pig markets in Busia District.

3.2 MATERIALS AND METHODS

3.2.1 Study area

3.2.1.1 Physical geography, climate and vegetation cover

The study was conducted in Busia District, which is situated in the Western Province of Kenya. The district is located approximately 500 Km from Nairobi and lies between latitudes 0°136' and 0° North of the equator and longitude 33°54' and 34°25' East of Greenwich meridian. The district covers an area of 1261.3 Km^2. It is made up of six administrative divisions, which are Budalangi, Funyula, Matayos, Township, Nambale and Butula. Recently, the district has been divided into three districts, including Bunyala (formerly Budalangi Division), Samia (formerly Funyula), and Busia (combining Matayos, Township, Nambale and Butula). However, for the purposes of this study, all the three districts were regarded as Busia. The district is bordered by the Butere-Mumias District to the East, Bungoma to the North East, Teso District to the North, Siaya to the South East, Bondo District to the South and Lake Victoria and the Republic of Uganda to the West (Fig 3.1).



Key*= Location of study sites/farms, Source: KARI-TRC GIS Lab
Fig 3.1. Map of Kenya showing Busia District and key study sites*

Most parts of Busia District receive 1270-1790 mm mean annual rainfall which generally decreases from North to South. The driest part of the district is found along the shores of Lake Victoria (covering Budalangi Division) and receives 760-1015 mm rainfall annually. About 50% of the annual rainfall is received during the long rains, which occur between March and May while 25% of the rain is during the short rains between August and October. The dry period is between December and February, but can vary from year to year. Appendix I shows a comparison of the average rainfall amount from three main meteorological stations (Port Victoria in Budalangi, Nangina in Funyula and Busia Farmers Training Centre in Township Division) in Busia District. The air temperature in the district ranges between 14-30°C. Due to the proximity to the Lake Victoria and drainage systems, humidity is relatively high, with evaporation rates of between 1800 mm to 2000 mm per year (GoK, 2002b).

Most parts of Busia District lie within the Lake Victoria basin. The district lies within the Low Midland zone (with 4 agro-ecological zones) and has an altitude ranging between 1,130-1,375 meters above sea level. The vegetation cover in most parts of the district is *Tithonia diversofolia* and *Lantana camara*, which are found in the fallow land, between hedges or along water banks. The central part of the district is a peneplain marked by low flat areas of approximately uniform height while the southern part of the district has some huge areas that are covered by the Yala swamp which is lowland. The latter has papyrus growth and is broken by regular water channels and occasional small lakes with grassy islands. The Samia and Funyula hills are also found in southern parts of Funyula and Budalangi divisions. The river drainage system runs into River Malaba which originates from Mt Elgon and forms the border with Uganda, north of Busia Town. In Nambale Division and south in Funyula Division, several rivers drain into river Sio, which also forms part of the Uganda border but south of Busia Town. All river courses in southern part of the district form part of the Lake Victoria basin drainage system (GoK, 2002b).

3.2.1.2 Crop farming

The upper parts of the district are high agro-ecological potential zones while southern regions near Lake Victoria are graded medium potential (GoK, 2002b; FITCA, 2001). The main staple foods grown in Busia District are: maize, cassava, millet, sorghum, beans, groundnuts, sweet potatoes, cowpeas and bananas. Crop farming is at subsistence level while sugarcane is the only cash crop, mainly grown in Nambale, Matayos and Butula divisions.

3.2.1.3 Human and animal populations

The total human population of Busia District is 405,388, and growing at a rate of 2.9% per annum (GoK, 2002b). The predominant tribe is Luhya, although other tribes including Teso and Luo can be found in areas bordering their respective ancestral lands. The district has high prevalence of poverty (66%) and Human Immunodeficiency Virus/Acquired Immunity Deficiency Syndrome (HIV/AIDS) (33%) (GoK, 2002b). The different types of domestic animals kept in the district are cattle, sheep, goats, donkeys, pigs, rabbits, with cats and dogs as companion animals. Poultry production is also an important engagement for the farmers. Fishing is a major income earner especially for Funyula and Budalangi Divisions, where fishing is undertaken in Lake Victoria and major rivers. Recent estimates indicate that there are 74,818 cattle, 28,194 sheep, 50,141 goats, 21,280 pigs, 2,118 donkeys, and 16,814 rabbits (FITCA-K, 2001). There has been no livestock census done since 1970s and the current figures are estimates by NGOs and the District Veterinary Office. The domestic animals are mainly of indigenous breeds reared under free-grazing conditions.

3.2.2. Cross-sectional farm questionnaire survey

3.2.2.1 Selection of study farms

The study was a cross sectional survey which carried out in the six Divisions (Township, Nambale, Matayos, Butula, Funyula, Budalang'i) of Busia District and was conducted between October and December 2006. The design of the study was undertaken as described in Fig 3.2. The study farms selection was carried out with the assistance of field extension officers of the Ministry of Livestock Development (MLD) and administrative officers in charge of the sub-locations (*liguluu*). The sampling unit of interest was individual smallholder pig farms and all the pig farms in the area were regarded as being smallholder with herd sizes of between 1 and 10 pigs. From each Division, five villages were selected on the basis of having high number of pigs. Two villages per division were then randomly selected (using random number table) from the sampling frame of the selected villages. At village level, household with pigs were established using the snowballing method and sampling to redundancy method which have been used previously in studies on cysticercosis (Sikasunge *et al.*, 2006). Snowballing is a technique for developing a research sample where existing study subjects recruit other subjects. The village chairman (*Liguluu*) identified the first few pig farmers, who helped in identifying the others until all the farmers in the village were covered. All the farms were geo-referenced using the geographical positioning system (GPS) machine (Garmin®, GPS 12 x L model, USA). The current study was undertaken as a prelude to the one on parasite prevalence (Chapter 4) and thus the latter acted as a guide for estimating the sample size. In the prevalence study (Chapter 4), a sample size of 185 pigs was estimated using formula stated by Martin *et al.*, (1987). Assuming each farm could have at least two pigs (Githigia *et al.*, 2005; Mutua *et al.*, 2007); a sample size of at least 93 farms (8 farmers per study site) was required. A total of 182 farmers were sampled.

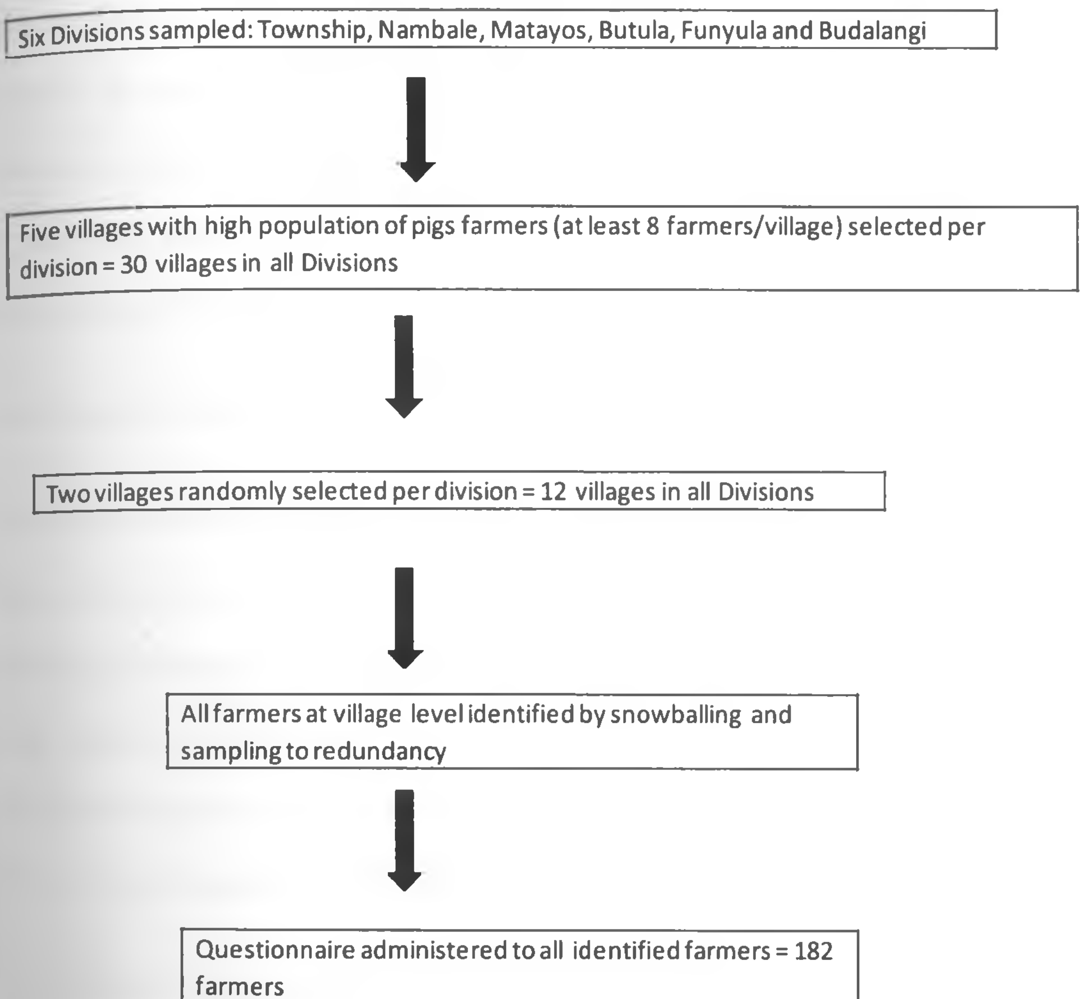


Fig 3.2. Sampling strategy used in the farm survey in Busia District

3.2.2.2 Questionnaire farm survey

The farm characteristics and constraints to pig production were obtained through face-to-face personal interviews using the structured questionnaire outlined in the Appendix II. In general, the aspects covered in the farm survey questionnaires included, level of education, occupation, pig keeping period, farm management practices, source and type of feeds and feeding practices, housing system, disease control practices, marketing, production and disease constraints (Appendix II).

3.2.3 Slaughter slab study

3.2.3.1 Retrospective analysis of veterinary data

The records (between year 2002 and 2005) at the District Veterinary Headquarters were analyzed to determine the number of livestock slaughtered, the personnel involved in meat inspection, and money generated from veterinary services. Key informant discussions were held with the District Veterinary Officer (DVO) regarding pig farming, trade and meat inspection in the district.

3.2.3.2 Questionnaire survey on pig trade

All the pork butcheries (16) in Funyula, Nambale, Matayos and Butula urban (shopping) centres were included in the study. These areas were chosen after discussion with the DVO, who indicated that they are the main markets for the local pigs. A structured questionnaire was administered to the butchers. In general, the aspects covered in the questionnaire included: age, sex and level of education of the butcher, other activities undertaken by the butcher, period of business operation, type of butchery structure, source of pig and transport mode to the butchery, consideration when buying pigs, constraints and types of costs incurred by the butchery business.

3.2.4 Data management and analysis

Data was entered into Ms Excel program and descriptive statistics analyzed using the Statview® for Windows Version 5.0.1 (SAS Institute Inc, 1995–1998, Cary, NC).

3.3 RESULTS

3.3.1 Farm survey

3.3.1.1 Location of the study farms

The questionnaire data was collected from a total of 182 farmers distributed across twelve villages in the six divisions as shown in Table 3.1.

Table 3.1. Distribution of 182 farmers interviewed in the questionnaire study across the six divisions of Busia District

Division	Village	Number of farmers	Percentage (%)
Budalangi	Bukani	18	10
	Mudembi	15	8
Township	Mauko B village	17	10
	Bulada village	14	8
Butula	Bumala Town	8	4
	Bujumba village	9	5
Funyula	Namioso	19	10
	Sichehe	20	11
Matayos	Nahakina	10	6
	Buriang	19	10
Nambaie	Makongeni	21	11
	Segero A	12	7
Total		182	100

3.3.1.2 Level of education, occupation and pig keeping characteristics of the study farmers

During the survey, the questionnaires were administered to the head of household (87/182, 48% male and 79/182, 43% female) or siblings (16/182, 9%). The average age of the household head was 43 years (range: 19-76). The level of education for household heads was categorized as none (21%), primary school (58%), secondary (19%), and tertiary (1%). Livestock and subsistence crop farming was undertaken by 67% of the household heads. Other activities undertaken by household heads included business (28%) and full time employment (2%). The periods during which farmers had kept pigs were less than one year (23%), one to five years (47%) and over five years (30%). Ninety percent (90%) of the farmers owned less than five acres of land, while 10% had more than 5 acres. The interviwed farmers owned a total of 654 pigs (Table 3.2). In descending order, the classes of pigs included growers/finishers (49.4%), piglets (30.1%), sows (16.8%), and boars (3.7%). The mean number of pigs per farm was 3.6, with 73% of the farms having 1-3 pigs.

Table 3.2. The structure and sizes of the pig herds on the 182 study farms in Busia District

Category of pigs	Number of pigs in all farms (% of total pigs)	Number of farms with pigs class (% of total farms)
Growers/Finishers	323 (49.4)	149 (82)
Piglets	197 (30.1)	30 (17)
Sows	110 (16.8)	82 (45)
Boars	24 (3.7)	22 (12)
Total	654	182

Sixty four percent (64%) of the pigs in the study area had white colour and were probably crosses of exotic and/or indigenous varieties (Fig 3.3). Thirty six percent (36%) of pigs had either black or black and white colours, and were often referred by the farmers as indigenous (*Kienyeji* in the Swahili dialect) (Fig 3.3).

Key: a = white boar, b= black and white sow, c = black boar, d= black and white grower

Fig 3.3. Variety of pigs kept by the farmers in Busia District

In descending order, other livestock kept by the pig farmers included cattle (509), goats (267) and sheep (115). Pigs accounted for 41.4% of the livestock population in the studied farms. The cattle, sheep and goats were found in 99 (54%), 68 (37%) and 36 (20%) farms. All the study farmers also kept chicken in variable numbers.

3.3.1.3 Production characteristics

The production characteristics are shown in Table 3.3. The pigs were kept as a source of income by 98% of the households. Based on the type of operation undertaken in the previous five years, the farmers were divided into four categories. The first category of farrow to weaner (12%) involved farmers with sows and reared piglets up to around two months of age. These farmers then sold the weaners to other farmers involved in weaner to finisher (36%), who then fattened the pigs before selling for slaughter. Another category of farmer was farrow to finisher (6%) who had sows and reared the pigs up to when they reached market weight. Other farmers were mixed (46%) and had a combination of the three systems (farrow to weaner, farrow to finisher and weaner to finisher). Management was measured in terms of the gender involved in pig rearing (daily provision of food and tethering). The pigs were managed by female spouses on 55% of the farms. The male spouses were only directly involved in pig rearing in 17% of the farms. The farmers obtained most of the advice on pig husbandry from other pig farmers (37%), with 14% being advised by government extension officers (Table 3.3).

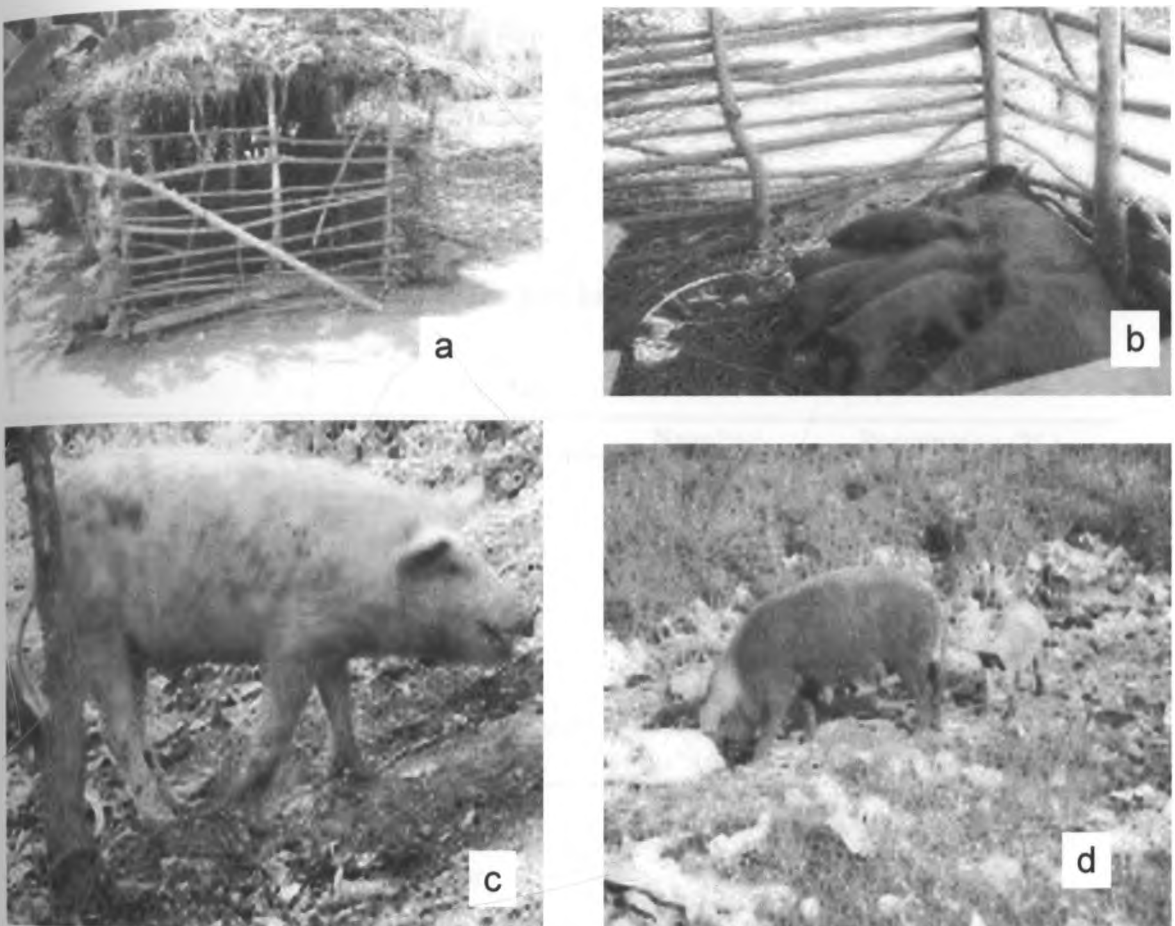
Table 3.3. Pig keeping practices on the 182 study farms in Busia District

Variable	Number	Percentage (%)
Reasons for keeping pigs (n=182)		
Source of income only	178	98
Income source + domestic consumption	2	1
Domestic consumption only	2	1
Type of operation (n=182)		
Mixed	83	46
Weaner to finisher	65	36
Farrow to weaner	22	12
Farrow to finisher	12	6
Farm management (n=182)		
Mother (female spouse)**	101	55
Mixed	37	20
Father (male spouse)	31	17
Siblings	10	6
Hired workers	3	2
Advice on husbandry (n=182)		
Other farmers	67	37
Extension officers	25	14
Over the counter sales people	7	4
Field days/seminars	7	4
Never received from any source	76	41

Key: *Mixed = This category consisted of farmers who had a history of keeping pigs in various categories including farrow to weaner, farrow to finisher, weaner to finisher,

**Mixed = Farm management by either mother , father or siblings

Housing was not provided in 61% of the farms, with the pigs staying under a tree or next to the homestead. Shelter was provided in 36% of households mainly as night boma made of timber on the sides and thatched roof shelter (Fig 3.4a and b). In 3% of the households, the pigs were housed either with other types of livestock or in one of the rooms in the homestead. Ninety six percent (96%) of the shelters had dirt floors that were rarely cleaned, while 4% were made of concrete. Sixty five percent (65%) of the pigs were tethered (Fig 3.4c) while 33% were kept in a mixed system. The latter was characterized by free-range during the dry season (Fig 3.4d) and tethering during the crop (rainy) season. Only 2% were kept permanently indoors.



Key: a = shelter made of timber and grass thatched roof
b= sow and its piglets in a typical pig shelter
c= tethered pig (note the extensive rooting around the tied area)
d= sow and piglet scavenging in a dumpsite

Fig 3.4. Types of shelters and systems of pig production in Busia District

The type of feeds provided to the pigs is shown on Table 3.4 and included leftover food from the households (58%), *Ugali* (57%), vegetables and sweet potato vines (52%) and swill collected from market places (50%) amongst other types of food. The farmers also gave Omena fish in low quantities (two to three spoonfuls) as they thought that it had deworming potential. The amount of feed given to the pigs was not quantified but the farmers indicated that it varied with availability leading to production of unthrifty pigs during the dry season. Only 5% of the farmers supplemented commercial pig feed to their animals. The sources of water for pigs were the local river (46%), borehole (34%), tap water (18%) and lake water (2%).

Table 3.4. Types of feed provided to the pigs on the 182 study farms in Busia District

Types of feed given (n=182)	Number	Percentage (%)
Left over from household	106	58
Ugali	103	57
Vegetables from farm and sweet potato vines	94	52
Swill collected from the market and hotels	90	50
Omena fish	61	34
Fruits (pawpaw, avocado, guava, jackfruit)	25	14
Cassava (peelings and tubers)	20	11
Busaa waste product (*machicha* in local dialect)	12	7
Commercial feeds	10	5

Busaa = local brew,
Ugali= made from spilt Posho mill flour

Fourty nine percent (49%) of the farmers did not castrate the pigs. For those who did, 96% castrated the pigs when they were more than four weeks of age, while 4 % castrated the pigs when they were less than four weeks of age. Thirty percent (30%) of the farmers weaned the pigs when they were 2 months old or more. Those who weaned at less than one month of age, and between 1 month and 2 months accounted for 19% and 24%, respectively. Twenty seven percent (27%) could not remember when they weaned their pigs.

3.3.1.4 Production constraints

The production constraints associated with pig farming on the study farms are shown on Table 3.5. In order of importance, the main production constraints as perceived by farmers included diseases (81%), high cost or lack of feed (81%) and conflict with neighbours (28%). The conflict with neighbours originated from damaging of crops by the pigs, and some farmers opted for nose ringing to prevent the rooting behaviour.

Table 3.5. Production constraints associated with pig farming in Busia District

Production constraints (n=182)	Number	Percentage (%)
Diseases	148	81
High cost or lack of feed	148	81
Conflict with neighbors	51	28
Limited marketing channels	50	27
Lack of extension services	46	25
Lack of breeding animals (mainly boars)	34	19
High cost of drugs	21	12
Lack of credit	12	7

3.3.1.5 Disease conditions and control strategies for parasites

In order of importance, the main disease constraints, according to the signs/ syndromes given by the farmers, included lice infestations (pediculosis) (71%); worms (58%), respiratory problems (39%), mange (38%) and African Swine Fever (31%). Farmers mainly diagnosed worm infection by observing unthriftness (71%), stool consistency and distension of abdomen (56%), distended abdomen (48%), coughing (10%) and poor appetite and vomiting (10%).

Sixty nine percent (69%) of farmers had a history of deworming their pigs. Of these, 46% dewormed their pigs after every three months while 35% of the farmers dewormed the pigs after periods of more than three months mainly when the pigs showed signs of worm infection. Nineteen percent (19%) dewormed at a period of less than two months.

The main dewormers used by the farmers in the current study included Levamisole (Wormicid® tablets, levamisole hydrochrolide 7.5%, Cosmos Ltd, Kenya) (37%), piperazine (Ascarex®, piperazine dihydrochloride 53%, Cosmos Ltd, Kenya) (17%) and combination of oxyclozanide and levamisole (Nilzan®, Coopers Ltd, Kenya) (3%). Those who could not remember the name of the dewormer used were 24%.

The cost of the conventional dewormers was low, mostly (41%) less than Ksh 10 per dosage (of one pig). Fifty one percent (51%) of the farmers bought the drugs after getting advice from either over the counter salesmen or after previous experience of the drug effectiveness (34%). Other considerations included the low price of the drug (12%), ease of administration (18%), advice from animal health personnel (26%), advice from other farmer (9%) and seminars (5%). Some farmers deliberately used *Omena* fish (13%) and pawpaw seeds (10%) as forms of dewormer.

Fifty five percent (55%) of the farmers indicated that ectoparasites were of great significance (Table 3.6). However, 62% of the farmers did not treat the pigs for ectoparasites. The commonly used conventional ectoparasiticides included amitraz (Tactik®, 12.5% amitraz, Intervet Ltd, Kenya) and Cabaryl, (Sevin®, Cabaryl 5%, Bayer

EA Ltd). The farmers, who sprayed after every week, every two weeks and more than two weeks, were 8%, 7%, and 23%, respectively. The pigs were mainly sprayed together with cattle. The farmers also used non-conventional treatments including crop insecticides and hand washing.

Table 3.6. Perception of the farmers on the ecto-parasites and control strategies within the study farms in Busia District

Significance of ectoparasite infestation (mange and *H. suis*) (n=182)	Number of farmers	Percentage (%)
Great significance	100	55
Mild significance	51	28
No significance	24	13
Did not know	7	4
Conventional drugs against ectoparasite (n=69)		
Tactik® (12.5% amitraz, Intervet Ltd, Kenya)	44	64
Sevin® (Cabaryl 5%, Bayer EA Ltd)	12	17
Decatix® (Deltamethrin 2.5%, Cooper Ltd, Kenya)	6	9
Ectomin® (Cypermenthrin 2.5%, Ultravetis Ltd, Kenya)	1	1
Could not remember	6	9
Unconventional methods used for ectoparasite treatment (n = 49)		
Hand washing with soap	24	49
Mud washing (shallow mud pools)	10	20
Crop insecticides (eg., Karate®, cyhalothrin 13.1%, Syngenta Ltd, US)	7	14
Petroleum products (Kerosene, grease or engine oil)	5	10
Insect sprays (eg., Doom®, deltamethrin 0.5%, Bayer EA)	3	6
Any unconventional method	49	27

3.3.1.6 Marketing of pigs by farmers

Ninety five percent (95%) of the farmers bought their replacement stock from other local farmers while 3% bought improved breeds from the local government owned Busia Farmers Training Centre (FTC). Only 2% obtained their stock from across the border in Uganda. Eighty nine percent (89%) of the slaughtered pigs were sold by farmers to local butchers (sometimes through middlemen/brokers), while 1% sold the pigs to Farmers Choice Company. Three percent (3%) of the farmers (from Port Victoria, Budalangi Division) sold their pigs directly to consumers, through renting butchery. In such an arrangement, the farmers (who formed a youth group) alternated in slaughtering their pigs. Seven percent (7%) had not sold any pig at the time of study.

Factors considered when selling pigs included: family needs (57%); body condition of the pigs (53%), age of the pigs (30%) and lack of feeds (3%). For the farmers who indicated to have sold slaughter pigs, 76% sold them at an age of more than 9 months, while 24% sold at less than 9 months of age.

Seventy eight percent (78%) of the farmers indicated that they had marketing constraints for either the piglets or pigs for slaughter. In order of importance, these marketing constraints included poor output prices (89%), inadequate market information (51%) and poor infrastructure (2%).

3.3.2 Slaughter-slab survey

3.3.2.1 Retrospective analysis of livestock slaughter trade in Busia District

Table 3.7 shows that the number of slaughtered pigs was second only to that of cattle. The DVO also indicated that some farmers and butchers slaughtered the pigs and other small stocks in homesteads.

Table 3.7. Number of livestock slaughtered in Busia District between 2002 and 2005

Year	Pigs numbers	Cattle numbers	Sheep numbers	Goats numbers
2002	1952	3492	55	420
2003	1716	3508	26	448
2004	1722	3998	41	703
2005	2987	7989	224	1569
Total	8377	18987	246	3140

According to the records available at the Veterinary Department, the pigs slaughtered were mainly males (58% in 2002, 63% in 2003, and 61% in 2004). A substantial number of pregnant females were also slaughtered (e.g. 15% in 2002, 19% in 2003 and 6% in 2004). The slaughter slabs for pigs in the district in 2005 were 47, while the number of meat inspectors in the district were 10. According to the DVO, the personnel were not enough and had inadequate means of transport. The revenue collection by the veterinary department in 2002, 2003, 2004 and 2005 was Ksh. 470,780, 492,635, 609,730 and 1,130,436, respectively. Most of the money originated from meat inspection charges. For example, in 2005, the revenue collected from meat inspection was Ksh 929,616 (82%), while other forms of collections included meat transportation certificate (Ksh 191,520, 17%) and licensing of slabs (Ksh 9,300, 1%). The records also indicated that lungs infected by lungworms (*Metastrongylus* spp) were the only organs condemned, and in 2005 an average of 21% of the lungs were condemned causing a loss of Ksh. 18,818.

The pigs slaughtered in the district were mainly sourced from the local farms. These pigs were slaughtered in the slaughter-slabs where they were inspected before being taken to butchery and then sold to consumers. In some cases, meat inspection was also undertaken at the butchery level. The slaughter slabs were owned by traders who charged a fee for the slaughtered pig.

3.3.2.2 Prospective slaughter-slab and butchery survey

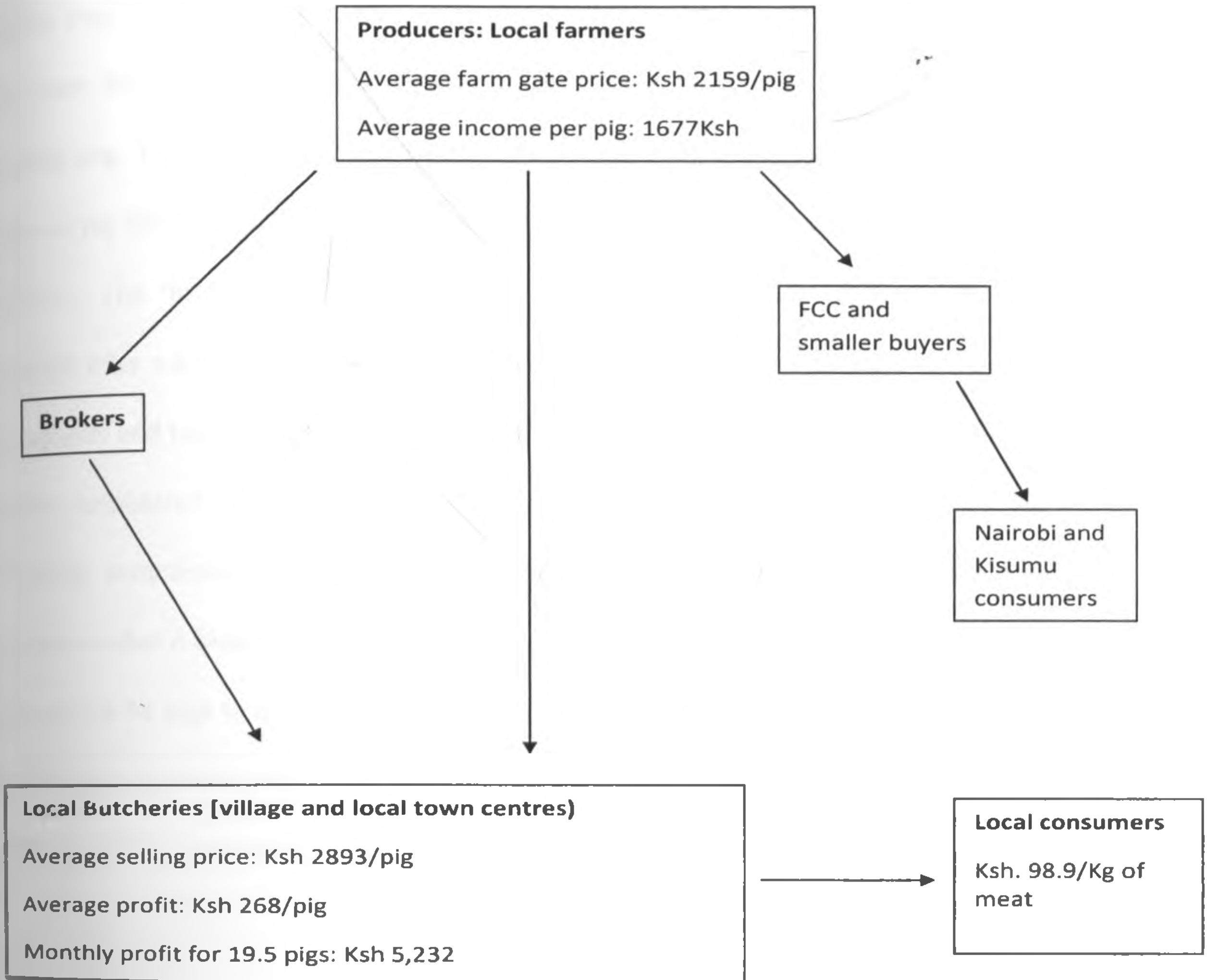
3.3.2.2.1 Characteristics of the butchers and trade

The butchers who were interviewed operated butcheries in the towns of Bumala (5), Matayos (5), Nambale (3) and Funyula (3). In 75% of the cases, the questions were answered by the owners of the butcheries. Other respondents included siblings (2/16, 12.5%) and employees (2/16, 12.5%) at the butchery. The respondents age ranged between 20 and 32 years and all had attained primary school education. Ninety four percent (94%) of the butchers were males. The mean age of the butchers was 39 (SD=±13) years and ranged from 21-71 years. Sixty three percent (10/16) butchers had primary level of education while 37% had either secondary or tertiary education. Only 3 (19%) were involved in butchery business alone, while the rest (81%) undertook other types of activities.

Seventy five percent (12/16) of the butchers had operated the business for period of more than five years. Thirteen (81%) of the butchers rented the building housing the butchery while the rest were the owners. The butchery structure was mainly made of concrete walls and iron sheet roof.

3.3.2.2.2 Market chain analysis

The marketing channel reported in this study was derived from interviews with the farmers, veterinary personnel and butchers involved in the pig trade. The pig meat market structure is shown in Fig 3.5.



NB: - Only the earnings of the main participants (butchers and producers) are shown

- FCC: Farmers Choice Company
- Butchers run the local butcheries, buying pigs from farmers and brokers

Fig 3.5. Pig meat market structure in Busia District

All the pigs slaughtered in the butcheries emanated from the local farmers living 1 to 20 Km from the butchery. In some few cases, pigs could be sourced from neighbouring districts (eg. Teso and Siaya) and Uganda. At village level, itinerant traders visited the home of pig farmers to buy pigs in small numbers. The traders either acted as 'brokers' or butchers. The 'brokers' usually bought the pigs from the farmers and sold them to butchers after adding a small margin in price. In this study, these 'brokers' were quite amorphous and their profit margins could not be ascertained. When buying the pigs, the traders considered the visual body weight and condition (16/16, 100%) of the pig. However, sometimes (3/16, 19%) butchers bought the pigs after being approached by farmers or after Africa Swine Fever (ASF) outbreak. From the farms, 88% of the butchers transported the pigs to the slaughter slab using bicycles. The pigs were tied and either put into boxes or on hand made stretchers before being loaded on the bicycle (Fig 3.6). Other methods (12%) of transporting pigs included trekking, hand carts and motor vehicles.

Key: Arrow = Slaughter slab is in the background

Note: The amount of discomfort on the pigs

Fig 3.6. A pig being transported to a slaughter slab in Busia District by bicycle

It was also reported that Farmers Choice Company (FCC), had a previous history (1990s) of buying pigs from 'pig brokers' in the district, but the occurrence of ASF stopped the company from undertaking this activity. There were also two buyers who frequently bought pigs from individual farmers and sold them to far fledged towns like Nairobi and Kisumu. As indicated above, some farmers from Budalang'i also sold meat directly to consumers. However, these three categories of channels were relatively few and are not considered further.

The profits earned by the farmers involved in fattening (who were the majority) and butchers were documented. It is important to note that farmers did not keep records and the study relied on their memory. However, the butchers, kept some form of records, which helped in preparing the profit margins reported in this study.

The farmers (involved in fattening) bought the piglets at a mean price of Ksh. 482 (range = Ksh. 400-800) from the breeders, while the average selling price (farm gate) per slaughter pig was Ksh. 2,159 (range = Ksh. 700-5,000). The farmers, who in the majority of cases sold only one pig a year, earned an average of Ksh. 1,677 per pig sold. The costs of drugs and any supplemental feeds were not assessed in the current study.

The costs and profit by the pig butchers is shown in Table 3.8. The butchers slaughtered and sold an average of 19.5 (range=12-30) pigs per month. The off-take was in terms of meat (sold at mean of Ksh. 98.9, range =90-110) and their products (eg., soup and fat); all earned a gross of Ksh. 2,893 per pig. The daily costs incurred by the butchers included: slaughter slab use charges (paid to the owner of the slab), inspection fees (paid to the inspector), workers' wages (paid to the workers involved in transportation, slaughtering and selling process), and consumables (eg., soap, fuel). The other longer term costs included rental and trade licenses (to the local council, and ministry of health). The butchers could not clearly remember the number of condemned organs (mainly lungs) and thus this type of losses was omitted in the analyses. From selling 19.5 pigs per month at a cost of Ksh. 2893 per pig, each butcher was able to raise 56,414. The monthly profit earned by each butcher was Ksh. 5, 232 (75 USD) and thus, the average profit per pig was Ksh. 268 (3.8 USD). The annual profit per butcher was Ksh 62,784 (897 USD) which translated to Ksh 1, 004, 544 (14,350 USD) for all the 16 butchers.

Table 3.8. The costs and income (in Ksh) generated by pig butchers from Busia District

Type of cost/income	Total amount (Ksh) per month (mean)
Expenditures:	
Buying price 19.5 pigs @ Ksh. 2159	42,101
Workers pay	2,688
Bora afya (health license)	1,700
Water + fuel	1,392
Renting cost	1,035
Inspection fee	1010
Slab fee	936
Trade license	270
Consumables (papers, soap, salt, etc)	50
Total expenditure:	51,182
Income:	
Selling price for 19.5 pigs @ Ksh. 2893 (meat) per pig	56,414
Average profit margin per month	5,232
Average profit margin per year	62,784

3.3.2.2.3 Constraints to pig butchery business

In order of importance, the main constraints included conflicts with regulatory authorities, lack of enough numbers of slaughter-pigs, long distances from farms to the butchery, seasonality of the market, competition with other meat businesses and poor mode of pig transportation. The constraints are summarized in Table 3.9.

Table 3.9. Constraints associated with pig butchery business in Busia District

Constraints	Number of farmers	Percentage
Conflicts with regulatory authorities and police	16	100
Lack of pigs after ASF outbreak	15	94
Seasonality of the market	14	88
Poor mode of transport	14	88
Competition with other meat businesses	10	81
Lack of credit to operate the business	9	56
Low purchasing power of the consumers	9	56
High cost of business premises	8	50
Condemnation of carcasses or organs	5	31
Unavailability of meat inspectors when required	3	19
High labour costs	3	19

Key: ASF = African Swine Fever

3.4 DISCUSSION

The spectrum of livestock kept by the farmers in this study has been reported before, where keeping of free range livestock is common in the district (GoK, 2002b; Karanja, 2005). The study farms had a low acreage, which could only support few livestock in addition to crop farming. The low farm acreage could also explain why the majority of farmers kept animals, which required less space e.g., chicken and pigs.

On the majority of farms, women who were in most cases the wives of household heads managed the pigs, with the household heads undertaking a lesser role. This is in contrast with results of surveys on pig and beef rearing in Botswana by Nsoso *et al.*, (2004) where majority of people participating in livestock rearing were found to be males. In Western Kenya, the increased role of women in poultry production has been emphasized by Okitoi *et al.*, (2007a). The pattern of chicken and pig daily management in Western Kenya could be linked to the women traditional roles within the family. The two species of livestock are reared in close proximity to the homesteads where women's involvement is critical in provision of feed and kitchen waste to supplement the scavenged feed. However, in spite of the fact that women manage the pigs, men dominate the sale of these animals since the latter is the gender mainly involved in cash related activities in most African communities (Okitoi *et al.*, 2007a). Consideration of the gender component will be important in projects involving technology transfer such as improvement of animal husbandry and parasite control.

In the current study, the low level of education amongst the pig farmers, which reflects the reported low literacy rates (55%, GoK, 2002b) in Busia District, could limit their knowledge on the management of pigs. Seventy two percent (72%) of the farmers had kept the pigs for a period of more than one year, showing a sustained interest in pig rearing. In contrast to the practice of keeping other species of livestock for subsistence (Karanja, 2005), most farmers in Busia District kept pigs purely for generation of income. In other countries, low-input pig production has both income-generating and socio-cultural functions and is driven by the availability of resources (Hide, 2003; Deca *et al.*, 2007; Lemke and Zarate, 2007, Ajala *et al.*, 2007).

It was found that majority of the farmers kept pigs which had a white coat and these could have been crosses of either exotics or exotic and 'local' breeds. However, 36% kept breeds which had other colours especially black and black/ white spotted pigs. The latter breeds were smaller in size and suspected to be indigenous or cross of indigenous and exotic breeds. Most of the pigs raised in developing countries are crosses or local breeds raised under traditional production system (Ajayi *et al.*, 1988; Permin *et al.*, 1999; Wabacha, 2001; Hide, 2003; Nsoso *et al.*, 2004). Improved pig breeds yield higher cash revenue due to pigs' higher output, but these breeds are also an economic risk for resource poor farmers due to higher input requirements (Hide, 2003; Lemke and Zarate, 2007). The productivity and health of the suspected indigenous pigs found in Busia District needs to be further evaluated.

In the present study, the pig farmers owned a total of 654 pigs, 70.5% of which were growers/finishers and piglets. The mean number of pigs per farm was 3.6 showing that pig farming is a smallholder concern. In most developing countries, the mean pig herd sizes are relatively small (e.g., in Vietnam = 6, Lemke and Zarate, 2007, Nigeria = 3, Ajala *et al.*, 2007). This is in contrast to higher number of pigs per farmer in Central Kenya (mean >10, range = 1-6000) (Gichohi *et al.*, 1988; Kagira, 2001; Wabacha, 2001) where pigs are kept intensively. The order of pig classes in this study was influenced by the structure of traditional system in Busia District. In the system, a smaller number of farmers were involved in keeping of sows for production of piglets. Sow keeping amongst the resource-poor farmers seems to be less common as they have a higher maintenance costs (Lemke and Zarate, 2007; Deca *et al.*, 2007). Majority of the farmers in Busia reared pigs of the weaner to slaughter category, which is similar to that reported in Vietnam and India (Lemke and Zarate, 2006; Deca *et al.*, 2007). Some other farmers also kept boars (either alone or together with other categories of pigs) which were rented out to other farmers for mating (breeding). As observed in other studies, the low number of boars amongst the small-scale farmers could lead to inbreeding (Wabacha, 2001; Ajala *et al.*, 2007; Lemke and Zarate, 2007).

Pigs in the current study were either tethered or kept in a mixed system characterized by free-range during the dry season and tethering during the rainy season. This is similar to other studies in Botswana and Nigeria by Permin *et al.*, (1999) and Ajala *et al.*, (2007), respectively, where tethering of free-range pigs is undertaken during the rainy season since pigs are prone to damaging of crops. As reported by Ajala *et al.*, (2007), the increased availability of feeds during the rainy season may also act as motivation for tethering of pigs.

Housing was not provided in most of the farms and where the shelter was provided; it appeared temporary and was rarely cleaned. Unhoused pigs in the study area could be adversely affected by adverse weather, which includes high environmental temperatures and high rainfall (Lekule and Kyvsgaard, 2003). The lack of provision of housing by most farmers is a manifestation of low-input traditional system of pig farming which is common in most developing countries (Hide, 2003; Nsoso *et al.*, 2006). For example in Botswana, 25% of pig farmers in traditional system did not provide any housing for their pigs, and even where shelter was present on the farm, only 50% of the pigs spent their time in night boma (Nsoso *et al.*, 2006). Although, this could be a manifestation of high levels of poverty in Africa, the use of local materials for house construction has not been exploited.

The pigs in the current study were supplemented with other types of feed including food leftovers, swill, vegetables from the farm, *Ugali* from spoilt maize and spoilt fish. This shows that there is a wide variety of feed in the study area; although the amount given per pig might not be adequate and can be of poor nutritional value. Most of the farmers were also not providing commercial pig feed to their animals, owing to high costs. This is similar to other studies where pigs are kept in a traditional production system (Permin *et al.*, 1999, Hide, 2003; Nsoso *et al.*, 2006, Lemke and Zarate, 2007). Even in intensive farms in central Kenya, poor nutrition status of pigs was observed, which was related to lack of provision of formulated feeds to pigs (Gichohi *et al.*, 1988; Kagira *et al.*, 2001; Wabacha, 2001). Since the high costs of feed in Kenya is a deterrent factor in livestock production, an alternative would be to formulate home based rations which combine some commercial ingredients with feeds which are available locally. This has already been tried in countries such as Papua New Guinea (Hide, 2003) and Tanzania (Lekule and Kyvsgaard, 2003). The feeding of swill and spoilt food may also act as source of mycotoxicosis in pigs which may cause immunosupression. Previous studies have shown that immunosupression due mycotoxicosis acts as a major risk factor for occurrence of diseases (Oswald *et al.* 2005). Future studies should evaluate the impact of mycotoxicosis in affecting the epidemiology of parasites of pigs kept under free-range conditions.

The farmers in the district obtained most of the advice on pig husbandry from other pig farmers with only 14% being advised by government extension officers; and this is similar to the intensive pig production in Central Kenya (Kagira, 2001). Indeed, limited government veterinary extension services have been reported in most studies in Africa owing to poor funding and change of government policies to privatization of veterinary services (Chema and Gathuma, 2004). This scenario has led to a situation where majority of the farmers lack proper pig husbandry skills. For example, in the current study castration was rarely practiced and when done, it was undertaken after a period of more than one month, instead of the recommended 2-3 weeks of age. In Busia District, weaning was dictated by sale of piglets, which mainly started at around two months of age and continued until all piglets were sold. The delayed weaning (and hence lactation) period may also prolong the inter-farrowing period and hence reduce sow performance.

Weaning of piglets when they are above two months of age has been reported in smallholder farms in Nepal (Gatenby and Chemjong, 1992) and Solomon Islands (de Fredrick and Osborne, 1977). Weaning of piglets at an earlier age will require provision of high quality feeds for piglets which may lack in free range systems (Taveros and More, 2001).

The main production constraints as perceived by farmers included diseases and lack or high cost of feeds, conflict with neighbours, poor marketing, lack of extension officers, lack of breeding animals. Similar constraints have been identified in other traditional and smallholder pig production systems (de Fredrick and Osborne, 1977; Gichohi *et al.*, 1988; More *et al.*, 1999; Wabacha, 2001). The main disease constraints in the current study included pediculosis, worm infections, respiratory problems, mange and ASF, all of which have been reported to be common in free range pigs (Ajala *et al.*, 2007). It is important to note that the diseases were mainly classified according to the signs/ syndromes given by the farmers. Thus, some worm infestations (eg. lung worms) could contribute to the occurrence of respiratory distress. Further, mange and lice infestations result in pruritus, although other signs including presence of lice and hyperkeratosis were used to differentiate the two diseases. The occurrence of *H. suis* (hog louse) and African Swine Fever in the free range pigs in Busia District could be related to the free-range system of production.

The farmers diagnosed worm infection mainly using signs such as unthriftness and occurrence of pendulous abdomen. However, in spite of relatively good knowledge of worm infection in pigs, most pigs in the study farms were in a poor body condition. This is possibly related to high level of poverty in the study area, where farmers might not be able to buy the dewormers. Most farmers had a history of deworming their pigs, mostly after every three or more months, mainly after observing the signs of worm infection. The main dewormers used by the farmers included piperazine and levamisole, as was the case in Central Kenya (Kagira, 2001). The farmers bought the anthelmintics after getting advice from either the over the counter salesmen or previous knowledge of anthelmintic effectiveness. The over-reliance on sales people for guidance on choice of drugs by the farmers has been noted by other authors (Bett *et al.*, 2003; Kagira *et al.*, 2003) and training of this category of sales people can help in the control of parasitic diseases.

In the current study, the non-conventional products used by the farmers to deworm pigs included *Omena* fish and pawpaw seeds. The use of natural remedies to deworm pigs has been reported in other studies (Lemke and Zarate, 2007). Although the farmers in the current study in Busia claimed that the non-conventional products were effective, it would be important to investigate the efficacy of non-conventional remedies. Some products with high protein contents, such *Omena* fish, may improve the resilience of hosts in control of gastrointestinal nematodes in livestock (Lemke and Zarate, 2007). The practice of giving pawpaw to pigs has also been described in Papua New Guinea (Hide, 2003). In this study, the efficacy of various pawpaw products against parasites was evaluated as reported in Chapter 6.

Most farmers in the current study indicated that ectoparasites were a common problem, but majority did not apply any treatment. Where treatment was applied, the commonly used drugs were amitraz or pyrethrin based compounds. The interval of usage of these drugs was haphazard with most farmers using them after a prolonged period of more than three months leading to poor parasite control. This may be responsible for the high prevalence of ectoparasites observed in the current study. Factors responsible for this practice could include lack of knowledge of their use or low purchasing power of the farmers. A substantial number of farmers also used non-conventional treatments, some of which have been reported in other studies (Nsoso *et al.*, 2000; Wabacha, 2001; Ajala *et al.*, 2007). Although the effectiveness of non-conventional treatments may be doubtful, farmers in the current study claimed they were effective. The use of petroleum products in control of pig mange is common in Africa (Wabacha, 2001; Ajala *et al.*, 2007) and it is important to determine whether they are effective. The presence of sulphur in these products could be responsible for their perceived efficacy (Mathius-Mundy and McCorkle, 1989).

Most farmers in the current study bought their replacement stock from other farmers, which is similar to what was reported in Nigeria by Ajala *et al.*, (2007). This shows that most farmers were either not aware of the Busia Farmers Training Centre (FTC) which sells exotic breeds, or could not afford the expensive breeds. In future, the role of FTC in providing training and providing replacement stock to pig farmers requires to be enhanced. In Vietnam, rural based pig breeding and training centres have played a critical role in upgrading of local breeds (Lemke and Zarate, 2007). The role of Farmers Choice

Company (FCC), the major buyer and processor of pig and pig products in Kenya was minimal in the district. This was mainly attributed to the high endemicity of ASF, risks of cysticercosis, poor body condition of local pigs and long distance to the processing facility in Nairobi (Procurement Manager, FCC, personal communication). In the current study, the pigs sold by farmers to butchers were over 9 months based mainly on body condition and this is possibly related to the slow growth rate of free-range pigs which has also been reported in other studies (Ajala *et al.*, 2007). It would be important for the government to facilitate the pig farmers in controlling the diseases and improving the husbandry conditions of pigs through extension services.

Marketing constraints were common amongst the farmers and mainly included poor prices and inadequate market information. Marketing as a constraint in smallholder pig farming has also been reported in several studies (Gichohi *et al.*, 1988; Gatenby and Chemjong, 1992; More *et al.*, 1999; Wabacha, 2001). It is recommended that small holder farmers form cooperative groups which would allow them to bargain for better feed and pig prices and in seeking for other markets. For example the Budalangi youth group had their own system (where they slaughtered and sold their pigs thereby eliminating brokers and butchers), which allowed them to have a higher income compared to the other farmers in other divisions.

The number of pigs slaughtered in the District was only second to cattle and is relatively high compared to most districts in Kenya (Langat, 1999). This is because pork consumption is popular amongst the local community and thus if well harnessed can improve the livelihoods of local farmers (GoK, 2002b). The substantial number of pregnant pigs slaughtered in the study is a source of concern and shows that farmers are not able to undertake pregnancy diagnosis before sale. Pregnant animals are often in good body condition and farmers might opt to sell them so that they can fetch better prices (Oyenkule *et al.*, 1992).

The pig marketing system in Busia District was made of farmers, middlemen, slaughter-slab owners and butchers, while the government officers played the role of meat inspection. A similar structure was also observed in other countries such as Nigeria, where the participants included producers (farmers), rural assemblers, wholesalers, and retailers (butchers) (Ajala and Adesehinwa, 2007). Most of the butchers in the current study had operated the butchery business for more than five years indicating that it was profitable. According to the study, the average net income per annum for each butcher was Ksh 62,784 (897 USD) which amounted to Ksh 1, 004, 544 (14,350 USD) for all the 16 butchers. This was relatively higher compared to the money earned by the local pig farmers, who normally sold 1-2 pigs a year at an average price of Ksh 2159 (31 USD) per adult pig. The profit earned per pig slaughtered in Busia was 3.8 USD which was relatively lower than that earned by pig butchers in Nigeria (9.2 USD) (Ajala and Adesehinwa, 2007). To improve the livelihoods of the butchers, it would be important for the local municipal authority to consider lowering the licenses and fees incurred in running of the butcheries.

The butcher-men also identified several constraints to their business, which mainly included conflicts with regulatory authorities, inadequate numbers of slaughter-pigs and poor mode of pig transportation. The use of bicycle to carry pigs is the most common method of transport in the area and although it is not legal, it will be important to determine the effects it has on the pig (eg. bruising). Due to their convenience, bicycles are also used to transport pigs and other types of small stock in slums in urban areas in Africa and India (Aklilu *et al.*, 2002; Deca *et al.*, 2007). Other humane ways of transpoting pigs including modification of the bicycle carrier, use of carts and vehicles should be considered. During the study, there was a scarcity of slaughter pigs, possibly due to high mortality associated with ASF which had just occurred (OIE/WAHID website, 2007). The observed competition of pig meat with other meat business has also been observed in Nigeria, where prices are higher during the dry season when there is partial scarcity of cattle (Ajala and Adesehinwa, 2007). In Busia District, the wet season is accompanied by abundance of fish (from the local rivers) and cattle for sale, and this leads to a decline in pig prices (DVO, personal communication). To increase the population of pigs in Busia District, farmers will need to be facilitated by veterinary authorities and local NGOs in terms of disease control and be educated on improved pig production. ASF control strategies targeting the butchers and pig brokers will be very important since the DVO (DVO, personal communication) indicated that the whenever there was a disease outbreak, butchers enhanced its spread through buying, transporting and selling sick animals to consumers.

In conclusion, this study has contributed to the understanding of small holder pig production system, and pig trade in Busia District, Kenya. The production system is characterized by low-input pig production with an objective of income generation and driven by availability of farm resources. The poor husbandry skills could be an impediment to the marketing of the pigs emanating from the study area. There is a need to improve access to quality extension services and to seek solutions to constraints using locally available means. This will result in increased number of heathy pigs, which can be a source of low cost protein for the local human population. Future research and development approaches should also focus on the integration of small-holders farmers and pig traders from Busia District, Kenya into the country's market chains.

CHAPTER FOUR: THE PREVALENCE AND INTENSITY OF PARASITES IN PIGS RAISED UNDER FREE-RANGE PRODUCTION SYSTEM IN BUSIA DISTRICT, KENYA

4.1 INTRODUCTION

Although it is illegal to keep pigs outdoors in Kenya, the free-range pig production system is common in Western and Nyanza provinces as well as slums in the urban areas. In studies undertaken in other countries, pigs raised under free-range system of production have higher parasite spectrum and intensity when compared with animals kept under intensive conditions (Permin *et al.*, 1999, Ajayi *et al.*, 1988). This is because of risk factors such as continuous exposure to infective stages of parasites in tropical environment, scavenging behaviour of pigs and presence of intermediate hosts and parasite vectors in the environment (Roepstorff and Nansen, 1994). In spite of the importance of the free-range system in parts of Kenya, the profile and intensity of parasites infecting free-range pigs had not been studied.

In Kenya, slaughterhouse surveys on pig parasites had only been undertaken in intensively kept pigs (Langat, 1999; Nganga *et al*, 2007). Although slaughterhouse surveys have some limitations, they are able to reveal a more definitive spectrum of parasites and pathologies associated with these parasites. The types of parasites which can be observed easily after post-mortem include parasite stages during the pre-patent period and visceral stages of parasites (eg., cysts). This study was designed to determine the spectrum and intensity of parasites in pigs kept on small-holder farms in Busia District, Kenya. A preliminary slaughter slab study was undertaken as a prelude to a larger cross-sectional farm survey.

4.2 MATERIALS AND METHODS

4.2.1 Slaughter-slab survey

4.2.1.1 Retrospective data analysis

The records at the District Veterinary Headquarters were analyzed to determine the prevalence of parasites identified at meat inspection in the year 2006. The number of pigs infected by the parasitic diseases was recorded.

4.2.1.2 Prospective slaughter-slab survey

The survey was undertaken on 37 pigs slaughtered at the six slaughter-slabs in Funyula, Nambale, Matayos and Butula urban centres, in December 2006. Skin lesions associated with mange were described as either acute or chronic (Soulby, 1982). Ticks and lice presence, location and intensity were recorded, then picked and stored in 70% alcohol for latter identification according to the keys provided by Soulsby (1982) and Kaufmann (1996). The intensity of lice was subjectively categorized as light (<10 lice, whole body count) and heavy (10 or more lice, whole body count).

Blood samples (4 ml) were collected from the jugular vein and put into heparinised and non heparinised tubes. The samples were labeled and transported to the laboratory in cool box. Heparinised blood was used to diagnose haemoparasites and determination of packed cell volume (PCV) using the haematocrit technique (Murray *et al.,* 1977). Samples with either low PCV or trypanosomes at buffy coat were sub-inoculated into Swiss white mice and where parasitemia developed, the species of parasite was determined. The blood smears were fixed in 70% absolute alcohol, stained with 10% Giemsa solution and examined for presence of haemoparasites as described by Permin *et al.*, (1999).

The non-heparinised blood was used to prepare serum which was then stored at -20°C until analysis. The serum samples were screened for the presence of antigens of *T. solium* cysticerci using the Ag-ELISA method described by Dorny *et al.*, (2004) (Appendix III). The Ag-ELISA was carried out at the School of Veterinary Medicine, University of Zambia, Lusaka, Zambia, which runs a regional diagnostic laboratory for the diagnosis of *T. solium* taenisois/cysticercosis in the East and South Africa (ESA) sub-region.

After slaughtering the pig, the whole carcass was examined for pathological lesions caused by parasites. *Cysticercus cellulosae* cysts were assessed following the incision of muscles at the predilection sites (Boa *et al.*, 1995). The lungs were incised up to the distal bronchioles and examined for the presence of lungworms (*Metastrongylus* spp.) and where present, the worms and the surrounding lung tissue were collected and stored in universal bottles containing 10% formalin. The samples were processed for histology using standard procedures. The lungworms were identified using identification keys provided by Soulsby (1982). The superficial white spots in the liver, which are caused by migrating *A. suum* were also counted. From each carcass, a piece of diaphragm was cut along the *pars lumbalis* and a sample compressed between two glass slides and examined microscopically for the presence of *Trichnella* spp (Soulsby, 1982). The large intestines of the slaughtered pigs were thoroughly examined for nodular lesions caused by *Oesophagostomum* spp. The number of pigs with these lesions were recorded.

Faecal samples were collected from the rectum using plastic gloves and processed for the GIT nematode and coccidia analysis as described under subsection 4.2.2.5. Cecal contents were also collected and analysed for the presence of other GIT protozoa parasites as described under subsection 4.2.2.5.

4.2.2 Farm survey

4.2.2.1 Study area and farms

The study was undertaken in April and May 2007 on pig farms selected from the six administrative divisions of Busia District. The study farms were the same as those which had participated in the socioeconomic survey (Chapter 3). However, out of 182 farms in the original socio-economic survey, only 135 were found to be keeping pigs. Pigs in the other farms had either been sold or had died from a suspected ASF outbreak.

An estimation of the sample size of the pigs required for the study was done using the formula $n = Z^2PQ/L^2$ (Martin *et al.*, 1987), where *n* is the required number of individuals to be examined (sample size), Z is the normal deviate (1.96) at 5% level of significance, P is a known or estimated prevalence, Q= (1-P), and L is the allowable error (precision) of estimation. In the current study, 95% confidence interval (CI) with an allowable error of estimation of 0.05 was used. Since the prevalence of most of the diseases under investigation has not been well established, a prevalence (P) value of 14 % was used, based on the prevalence of cysticercosis reported in a preliminary study in Busia District (Githigia *et al.*, 2006). Thus $n = 1.96^2 \times 0.14 \times 0.86/0.05^2 = 185$ pigs. The final sample size was 306 using the snow balling method (Sikasunge *et al.*, 2006) described in Chapter 3.

4.2.2.3 Physical examination for external parasites

All pigs in the study were examined for ectoparasites and skin lesions as described in the slaughter survey (section 4.2.1.2).

4.2.2.4 Blood sample collection and analysis

Apart from piglets less than four weeks old and sows with advanced pregnancy, all pigs were sampled for blood. Blood was collected and analyzed for PCV, haemoparasites, and cysticercosis antigen as described in the slaughter-slab survey (section 4.2.1.2).

4.2.2.5 Faecal samples collection and analysis

Apart from piglets less than four weeks old and sows with advanced pregnancy, all pigs were sampled for faeces. Faecal samples were collected per-rectum using plastic gloves, put into faecal pots, labelled and kept cool before transportation to the laboratory where they were quantitatively analysed to determine the eggs per gram (EPG) of faeces using a modified McMaster technique (MAFF, 1986). Larval cultures for samples containing strongyle type nematode eggs were set up for species differentiation as described in the MAFF (1986) manual.

Quantification of coccidia oocysts was done as described in the MAFF manual (1986). Briefly, samples with an OPG of more than 1000 were pooled, thoroughly mixed with aqueous 2.5% (w/v) potassium dichromate solution, placed in thin layers in Petri dishes and allowed to sporulate for 14 days at room temperature. To harvest oocysts, the solution was centrifuged at 1200 rpm for 10 min and the supernatant discarded. The sediment was put into a test tube, which was filled with saturated Sodium chloride until a positive meniscus had formed. A cover slip was placed on the tube, allowing 10 min for oocysts to float onto the cover slip. The cover slip was removed carefully, placed on a microscope slide and the slide examined under the microscope. The first 200 oocysts seen were identified using the features described by other authors (Soulsby, 1982; Daugschies *et al.*, 1999).

Faecal smears from fresh faeces were also made on glass slides and examined for the presence of trophozoites and cysts of GIT protozoans parasites. The latter were diagnosed using features described by Soulsby (1982) and Kauffmann *et al.*, (1996).

Photographs on the appearance of the parasites and lesions under the microscope were taken using digital camera as described by Maude *et al.*, (2008).

4.2.3 Statistical analysis

Data was entered and managed in Ms Excel®, 2003 (Microsoft corporation, USA). The data files were screened for any errors that could have occurred during data entry and the observed errors corrected by cross checking against the original data forms. Data analysis was conducted using Ms Statview® (SAS Institute Inc, 1995-1998, Cary, NC, USA), Ms Excel® (Microsoft Corporation, USA). Descriptive statistics were calculated and presented as tables and graphs.

The prevalence (p) of each parasite was calculated as p = d/n, where d is the number of pigs diagnosed as having a given parasite at that point in time and n = number of pigs at risk (examined) at that point in time (Thrushfield, 1995). A pig was considered infected if it had an EPG of 200 or more. Farm prevalence was computed by dividing the number of farms that had at least one pig infected with a given parasite divided by the number of farms visited. The intensity of nematode and coccidia infection was defined as mean egg per gram (EPG) and oocysts per gram (OPG). The 95% confidence intervals of the prevalences and intensities of parasites were also calculated, while the significance level was set at 0.05. The 95% confidence intervals were calculated using the formula: Estimate ± 1.96 x standard error. Correlations between occurrences of various parasites were undertaken using Pearson correlation coefficient (Rho). The PCV of the pigs was compared with the mean EPG of the nematodes using one-way analysis of variance (ANOVA) test.

4.3. RESULTS

4.3.1 Slaughter slab survey

4.3.1.1 Retrospective data analysis

In 2006, out of the 2095 pigs slaughtered, the prevalence of lungworms and liver spots was 21% (n=440) and 12% (n=245), respectively. Other lesions observed at slaughter included hydronephrosis (18.2%), undefined liver cysts most probably hydatid or cysticercosis (4.4%), lung hemorrhages (6%) and renal calculi (5.2%).

4.3.1.2 Prospective slaughter-slab parasite survey

The number of males and females pigs was 19 and 18, respectively. According to the information given by the butchers, all the pigs slaughtered were sourced locally and older than seven months. However, this could not be ascertained as they were not the owners of the pigs. In descending order, the categories of slaughtered pigs were finishers (65%), sows (24%) and boars (8%).

All the pigs examined were found to shed eggs of either one or more species of nematodes (Table 4.1). In descending order, the nematodes observed included: *Oesophagostomum* spp, *Metastrongylus* spp, *Physocephalus sexalatus*, *Ascaris suum*, *Strongyloides ransomi*, *Trichuris suis* and *Globocephalus urosulubatus*. The occurrence of *Oesophagostomum* eggs in all pigs was corroborated by the findings of cecum and colons with heavy infestation of nodules in 24 pig carcasses (69%).

Table 4.1. Prevalence and intensity of nematodes observed by faecal examination of 37 pigs from Busia District

Nematode	Prevalence (%)	Mean EPG	(range)
Oesophagostomum spp	100	1778	(200-10,600)
Metastrongylus spp	54	589	(0-2,200)
Physocephalus sexalatus	24	164	(0-2,400)
Ascaris suum	19	45	(0-400)
Strongyloides ransomi	16	97	(0-2,400)
Globocephalus urosulubatus	6	6	(0-200)
Trichuris suis	3	5.4	(0-200)
All	100	2617	(200-10,800)

A total of 34 lungs were examined, of which 33 (97%) were heavily infested with lungworms. The resulting verminous pneumonia was mainly observed in the diaphragmatic lobes. Grossly, the lesions were characterized by congestion, haemorrhages, consolidation, marginal emphysema leading to formation of white to grey patches (Fig 4.1a), marbling (nodular areas) and froth within the respiratory tract. The bronchial lymph-nodes were enlarged and sometimes congested. On incision of the infected lungs, there were small whitish lungworms in the bronchioles (Fig 4.1b) and these worms mainly occurred in batches. All (100%) the butchers could identify the affected lungs (mainly based on the characteristic marginal emphysema and marbling of the diaphragmatic lobes) even before the inspection.

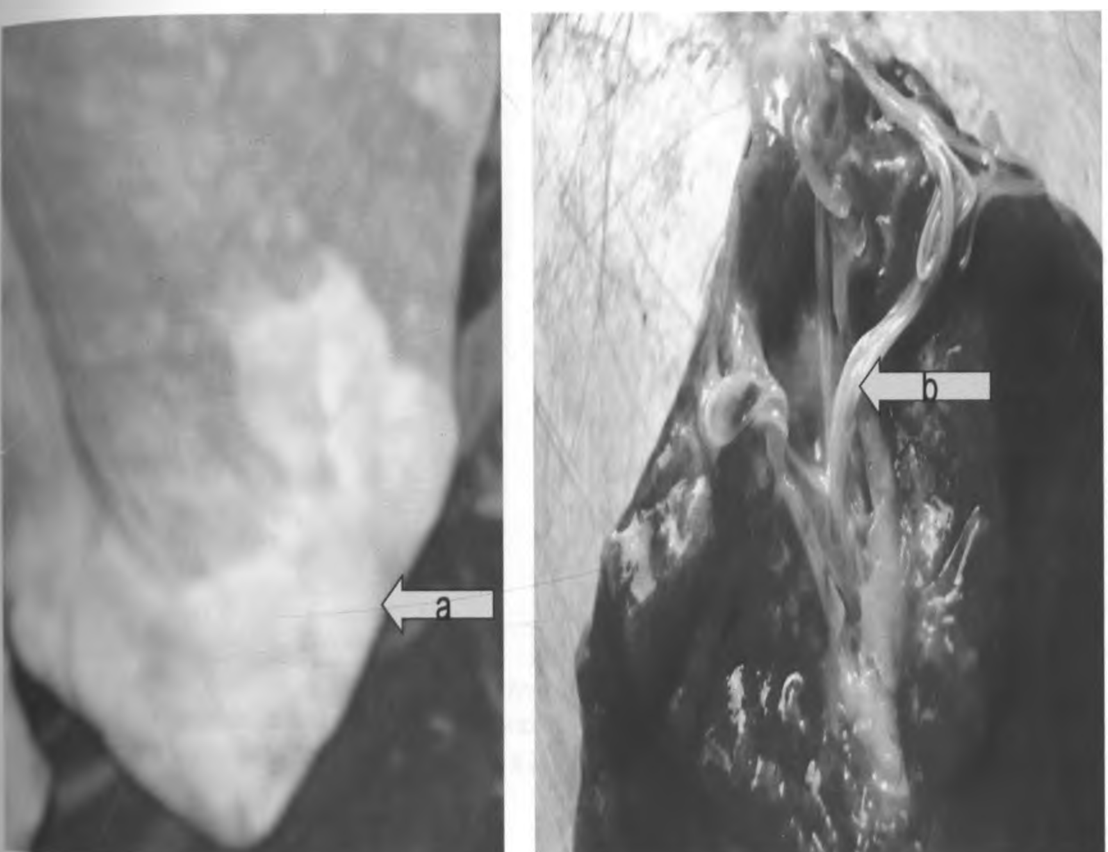


Fig 4.1. Lesions caused by lungworms in a pig slaughtered at the Bumala slaughterslab in Busia District. Note the white patches (a) at the tip of diaphragmatic lobe and lungworms (b) in bronchioles of the cut section of the lung

Lungworms from 10 pigs were collected from the affected lungs and pooled together. From the pool sample, the species observed included *Metastrongylus elongatus* (75.9%) and *M. pudendoctus* (24.1%). The identifying features of these two species of *Metastrongylus* as described by Soulsby (1982) are shown in Figures 4.2, 4.3 and 4.4. The mean length of *M. elongatus* females and males were 41 mm and 22 mm, respectively. The mean length of the *M. pudendoctus* females and males were 43 mm and 19 mm, respectively.

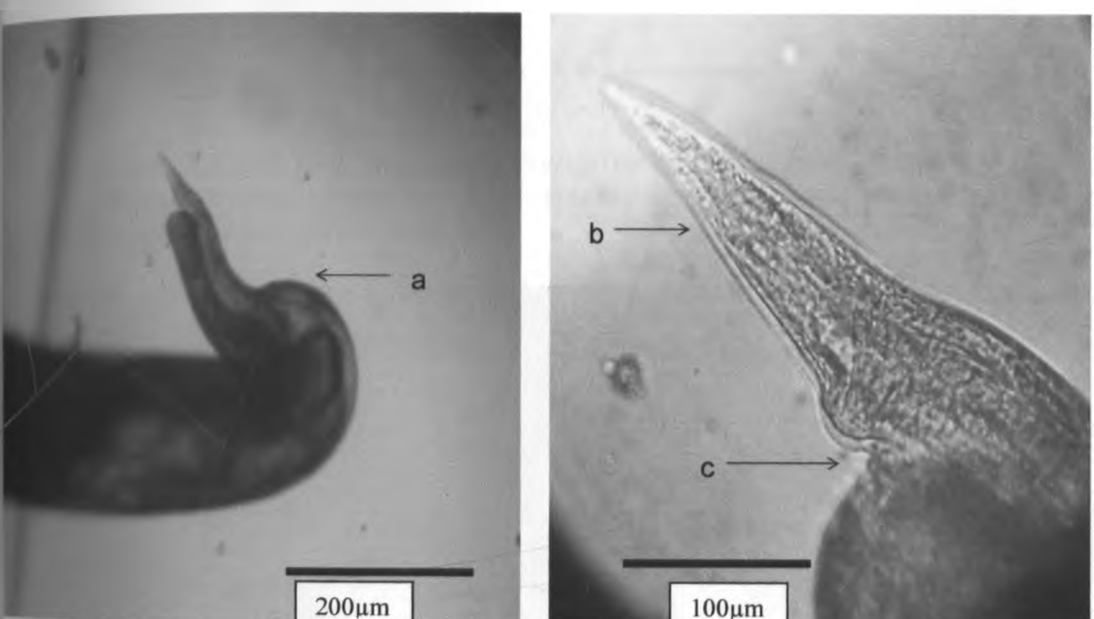


Fig. 4.2. Posterior end of a female *Metastrongylus elongatus* isolated from a pig in Busia District. Note the lateral flexing of the posteriour end (a), pointed tail (b) and anus (c). Images taken at x250 (a) and x 400 (b, c) magnifications.

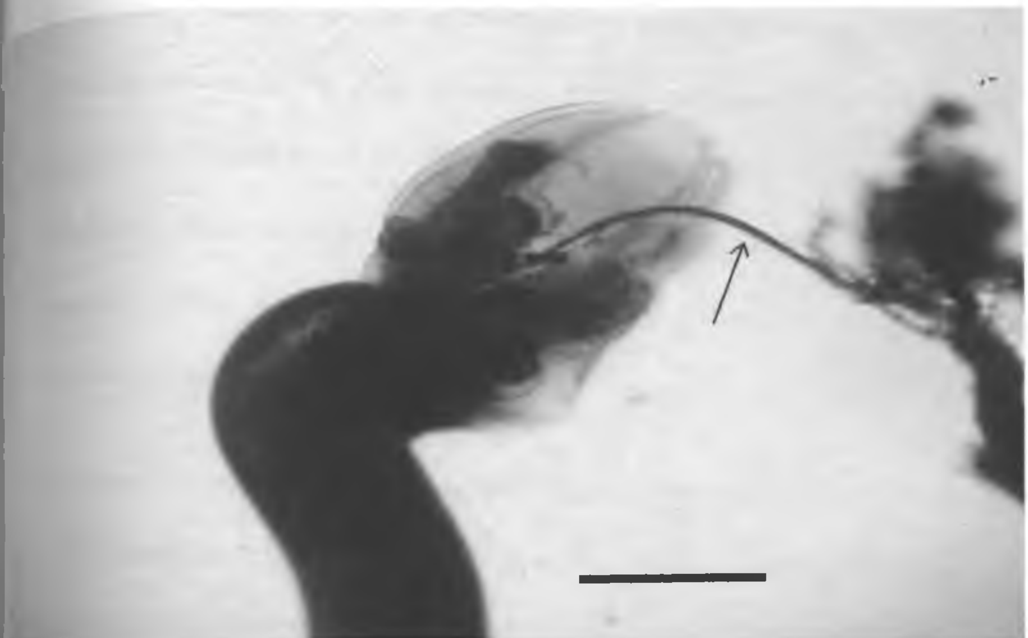


Fig. 4.3. Posterior end of a male *Metastrongylus elongatus* isolated from a pig in Busia District. Note the long spicule (arrow). Image taken at x250 magnification. Bar = 200µm

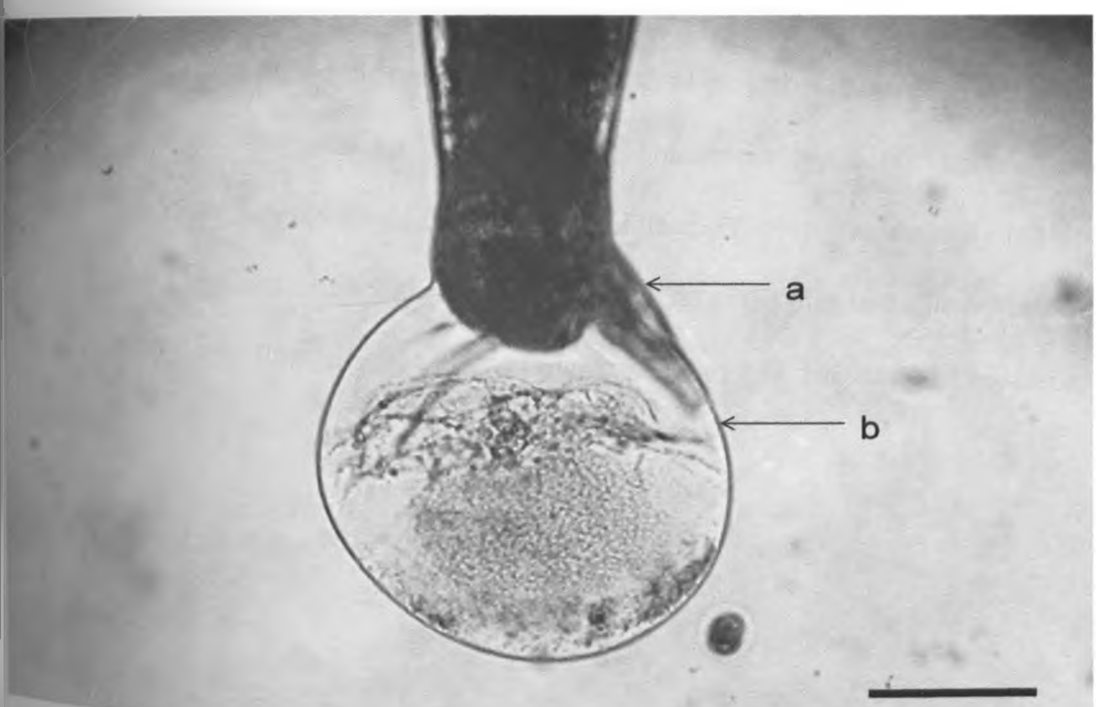


Fig. 4.4. Posterior end of female *Metastrongylus pudendoctus* isolated from a pig in Busia District. Note the tail (a) and bulbous enlargement (b). Image taken at x250 magnification. Bar = 200µm

Milk spots, possibly due to *A. suum* migration were observed in 15 (43%) of the pigs. Seventeen percent (17%) of the pig livers had an average of 3 spots and none had more than 5 spots. Ninety seven percent (97%) of the pigs had liver spots which were healed and fibrotic with no migratory worms. Other lesions observed in the liver included: abscesses (9%), severe cirrhosis/fibrosis (6%), capsular haemorrhages (9%) and discoloured black/pale livers (9%).

The mean PCV of all the pigs slaughtered was 37.9% (95% CI = 35.97-39.83%). There were no significant ($p<0.05$) differences in PCV when cut-off of EPG was set at values less than 2000. However, pigs with EPG of more than 2000 had significantly ($p<0.05$) lower PCV than those with less than 2000.

All the pigs examined had at least one gastro-intestinal protozoan parasite (Table 4.2). In descending order, the parasites found included: *Entamoeba* spp. (100%), *Balantidium coli* (89%), *Tritrichomonas suis* (89%) and coccidia spp (86%). The majority (68%) of the pigs had a light infection with coccidia. Most (72%) of the pigs had a heavy infection of *B. coli* (Fig 4.5). Similarly, majority of pigs had a heavy *Tt. suis* and *Entamoeba* spp. infection.

Table 4.2. **Prevalence and intensity of gastrointestinal protozoan infections observed in 37 slaughtered pigs from Busia District**

Parasite	Prevalence (%)	Intensity of parasites				
		Mean OPG	Min	Max	Light%	Heavy%
Entamoeba spp	100	-			8	92
Balantidium coli	89	11.5	0	32	28	72
Tritrichomonas suis	89	-	-	-	35	65
Coccidia	86	7,687	0	28,000	68	32

Key:

I Coccidia oocysts were observed in rectal faeces using the McMaster method and classified as: 1- <2000 oocysts per gram (OPG) = light infections, >2000 OPG = heavy infection

II *Balantidium coli* observed using a wet preparation of caecal contents and classified as 1-<5 trophozoites = light infection, > 5 trophozoites=heavy infections

III *Entamoeba* spp. and *Tritrichomonas suis* oocysts were observed on a wet preparation of caecal contents. Light infection = < 20 oocysts/microscope field, Heavy infection= >20 oocysts/microscope field

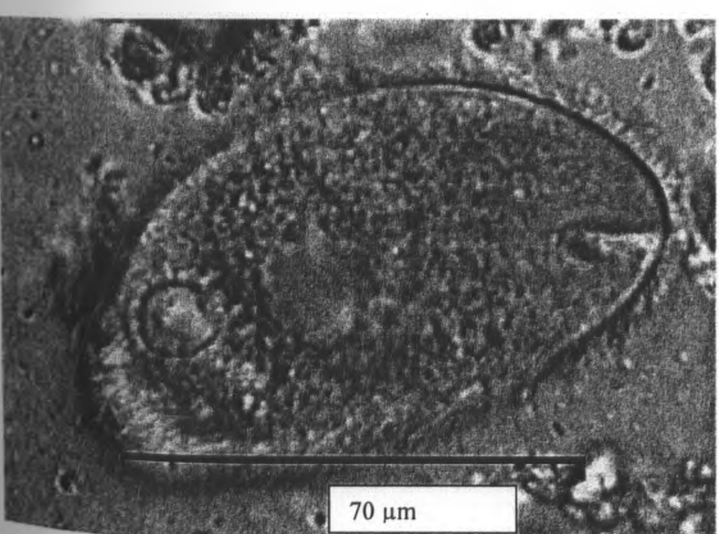
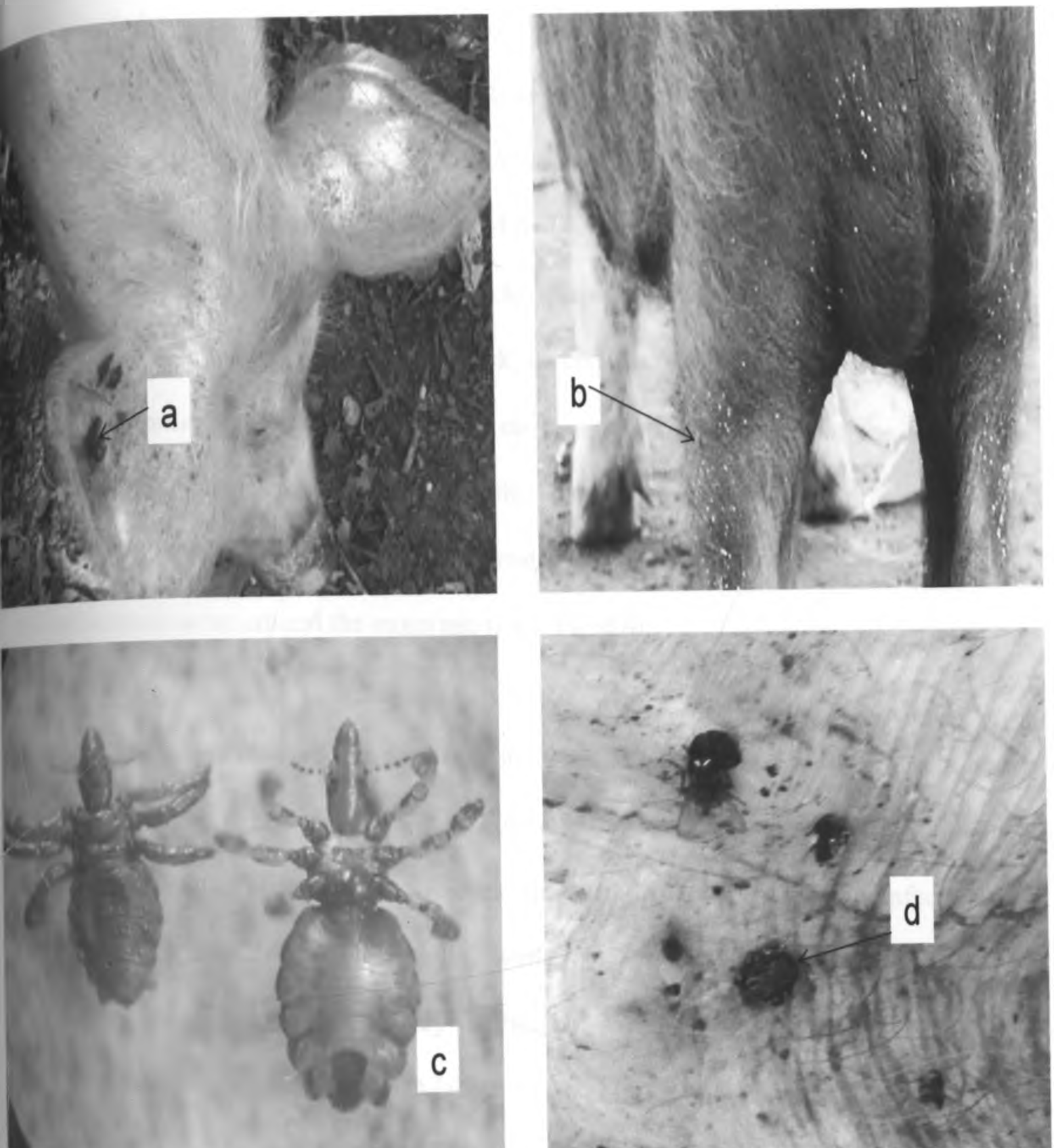


Fig. 4.5. ***Balantidium coli*** **trophozoite from cecum sample of a pig from a slaughter slab in Busia District. Image taken at x1000 magnification.**

Skins from thirty three (33) carcasses were examined for the ectoparasites. Four types of external parasites were observed and included mites, hog louse, ticks and jiggers. The majority (65%) of pigs had lesions caused by *Sarcoptes scabiei* mites, but only 32% were positive by microscopy. Out of 65%, 58% were either chronic (58%) or acute (7%) (as judged by skin lesions) and were mainly found along the ears, backline and flank (Fig 4.6). The chronic lesions were characterized by hyperkeratinization, encrustations and flakes.

A total of 28 pigs (85%) were found to be infested with the *H. suis* (hog lice), majority (61%) had a heavy infestation. The adults and nymphs were found on the ears and neck region in 73% of the cases, while others were distributed in other parts of the body. The nits were found in a fewer number of pigs (58%) and were located along the pre-scapular, inguinal area and hind quarters (buttocks) (Fig 4.6). A total of 5 pigs (15%) were infested with jiggers, whose number ranged between 1 and 17 per pig. The jiggers were mostly (3/5) located in the hoof skin junction (corneum). Other locations included the skin of the axillae and teats' region. An antemorten examination of one the heavily infested pigs (with 17 jiggers) showed that the toes in the hind limb were malformed by the bulging jiggers which caused the necrosis of the neighbouring tissues and caused the pig to limp.



Key: a= lesions associated with mange (note the bleeding ear due to aggressive scratching)
b= nits of lice on a boar
c= *Haematopinus suis* showing the dorsal and ventral sides
d= *Amblyomma variegatum* ticks attached to the skin (note the prominent inflammation)

Fig 4.6. Some of the ectoparasites and associated lesions observed on the pigs in Busia District

A total of 13 pigs (40%) were found to be infested with tick of various species including *Rhipicephalus appendiculatus* (27%), *Boophilus decoloratus* (15%) and *Amblyomma variegatum* (6%). The mean numbers of ticks per pig were 2 (range = 0-22) for all types of ticks, 1.1 (range =0-7) for *Rhipicephalus appendiculatus*, 0.2 (range = 0-2) for *Boophilus decoloratus* and 0.7 (range = 0-17) for *Amblyomma variegatum* (6%). Most of the pigs had only a light infestation of ticks, mainly (33%) of one species. Concurrent infestations with either two or three tick species were only found in two pigs. In descending order, the ticks were located on the inner or outer side of the ear pinnae (92%), around the eye margins (15%), on the udder (8%), neck (8%), and inguinal areas (1%). The ticks caused a prominent inflammation on the skin characterized by reddening and edematous areas around the engorged ticks (Fig 4.6).

Trypanosomes were observed in only one pig (3%). The trypanosomes were preliminary diagnosed as belonging to the *T. brucei* sub-group, based on the morphological characteristics. Other post mortem lesions observed in the trypanosome infected pig included splenomegally, hepatomegally, white patches/spots on the epicardium and petechial haemorrhages in the kidney capsule. In particular the liver was flabby and oedematous, had many white patches (about 50), and white nodules were observed at the surface but also extended into parenchyma. At histology, mononuclear cellular infiltrations, mainly lymphocytes, were observed in the liver, kidney and heart. The pig was also infected with *Oesophagostomum* spp, *A. suum*, coccidia oocysts, lungworms, *B. coli*, *Tt. suis*, *Entamoebae* and *Sarcoptic scabiei*. The PCV in the pig was also relatively lower (33%) compared to the average of 38% in the others.

None of the carcasses was found to have cysticercosis and all the serum samples collected were negative for *Taenia solium* cysticerci antigens. All the diaphragms were inspected for *T. spirallis* larvae using the trichnoscopy method and none of the sections had any larvae.

4.3.2 Farm survey

4.3.2.1 Origin of sampled pigs

Eleven (11) villages from the six divisions in Busia District were visited during the survey (Table 4.3.). A total of 306 pigs from 135 farms were sampled.

Table 4.3. Number of pigs and farms sampled in different villages in Busia District

Division	Village	Farms sampled	Animals sampled	Mean no. of pigs sampled per farm
Township	Mauko B	9	27	3 (1-6)
	Bulada	8	31	3.9 (1-6)
Nambale	Segero A	13	26	2 9 (1-4)
	Makongeni	17	39	2.3 (1-7)
Matayos	Buriang	14	35	2.5 (1-4)
	Nahakina	19	39	2.1 (1-4)
Butula	Bumala town	8	17	2.1 (1-5)
	Bujumba	13	23	1.8 (1-4)
Funyula	Namioso	13	25	1.9 (1-5)
	Sichehe	10	14	1.4 (1-3)
Budalang'i	Bukani	12	30	2.5 (1-7)
Total		135	306	2.2 (1-7)

In Budalang'i Division, a previous outbreak of African Swine Fever (OIE/WAHID, 2007) decimated the pig population resulting in very low populations and thus only one village was selected.

4.3.2.2 Nematodes infections in pigs

4.3.2.2.1 Overall prevalence and intensity

The prevalence of nematodes in the sampled pigs is shown in Table 4.4. The overall prevalence of any nematodes (as determined by faecal egg shedding, including *Metastrongylus* spp) infection was 84.2%. *Oesophagostomum* spp was the most prevalent (74.8%) nematode followed by *S. ransomi* (36.6%) while *P. sexalatus* was the least prevalent (3.6%). Only 3 % of the pigs were found to shed trematode eggs, whose species could not be identified. These trematodes eggs were of similar morphology to that described for *Fasciola* spp.

Table 4.4. Prevalence of nematodes in pigs from 135 farms in Busia District

Nematode	Prevalence (%) of infected pigs	Prevalence (%) of infected* farms
All nematodes (one spp or mixed)	84.2	89.6
Oesophagostomum spp	74.8	84.4
Ascaris suum	17.6	30.4
Trichuris suis	6.5	14.1
Strongyloides ransomi	36.6	56.3
Metastrongylus spp	9.8	18.5
Physocephalus sexalatus	3.6	5.2

***Infected**= at least one pig in a farm with an infection

The percentage of farms with pigs infected with any of the nematodes (single or mixed species) was 89.6%. The farm prevalences were *Oesophagostomum* spp (84.4%), *S. ransomi* (56.3%), *A. suum* (30.4%), *Metastrongylus* spp (18.5%), *T. suis* (14.1%) and *P. sexalatus* (5.2%).

There was a positive correlation between prevalences of *Oesophagostomum* spp., *A. suum*, *T. suis* and *S. ransomi* at animal level. This correlation was only significant when the prevalence of *Oesophagostomum* spp was compared with that of *S. ransomi* (r^2 = 0.58, p<0.001).

The intensities of nematode infections are shown in Table 4.5. The overall mean EPG of all (one or mixed species) nematodes was 2,355 and ranged from 0-35,000 EPG in individual pigs. The highest mean EPGs were observed for *Oesophagostomum* spp (mean = 1,060, range=0-22,800), *S. ransomi* (840, 0-26,600) and *A. suum* (345, 0-15,200). The lowest mean and range EPGs was observed for *T. suis* (57, 0-4400), *Metastrongylus* spp (39, 0-2,000) and *P. sexalatus* (16, 0-1,200). Fifty two percent (52%) of pigs were excreting nematode eggs (single or mixed species) of less than 1000 EPG. However, a substantial number (10%) of pigs were found to be excreting a high number (>5,000) of nematode eggs. *Oesophagostomum* spp and *S. ransomi* were major contributors to the occurrence of high mean EPGs.

Table 4.5. Distribution of nematode EPG values in 306 pigs sampled in Busia District

Range of EPG counts	Number of pigs infected with a given nematode (proportion)					
	All spp	Oeso	*S. ransomi*	*A. suum*	*T. suis*	Meta
<200	48 (16%)	77 (25%)	193 (63%)	252 (82%)	286 (94%)	275 (89%)
200-900	103 (34%)	95 (31%)	74 (24%)	38 (12%)	16 (5%)	27 (9%)
1000-1900	64 (21%)	70 (23%)	13 (4%)	4 (1%)	2 (1%)	2 (1%)
2000-2900	31 (10%)	22 (7%)	3 (1%)	5 (2%)	0 (0%)	1 (<1%)
3000-3900	8(3%)	8 (3%)	5 (2%)	1 (<1%)	0 (0%)	0 (%)
4000-4900	15 (5%)	8 (3%)	4 (1%)	0 (0%)	2 (1%)	0 (0%)
5000 and more	30 (10%)	6 (2%)	13 (4%)	1 (<1%)	2 (1%)	0 (0%)

Key: Oeso. = *Oesophagostomum* spp, *S.ransomi* = *Strongyloides ransomi*, *A. suum* = *Ascaris suum*, *T. suis* = *Trichuris suis*, Meta = *Metastrongylus* spp,

4.3.2.2.2. Multiple nematode infections in sampled pigs

Majority of the pigs had monospecific infections of *Oesophagostomum* spp (Table 4.6). It was only in a few animals that monospecific infections of *S. ransomi, A. suum* and *P. sexalatus* were observed. Multiple nematode infections were also observed, with the highest combination being that of *Oesophagostomum* spp and *S. ransomi* (16.7%). Only 2 pigs were infected with 5 types of nematodes, and none was infected with all types of nematodes.

Table 4.6. Distribution of nematodes infections in 306 pigs from 135 farms in Busia District

Nematode combination	Animals	Percentage (%)
Mono-infections		
Oesophagostomum spp alone	105	34.3
Strongyloides ransomi alone	18	5.9
Ascaris suum alone	4	1.3
Physocephalus sexalatus alone	1	0.3
Multiple infections	178	58

4.3.2.3 *Taenia solium* cysticercosis

The sera of 284 pigs were examined for antigen of *T. solium* cysticerci. The total number of pigs testing positive was 11, resulting in a pig prevalence of 4% (95% CI: 1.9-6.2%). These infected pigs originated from Township (2 pigs), Nambale (3), Matayos (2), Budalangi (2), Funyula (1) and Butula (1) divisions. The percentage of farms with a pig infected with cysticercosis was 9%.

4.3.2.4 Gastrointestinal protozoan infections

The GIT protozoan infections observed included *Entamoeba* spp. (87%), *B. coli* (64%), *Tt. suis* (42%) and *Coccidia* spp. (33%). The mean coccidial oocysts per gram (OPG) of all the sampled pigs was 1,276 (range = 0-28,000 OPG). From a pooled sample, the coccidial oocysts were of *Eimeria* spp and the proportions of species observed in descending order included *E. debliecki* (40%), *E. suis* (26%), *E. porci* (16%), *E. scabra* (13%) and *E. polita* (5%). The only significant correlation was that between prevalences of *B. coli* and *Entamoeba* (r^2=0.21, p=0.0002) and *Tt. suis* and *Entamoeba* spp (r^2= 0.28, $p<0.0001$). The percentage of farms infected with *Entamoeba* spp, *B. coli*, *Tt. suis* and coccidia spp were 94.1%, 77%, 56.3% and 45.9%, respectively.

4.3.2.5 Ecto-parasite infestations

The types of ectoparasite infestations observed in this study included ticks (*Ixodid* spp), *H. suis* (hog lice) and *Sarcoptes scabiei* (mange). The overall prevalence of ticks was 29.7% (95% CI = 24.6-34.9%) and the observed species included *Rhipicephalus appendiculatus*, *Boophilus decoloratus* and *Amblyomma variegatum*, in proportions of

70%, 31% and 12%, respectively. The tick specific-herd prevalence was 46.7% (95% CI =38.1-55.2%).

At animal level, the overall prevalence of *H. suis* (pediculosis) and clinical mange was 96.1% (95% CI = 93.9-98.3%) and 63.7% (95% CI = 58.3-69.1), respectively. The percentage of farms infested with *H. suis* and clinical mange was 99% and 78.7%, respectively. Pigs with heavy infestation (90%) of *H. suis* were more than those with light (10%) infestation.

4.3.2.6 Trypanosome infection

Only one (0.3%) case of trypanosome infection (*T. brucei* spp) was observed using the buffy coat method. The infected pig originated from Township Division and the trypanosomes were only observed using the buffy coat method, while sub-inoculation in mice did not produce a patent infection. The pig was a female, white grower, which appeared healthy but lethargic.

4.4 DISCUSSION

The current study consisted of slaughter slab and farm surveys which showed that pigs kept extensively on small holder farms in Busia District, Kenya have a wide variety of parasites. The two types of studies were complimentary. Although slaughter slab surveys has some limitations including non-determination of the origin of pigs and slaughtering of only a specific age group, the occurrence and importance of some parasites such as lungworms and ascarids are more appreciated at post mortem.

There is limited literature on parasites affecting pigs in Africa, although a few detailed studies have been conducted in Nigeria (Ajayi *et al*., 1988) and Ghana (Permin *et al*, 1999). The wide spectrum of parasites reported in this study is similar to that reported for free range pigs in other African countries but is in contrast with studies on pigs kept indoors (Roepstorff *et al*., 1998, Kagira, 2001), in which only a limited number of parasites have been reported. It is possible that the aggressive chemotherapy and improved husbandry conditions in the farms keeping pigs under intensive indoor conditions drastically reduces the spectrum of parasites. On the other hand, there is high parasite risk in pigs kept under the traditional production system where poor husbandry, warm climate and high rainfall allow for the continuous proliferation of parasites in the enviroment.

For the slaughter-slab survey, all (100%) the pigs were shedding eggs of *Oesophagostomum* spp, which was in contrast to the farm survey where 75% of the pigs were infected with this nematode. Other studies have also reported that the nodular worms are the most common nematodes on pig farms, worldwide. In these studies, a prevalence of 28- 50% of *Oesophagostomum* spp has been reported in indoor pigs in Central Kenya (Langat, 1999, Kagira *et al*., 2001), outdoor pigs in Nigeria at 70% and 61% in Ghana (Ajayi *et al*., 1988, Permin *et al*., 1999). The higher prevalence reported at slaughter slab level could be related to the fact that all the pigs slaughtered were adults, which have a longer exposure period compared to other categories of pigs. *Oesophagostomum* spp has low immunogenicity and thus infected animals only develop limited immunity (Roepstorff and Nansen, 1994). In the slaughter survey, the nodular lesions caused by this worm were observed only in 69% of pigs, possibly due to complete healing of some lesions or missing out of small nodules. These lesions are caused by mucosal larval migration and burrowing of nodular worms and in some cases, these nodules may be supra-infected by other gut flora including bacteria and protozoa leading to development of enteritis (Steenhard *et al.*, 2000). Although, larval stages of the nodular worm are hard to distinguish, a recent abattoir study examining adult worms found that both *O. quadrispinalatum* and *O. dentatum* exist in indoor pigs in Kenya (Ng'ang'a *et al.*, 2007).

A substantial number of pigs in the current study had a high intensity of *Oesophagostomum* spp infection, which could warrant deworming. The mean EPG of *Oesophogostomum* spp (1,060-1,778) observed in this study was higher than that reported in outdoor pigs in Ghana (262) (Permin *et al.*, 1999) and indoor pigs in Kenya (Kagira *et al.*, 2001). The threshold EPG needed before treatment of GIT nematodes of pigs has been indicated as 500 (Soulsby, 1982; Roepstorff and Nansen, 1994) and thus most of the pigs in the current study required deworming. A high intensity of *Oesophagostomum* spp infection causes amongst others reduced weight gains (Hale *et al.*, 1981) and thus causes economic loss to the farmer. There are several factors which could explain the high prevalence and intensity of *Oesophagostomum* spp in pigs raised on pasture including favourable conditions for continuous exposure and poor nutrition (Pattison *et al.*, 1980). Majority of the pigs in the current study were also tethered, often in one spot for prolonged periods, which may increase exposure to infected vegetation.

The prevalence of *A. suum* recorded in the slaughter slab and farm surveys was 19% and 18%, respectively. In studies which have examined all categories of pigs, high prevalences (53-90.4%) of *A. suum* have been reported in outdoor pigs from Nigeria (Ajayi *et al.*, 1988), Ghana (Salifu *et al.*, 1990), and Denmark (Roepstorff and Jorsal 1989). However, the prevalence of *A. suum* reported in the current study was more than that reported in Tanzania (12%) (Esrony *et al.*, 1997), Ghana (12.7%) (Permin *et al.*, 1999) and Kenya (8.3-11%) (Langat, 1999; Kagira *et al.*, 2001). The differences in prevalence could be attributed to different production systems in pigs in the different studies. The intensity of *A. suum* (mean EPG=345) in the farm survey was higher than that reported for the slaughter slab survey (mean EPG=45) and this could be due to the sampling of only adults in the slaughter survey which are more resistant to *A. suum*. Ascarids are known to cause economic losses mainly through reduction in production potential and condemnation of infected livers at inspection.

In the slaughter slab survey, 43% of the pigs had milk spots which could be associated with migratory stage of ascarids. However, in the study area, livers were only trimmed and were rarely condemned due to the presence of these spots (DVO, personal communication). The spots observed were chronic in nature and no parasites were observed in the livers. Although it is possible for migrating flukes and lungworms to cause similar lesions in the liver, most authors have postulated that *A. suum* larvae are the main causes of milk spots in the liver (Wagner and Polley, 1997; Bernado *et al.*, 1990). These spots are a manifestation of host immune response towards all the migratory sites of the ascarid where the larvae are eliminated during inflammatory process (Bernado *et* al., 1990). The variation between prevalence of pigs with milk spots and those excreting eggs have been reported previously (Bernado *et al.*, 1990). In the studies by Bernado *et al* (1990) and Langat, (1999) milk spots were found to occur in the absence of intestinal ascarids in 51% and 16% of the pigs, respectively. The possible causes of the lack of correlation in the two measurements include: presence of only male ascarids in some of the pigs (sex ratio imbalance, thus no eggs were shed in some pigs), recent uptake of eggs and larval migration, but not enough time had elapsed to allow for the adult ascarids to produce the eggs, and the infection could have been cleared by the host immune response, but the lesions were yet to resolve.

Pigs which were found to be shedding the eggs of *Metastrongylus* spp at the slaughter slab and farm level were 54% and 9.8%, respectively. The two species of lungworms which were found in the slaughtered pigs were *Metastrongylus elongatus* and *M. pudendoctus*. This is the first report showing the presence of lungworms in pigs in Kenya and is posiibly because other studies in the country have only focussed on pigs kept indoors (Kagira *et al.*, 2001, Wabacha, 2001) which might not access earthworms, the obligatory intermediate stages of lungworms (Soulsby, 1982). *Metastrongylus* spp has however, been reported in outdoor pigs in Nigeria (66%) (Ajayi *et al.*, 1988) and Ghana (19%) (Permin *et al.*, 1999). The intensity of *Metastrongylus* spp was higher in the slaughter slab (mean EPG =589) than farm survey (mean EPG=39). This can be compared to mean EPG of 107, observed in outdoor pigs in Ghana (Permin *et al.*, 1999).

The contrasting prevalences and intensities of *Metastrongylus* spp between the slaughter slab and farm surveys could be related to several factors including: category of animal slaughtered and seasonal influence. It is important to note that although 54% of the pigs in slaughter slab study were excreting the lungworm eggs, 97% of the pigs had lungworms in the diaphragmatic lung lobes. This shows that diagnosis at meat inspection is more sensitive than the faecal examination. Similar findings have been recorded by other authors (Corwin *et al.*, 1986; Permin *et al.*, 1999). In the study by Permin *et al.*, (1999) in free range pigs in Ghana, a prevalence of 19% and 83% was reported for faecal and post-mortem lung examination, respectively. The variation between the faecal examination and presence of worms in the lungs emanates from the fact that in some pigs the lungworms had not yet completed their life cycle and eventual production of eggs. Further, lungworms are known to produce relatively fewer eggs, and thus it is sometimes harder to find the eggs in faeces when using the regular detection methods (Roepstorff and Nansen, 1994). The lung pathology due to lungworms observed in the current study could severely constrain the productivity of the free range pigs. The occurrence of pneumonia due to lungworms has been reported from other studies (Soulsby, 1982), and may be a cause of persistent coughing reported by the farmers (see chapter 3).

The prevalence of *S. ransomi* in slaughter slab and farm survey was 16% and 37%, respectively. This prevalence was higher than that reported in intensive farms in Kenya (2%) (Kagira *et al.*, 2001) and Tanzania (9%) (Esrony *et al.*, 1997) but was lower than that reported in free range pigs in Nigeria (55%) (Ajayi *et al.*, 1988). The intensity of *S. ransomi* infection was higher in farm survey (mean EPG=840) than slaughter slab survey (mean

EPG=97). The observed differences in prevalences and intensities could be due to the variation in age groups of pigs sampled by the different studies. The effects of *S. ransomi* are maximal in piglets and weaners where it causes profuse diarrhoea, decrease in daily weight gain, PCV reduction and feed conversion inefficiency in piglets (Hale and Marti, 1984).

The prevalence of *T. suis* in the slaughter slab and farm survey was 3% and 7%, respectively. Low prevalence of *T. suis* has been reported in indoor pigs in Kenya (7%) (Kagira *et al.*, 2001) and outdoor pigs in Ghana (5%) (Permin *et al.*, 1999) and Nigeria (6%) (Ajayi *et al.*, 1988). The development of *T. suis* in the pastures is relatively slow and it has been observed that although a fraction of eggs may develop within few weeks, majority of the eggs may take months or a year before full development (Roepstorff and Murrell, 1987). Also the nematode has a longer pre-patent period when compared with the strongyles and strongyloids and may thus take long before eggs are shed by infected pigs (Roepstorff and Murell, 1987).

The prevalence of *P. sexalatus* in the slaughter slab and farm surveys was 24% and 4% respectively. In other studies, *P. sexalatus* was reported in outdoor pigs in Ghana (17%) (Permin *et al.*, 1999), and Nigeria (57%) (Ajayi *et al.*, 1988) and Belize (1%) (Gibbens *et al.*, 1989). The intensities of *P. sexalatus* was higher in slaughter slab (mean EPG=164) than farm survey (mean EPG=16). *Physocephalus sexalatus* is a stomach nematode which is common in free-range pigs, which become infected by eating the infected coprophagous beetle. Infected pigs do not exhibit any noticeable symptoms, although gastritis and anemia has been reported (Soulsby, 1982). *Globocephalus urosubulatus* was observed in 6 % of the slaughtered pigs, while in outdoor pigs in Nigeria and Belize, the parasite was observed in 66% and 25% of the pigs (Ajayi *et al*., 1988; Gibbens *et al*., 1989), respectively.

In the farm survey, 3% of the pigs were found to shed trematode eggs, whose species could not be identified. The morphology of the eggs was similar to that described for *Fasciola* spp in ruminants. In a study by Permin *et al*., (1999) in Ghana, a low prevalence of *Schistosoma suis* (0.4%) and *Paragonimus* spp. (0.8%) was observed. Boes *et al*., (2000) reported a trematode prevalence of 1% in pigs in China, while Capucchio *et al*., (2009), reported a fasciolosis prevalence of 2% in feral pigs slaughtered in Italy. Compared to ruminants, pigs are regarded as highly resistant to infection with *F. hepatica* (Nansen *et al*., 1974). Further, McMaster technique has a low sensitivity for detection of trematode eggs. Sedimentation techniques as described by Roepstorff *et al* (1998) would be appropriate for detection of trematode eggs and should be included in future studies to give a better picture of the prevalence. Ruminant fasciolosis is highly endemic in Busia District, where up to 30% of cattle livers are routinely condemned due to fasciolosis (Busia DVO, unpublished information).

Apart from *Oesophagostomum* spp, the occurrence of other nematodes can be described as overdispersed since they were only parasitizing a few hosts. Similar observations have been made for a number of nematodes such as *A. suum*, *H. rubidus*, *M. apri*, *P. sexalatus* and *T. suis* (Boes *et al*., 1998; Permin *et al*., 1999, Nejsum *et al*., 2009). Overdispersal has also been reported in *Oesophagostomum* spp in indoor pigs (Christensen *et al*., 1995; Kagira, 2001). The major factors thought to contribute to this negative binomial distribution include: heterogeneity in the host behaviour influencing the uptake of infective stages, high heritability in genes responsible for specific infection, spatial heterogeneity in the distribution of infective stages and heterogeneity in effective immunity within the host population due to the past experiences of infection (Boes *et al.*, 1998, Nejsum *et al.*, 2009). The present study showed that most of the sampled pigs had monospecific infections of *Oesophagostomum* spp, which is similar to other studies (Dangolla, 1994; Kagira, 2001). The highest association of concurrence in indoor pigs were found between *Oesophagostomum* spp and *A. suum* (Dangolla, 1994; Kagira, 2001), which is different from the current study where *Oesophagostomum* spp and *S. ransomi* concurrent infections were the commonest.

Cysticercosis was only recorded in the farm survey in the current study, where a prevalence of 4% was noted. The lack of cysticercosis in slaughtered pigs even when both visual and Ag-ELISA were used could be due to the low number of pigs sampled. The prevalence of *T. solium* reported in the farm survey was lower than that reported by Githigia *et al.*, (2006) (11%) and Mutua *et al.*, (2007) (7%) in Busia and Teso districts, respectively. The antigen ELISA method used (which detects ongoing infection) in the current study has a sensitivity of between 85 and 97% while that of lingual palpation is 21% (Sikasunge *et al.*, 2006) and thus the results of this study are more definitive. Improper identification of lingual cysts of pig cysticercosis has been observed in previous studies in Western Kenya (Downie-Ngini, 2007). In general, the prevalence of *T. solium* reported in Western Kenya is relatively lower than that reported in other sub-Saharan countries such as Tanzania (up to 17%) by Ngowi *et* *al.*, (2004), Uganda (up to 45%) by Mafojane *et al.*, (2003) and Zambia (51%) by Sikasunge *et al.*, (2006). The differences could be due to factors including higher levels of risk factors and higher numbers of free-ranging pigs in those countries.

In the current study, four gastrointestinal protozoan parasites including coccidia, *B. coli*, *Tt suis* and *Entamoeba* spp were detected in the faecal samples of the pigs. The prevalence of coccidia was higher in the slaughtered pigs (86%) than in those sampled at farm survey (33%). Majority of pigs in both cases had a light infection. The prevalence reported at farm level in this study was lower than that reported in free-range pigs in Ghana (77%) (Permin *et al.*, 1999) and Nigeria (81%) (Ajayi *et al.*, 1988) but was higher than that reported for indoor kept pigs in Kenya (15% - 20%) (Langat, 1999, Kagira *et al.*, 2001) and Nordic countries (17%) (Roepstorff *et al.*, 1998). It is important to note that in Ghana and Nigeria where a high prevalence of coccidiosis was reported, the pigs are kept in free-range conditions, showing that outdoor environments are conducive for the proliferation of coccidia. In the current study, the mean OPG was high (7,687 for slaughter slab survey and 1,267 for farm survey), with several pigs having high number of oocysts (over 20,000 OPG). Similar high levels of coccidian OPG have been reported in free-range pigs in Papua New Guinea (Varghese 1986) and Ghana (Permin *et al.*, 1999). Five species of *Eimeria* were observed in this study, with *E. debliecki* and *E. suis* being the most common. Similarly, *E. debliecki* was observed to be the most common species of coccidia in free range pigs in Zimbabwe and Papua New Guinea (Chhabra and Mafukidze, 1992, Varghese, 1986). Although *Eimeria* spp are not a major cause of diarrhoea in pigs (compared to *Isospora suis*), some species of including *E. debliecki* cause intestinal inflammation, atrophy of villi, diarhoea and weight loss (Lindsay *et al.*, 1987, Hill *et al.*, 1985).

The prevalence of *B. coli* in the slaughter slab and farm surveys was 89% and 64%, respectively, with most pigs having a heavy infestation. There are no previous reports of this parasite in pigs or other livestock in Kenya. The prevalence of *B. coli* in this study was higher than reported in pigs in China (47%) (Weng *et al.*, 2005) and Ghana (19%) (Permin *et al.*, 1999), but lower than that reported in wild pigs in Iran (100%) by Solaymani-Mohammadi *et al.*, (2004). This high prevalence in the slaughter slab survey in the current study could be due to the fact that the sampled animals were adults (having prolonged exposure) and the samples used were from ceca. *Balantidium coli* mainly live in the cecum, where it causes minimal pathology, but is of zoonotic significance (Schuster and Visvesvara, 2004). The pig to human transmission occurs in areas where pigs are in close contact with humans, and there is lack of sewage and sanitary facilities (Schuster and Visvesvara, 2004); conditions that were common in the study area.

Entamoeba species was observed in pigs at both the slaughter slab (100%) and farm levels (87%), with majority of the animals having a heavy infection. Although this parasite was not characterized to species level, the species of *Entamoeba* which can be found in pigs include *E. suis* and *E. polecki* (Ajayi *et al.*, 1988; Salifu *et al.*, 1990). These species are morphologically similar to and often confused with *E. histolytica*. *Entamoeba polecki*, is of high zoonotic potential and is a frequent inhabitant of the intestine of pigs worldwide, with a prevalence of up to 25% reported in some countries (Barnish and Ashford, 1989; Pakandl, 1994; Solaymani-Mohammadi *et al.*, 2004).

Tritrichomonas suis was found in pigs at both the slaughter slab (89%) and farm studies 43%, with most pigs having heavy infection. The high prevalence in slaughtered pigs could be due to the fact the *Tt. suis* mainly inhabits the cecum (Tachezy *et al.*, 2002), from where the samples were obtained. Natural infection of pigs with *Tt. suis* has been reported from several studies (Packandl, 1994; Solaymani-Mohammadi, *et al.*, 2004). The prevalence in the current study was lower than that reported in pigs in Czech Republic (90%) (Packandl, 1994), but higher than that reported in wild boars in Iran (25%) (Solaymani-Mohammadi, *et al.*, 2004). The parasite can cause occasional rhinitis but the intestinal pathogenicity remains uncertain (Tachezy *et a*l., 2002; Solaymani-Mohammadi *et al.*, 2004). *Tt. suis* and *Trichomonas foetus* are closely related and recent genomic studies have shown that they are one species with pigs acting as a natural reservoir (Tachezy *et al.,* 2002). The zoonotic significance of the trichomonads was highlighted recently where fatal human meningitis caused by *T. foetus* occurred in an immuno-suppressed human being indicating that it could be important as a concurrent infection in HIV/AIDS (Okamoto *et al.*, 1998).

The ectoparasites found in pigs in the two surveys included mites (*S. scabiei*), lice (*H. suis)*, jiggers (*T. penetrans)* and ticks (*Ixodid* spp). The prevalence of *Sarcoptes scabiei* mange in the slaughtered pigs by lesions was 66% while in farm survey it was 64%. The specificity of the dermatitis lesions due to *S. scabiei* as an indicator of sarcoptic mange has been estimated to be between 79% and 92% (Davies, 1995) and the occurrence of these lesions in slaughter pigs have been used previously to advice farmers on control measures (Cargill *et al.*, 1997). The prevalence of clinical mange pigs reported in the current study was higher than that reported in other studies in Ghana 38% (Permin *et al.*, 1999), Botswana 40% (Nsoso *et al.*, 2000) and Tanzania 52% (Kambarage *et al.*, 1990), but was lower than that reported in breeding farms in Spain 93% (Alonso *et al.*, 1998). Several factors suspected to be the cause of high prevalence of sarcoptic mange in pigs include free-range conditions and poor husbandry skills eg., lack of spraying. *Sarcoptes scabiei* is known to be pathogenic to pigs, causing skin lesions and pruritus, and can reduce the production efficiency in both breeding and fattening pigs (Davies, 1995). It will be important to devise an effective control strategy for control of mange in pigs in the extensive system of production.

The prevalence of pediculosis (*H. suis* infestation) in the slaughter slab and farm survey was 85% and 96%, respectively. This study is the first report on occurrence of *H.suis* in pigs in Kenya. The animal prevalence reported in this study was higher than that reported in Ghana (66.7%) by Permin *et al.*, (1999), but of similar range with that reported in indigenous pigs from Botswana (100%) (Nsoso *et al.*, 2006). Although knowledge on the possible risk factors for transmission of *H. suis* is scanty, pasturing of pigs, purchase of replacements from infected farms, keeping of pigs in dirty and unhygienic conditions have been indicated to cause an increase prevalence of hog louse on a farm (Damriyasa *et al.*, 2004; Nsoso *et al.*, 2006). Hog louse is a major cause of pruritis and anemia in pigs and may thus cause a reduction in animal productivity (Davis and Williams, 1986; Nsoso *et al.*, 2006). Further, *H. suis* are potential vectors of several parasites including *Mycoplasma suis* (formerly *Eperythrozoon*) (Permin *et al.*, 1999; Damriyasa *et al.*, 2004). The suggested transmission of African Swine Fever by hog louse has however not been confirmed; it would be important to determine their importance based on the fact that Busia District is endemic for ASF (OIE-WAHID, 2007).

The prevalence of tick infestation in slaughter slab and farm survey was 40% and 30%, respectively. The pigs were found to be infested with ticks of various species, including *R. appendiculatus*, *B. decoloratus* and *A. variegatum*. This study is the first report on occurrence of ticks in pigs in Kenya. Most of the pigs had only a light infestation, mainly of one species. Literature on occurrence of ticks in pigs is scanty possibly because most studies have been undertaken in indoor pigs, where infestation with ticks is expected to be minimal. However, in extensive systems where pigs are reared in pasture or are scavengers, tick transmission either between pigs or from other livestock is common (Holness, 1999). The prevalence reported in this study was lower than that reported in free-range pigs in Botswana (100%) (Nsoso *et al.*, 2006) and Ghana (58%) (Permin *et al.*, 1999) and wild pigs in USA (99%) (Greiner *et al.*, 1994). Permin *et al.*, (1999) reported three species of ticks (similar to the current study), while Nsoso *et al.*, (2006) observed only *Rhipicephalus evertsi evertsi* and *Amblyomma hebraeum*. The tick-load even in other studies was low, ranging between 1 – 10 ticks per pig (Nsoso *et al.*, 2006, Labruna *et al.*, 2002). It is highly possible that pigs could be getting these ticks mainly from other livestock since in most cases the animals are grazed and kept together. A study by Karanja (2005) in the Busia District showed the same spectrum of ticks in cattle. Ticks are known transmitters of tick-borne diseases including ASF, classical swine fever, babesiosis, anaplasmosis and eperythrozoonosis (Heuschele and Coggin, 1965; Permin *et al.*, 1999; Nsoso *et al.*, 2006).

Another ectoparasite which was found in slaughtered pigs was the jigger (*Tunga penetrans*). In the slaughter survey, 15% of the pigs were infested with jiggers mostly located in the hoof skin junction (coronet). *Tunga penetrans* is one of the neglected zoonosis which is endemic in Latin America, the Caribbean and sub-Saharan Africa, with point prevalence rates in human populations of 21–83% reported from poor communities (Ade-Serrano and Ejezie, 1981; Chadee, 1998; Heukelbach *et al.*, 2004). Although the disease is common in the human populations in Kenya (Website: www.jigger.co.ke), no systematic studies have been conducted. Literature is scarce on systematic studies on animal reservoirs, although various incidental reports have found both domestic and wild animals to be infected (Heukelbach *et al.*, 2004). Heavy infestation with jiggers may cause locomotor problems in pigs (Heukelbach *et al.*, 2004), and where sow teats are affected agalactia and subsequent starving of the piglets (Verhulst, 1976) is common. Recent work in Nigeria (Ugbomoiko *et al.*, 2007) showed that the presence of the sandy soil and pigs within a human compound were most important risk factors for tungiasis; and these factors are common in Busia District. Any successful tungiasis control will require combined control of the infestation in infested people and animal reservoirs.

In both the slaughter-slab and farm survey, trypanosomes were observed in one pig. The trypanosomes were morphologically diagnosed as *T. brucei*. In the slaughtered infected pig, features typical of trypanosomosis were observed including splenomegally, hepatomegally, cardiac and renal changes. The presence of trypanosome infections in pigs in Busia District has been highlighted by Angus, (1996) and Ng'ayo *et al.*, (2005), where a prevalence of 2-5% was reported. It would be important to clarify if the *T. brucei* parasite observed was the zoonotic *T.b. rhodesiense* since in neighboring Uganda; pigs have already been shown to be a major reservoir for the *T.b. rhodesiense* (Magona *et al.*, 1999; Waiswa *et al.*, 2003). The pig had lower PCV than other non-infected pigs possibly due to trypanosome infection, although the occurrence of concurrent parasitic infections in pigs is thought to increase the pathogenicity of the trypanosomes (Stephen, 1986).

Based on the diagnostic methods used in the slaughter slab survey, there were no cases of richnellosis. Trichnellosis was previously reported in Kenya in 1960s and 1970s (Nelson and Mukundi, 1963; Hutcheon and Pamba, 1972). A slaughter survey by Langat (1999), did not detect any trichnellosis cases in indoor Kenyan pigs. Further studies should be conducted using the sensitive serological or molecular methods. A recent trichnosis case of a Japanese tourist who had eaten some wild meat (from alligator, pigs, zebra, ostrich) in Kenya indicated that the disease could still be circulating amongst the wild animals in the country (Nakamura *et al.*, 2003).

CHAPTER FIVE: ASSOCIATION BETWEEN RISK FACTORS AND OCCURRENCE OF PARASITES IN PIGS

5.1 INTRODUCTION

The occurrence of parasites in livestock has been associated with several risk factors which could be related to the host or environment. For example, the development and survival of the infective stages of most helminths is dependent upon microclimate (Roepstorff and Nansen, 1994; Larsen and Roepstorff, 1999). Thus, transmission rates may be associated with factors such as rainfall, temperature, humidity and a variety of husbandry conditions (Roepstorff and Nansen, 1994). Based on these facts, pigs raised on pasture would be expected to have a wide diversity of parasites. The host characteristics have also been associated with the occurrence of pig parasites, where some classes of pigs have higher burdens of parasites than others (Roepstorff and Nansen, 1994). Such associations are mainly due to interactions between the parasites and the immune system of the host. Most of the current known associations between occurrence of parasites and risk factors are based on studies in indoor kept pigs and it would be important to examine these associations in outdoor pigs.

The occurrence of *T. solium* cysticercosis has been reported to be common in pigs kept under free-range system of production, mainly due to access to infected human faeces (Ngowi *et al.*, 2004, Copado *et al.*, 2004). The occurrence of risk factors and their association with the prevalence of porcine cysticercosis is important in devising strategies which can be used in the control of the zoonosis. The current study was thus aimed at examining the association between host, environmental and husbandry factors and the prevalence of parasites in pigs on small-holder farms in Busia District, Kenya.

5.2 MATERIALS AND METHODS

5.2.1 Farm survey

The farm survey was undertaken in pig farms in Busia District as described in Chapters 3 and 4. During the parasitological survey, a total of 306 pigs were sampled from 135 pig farms. The samples collected included blood and faecal samples. The methods used in sample collection and analysis are described in detail in the Material and Methods section of Chapter 4 (subsections 4.2.2.3 to 4.2.2.5).

The host related attributes which accompanied the sampling of each pig included sex and age (category). The categories from which samples were obtained included: piglets (23), growers (135), finishers (53), sows (84) and boars (11). In the pig production system in Busia District, it was very common to find piglets aged two months or more which were still suckling and grazing at the same time. The growers were regarded as the class of pigs which were already weaned but were less than 16 weeks (4 months) old. Thus, the growers were a combination of weaners and porkers. The finishers (also referred to as baconers) were the pigs aged between 4 and 10 months. Sows were regarded as the breeding females which were either pregnant or had previously farrowed. Boars were very few (11) and were excluded from the statistical analysis on relationship between occurrence of parasites and categories.

A structured questionnaire regarding the husbandry and management practices was also undertaken (Appendix II) on every farm. For comparison with the occurrence of helminths and ectoparasites, the farmers were interviewed on history of anthelmintic treatments (deworming), anthelmintics used and provision of housing. The questionnaire also recorded the occurrence of risk factors for porcine cysticercosis including provision of housing, rearing method, presence or absence of latrines, eating of pork, home slaughtering, history of cysticercosis and taeniosis, and knowledge of taeniosis transmission. The data reported on risk factors which can be associated with cysticercosis (section 5.3.2) was collected through a questionnaire survey on 182 farms described in Chapter 3.

Since the definition of a division was general, the amount of annual rainfall was also considered. In the current study, it was only possible to obtain rainfall data for Budalangi, Funyula and Township using the Port Victoria, Nangina and Busia Farmers Training centre meteorological stations. Pigs sampled from areas close to these locations, included Bulada/Mauko B (close to Busia FTC station), Nimioso/Sichehe (close to Nangina station) and Bukani (close to Port Victoria) villages. The mean annual rainfall for Budalangi, Funyula and Township divisions for the year 2006 were 942, 1473, 2111 mm, respectively and exhibited a bimodal pattern (Appendix I). For comparisons with occurrences of parasites, rainfall data for April 2007 when the study was undertaken was used.

5.2.2 Questionnaire survey on occurrence of *T.solium* cysticercosis at slaughter-slab/butchery level

A questionnaire and observations study was undertaken both at the slaughter slab and butchery level. All the slaughter-slabs (6) and pork butcheries (16) in Funyula, Nambale, Matayos and Butula shopping centers were included in the study. These areas were chosen after discussion with the District Veterinary Officer, who indicated that they are the major markets for the local pigs and were easily accessible by the investigator.

Through direct observation, the risk factors for *T. solium* cysticercosis observed at slaughter slab level were assessed. The factors assessed included: presence of latrines, discharge of offals and meat inspection process. A structured questionnaire on presence of risk factors and knowledge of zoonoses was administered to the butchers. The administered questions included: source of water for butchery, presence or absence of latrines, eating of pork/mode of preparation, home slaughtering, history of cysticercosis and taeniosis, and knowledge of taeniosis transmission.

5.2.3 Statistical analysis

The collected data was entered into Ms Excel spreadsheets before being exported to Statview® package where statistical analyses were undertaken. Associations between the categorical variables namely sex, age, divisions, deworming history, anthelmintic used and housing and continuous variables i.e. nematode EPG, coccidian OPG were examined by one-way analysis of variance (ANOVA). The EPG and OPG were transformed to their natural logathrims, ln (count +1), before analysis. Associations between categorical variables as indicated above and prevalence of parasites were examined using chi-square (χ^2). The 95% confidence intervals of the prevalences and intensities of parasites were also calculated, while the significance level was set at 0.05. The 95% confidence intervals were calculated using the formula: Estimate ± 1.96 x standard error. Pearson correlations (r) were calculated between the amount of rainfall and the measures of parasite burdens. The association between the prevalence of porcine cysticercosis and categorical variables namely presence of latrines, pork consumption, slaughtering of pigs at home, taeniosis history, knowledge of transmission of taeniosis was undertaken using the Chi square and risk factors, which had p value of less than 0.05, were considered significant.

For multivariate analysis, the association between independent and dependent variables was undertaken. The most important parasites (with heavy burdens) that were considered consisted of GIT nematodes and lice infections. A multivariate logistic regression using a backward stepwise analysis was used to test for association between prevalence of GIT nematodes and the independent variables ie division of origin, sex, age, deworming history, anthelmintic used and housing. Similar comparisons were made between the prevalence of lice in pigs and the independent variables (division of origin, sex, age, spraying history and housing). The first multivariable model included the variables with $p<0.10$, based on the univariate analysis. Multivariate regression analysis was then performed to quantify the relation, adjusted for the significant variables, between the prevalence of GIT nematodes and lice and the significant independent variables at the univariate analysis. The level of significance was determined at 95% (P value < 0.05), and all tests were two-sided.

The strength of association between independent and dependent variables was estimated by odds ratios (OR) which were directly derived from estimates of logistic regression. The OR measures the strength of association between the risk factor and the outcome (OR) (Thrushfield, 1995). The OR is a relative measure of risk that describes by how much more likely a subject that is exposed to a factor under study will develop the outcome as compared to a subject that is not exposed. An OR of 1 means that there is no association, an OR>1 means increased risk and OR<1 means decreased risk.

5.3 RESULTS

5.3.1 Nematodes

5.3.1.1 Univariate association between burdens of nematodes and farm factors

The prevalence of various nematodes was not significantly (p>0.05) associated with history of deworming, anthelmintic drug used previously or provision of housing.

The intensity of various nematodes excreted by the sampled pigs and their relationship with husbandry factors is shown on Table 5.1. History of previous deworming was not significantly (p>0.05) associated with mean EPGs of total nematodes (mixed), *Oesophagostomum* spp, *Metastrongylus* spp, *Trichuris suis*, *Strongyloides ransomi*, and *Physocephalus sexalatus*. The mean EPGs of *Ascaris suum* were significantly (F=4.09, p=0.04) associated with deworming, being lower on farms with a history of deworming. Piperazine and levamisole were the anthelmintics used previously by farmers, and the use of either drug was not significantly (p>0.05) associated with mean EPGs for any of the nematodes. The provision of housing (night bomas) was not significantly (p>0.05) associated with mean EPGs of *Oesophagostomum* spp, *A. suum*, *T. suis* and *P. sexalatus*. However, the mean EPGs for *S. ransomi*, *Metastrongylus* spp, and total nematodes were significantly lower (p<0.05) in pigs from farms where housing was provided than in those where housing was not provided.

Table 5.1. **Mean EPG of nematodes in 306 pigs from Busia District, exposed to various farm factors**

Variable	Mean EPGs of nematodes (95% CI)						
	Oeso	***A.suum***	**T.suis**	**S.rans**	**Meta**	**Physo**	**All**
Deworming history							
Yes	995 (740-1250)	198[a] (61-335)	62 (1-123)	924 (485-1363)	38 (14-62)	20.3 (2-38)	2248 (1684-2812)
No	1173 (645-1703)	624[a] (67-1181)	55 (2-108)	712 (179-1245)	41 (4-78)	7 (0-15)	2612 (1442-3782)
Drugs used							
Levamisole	937 (643-1271)	249 (78-420)	25 (1-49)	697 (370-1024)	41 (16-66)	25 (3-47)	1975 (1487-2463)
Piperazine	1667 (912-2422)	57 (0-133)	29 (0-70)	104 (0-236)	0	0	1950 (1162-2738)
Housing							
Provided	772 (572-972)	251 (24-478)	28 (0-63)	372[b] (151-593)	23[c] (9-37)	22 (0-44)	1528[d] (1107-1949)
Not provided	1229 (876-1582)	424 (171-677)	31 (9-53)	1011[b] (552-1470)	69[c] (0-44)	13 (0-27)	2730[d] (2020-3440)

Key: a= significant ($F=4.09$, $p=0.04$), b= significant ($F=4.0$, $p=0.046$), c=significant ($F=4.7$, $p=0.03$), d=significant ($F=5.7$, $p=0.02$).
Oeso. = *Oesophagostomum* spp, Meta = *Metastrongylus* spp, S.rans=*S. ransomi*, Physo = *P. sexalatus,* All = Single or mixed app of nematodes

5.3.1.2 Univariate analysis on the relationship between occurrence of nematodes and divisions of origin

In descending order, the prevalence of the nematodes (pigs infected with any nematode) was highest in Nambale, Matayos, Township, Butula, Funyula and lowest in Budalang'i division (Table 5.2). Among the divisions, there were significant statistical differences in the prevalences of pigs infected with mixed nematode (χ^2=25, p=0.0001), *Oesophagostomum* spp. (χ^2=59.1, p<0.0001), *T. suis* (χ^2=11.8, p=0.0375), and *P. sexalatus* (χ^2=20.4, p=0.001), while there were no statistically significant (p>0.05) differences in prevalences of *A. suum*, *S. ransomi* and *Metastrongylus* spp.

Table 5.2. Prevalence of nematodes in pigs sampled from various divisions in Busia District

Division	Prevalence (%) of nematodes						
	All	Oeso spp	*S. ransomi*	*A. suum*	Meta. Spp	*T. suis*	Physo
Nambale	92.4	89.4	39.4	16.7	6.1	10.6	6.1
Matayos	91.8	86.5	37.8	23	8.1	2.7	0
Township	89.3	78.9	38.6	21.1	21.1	7	12.3
Butula	77.5	75	27.5	7.5	5	2.5	0
Funyula	74.4	61.5	33.3	17.9	7.7	15.4	0
Budalang'i	60	23.3	40	13.3	10	0	0
All divisions	84.2	74.8	36.6	17.6	9.8	6.5	3.6

Key: Oeso. spp = *Oesophagostomum* spp, *S. ransomi* = *Strongyloides ransomi*, *A. suum* = *Ascaris suum*, Meta. *spp* = *Metastrongylus* spp , Physo. spp = *Physocephalus sexalatus*, *T. suis* = *Trichuris suis*, All = single or mixed species

In terms of intensity (Table 5.3), the highest mean EPGs of any nematode were recorded in pigs from Matayos Division and lowest in those from Budalang'i Division. A comparison of intensities for specific nematodes across the divisions showed statistical significant differences in *Oesophagostomum* spp (F=2.7, p=0.02) and *T. suis* (F=3.7, p=0.003), while no significant (p>0.05) difference was observed in intensities of the other nematodes.

Table 5.3. Mean faecal egg counts of different nematodes in pigs from various divisions of Busia District

	Mean EPG of nematodes						
Division	**All**	**Oeso**	***A. suum***	***T. suis***	***S.ransomi***	**Meta**	**Physo**
Matayos	3073	1457	887	8	692	30	0
Nambale	3041	1424	239	59	1264	21	33
Funyula	2774	698	410	292	1328	46	0
Township	2061	902	77	24	884	91	46
Butula	1205	1015	15	5	160	10	0
Budalang'i	687	106	107	0	447	27	0
All divisions	2362	1060	345	57	839	39	16

Key: All = Single or mixed species of nematodes, Oeso = *Oesophagostomum* spp, *A. suum* = *Ascaris suum, T. suis* = *Trichuris suis, S. ransomi* = *Strongyloides ransomi*, Meta = *Metastrongylus* spp, Physo = *Physocephalus sexalatus*

5.3.1.3 Univariate analysis on the relationship between nematodes and rainfall

The occurrence of the nematodes was also related to amount of annual rainfall for a given division. The rainfall distribution in the year 2006 is shown in Appendix I. The relationship between amount of rainfall and prevalence of GIT nematodes is shown in Figure 5.1. There was a positive correlation between the amount of rainfall in a given division and prevalence of *Oesophagostomum* spp (r=0.99, p<0.05), *A. suum* (r=0.98, p<0.05), *Metastrongylus spp* (r=0.58, p<0.05) and *P. sexalatus* (r=0.71, p<0.05). However, there was negative correlation between the amount of rainfall and prevalence of *S. ransomi* (r=-0.448, p<0.05). The mean EPGs of *Oesophagostomum* spp, *Metastrongylus* and *P. sexalatus* were also significantly correlated ($r>0.80$, $p<0.05$) with rainfall abundance.

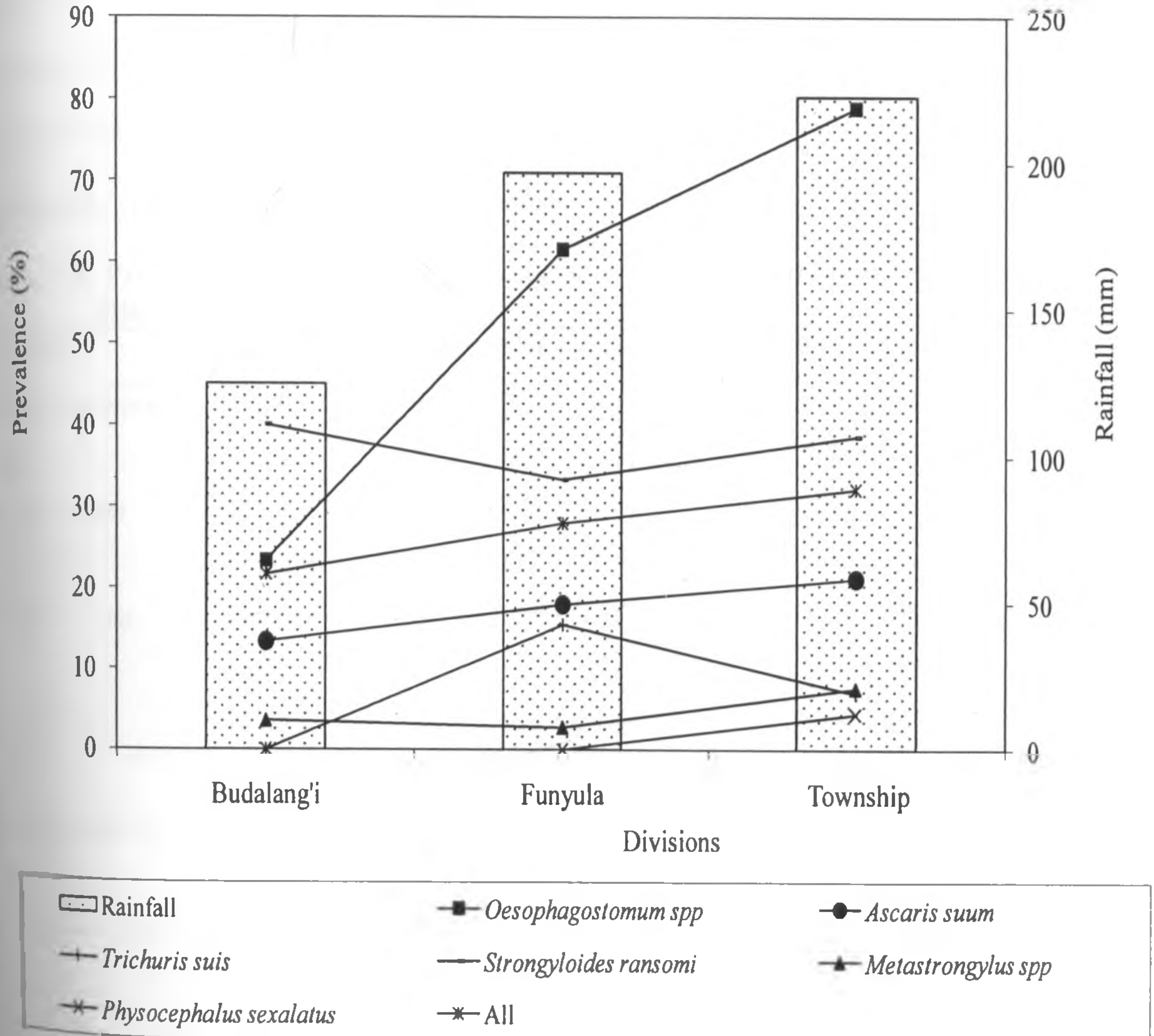


Fig. 5.1. Relationship between amount of rainfall in April 2007 and prevalence of nematode eggs in pigs in Busia District

5.3.1.4 Univariate relationship between host sex and nematodes

The relationship between sex of the pig and prevalence of nematodes is shown in Table 5.4. The number of female pigs were 209, while males were 97. The prevalences of total nematodes and *P. sexalatus* were significantly ($p<0.05$) higher in males than females. The prevalence of *T. suis* and *Metastrongylus* spp was higher in females than males, but this was not statistically significant ($p>0.05$). The EPGs of *S. ransomi* was only significantly ($p<0.05$) higher in males than females.

Table 5.4. Prevalence and intensity of nematodes in pigs of different sex in Busia District

Parasite	Sex	Prevalence (%)	(95% CI)	EPG	(95% CI)
Oesophagostomum spp	Male	78.4	(70-86.7)	913	(666-1161)
	Female	73.2	(67.2-79.3)	1127	(813-1442)
Ascaris suum	Male	18.6	(10.7-26.4)	239	(0-533)
	Female	17.2	(12.1-22.4)	394	(143-646)
Trichuris suis	Female	6.7	(3.3-10.1)	33	(9-56)
	Male	6.2	(1.3-11.1)	110	(0-238)
S. ransomi	Male	44.3	(34.3-54.4)	1357	(551-2173)
	Female	33	(26.6-39.4)	598	(286-910)
Metastrongylus spp	Female	10	(5.9-14.2)	38	(17-59)
	Male	9.3	(3.4-15.2)	39	(0-82)
P. sexalatus spp.	Male	7.2	(2-12.5)	35	(2-69)
	Female	1.9	(0-3.8)	6.7	(0-14)
All Nematodes (single or mixed)	Male	90.7	(84.8-96.6)	2694	(1672-3716)
	Female	81.2	(75.8-86.5)	2208	(1629-2787)

Key: *S. ransomi* = *Strongyloides ransomi*, *P. sexalatus* = *Physocephalus sexalatus*

5.3.1.5 Univariate relationship between category of pigs and nematodes

The occurrence of various nematodes in different categories of pigs is shown in Table 5.5. The prevalence of infection with any (single or mixed species) nematodes was highest amongst piglets (91.3%), growers (86.5%), sows (81%) and lowest in finishers (79.2%). The differences were not statistically significant (F=3.4, p= 0.49). The prevalences of *Oesophagostomum* spp and *T. suis* were highest in sows and finishers respectively, while the prevalence of the rest of the nematodes were highest in piglets. The differences attributable to category of pigs, which were statistically significant ($p<0.05$) were those for prevalences of *S. ransomi*, *P. sexalatus* and *Metastrongylus* spp infections.

In descending order, the intensity of total nematodes (single or mixed species) was highest amongst the piglets and lowest in sows (Table 5.5). The intensities of *Oesophagostomum* spp., *A. suum*, *S. ransomi* and *P. sexalatus* were highest in piglets while the intensities of *T. suis* and *Metastrongylus* spp were highest in growers, respectively. The only significant ($p<0.05$) category differences were for intensities of any nematode (single or mixed species), *S. ransomi* and *Metastrongylus* spp.

Table 5.5. The prevalence and intensity of various nematodes in different categories of pigs from Busia District

Parasite	Category	Prevalence (%)	(95% CI)	EPG	(95% CI)
All nematodes (mixed)	Piglets	91.3	(78.8-100)	6078	(2781-9376)
	Growers	86.5	(80.6-92.4)	2510	(1627-3392)
	Sows	81	(72.4-89.5)	1407	(999-1815)
	Finishers	79.2	(68-90.5)	2060	(1108-3013)
Oesophagostomum spp	Sows	79.8	(71-88.5)	1058	(815-1294)
	Piglets	73.9	(54.5-93.3)	1444	(0-3226)
	Growers	73.3	(65.8-80.9)	994	(627-1361)
	Finishers	69.8	(57-82.6)	1064	(666-1462)
Ascaris suum	Piglets	21.7	(3.5-40)	800	(0-2169)
	Sows	17.9	(9.5-26.2)	248	(0-538)
	Finishers	17	(6.5-27.4)	366	(0-824)
	Growers	16.3	(10-22.6)	339	(47-631)
Trichuris suis	Finishers	9.4	(1.3-17.6)	53	(0-123)
	Growers	7.4	(2.9-11.9)	93	(0-186)
	Sows	4.8	(0.1-9.4)	24	(0-51)
	Piglets	4.3	(0-13.4)	9	(0-27)
Strongyloides ransomi	Piglets	78.3	(60-96.5)	3617	(1200-6035)
	Growers	47.4	(38.9-55.9)	1039	(479-1598)
	Finishers	35.8	(22.5-49.2)	528	(0-1206)
	Sows	11.9	(4.8-19)	48	(14-81)
Metastrongylus spp	Piglets	30.4	(10.1-50.8)	70	(20-119)
	Finishers	9.4	(1.3-17.6)	45	(0-101)
	Sows	8.3	(2.3-14.4)	33	(5-62)
	Growers	5.2	(1.4-9)	16.3	(0-32)
Physocephalus sexalatus spp	Piglets	21.7	(3.5-40)	139	(0-283)
	Growers	3.7	(0-6.9)	10	(1-20)
	Finishers	1.9	(0-5.7)	4	(0-11)
	Sows	0		0	

5.3.1.6 Multivariate analysis for prevalence of nematodes

Table 5.6 shows the multivariate association between variables (division, host and farm) and prevalence of nematodes. Division of origin was the only variable which was significantly associated with prevalence of *Oesophagostomum* spp. Thus, pigs originating from Nambale, Township, Matayos, Butula and Funyula divisions were 26.8, 18.7, 12.3, 9.9, 4.9 times likely to have an *Oesophagostomum* spp infection than those from Budalang'i Division. Division of origin was not an important predictor in the occurrence of the other nematodes.

For *Metastrongylus* spp, the multivariate analysis showed that age category was the only statistically significant ($p<0.05$) variable associated with prevalence of the nematode. Thus, piglets were 3.2 times more likely to have *Metastrongylus* spp compared to adults, while growers were approximately 2 times less likely to have *Metastrongylus* spp compared to adults.

For *S. ransomi*, age category and dewormer used were the only significant variables associated with the prevalence of the nematode. Thus, growers and piglets were 38 and 7 more times likely to have an *S. ransomi* infection than adult pigs. Pigs dewormed with piperazine were 3.3 times less likely to have *S. ransomi* infection compared to those treated with levamisole.

For *P sexalatus*, *T. suis* and *A. suum*, multivariate analysis showed that the variables studied were not significant predictors of the prevalence of these nematodes.

Table 5.6. Multivariate analysis showing significant factors (p=0.05) associated with prevalence of nematodes in pigs from Busia District

Variable	Levels	OR (95%CI) *Oesophagostomum* spp		OR (95%CI) *Metastrongylus* spp		OR (95%CI) *S. ransomi*	
Division of origin	Township	12.3	(4.2-36.1)	-		-	
	Butula	9.9	(0.17-30)	-		-	
	Funyula	4.9	(1.6-15)	-		-	
	Matayos	18.7	(6.35-55.1)	-		-	
	Nambale	26.8	(8.4-85)	-		-	
	Budalangi	1		-		-	
Age category	Grower	-		0.51	(0.22-1.2)	38	(8.3-174.6)
	Piglet	-		3.2	(0.94-10.9)	7	(2.8-17.7)
	Adult	-		1		1	
Dewormer used	Piperazine	-		-		0.3	(0.078-1.2)
	Levamisole	-		-		1	

Key: OR=odd ratios in the final model, 95% CI = 95% confidence intervals,

NB: where OR was 1 (one), the variable acted as reference

5.3.2 *Taenia solium* cysticercosis

5.3.2.1 Risk factors for occurrence of *T. solium* cysticercosis at farm level

The risk factors were assessed on 182 farms. Sixty eight percent (68%) of the farmers in the area consumed pig meat at least once a month, while 23% consumed pork after a period of more than one month. Those who consumed the pork either fried (93%) or boiled (7%) it and 9% of farmers did not consume pork at all.

Ninety one percent (91%) of the farmers indicated that they had latrines in the homestead, while 9% did not have. However, it was observed that only 154 (85%) homesteads had latrines, 82% of these with recent usage. These latrines were mainly mud-walled and thatch roofed. However, 50% of them did not have doors while 2% were shallow pits which pigs could access. The usage of toilets by any member of the family was high at 90%. The average age at which children started to use toilet was 3 years, before then, they defaecated in the compound, bush or garden.

Only 36 (20%) farmers slaughtered the pigs at home and majority of them (27/36, 75%) did not consult a meat inspector (Table 5.7). For a period of two years before the study, cysticercosis had only been recorded in pigs sold from only 5 (3%) farms. The infected pigs originated from Budalangi (3), Funyula (1), and Township Division (1). Out of the 5 carcasses, which were infected with cysticercosis, only one was condemned. The other four were sold to consumers at either a lower or normal price. Ninety nine percent (99%) of the farmers had poor knowledge of transmission of cysticercosis with only two farmers (1%) suggesting that porcine cysticercosis can originate from infected human faeces.

Eighty five percent (85%) of the farmers had seen tapeworm infection in the previous 10 years (Table 5.7). In order of importance, the farmers had seen tapeworms from an infected family member (51%), school going children (20%) and neighbor/villager (23%). Ninety percent (90%) farmers indicated that the number of tapeworm infections had declined in recent years but were quite common two or three decades ago. In descending order, the diagnosis of tapeworm infection was mainly attributed to seeing proglottids in stools (54%), skin infections (7%) and digestive problems (4%). The source of infection was attributed to eating undercooked meat (24%), eating undercooked/raw vegetables (18%) and eating soil (7%) (Table 5.7). The farmers, who did not know the diagnosis or source of tapeworm infection, were 31% and 15% respectively (Table 5.7).

Table 5.7. Risk factors for cysticercosis and knowledge on tapeworm infection amongst farmers keeping pigs in Busia District

Variable	Frequency	Percentage (%)
Slaughter at home (n=182)		
Never	146	80
Less than once a month but at least once a year	21	12
Less than once a year	13	7
At least once a month	2	1
Meat inspection (n=36)		
Always	6	17
Sometimes	3	8
Never	27	75
Source of information on tapeworm infection (n=154)		
Infected family member	92	51
Neighbour/villager	42	23
School going children	36	20
Source of tapeworm infection (n=154)		
Eating undercooked pork/beef	43	24
Eating raw/undercooked vegetables/tubers/fruits	33	18
Eating soil (pregnant mothers)	12	7
Drinking contaminated water	5	3
Not washing hand before eating	4	2
Eating omena	4	2
Drinking raw milk	2	1
Other sources	52	29
Do not know	27	15

5.3.2.2. Risk factors for occurrence of zoonoses at slaughter-slab level

Of the six slaughter-slabs visited, only 3 (50%) had roofing, while the rest were open (Fig 5.2 a and b). The risk factors for zoonoses observed at slaughter slab level included lack of toilets (4, 67%) and discharge of offals (3, 50%) into open grounds or nearby streams. In five slaughter slabs, the investigator found that some un-inspected organs were consumed before inspection, by the people hired during the slaughtering process.

Key: a = Roofed slaughter slab
b = Open slaughter slab

Fig 5.2. Types of pigs' slaughter-slab in Busia District

All (100%) the butchers ate fried pig meat at least every week and all (100%) had toilets at their butcheries. In terms of meat inspection, all (100%) butchers indicated that they consulted meat inspectors whenever they slaughtered and only one (1/16; 6%) indicated to have ever seen white nodules in the meat. All (100%) the butchers had poor knowledge on either the diagnosis of cysticercosis in a live pig or mode of infection. Eighty one percent (81%) of the butchers also indicated that they had heard or seen human beings shedding tapeworm infections mainly from school going children (31%), family member (25%) or within the village (3%). The butchers also indicated that the source of these infections could be from eating uncooked beef or pork (56%), not washing hands before eating (13%), eating spoilt food (13%), or did not know (31%).

5.3.2.3 Association between prevalence of *Taenia solium* cysticercosis and risk factors

This was a prospective study conducted in 306 pigs from 135 farms and was based on the antigen ELISA. The prevalence of cysticercosis was not significantly ($\chi2=1.4$, $p=0.92$) related to division of origin. The infected pigs included 7 sows (8%), 2 growers (2%) and 2 finishers (4%), and there were no significant class differences ($\chi2=6.6$, $p=0.16$). The females and males infected with *T. solium* were 8 (4%), and 3 (3%) respectively, and there was no sex related differences ($\chi2=0.1$, $p=0.75$). The farm variables including housing, rearing method, pork eating, home slaughter and taeniosis knowledge were not significantly ($p>0.05$) associated with occurrence of cysticercosis. Lack of provision of housing was marginally associated ($p=0.07$) with occurrence of the cysticercosis. The only significant ($\chi2=4.4$, $p=0.034$) risk factor was lack of latrines at homestead level, where 36% of the infected pigs originated from homesteads without toilets. Multivariate analysis also revealed that lack of latrines as the only significant variable associated with porcine cysticercosis (Odds ratio: 3.8). Thus, farms which did not have latrines were 3.8 times more likely to have a pig positive for cysticercosis.

5.3.3 GIT protozoan parasites

5.3.3.1 Prevalence and intensity of GIT protozoan parasites as related to divisions and rainfall

The prevalence and intensity of GIT protozoan parasites as related to divisions is shown in Table 5.8. The prevalence of coccidia spp, was highest in pigs from Budalang'i Division (53.3%) but lowest in those from Butula Division (20%), and the divisions-related differences were statistically significant (χ^2=15.2, p=0.009). In descending order, the mean coccidia OPG of pigs from Funyula, Township, Budalang'i, Nambale, Matayos and Butula divisions were 1951, 1651, 1453, 661, 434, and 145 OPG, respectively. The differences in mean OPGs of pigs from various divisions were statistically significant (χ^2=2.96, p=0.013).

The prevalence of *Entamoeba* spp ranged from 97% in Budalang'i to 77% in Nambale Division and the differences were not statistically significant (χ^2 =9.97, p=0.0760). In contrast, the highest prevalence of *B. coli* was recorded in Butula Division (78%) and was lowest in Budalang'i Division (37%) and the differences were statistically significant (χ^2 = 13.9, p=0.016). *Tritrichomonas suis* was commonest in pigs from Budalang'i (73%) Division and lowest in those from Matayos Division (27%) and the differences were statistically significant (χ^2 =32.3, $p<0.0001$).

There was negative correlation between amount of rainfall and prevalence of coccidia spp (r=-0.571), *Tt. suis* (r=-0.549) and *Entamoeba* spp (r=-0.973), but a positive correlation with prevalence of *B. coli* (r=0.961). All these correlations were statistically significant ($p<0.05$).

Table 5.8. **Prevalence of gastrointestinal protozoan parasites in pigs from various divisions in Busia District**

Division	Prevalence (%) of GIT protozoa (95% CI)			
	Coccidia	***Balantidium coli***	***Tritrichomonas suis***	***Entamoeba* spp**
Budalangi	53.3 (34.4-72.3)	36.6 (18.4-55)	73.3 (56.5-90.1)	96.7 (89.8-100)
Township	45 (32.3-58.9)	68.4 (56-80.9)	61.4 (48.4-74.4)	89.5(81.3-97.7)
Funyula	30.8 (15.6-45.9)	69.2 (54-84.4)	35.9 (20.1-51.7)	89.7 (79.8-99.7
Nambale	29.2 (17.9-40.6)	61 (47.8-72.2)	41.5 (29.2-53.8)	76.9 (66.4-87.4)
Matayos	25.7 (15.5-35.9)	64.9 (53.7-76)	27 (16.7-37.4)	85.1(76.7-93.4)
Butula	20 (7-33)	76.9 (63-90.8)	28.2 (13.4-43)	92.3 (83.6-100)

NB: The figures are indicated as: prevalence and 95% confidence interval in brackets

5.3.3.2 Relationship between prevalence of GIT protozoa and sex of pig

There were sex-related differences in the occurrence of the protozoan parasites (Table 5.9). The prevalences of coccidian spp, *Entamoeba* spp and *Tt. suis* were higher in males than females; but it was only the differences for *Tt. suis* which was statistically significant ($p<0.05$). The prevalence of *B. coli* was higher in females than males, although the difference was not statistically significant ($p>0.05$).

Table 5.9. **Comparisons of prevalence of different protozoan infections with sex of pigs from Busia District**

Parasite	Sex	Prevalence (%) (95% CI)	χ^2 comparisons
Coccidia	Male	36.1 (26.4-45.8)	χ^2 =0.75, p=0.38
	Female	31.1 (24.8-37.4)	
Balantidium coli	Female	64.9 (58.4-71.4)	χ^2 =0.34, p=0.5627
	Male	61.5 (51.5-71.4)	
Tritrichomonas suis	Male	53.1 (43-63.3)	χ^2 =6.6, p=0.01*
	Female	37.5 (30.9-42.1)	
Entamoeba spp	Male	87.5 (80.8-94.2)	χ^2 = 0.05, p=0.82
	Female	86.5 (81.9-91.2)	

*= Statistically significant

5.3.3.3 Relationship between prevalence of GIT protozoa and category of pigs

The prevalences of the GIT protozoan parasites were weakly related to the category of pigs (Table 5.10). Thus, the prevalences of *B. coli* and *Coccidia* spp were highest in growers and sows, respectively. However, the prevalence of both *Tt. suis* and *Entamoeba* spp was highest in piglets. The prevalences of *Tt. suis* in sows was statistically significant lower than that of growers (p = 0.0464) and piglets (p= 0.0179). Other comparisons were not statistically significant (p>0.05).

Table 5.10. Category differences in the prevalence of various GIT protozoa parasites of pigs from Busia District

Parasite	Category	Prevalence (%)	(95% CI)
Balantidium coli	Piglets	69.6	(49.2-89.9)
	Finishers	69.2	(56.3-82.2)
	Sows	66.7	(56.4-77)
	Growers	57.5	(49-65.9)
Tritrichomonas suis	Piglets	60.9	(39.3-82.4)
	Growers	47	(38.5-55.6)
	Finishers	40.4	(26.6-54.2)
	Sows	33.3	(23-43.6)
Entamoeba spp	Piglets	95.7	(86.6-100)
	Finishers	88.5	(79.5-97.4)
	Sows	85.7	(78.1-93.4)
	Growers	85.1	(79-91.2)
Coccidia	Sows	35.7	(25.3-46.2)
	Growers	33.3	(25.3-41.4)
	Finishers	32.1	(19.1-45.1)
	Piglets	26.1	(6.7-45.5)

5.3.4 Ecto-parasites

5.3.4.1 Prevalence of ticks as related to division of origin

In descending order, the prevalence of pigs infected with ticks was highest in Nambale and lowest in Budalang'i division (Table 5.11). There were statistically significant ($\chi^2 = 17.98$, $p=0.003$) differences in divisional specific tick prevalence. The prevalences were significantly lower ($p<0.005$) in pigs from Budalang'i and Butula divisions than those from the other divisions. There was a strong positive correlation ($r=0.98$, $p=0.0032$) between the prevalence of ticks and the amount of rainfall.

The proportion of pigs infested with various types of ticks is shown on Table 5.11. The proportion of pigs infested with *Rhipicephalus appendiculatus* was highest in pigs from Matayos Division but lowest in pigs from Nambale Division. However, the proportion of pigs infested with *Boophilus decoloratus* and *Amblyomma variegatum* was highest in pigs from Nambale Division

Table 5.11. Prevalence of ticks infesting pigs from the six divisions in Busia District

Division	Prevalence (%) (95% CI)		Proportion (%) relative to total infected		
			Rhipicephalus	***Boophilus***	***Amblyomma***
Township	33	(20.7-46)	68	37	0
Nambale	39	(27.3-51.5)	54	46	35
Funyula	31	(15.6-45.9)	75	25	0
Butula	10	(0.3-19.7)	25	25	0
Matayos	37	(25.3-47.7)	93	11	4
Budalangi	10	(0-21.4)	66	0	33
Overall	29.7	(24.6-34.9)	70	31	12

Rhipicephalus = *Rhipicephalus appendiculatus*, *Boophilus* = *Boophilus decoloratus*, *Amblyomma* =*Amblyomma variegatum*

5.3.4.2 Prevalence of ticks as related to sex and category of pigs

There were more male (35.1%) infested with ticks than female animals (27.3%), although the difference was not statistically significant ($\chi2=1.919$, p=0.1803). In descending order, the ticks prevalence was highest amongst the finishers (35.9%) followed by growers (28.1%), sows (27.4%) and piglets (21.7%); the differences were not statistically significant ($\chi2=5.237$, p=0.2599).

5.3.4.3 Relationship between prevalence of *H. suis* infestation and division, rainfall, sex and category of pigs

All (100%) the pigs from Funyula, Butula, Matayos and Budalang'i Divisions were infested with lice. Univariate analysis showed that the prevalences of lice infestation in these divisions were higher ($p<0.0001$) than that recorded in pigs from Township Division (82.5%, 95% CI = 72.3-92.6%). The prevalence of lice was negatively correlated (r=0-0.71) with the amount of rainfall in the respective division of sampling. In descending order, the pigs with heavy lice infestation were recorded in Nambale (96.8%), Funyula (94.9%), Matayos (91.9%), Butula (90%), Budalang'i (83.3%) and Township divisions (76.6%) and the differences were statistically significant ($\chi^2 = 53.3$, $p<0.0001$).

The prevalence of lice in males and female pigs was 96.7% and 94.8%, respectively; and the differences were not statistically significant ($p>0.05$). There were no ($p>0.05$) sex differences in terms of intensity of lice infestation. In descending order, the prevalence of lice was highest in finishers (98.1%), growers, (97%), sows (95.2%) and lowest in piglets (91.3%), and the differences were not statistically significant ($p>0.05$). The proportion of pigs with heavy lice infestation was recorded in sows (92.5%), growers (92.4%), finishers (86.5%), piglets (76.2%); and the differences were statistically significant (χ=36.2, p<0.0001).

5.3.4.4 Relationship between prevalence of mange and division, sex and category factors

The prevalence of mange was highest in Funyula Division and was lowest in Budalang'i Division, and the differences were statistically significantly (p<0.05) (Table 5.12). There was a negative correlation (r=-0.78) between the amount of rainfall and prevalence of mange.

Table 5.12. Distribution of division-specific prevalence of clinical mange in pigs from Busia District

Divisions	Prevalence (%)	95% CI
Budalangi	23.3	7.3-39.4
Township	77.2	66-88.4
Butula	77.5	64-91
Funyula	84.6	72.8-96.5
Matayos	54.1	42.4-65.7
Nambale	60.6	48.5-72.7
Overall	63.7	58.3-69.1

Clinical mange: as determined by the characteristic lesions

The prevalence of mange was higher in female (65.6%) than male (59.8%) pigs, but the difference was not statistically significant (χ^2=0.449, p=0.3314). In descending order, mange infestations were more common in sows (71.4%), finishers (69.8%), growers

(58.5%) and piglets (43.5%). The prevalence of mange was significantly ($p<0.05$) lower in piglets than finishers and sows. The rest of comparisons were not statistically significant ($p>0.05$).

5.3.4.5 Relationship between occurrence of ectoparasites and farm factors

The prevalence of mange, hog lice and ticks was not significantly ($p>0.05$) associated either history of spraying or drug used previously for spraying. At univariate level, the prevalence of pigs infested with hog lice and ticks was significantly ($p<0.05$) associated with housing, where pigs from farms with housing bomas (night shelters) had lower infestation than those from farms where housing was not provided. On multivariate analysis, pigs in farms where housing was provided were 2.3 less (OD=0.4413, 95% CI= 0.2-9.8) likely to have lice infestation than those where housing was not provided.

5.4 DISCUSSION

The current study described the relationship between different variables and prevalence and intensity of pig parasites. The investigated farm factors had variable impact on the prevalence of parasites. In the current study, a substantial number (31%) of farms did not have a history of deworming, and this could have led to the observed high burden of nematodes. However, only the EPG levels of *A. suum* were significantly ($p<0.05$) negatively associated with history of deworming, showing that for this parasite the treatments were effective. Lack of significant association between anthelmintic used and the burden or prevalence of nematodes in the current study is different from what has been reported in indoor pigs (Roepstorff and Nansen, 1994; Kagira, 2001). This is possibly because in indoor pigs, there is a more aggressive deworming programme

(Roepstorff and Nansen, 1994). The broadspectrum dewormers which have been successfully used in the control of gastro-intestinal nematodes of pigs include benzimidazoles, levamisole and avermectins (Conder and Campbell, 1995).

In the current study, provision of housing was significantly associated with lower burdens of total nematodes, *Metastrongylus* spp and *S. ransomi*. The houses, made of local materials, were mainly for use at night, with pigs being tethered and or allowed to scavenge for feed during a significant part of the daytime. It is possible that these pigs were better managed leading to lower burden of nematode infections. Other studies have also reported that housing contributes in reducing transmission of helminths in pigs (Roepstorff and Nansen, 1994; Kagira, 2001), and thus pig farmers in the study area should be encouraged to house their pigs.

The prevalence of *Oesophagostomum* spp ranged from 85% in Nambale to 23% in Budalang'i Division. Nambale Division has high rainfall which could favour the survival and development of *Oesophagostomum* spp eggs to infective larvae. However, Budalangi Division is relatively drier and thus the environment may not favour the development of these eggs. It has been observed that the eggs and free-living larvae of *Oesophagostomum* spp are sensitive to desiccation, and a humid microclimate on pastures is beneficial to transmission of *O. dentatum* (Pattison *et al.*, 1980; Larsen and Roepstorff, 1999). Infections with nodular worms stimulate only limited immunity, which moderately regulates the intestinal worm burden and fecundity (Roepstorff and Nansen, 1994). Thus, as observed in this study, pigs with prolonged exposure (eg. adult) have the highest worm burden

(Roepstorff and Nansen, 1994; Permin *et al.*, 1999; Kagira, 2001) and thus control strategies targeting the adults can prevent infections in the piglets (Joachim *et al.*, 2001).

The prevalence of *A. suum* in this study was higher (21%) in pigs from Township Division and lowest (8%) in pigs from Butula Division. In previous studies, the prevalence and re-infection levels of *Ascaris* spp have been strongly correlated with rainfall, temperature and number of wet-days (Gunawardena *et al.*, 2004), climatic features which were more common in the Township Division. Warm and moist environments facilitate the survival of *Ascaris* eggs with moisture making more eggs viable and infective (Gaasenbeek and Borgsteede, 1998). On the contrary, exposure to hot, dry environments leads to rapid desiccation of ascaris eggs (Gunawardena *et al.*, 2004). In the current study, the prevalence of *A. suum* was highest in piglets while in others it is higher in weaners and growers, but lowest in adults; this being related to strong acquired immunity which results in an expulsion of immature worms (Roepstorff and Nansen, 1994). The piglets in the current study were sometimes aged more than two months (and could have bio-characteristics of weaners) and could thus have patent *A. suum* infections.

The prevalence of *Metastrongylus* was highest in pigs from Township Division which had high rainfall and lowest in Budalang'i Division, which was relatively drier. Forrester *et al.*, (1982) reported that the prevalence of *Metastrongylus* spp in pigs was higher during the wet compared to dry seasons in USA. Abundant rainfall is important for the survival and development of earthworms, the intermediate hosts of *Metastrongylus* spp

(Forrester *et al.*, 1982). In the current study, the higher prevalence and intensity of *Metastrongylus* spp in piglets than other categories of pigs could be associated to development of immunity, which has also been reported to occur elsewhere (Foata *et al.*, 2006). The prevalence of *Metastrongylus* spp was also higher in males than females. This could be associated with the relatively higher rooting behaviour of males when compared with females, and this can lead to higher accessibility of infected earthworms by male pigs when compared to females (Forrester *et al.*, 1982).

Strongyloides ransomi prevalence was highest in Budalangi Division, which was drier than the rest of the divisions. This shows that for this parasite, the local transmission factors could be more favourable in this division than the rest. In indoor farms, this worm is favoured by the warm environment in a pen (Roepstorff and Nansen, 1994). In the current study, the prevalence of *S. ransomi* was highest in piglets but lowest in sows and boars, which was similar to the case of indoors pigs in Kenya (Kagira *et al.*, 2001). The decline in prevalence in adult pigs seems to be closely related to the fast development of acquired resistance as well as the prevalent trans-colostral transmission from sow to piglet (Roepstorff and Nansen, 1994). The effects of *S. ransomi* are maximal in piglets and weaners where it causes profuse diarrhoea, decrease in daily weight gain, PCV reduction and feed conversion efficiency in piglets (Hale and Marti, 1984). Male pigs had a significantly higher prevalence and intensity than female ones and this could be related hormonal effects of the host on the parasites.

Prevalence and intensity of *Trichuris suis* were highest in Funyula Division, while pigs in Budalang'i Division had no infection with this nematode. In Budalang'i Division, the survival and development of *T. suis* eggs could be affected by the hot and dry environments which reportedly cause high egg mortalities (Nansen and Roepstorff, 1999). The age pattern of infection where growers and finishers had highest level of infection has been reported previously (Nsoso *et al.*, 2000; Kagira *et al.*, 2001) and is related to development of immunity in adult animals. As reported for other nematodes, the male animals had a higher predisposition to *T. suis* infection. The prevalence of *P. sexalatus* was highest Township Division which as observed above, has high rainfall that can cause the abundance of coprophagous beetles (Nansen and Roepstorff, 1999). In the current study, the parasite was more common in male animals than females, while piglets had a relatively higher prevalence and intensity when compared to other categories of pigs. This could be indicative of age-related occurrence and is not previously reported in literature.

The risk factors which can be associated with transmission of *T. solium* were investigated in the current study. Pig meat consumption was high, possibly due to the fact that the surveyed people were involved in either pig rearing or trade. Before consumption, the meat was mainly boiled and this could kill most of the micro-organisms including cysts of *Cysticercus cellulosae*. However, frying meat may not completely destroy cysts, and this may lead to active transmission of the disease (Mafojane *et al.*, 2003).

The percentage (15%) of homesteads lacking a toilet in this study was lower than the 24% reported in Teso (24%) by Mutua *et al*., (2007), but was higher than the 9% reported by Githigia *et al.,* (2006) in three divisions in Busia District. The overall coverage of functional toilets in Busia District has been indicated as 71% (GoK, 2002b). The reported environmental defecation by non-school going children, could act as transmission route, if pigs consume faeces infected with *T. solium* eggs. In the prospective study, lack of latrines was the only significant risk factor associated with prevalence of porcine cysticercosis, where 36% of the homesteads where pigs were infected did not have toilets. In Teso District, a higher percentage (42%) of homesteads with positive cases of porcine cysticercosis did not have toilets (Mutua *et al*., 2007). It has been observed that defaecation in areas where pigs can access the infected human faeces can lead to increased transmission of *T. solium* (Ngowi *et al*., 2004; Sikasunge *et al*., 2006). A lower prevalence of cysticercosis was marginally associated with provision of housing (night shelter), showing that these pigs could have less access to transmission factors. Similarly, Sikasunge *et al*., (2006), observed that free range pigs had higher risks of acquiring cysticercosis when compared to those reared under semi-intensive conditions.

The proportion of farmers (20%) who undertook slaughter of pigs at home level in the current study were higher than the 11% reported by Wabacha (2001) in Kikuyu Division of Central Province, Kenya. As reported by Ngowi *et al*., (2004) from Tanzania, most farmers who undertook home slaughter of pigs did not consult a meat inspector as the meat is assumed to be harmless (Ngowi *et al*., 2004). The low prevalence of porcine cysticercosis may have led to the poor knowledge of the disease witnessed amongst the farmers and butchers. This can be contrasted to pig farmers in Tanzania and Uganda, where cysticercosis is highly prevalent in pigs kept under traditional settings and thus pig farmers and traders are highly knowledgeable of the disease (Mafojane *et al.*, 2003; Ngowi *et al.*, 2004). However, due to the cross-border pig trade between Kenya and other East African countries (Mafojane *et al.*, 2003; Mutua *et al.*, 2007) farmers in border districts need to be aware of cysticercosis.

In contrast to the low occurrence of cysticercosis, majority of the farmers and butchers had seen tapeworm infection in the previous decade. The infection was mainly observed in an infected family member or school going children. The observation of the proglottid segments in stool (as mentioned by some farmers with a specific local dialect name) could indicate that indeed the farmers and traders were describing a tapeworm infection. The farmers also indicated tapeworm infections had declined in recent years possibly due to increased deworming of children at schools by a local NGO (International Child Support) (Brooker *et al.*, 2000). However, a substantial number of the people in the current study could not correctly determine the source of tapeworm infection which warrants public health education. The tapeworm infections could be either *T. saginata* or *T. solium* since both of them are associated with shedding of proglottid segments. According to annual reports of the Ministry of Health in Kenya, the prevalence of human taeniosis in Busia District was estimated at between 4-10%, which was higher than the national average of 2% (GoK, 2001). However, it is important to note that all these are hospital data which could have some limitations; and higher figures are expected if a prospective epidemiological study is carried out.

A relatively higher prevalence of *T. solium* cysticercosis was observed in the sows. This is similar to what has been reported in other studies, where prevalence of the disease increases with age (Morales *et al.*, 2002). Some of the proposed reasons include prolonged exposure of sows to the parasite, high frequency of faeces consumption by sows compared to other groups of pigs, and influence of pregnancy hormones (Morales *et al.*, 2002, Copado *et al.*, 2004). It has been recommended that sows should be housed during the gestation period to reduce exposure to *T. solium* eggs (Morales *et al.*, 2002).

The prevalence of Coccidia was highest in pigs from Budalang'i Division but lowest in those from Butula Division. The optimum ambient temperature and relative humidity for sporulation of *Eimeria* spp has been indicated to be 25°C -34 °C and 75% (Varghese, 1986), respectively; and these conditions were common in Busia District with Budalang'i Division having higher temperatures than other divisions. Age and sex were not significantly associated with prevalence and intensity of coccidia spp, and this is different from age-related difference observed in indoor pigs (Kagira, 2001).

In contrast to *B. coli*, *Entamoeba* spp and *Tt suis* were commonest in the dry humid Budalang'i Division. The relationship between occurrence of these parasites and climatic variations has not been reported before. Females had a higher prevalence of *B. coli* than males, and this was in contrast with *Entamoebae* spp and *Tt suis.* Hormonal differences have been implicated in occurrences of *B. coli* (Solaymani-Mohammadi, *et al.*, 2004).

Equal numbers of male and females wild pigs were found to be infected with *Tt. suis* (Solaymani-Mohammadi *et al.*, 2004).

The prevalence of ticks was higher in divisions with high rainfall such as Nambale. As reported by Knopf *et al.*, (2002), high rainfall and humidity could increase the survival and fecundity of ticks. Age-related occurrence of ticks was noted in this study and has been reported among the wild pigs in USA, where piglets had lower prevalence than the other groups (Greiner *et al.*, 1994). Further, pigs which were not provided with housing had higher prevalence of ticks, possibly because they were highly exposed to ticks and were less sprayed with acaricides.

The prevalence of clinical mange varied with divisions being lowest in the drier Budalang'i Division, showing that the survival of sarcoptic mites may be affected by the dry conditions in this division. The prevalence of clinical mange was related to the age of pigs being highest in adults (sows) but lowest in piglets and this has been reported before (Alonso *et al.*, 1998). Pigs from all divisions had high prevalence of *H. suis*, showing that the environment in whole District was highly suitable for the parasite transmission. Majority of the pigs with heavy infestation originated from the wetter Divisions (eg. Nambale). The level of *H. suis* infestation was significantly associated with category of pigs; with heavy infestation being more common in sows. This shows that sows can be a major source of infestation to the piglets and could explain the high overall prevalence observed in the current study. Although knowledge on the possible risk factors for transmission of *H. suis* is scanty, pasturing of pigs, purchase of replacements from infected farms, keeping of pigs in dirty and unhygienic conditions have been indicated to cause an increase prevalence of hog louse in a farm (Damriyasa *et al.*, 2004; Nsoso *et al.*, 2006). All these conditions, together with inadequate ectoparasite control, were evident in the study area, where high prevalence of mange was associated with lack of acaricide spraying and housing.

CHAPTER SIX: EVALUATION OF EFFICACY OF NEEM AND PAWPAW PRODUCTS AGAINST *OESOPHAGOSTOMUM* SPP AND *HAEMATOPINUS SUIS* IN FREE RANGE PIGS: *IN VITRO* AND *IN VIVO* TRIALS

6.1 INTRODUCTION

The control of pig nematodes relies almost exclusively on multiple and regular dosing with anthelmintics (Kagira, 2001). However, the use of anthelmintics has several drawbacks including: the negative effect of preventive treatments on the development of natural immunity against helminths; consumer concerns regarding drug residues in food products and in the environment and the increasing incidence of parasite resistance against the available anthelmintics (Van wyk, 2001; Kagira *et al.*, 2003). Further, no new class of anthelmintics has been introduced into the world market in recent years. Consequently, livestock farmers are encouraged to adopt alternative or novel parasite control methods, plant-based anthelmintics being chief amongst them. The use of ethno-veterinary plant preparations has been documented in different parts of the world (reviewed by Githiori *et al.*, 2006). However, the importance of ethnoveterinary products in control of pig parasites has not been evaluated in Kenya. This is in spite of the fact that pig farmers are known to use several preparations to control the parasites. As reported in the socioeconomic survey, substantial number of farmers in Busia District, used products such as paw paw seeds (10%) to control nematode parasites.

Pawpaw products, notably, latex showed effects against *Heligmosomoides bakeri* (a strongyloid previously referred to as *H. polygyrus*, *Nematospiroides dubius*), *Aspiculuris tetraptera* and *Hymenolepis nana* infections in mice (Satrija *et al.*, 1995; Stepek *et al.*, 2007). Plant based latex was observed to have significant effects on gastrointestinal nematodes of humans and *A. suum* infection in pigs (Hansson *et al.* 1986; Satrija *et al.* 1994). The efficacy of papaya latex against other pig parasites has however not been tested.

The use of neem extracts in control of external and internal parasites of livestock has also gained importance in research. The efficacy of neem extracts in the control of gastrointestinal nematodes of livestock has given inconsistent results, with some studies claiming good results while others indicating lack of activity (Hordegen *et al.*, 2003; Githiori, 2004; Chandrawathani *et al.* 2006). The activity of *Azadirachta indica* on external livestock parasites was observed in *Bovicola ovis* (Heath *et al.*, 1995), *Hyalomma anatolicum excavatum* (Abdel-Shafy and Zayed, 2002) and biting louse *Damalinia ovis* (Habluetzel *et al.*, 2007). With the observed activity of neem on *Damalinia ovis*, it would be important to examine whether this product has similar efficacy on other lice including the pig louse, *Haematopinus suis*. In Western Kenya, neem was recently recognized as one of the plants which farmers use in treatment of viral diseases and external parasites of chicken (Okitoi *et al.*, 2007b).

The current study was aimed at evaluating the efficacy of pawpaw and neem products in the control of *Oesophagostomum* spp and hog louse infections of pigs reared under free-range system of production. As reported earlier (Chapter 4), these two parasites occur at high prevalences and intensities in pigs reared in Busia District, Kenya.

6.2 MATERIALS AND METHODS

6.2.1 Products and their preparation

The pawpaw latex and seeds were obtained from pawpaw plants (*Carica papaya*) from a farm in Esikulu village in Busia District, Kenya. The village was purposively selected due to its accessibility and closeness to the Trypanosomiasis Research Centre-Alupe, where some of the analyses were undertaken. Paw latex was collected and stored as described by Sartrija *et al.*, (1995). A V-shaped incision was made on unripe pawpaw fruits and the latex was collected on the ventral aspect of the incision into universal plastic bottles, weighed, and stored at 4°C until use. Pawpaw seeds were obtained from ripe pawpaw fruits, dried and ground into powder using a grinding machine. The powder was then put into universal bottles and stored at 4°C until use.

The aqueous extract of the pawpaw seeds was prepared using a method as described by other authors (Githiori, 2004; Houzangbe-Adote *et al.*, 2005). Briefly, the aqueous extract of the pawpaw seeds was prepared by dissolving 500g of pawpaw powder into 1litre of distilled water. The contents were heated in water bath for 2 hrs at 60°C. The mixture was then kept at room temperature for 12hrs (overnight). It was then filtered using Whatman No 1 filter and freeze-dried using a freeze drier. The final extract was weighed, recorded and then stored at 4°C.

Neem oil was obtained from a local company (Biolinet-Kenya Ltd, Nairobi, Kenya). The oil was prepared from neem seeds by cold-pressing whole seeds using a vegetable oil expeller (Hördegen *et al.*, 2003). The oil was kept at 4°C before use. Other products which were used as controls in the studies included Levamisole (Levamisole HCl, Sigma Ltd, USA; Wormicid®, Cosmos Ltd, Kenya) and Papain (Cysteine protease, Sigma Ltd, USA).

For *in vitro* assays, the pawpaw seed powder, aqueous extract of seed powder and also latex were dissolved in distilled water to form stock solutions having concentration of 30 mg/ml. Papain and Levamisole were dissolved in distilled water to make a solution of 10mg/ml and 0.5mg/ml, respectively.

6.2.2 Evaluation of efficacy of herbal products on *Oesophagostomum* spp using *in vitro* assays

The effects of various herbal products on *Oesophagostomum* spp was tested using the egg hatch assay, larval mortality assay, larval development assay and adult mortality assay. However, neem oil was only tested for its efficacy against the larvae and adult worms.

6.2.2.1 Faecal collection and egg extraction

Faecal samples were collected from the rectum of naturally infected pigs slaughtered at the Ndumbo-ini slaughter house in Nairobi, Kenya. *Oesophagostomum* spp eggs were extracted using the method described previously by Hurbert and Kuberof (1992). Briefly, the faecal samples were mixed with water and sieved through a tea strainer to remove the big debris. The samples were then centrifuged at 1000 rpm for 10 minutes in tap water. The supernatant were discarded and pellet re-suspended with saturated salt solution. The latter mixture was then centrifuged at 500 rpm for 5 minutes. The top one third of the supernatant was pipetted and transferred into a test tube. The sample was then run through a 38 µm sieve and cleaned with excess tap water. The cleaned eggs were then collected in a test tube and concentration of the eggs adjusted to 50 eggs per 50µl of distilled water, ready for use in the experiment.

6.2.2.2 Egg Hatch Assay (EHA)

The Egg Hatch Assay (EHA) was conducted as described by Hurbert and Kuberof (1992) and modified by Maingi *et al*., (1998) and Thoithi *et al*., (2002). Serial dilutions of pawpaw aqueous extract, powder, latex were carried out in 96 well plates to give dilutions ranging from 30,000 µg/ml to 15µg/ml of the extracts. Serial dilutions of papain were also carried out in 96 well plates to give solutions containing 30,000 µg/ml to 4.9 µg/ml. Serial dilutions of levamisole contained dilutions ranging from 500 µg/ml to 0.244 µg/ml. Control tubes containing distilled water were included in the set-up. 50µl of *Oesophagostomum* spp eggs solution were added into each well and incubated at 27°C for 48hrs. The number of eggs which had not hatched and number of hatched larvae were counted using the inverted microscope and percent hatching calculated.

6.2.2.3 Larval Mortality Assay (LMA)

The assay was set-up as described by Thoithi *et al*., (2002). The initial aspects were undertaken as described for EHA in section 6.2.2.2. After determination of the EHA, the plates were incubated for a further 48hrs. The number of live and dead larvae was determined using the inverted microscope and percent death calculated.

6.2.2.4 Larval development assay (LDA)

The LDA was conducted as described by Hurbert and Kuberof (1992) and modified by Maingi *et al*., (1998). The assay was initially set-up as described for the EHA described in section 6.2.2.2. The plates were then incubated for 7 days at 27°C. Then, 10 μl of Lugols iodine was added to each well and the number of eggs and larvae that developed to L_3 in each well counted using a dissecting microscope.

6.2.2.5 Adult Worm Mortality Assay (AWMA)

Adult *Oesophagostomum* spp were collected from the large intestine of naturally infected pigs slaughtered at the Ndumbo-ini slaughter-slab, Nairobi, Kenya. Only worms from pigs without previous anthelmintic treatments were used in this study. The collected worms were then washed and kept in phosphate buffered saline. The AWMA was undertaken as described by Houzangbe-Adote *et al*., (2005) and in Appendix IV. Briefly, the adult worms were placed in petri dishes filled with 4ml of the test products. The pawpaw aqueous extract and powder were prepared in concentrations ranging from 50mg/ml to 1.75mg/ml, while the pawpaw latex and papain preparations ranged from 10mg/ml to 0.125mg/ml. Serial dilutions of Levamisole HCl acted as positive control. After 24 hours the number of motile (alive) and immotile (dead) worms was counted under the dissecting microscope and percentage of dead worms calculated. Morphological changes on the worm were also noted.

6.2.2.6 Electron microscopy on *Oesophagostomum* spp exposed to pawpaw latex

The electron microscopy work was undertaken as described by Beugnet *et al*., (1996). Twenty (20) adult *Oesophagostomum* spp live worms were obtained and exposed to 10 mg/ml of pawpaw latex for 10 minutes as described in section 6.2.2.5. Another 20 adult live *Oesophagostomum* worms were kept under physiological saline for 10 minutes and acted as control. The worms were then removed from the petri dishes and fixed in 2%

glutaraldehyde diluted in 0.15 M phosphate buffered saline (PBS) at pH 7.2 for 48 hours at 4°C. The worms were then rinsed for 2 hrs at 4°C in three changes of 0.1M PBS buffer pH 7.2. Post fixation of the worms was then undertaken using 1% osmium tetroxide (in phosphate buffer) for 2 hours as a secondary fixative. The worms were then washed twice in phosphate buffer for 15 minutes after which they were dehydrated in increasing concentrations of ethanol (ethanol 70% for 10 minutes, 95% for 20 minutes, 100% for 20 minutes). After dehydration, the worms were placed in two changes of 100% propylene oxide for 15 minutes each. The worms were then placed in embedding capsules containing 'Epon 812' resin (made of a mixture containing EPON 812, DDSA, NMA and DMP-30) after which they were allowed to sink at the bottom of the capsule. The EPON 812 resin containing the worms was then polymerized at 60°C for 18 hours. The resin blocks were then thick sectioned at 1-2 microns using glass knives and stained with toluidine blue. These sections were examined under light microscope and were used as a reference to trim blocks for thin sectioning. The blocks were then sectioned using a Reichert ultramicrotome (Reichert Jung, Vienna, Austria) at 70-90nm and sections were placed on copper grids (mesh 200). After drying on filter paper for a minimum of 1 hour, these sections were stained with 4% uranyl acetate (in 30% ethanol)) for 20 minutes followed by 0.4% lead citrate (in distilled water) for 10 minutes. Observations were performed using a Philips 201C Transmission Electron Microscope (TEM) and changes described.

6.2.3 *In vivo* evaluation of effect of herbal remedies on *Oesophagostomum* spp

6.2.3.1 Pigs and study herds

A total of 30 grower pigs from 26 households in the Esikulu village in Busia District were used in the field trial. The pigs used in the study had not been treated with any anthelmintic in the previous 8 weeks, were of either sex, aged between 3 and 6 months of age.

6.2.3.2 Experimental design

This was a randomized, controlled efficacy study. At the start of the experiment, the pigs were screened for nematode infections and shown to be infected with *Oesophagostomum* spp (100%). The treatment groups were randomized based on the *Oesophagostomum* spp EPG counts.

The study pigs were ear-tagged, screened for nematode egg counts using the McMaster method (MAFF, 1986). Pigs with an EPG of more than 500 were then randomly allocated into 6 groups of 5 pigs each as detailed in Table 6.1. The dosages used in the current study were determined from literature review (Fajimi *et al*., 2002, Boeke *et al*., 2004). For pawpaw powder, the amount of seeds used by farmers to feed the pigs was quite variable (indicated as handful) and thus the amount of seeds (600mg/kg) used in the study by Fajimi *et al*., (2002) was used as a guide. In the current study, a higher level amount of seeds (1g/kg) were used as the amount used by Fajimi *et al*., (2002) did not cause 100% efficacy. For ease of comparison with the pawpaw powder, 1 g/kg pawpaw latex was used in the current study. Since papain is purified from pawpaw latex, a lower dose

(0.3g/kg) of commercial papain was used. This was expected to reduce any occurrence of toxicity in the pigs. Neem dosage of 0.2ml per kg was chosen because doses higher than 0.2ml/kg were indicated to be toxic to other animals eg., man (Boeke *et al.*, 2004).

After administration of the herbal doses, the pigs were then screened for nematode egg counts at days 7, 14, 28 and 56 days, using McMaster egg counting technique (MAFF, 1986).

Table 6.1. Treatment regimens for pigs infected with *Oesophagostomum* spp

Group	Treatment	Dosage
1	Pawpaw powder	1g/kg orally
2	Pawpaw latex	1g/kg orally
3	Papain	0.3g/kg orally
4	Neem oil	0.2ml/kg orally
5	Levamisole	7.5mg/kg orally
6	Distilled water	10 ml/pig

To determine any weight changes during the treatment period, the weight of the pigs during sampling points was determined using heart girth tape measure method (Carlson, 2006). The length (L) was measured from base of the tail to crown of the head between the ears while the girth (G) was measured by wrapping the tape measure at the heart girth area just behind the front legs. All measurements were made in centimeters. The weight of the pig was then determined using the formula: Wt (kgs) = $(LxG^2)/13781$ (Carlson, 2006).

During the administration of the herbal products and sampling days, the pigs in the six groups were examined for clinical signs which can be associated with toxicity (eg., change in stool consistency, raised hair coat, respiratory distress) of the products. Blood was also collected from the jugular vein and analyzed for PCV using the Haematocrit technique (Murray *et al.*, 1977).

6.2.4 *In vitro* evaluation of effect of herbal remedies on lice

6.2.4.1 Source of *Haematopinus suis*

Adult *H. suis* were obtained from 5 pigs in Esikulu village, Matayos Division, Busia District. The lice were handpicked from heavily infested parts of the pig body (Fig. 4.8). The lice were then put into universal bottles, protected from heat and light and delivered to the laboratory within 1 hour of capture. In the lab, the lice were identified to be *H. suis* using morphological features (Soulsby, 1982). The effect of the various products was tested within 2 - 6 hours of collection of the lice.

6.2.4.2 Experimental design for *H.suis in vitro* study

The study was undertaken as described by Canyon and Speare (2007) and Holdsworth *et al.* (2006). The products tested for their effects on lice are shown in Table 6.2. Three (3) ml of the respective test product was placed in petri-dish and batches of 10 lice were dipped (immersed) into the mixture for 3 minutes. They were then removed from the test product solutions, blotted gently dry on a tissue paper and returned to the original petridish, then incubated at 31°C. To improve on the humidity, a piece of cotton soaked in 50µl of water was also placed in the petri-dish. The number of living and dead lice was determined at 5, 10, 20, 40, 60, 90 and 120 minutes and 24 hrs post-dipping. Death was determined when all the limb movements had ceased. The tests were done in duplicate and average counts considered. Percent mortality was then calculated and plotted against time.

Table 6.2. Products tested for *in vitro* effects against lice

Product	Concentrations used	Solvent
Neem oil (Biolinet Ltd, Kenya)	100, 50, 25, 12.5, 6.25%	40% ethanol
Papaya latex *	30, 15, 7.5, 3.75mg/ml	Distilled water
Papain (Sigma Ltd, UK)	30, 15, 7.5, 3.75mg/ml	Distilled water
Amitraz (Taktik, Intervet Ltd, Kenya)	5, 2.5, 1.25, 6.25mg/ml	Distilled water
Levamisole (Levacide, Norbrook Ltd, Kenya	7.5, 3.75, 1.88, 0.93mg/ml	Distilled water
40% ethanol (Control group)	-	-
Distilled water (Control group)	-	-

*= Obtained from raw pawpaw fruits in Busia, Kenya

6.2.5 *In vivo* evaluation of effects of neem, amitraz and levamisole on *H. suis*

6.2.5.1 Pigs and study herds

This was a randomized, controlled study which was conducted in Esikulu village in Matayos Division. Thirty (30) pigs heavily infested with lice (>100) were used. The pigs in the study were tethered within the homesteads where they grazed and were sometimes supplemented with household leftovers, vegetables from the farms and feed from spoilt flour. The pigs were mainly growers, of both sexes and were three to six months of age.

6.2.5.2 Experimental design for *in vivo* studies

The lice infested pigs were ear-tagged, weighed and randomly allocated into five treatment groups of 5 pigs each treated as follows: Neem oil 3%, 5% and 10%, Amitraz (Taktic®,12.5%w/v, Hoechst, Germany), Levamisole (Wormicid tablets, Cosmos Ltd, Kenya) 7.5mg/kg once. The control group (involving 6 pigs) was sprayed with water mixed with ethanol (2%) and commercially available soap (5g of bar soap in 500ml of water), as these were the solvent used to dissolve neem oil. The pigs were administered with the treatments at day 0 and 7. Neem oil was dissolved in solvent made up of water mixed with ethanol (2%) and commercially available soap (bar soap) (10g in 500ml of water). Ethanol allows oil to dissolve in water, while soap enabled formation of a uniform aqueous emulsion. Amitraz was dissolved in tap water using the manufacturers' recommendation. The animals were sprayed with the test products and care was taken to ensure they were thoroughly drenched. The animals were then monitored for lice counts on day 7, 14, 28 and 56 post treatment.

6.2.5.3 Statistical analysis

The results were entered into Microsoft Excel® worksheets before being imported into StatsView® statistical software where statistical analysis was undertaken. Descriptive statistics were calculated and presented as tables and graphs. The percentages of eggs hatched, larval mortalities and larvae developed to L3 were transformed to probits and compared against logathrim of the product concentration. The concentrations required to inhibit 50% (ED_{50}) egg hatching, cause 50% (LD_{50}) larval mortality, and prevent 50% (LD_{50}) larvae from developing to L3 were calculated after correction was made for natural mortality by probit analysis using the StatView® statistical software. Comparisons of the mean percentages of egg hatch inhibition and mortality of larval/adult parasites at different concentrations with the control was performed by one-way ANOVA. For *in vitro* lice studies, background mortality values were taken from the solvent treated control.

For the field study evaluating efficacy of various products against *Oesophagostomum* spp, the WAAVP guidelines outlined by Hennesy *et al.*, (2006) were used. For *H. suis* field studies, mean counts of lice per group were calculated. Further, the counts were also arbitrary categorized in terms of intensity of +1 (1-10), +2 (11-50), and +3 (>50) lice. The significance of the differences among treatment groups on the mean lice counts and mean EPGs was determined using one way ANOVA.

6.3 RESULTS

6.3.1 Effect of plant products on *Oesophagostomum* spp *in vitro*

All the products had varied effects on *Oesophagostomum* spp and exhibited a linear dose-response relationship. The effective doses of various products on *Oesophagostomum* spp eggs, larvae and adult worms are shown in Table 6.3. In descending order, the most effective products against the eggs (EHA) were levamisole, papain, latex, aqueous extract and powder. For L_1 mortality (LMA) and larval development (LDA), the most effective products were levamisole, latex and papain, while the least effective products were seed aqueous extract and pawpaw seed powder. Papain and latex were the most effective products against adult worms. For the EHA, LMA and LDA, the effective dose all the products were significantly ($p<0.05$) different from each other. For AWMA, the ED_{50} for latex and papain were significantly ($p<0.05$) different from those of powder and aqueous extract. However, there was no significant ($p>0.05$) difference between the ED_{50} (EHA, LMA and AWMA) of powder and aqueous extract.

Table 6.3. Anthelmintic activity (ED_{50}) of various pawpaw plant products

	ED $_{50}$ (µg/ml) values of different assays			
Plant product/anthelmintic	**EHA**	**LMA**	**LDA**	**AWMA**
Pawpaw aqueous extract	234	3750	2362	2572
Pawpaw powder	554	3050	938	4743
Pawpaw latex	59	117	371	25
Papain	5	31.25	20.8	12.5
Levamisole	0.8	0.5	0.18	ND

Key: EHA=Egg hatch assay, LMA= Larval (L_1) mortality assay, LDA= Larval development assay, AWMA = Adult worm mortality assay, ND = Not done

On examination under the light microscope, the pawpaw latex and papain appeared to affect the cuticle of the adult worms leading to erosion of outer cuticle of the worm (Fig 6.1 and 6.2), weakening and formation of crinkled ridges (Fig 6.1). In some worms, the cuticle weakened and burst at some points leading to exposure of internal organs and eggs (Fig 6.3). At high doses of 10 - 30 mg/kg, the latex and papain completely dissolved the worm within a period of 12 hours.

At electron microscopy (EM) a similar process was observed to occur within the cuticle structure. Apart from features described above, at EM the cuticle was also observed to become weak and underwent disintegration and this lead to formation of empty halos within all the layers of cuticle. The hypodermis layer (thin layer that lies between the cuticle and somatic muscle) also appeared to become lighter, while in other sections there was separation from both the upper cuticle and lower muscular layers. The separation of cuticle and hypodermis is shown in Fig 6.4 and 6.5 taken after histological and EM sectioning respectively. Fig 6.6 shows the cuticle of normal *Oesophagostomum* spp at EM.

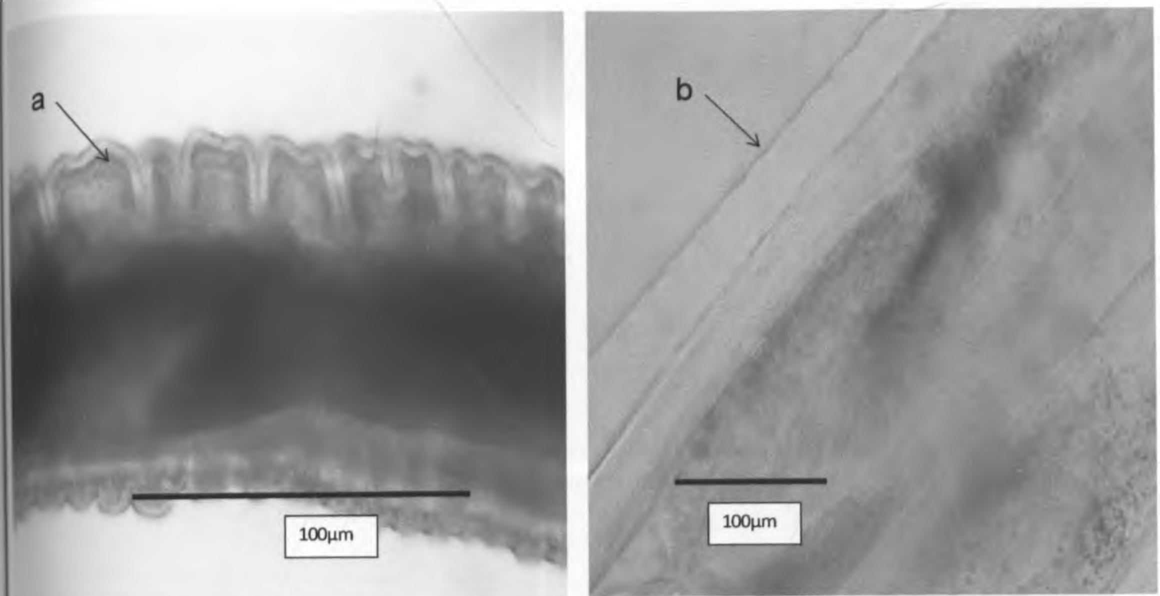


Fig 6.1. Effects of pawpaw latex on the cuticle of *Oesophagostomum* spp. Note the formation of wrinkles along the cuticle (b). A normal cuticle (a) is shown. Images taken at magnification of x400 (a) and x250 (b).

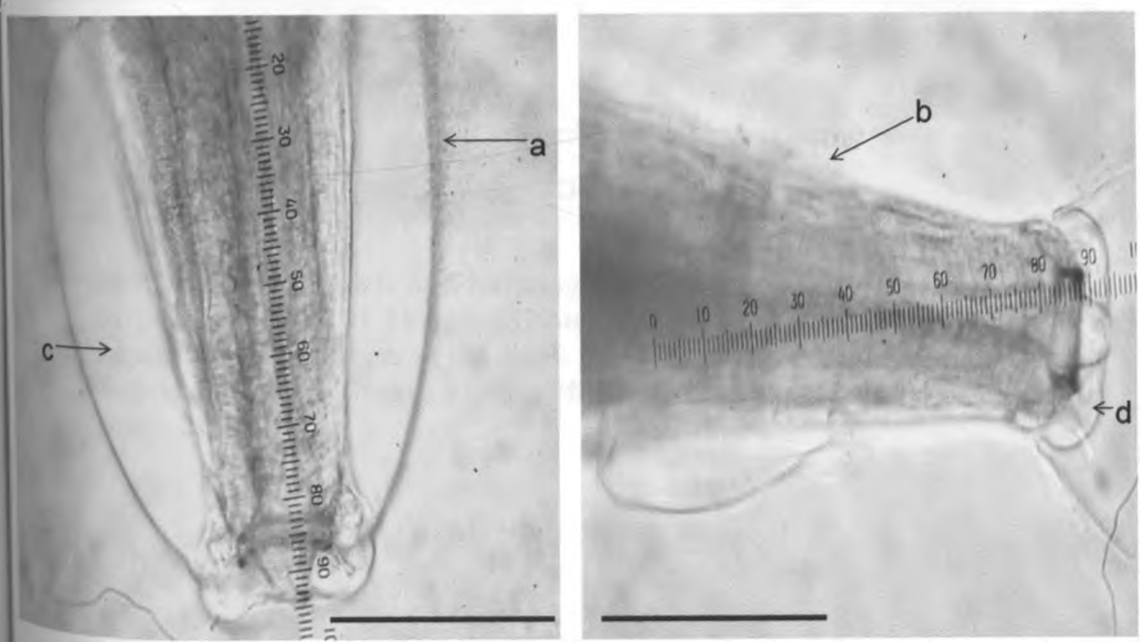


Fig 6.2. Effects of the papaya latex on the cephalic vesicle of *Oesophagostomum* spp. Note the erosion (a) and loss of cephalic vesicle (b), normal cephalic vesicle (c) and leaf crown (d). Images taken at 250x magnification, Bar = 250µm

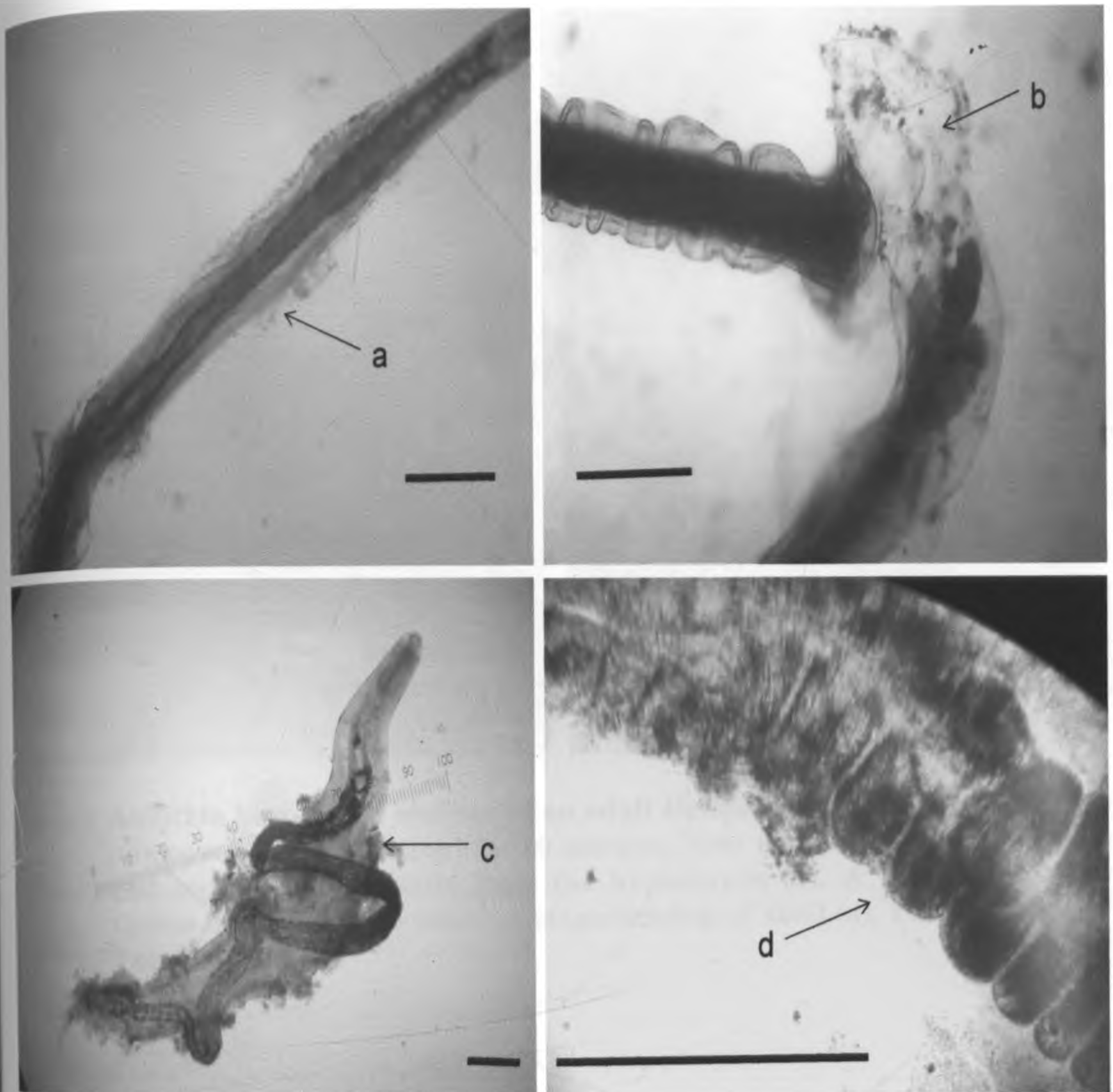


Fig 6.3. Disfiguration of cuticle and bursting of *Oesophagostomum* spp on exposure to pawpaw latex at 10mg/ml. Note the loss of normal architecture (a), rupture at of the worm at the weak point (b, c) and complete erosion of the cuticle (d). Images taken at magnification of x50 (a, c), x100 (b), x250 (d). Bar = 200μm

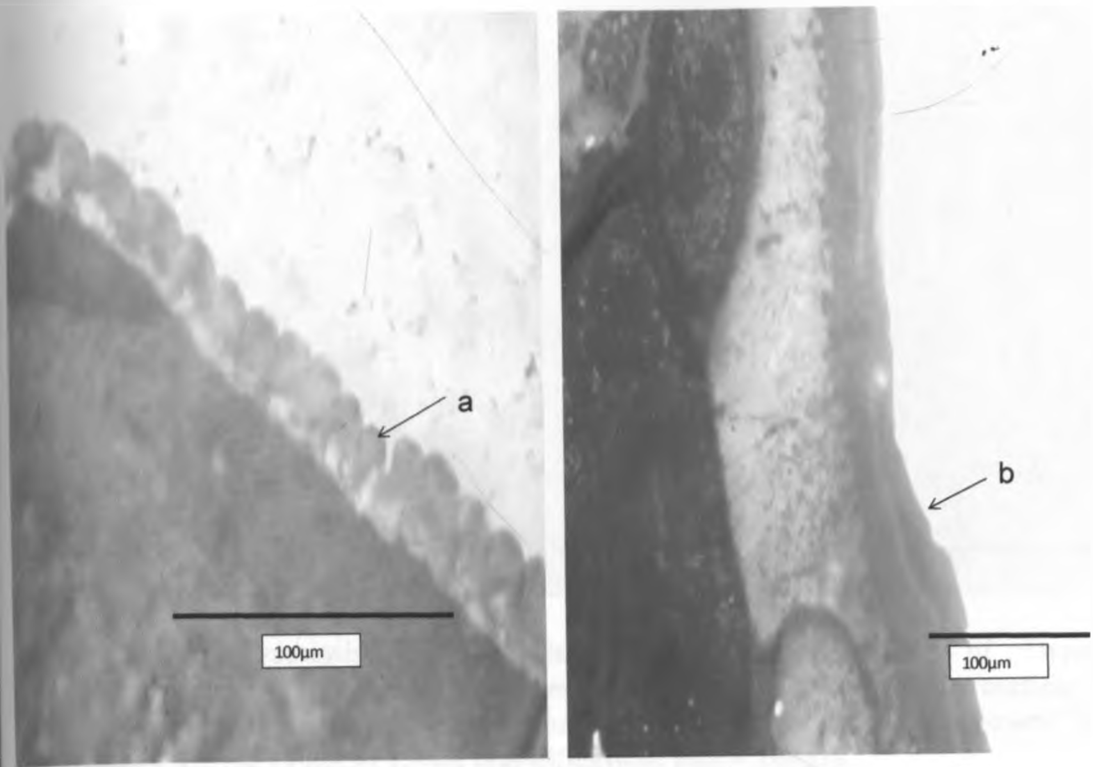


Fig. 6.4. Semithin histological sections of an adult *Oesophagostomum* spp exposed to pawpaw latex at 10 mg/ml for 10 minutes. Note that the wrinkle formation and separation of cuticle from the hypodermis (a). A normal cuticle is shown in (b). Images taken at magnification of x600 (a), x400 (b), Stain = Toluidine blue

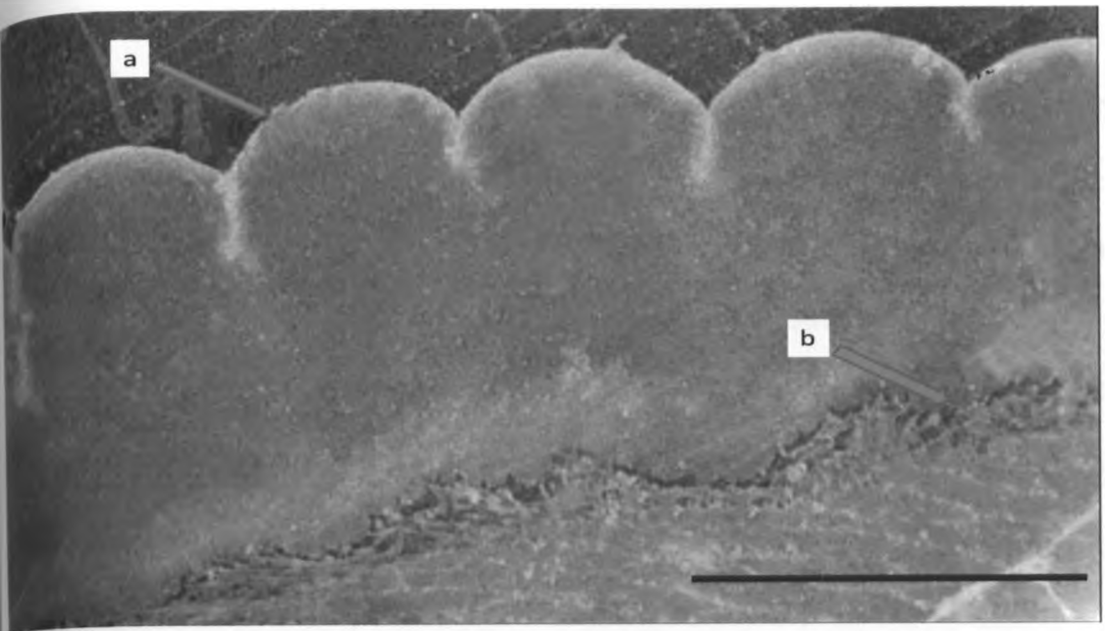


Figure 6.5. A transmission electron micrograph showing the effects of pawpaw latex on the *Oesophagostomum* spp cuticle. Note the formation of wrinkles (a) and separation between cuticle and hypodermis (b). Image taken at x3000 magnification. Bar = 200µm

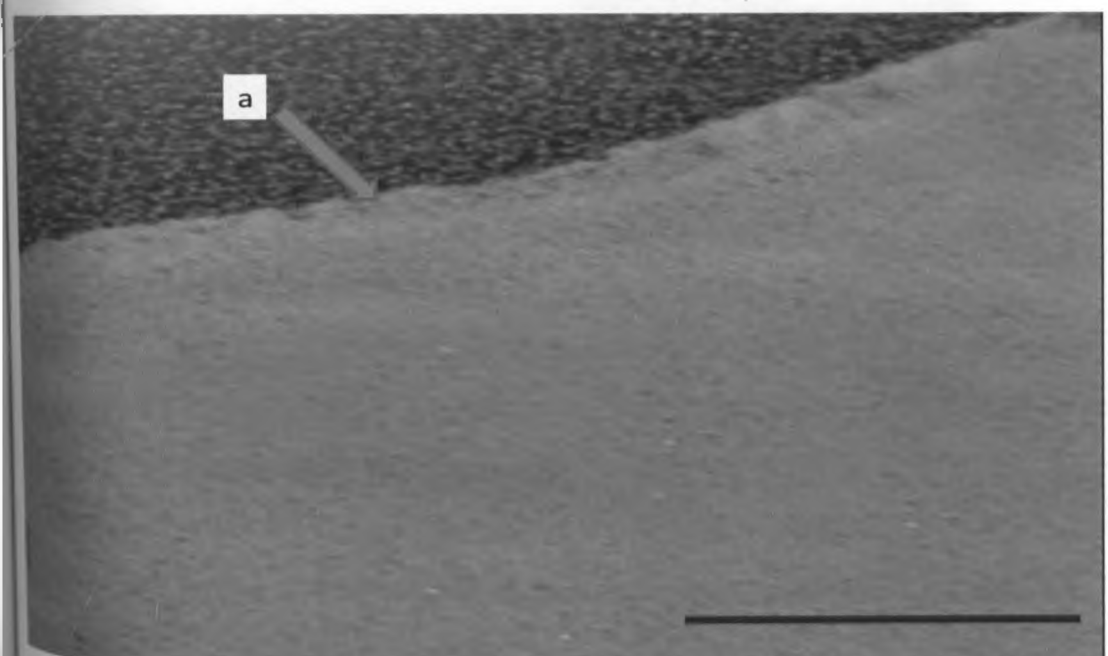


Figure 6.6. A transmission electron micrograph showing the normal cuticle of *Oesophagostomum* spp. Note the cuticle (a) without distinct separation from hypodermis. Image taken at x2000 magnification. Bar = 200µm

For adult worms, the LD_{50} of neem oil was 6.25% (24 hrs). A 100% mortality was observed in adult worms exposed to neem oil concentrations of 25% or higher. For larvae, the LD_{50} was achieved by neem oil at a concentration of 25%. A 100% mortality was only achieved when larvae were exposed to neem oil at concentrations of 50% or higher. Neem oil appeared to form oil droplets along the cuticle of the worm, causing disruption of the cuticle (Fig 6.7). However, the disruption of the cuticle was not as severe as that of the pawpaw latex.

Fig 6.7. Anterior end of *Oesophagostomum* spp showing the effects of neem oil on cuticle. Note the disruption of cephalic vesicle by the neem oil (a) and neem oil surrounding the mouth parts (b). Image taken at x 250 magnification, Bar = 200μm.

6.3.2 Effects of herbal remedies on *Oesophagostomum* spp infection in pigs

The EPG levels in pigs treated with various products are shown in Fig. 6.8. At day 0, there were no significant differences (p=0.68) in the *Oesophagostomum* spp EPGs of the various groups. In all the treated groups, the mean EPGs declined when compared with those at day zero. Thus, the overall decline in mean EPGs were statistically significant for the groups treated with pawpaw powder (F=5.12, p=0.007), latex (F=3.95, p=0.02), papain (F=2.96, p=0.052), neem oil (F=3.78, p=0.02) and levamisole. However, there was a significant (F=3.04, p=0.048) increase in mean EPG counts in pigs in the untreated control group during the monitoring period. At 56 days post treatment, there were significant differences (p<0.05) between mean EPGs in the control group and those of latex, levamisole and neem oil groups. Further, there was significant difference (p=0.03) in mean EPGs of the levamisole and powder treated groups at 56 days post treatment.

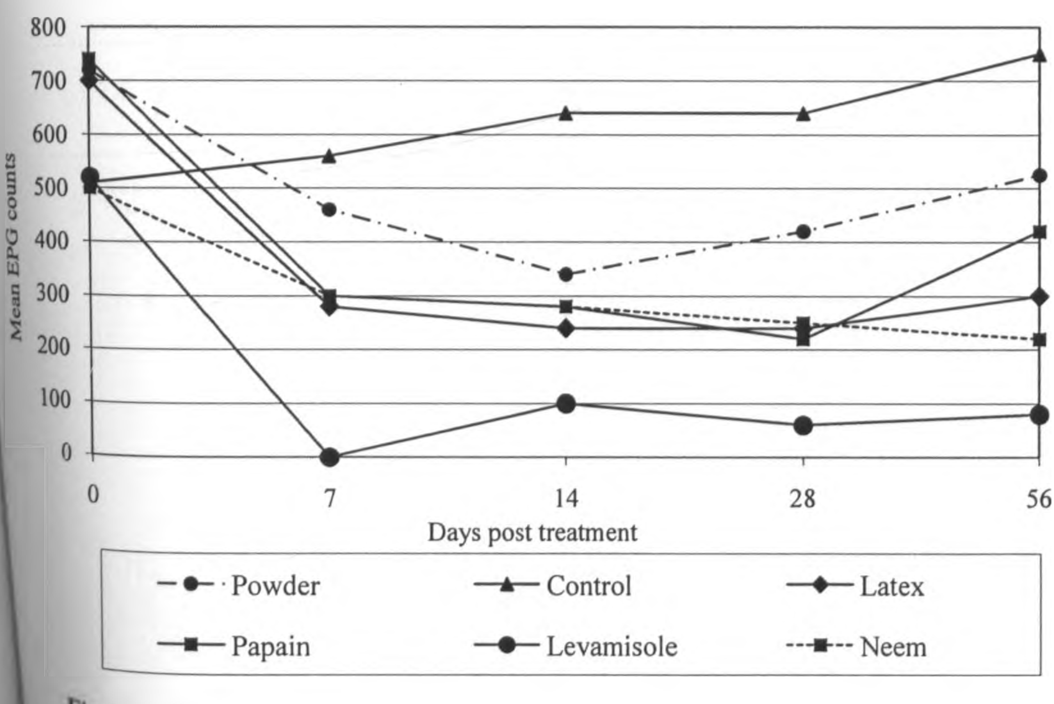


Fig 6.8. EPG counts in pigs treated with various plant products

The percentage reduction in mean EPGs were also analysed across the different treatment groups as shown in Fig. 6.9. The maximum reduction in mean EPGs for the treatment groups included: Levamisole group at day 7 (100%), papain group at day 28 (70.8%), pawpaw latex group at day 14 and 28 (65.7%), pawpaw powder at day 14 (52.8%) and neem oil group at day 28 (50%). At the end of the study (56 days post treatment), the percentage reductions in mean EPG in the levamisole, latex, neem oil, papain, powder treated groups was 84.6%, 57.1%, 56%, 43.2%, 27.1%, respectively. The increase in mean EPG counts in pigs in the untreated control group was 21.8%, 39.1%, 39.1% and 47% at 7, 14, 28 and 56 days post treatment, respectively.

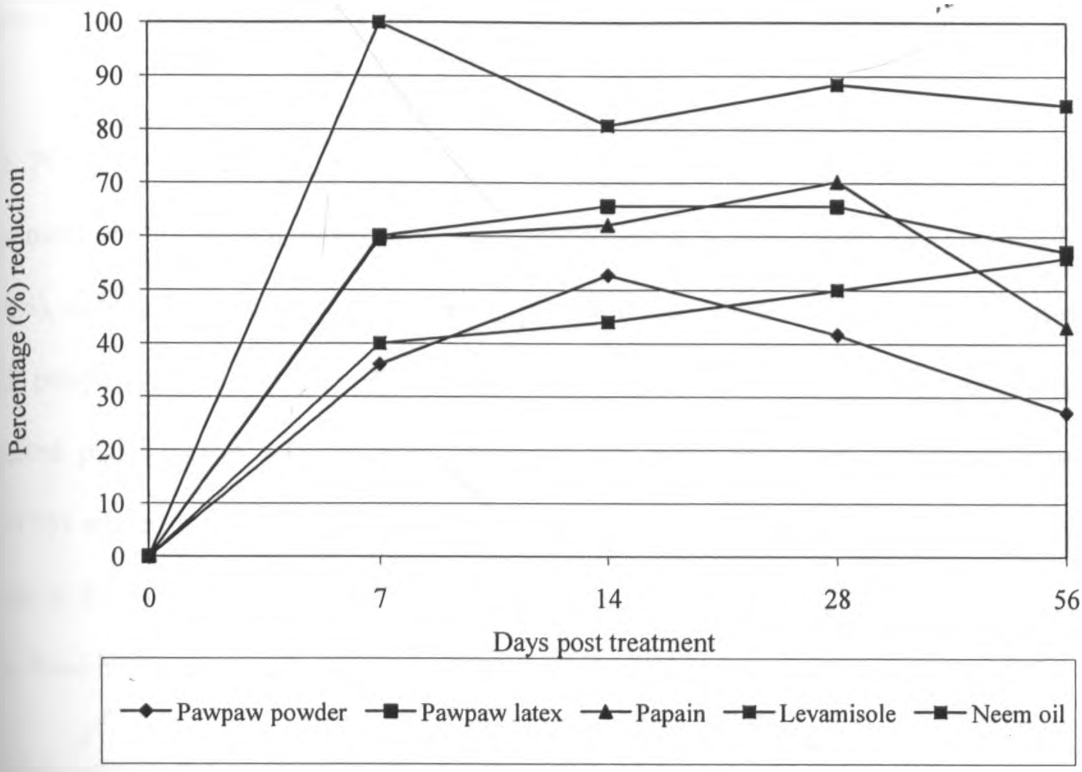


Fig 6.9. Percentage reduction of EPG counts in pigs administered various plant products

In all the groups, there was an increase in body weights by day 56, where a mean increment of 8.1, 7.7, 6.8, 6.3, and 6.2 Kgs was observed in pigs treated with neem oil, powder, papain, levamisole and latex, respectively. Thus, the daily weight gain ranged from 145g to 111 g/pig/day. The control group had a mean increment of 5.5 Kgs (98g/pig/day). The increase of weight was significantly ($p<0.05$) related to days post treatment in the neem oil, powder, papain and levamisole treated groups, but not ($p>0.05$)

in latex and control groups. However, comparisons of the weight changes in the different groups were not significant ($p>0.05$).

The PCV of all the groups increased with time. In descending order, the highest increment of PCV at 56 days post treatment was observed in pigs treated with levamisole (3.8%), neem (3%), papain (2.1%), latex (2%) and lowest increment was in pigs treated with pawpaw powder (1.6%). There was also an increment of PCV in the untreated infected pigs (1%) at 56 days post treatment. The mean PCV was not significantly ($p>0.05$) related to sampling date in all the treatment groups. However, the mean PCV levels at 28 days post treatment in the control group were significantly ($p<0.05$) lower than those in the levamisole and neem groups.

6.3.3 *In vitro* evaluation of the effects of herbal remedies on *H. suis*

The *in vitro* effects of the products on lice are shown in Fig 6.10 - 6.14 and in Appendix V. At between 25-100% of neem oil, all the hog lice were killed immediately they came into contact with the oil and thus mortalities are not indicated in the charts referred above. At a concentration of 12.5%, neem oil killed 90% of the lice within 5 minutes, while all lice were killed by the 90th minute (Fig 6.10, Appendix V). At concentrations of 6.25% and 3.125% neem oil, 93% and 86% of the lice were killed within 24 hours of incubation. Thus, neem oil effects were both dose and time dependent, with doses above 12.5% having 100% efficacy within 24 hours. There were significant effect of time ($F=4.34$, $p=0.0023$) and dosages ($F=239.31$, $p=0.000$) on lice mortality.

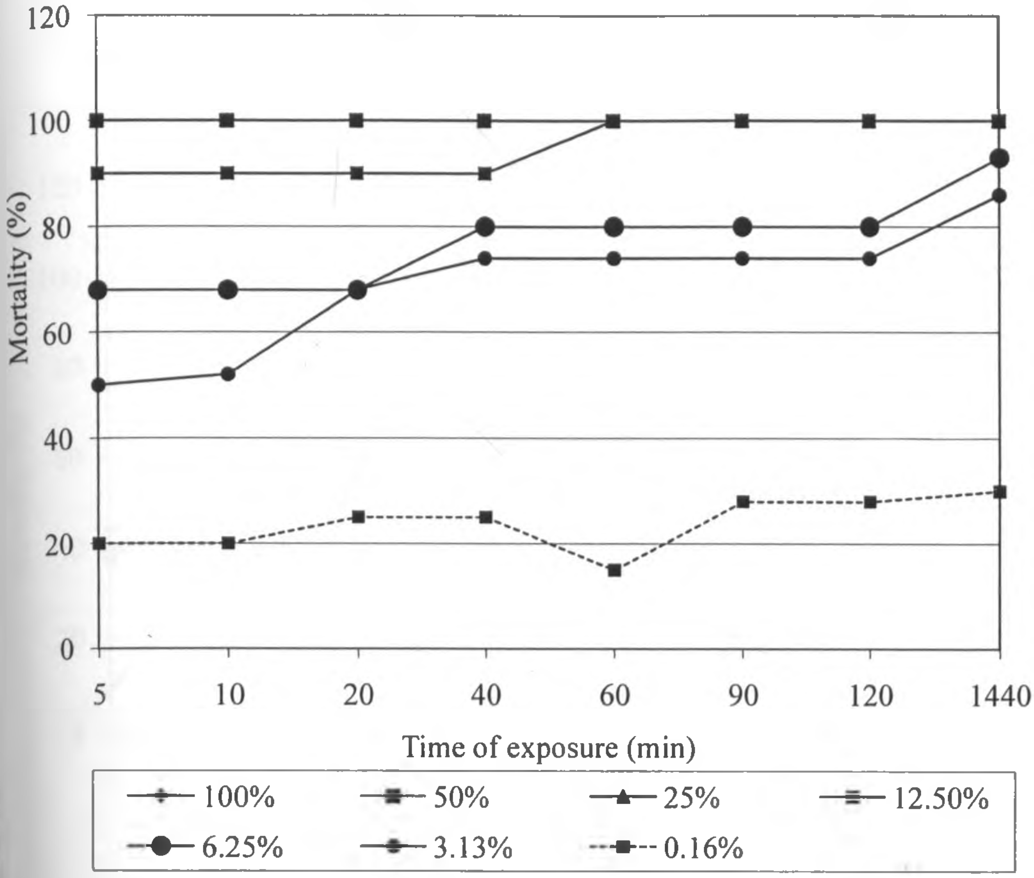


Fig 6.10. Percentage mortality of *Haematopinus suis* to at various concentrations of neem oil

At the highest concentration of amitraz (5 mg/ml) used, only 50% of the lice were killed in 20 minutes, while all lice were killed by the 24th hour of incubation (Fig. 6.11, Appendix V). However, at the concentration of 2.5, 1.25 and 0.625mg/ml Amitraz, 80%, [illegible]% and 30% of the lice were killed by 24hours of incubation. Thus, the effects of amitraz on lice were dose and time dependent, and 5mg/ml was the only dose which was able to kill all the lice in 24 hours. Statistically, there was a significant effect of time (F=5.83, p=0.0007) and dosages (F=46.01, p=0.000) on lice mortality.

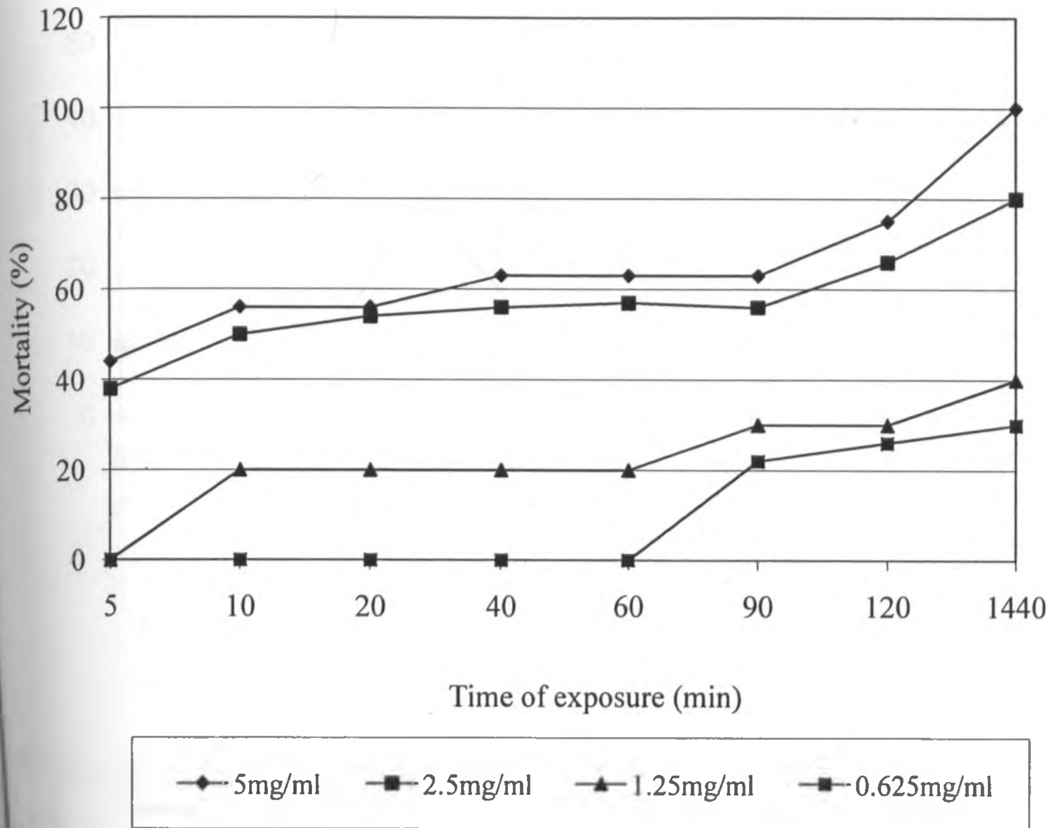


Fig 6.11. Percentage mortality in *Haematopinus suis* exposed to different concentrations of Amitraz

For latex, the concentration of 30, 15, 7.5, 3.75mg/ml respectively caused a mortality of [illegible], 55, 40 and 23% within 24 hours (Fig 6.12, Appendix V). Mortality was significantly affected by the differences in latex dosages (F=115.52, p=0.000) and time of measurement (F=10.32, p=0.0007).

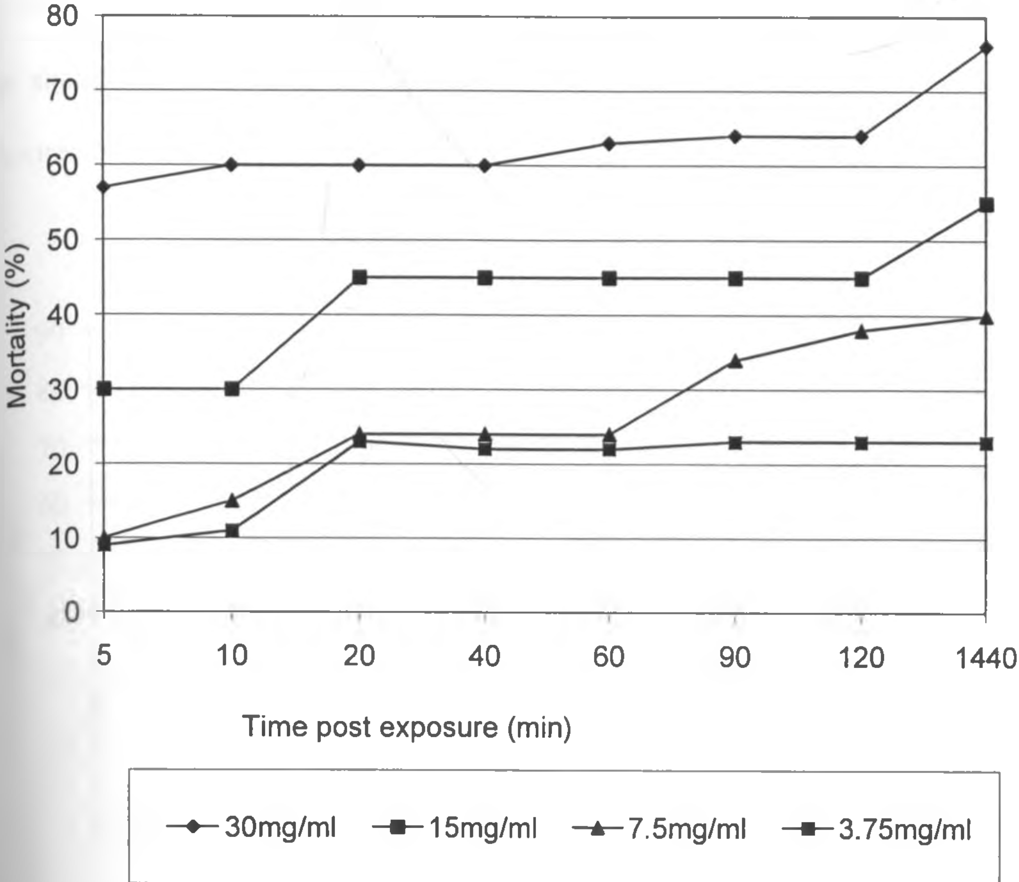


Fig 6.12. **Percentage mortality in *Haematopinus suis* exposed to various concentrations of latex**

Papain at a concentration of 30, 15, 7.5, 3.75mg/ml respectively caused a mortality of 83, 64, 50, and 17% after 24 hours of incubation (Fig. 6.13, Appendix V). Lice mortality was thus significantly affected by papain dosages (F=30.43, p=0.000) as well as time of measurement (F=4.60, p=0.003).

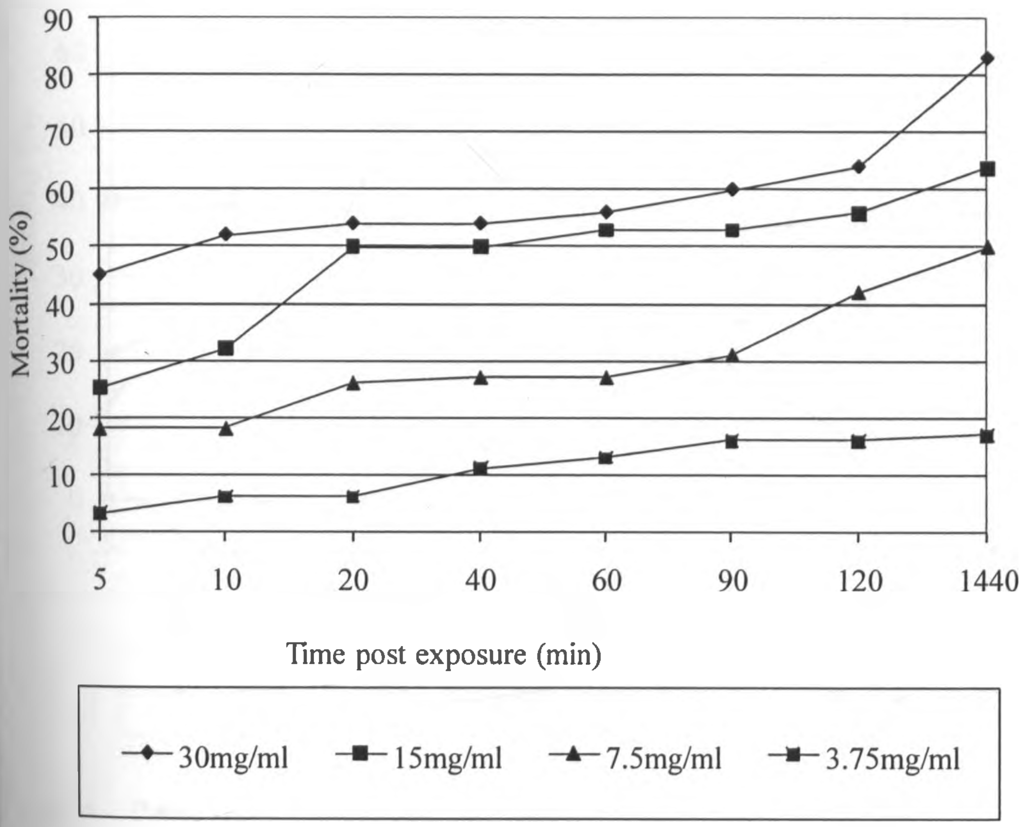


Fig 6.13. Percentage mortality in *Haematopinus suis* exposed to various concentrations of papain

Levamisole HCl at a concentration of 7.5, 3.75, 1.875 and 0.9325mg/ml caused a mortality of 83%, 70%, 40 and 33% respectively after 24 hours of incubation (Fig 6.14, Appendix V). Lice mortality was significantly affected by differences in Levamisole dosages (F=36.62, p=0.000) and time of measurement (F=6.30, p=0.0005). Although, the effects of latex, papain and levamisole were time and dose dependent, none of the dosages applied caused a 100% efficacy even after 24 hours of incubation.

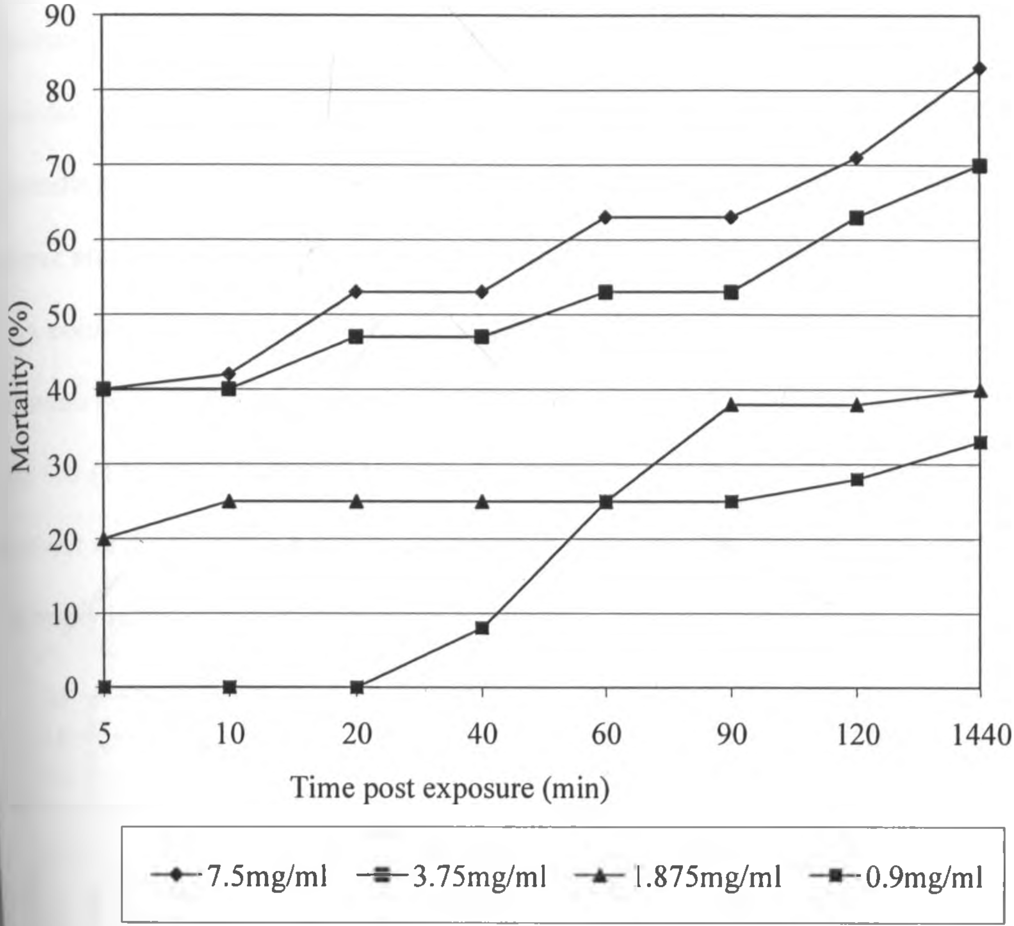
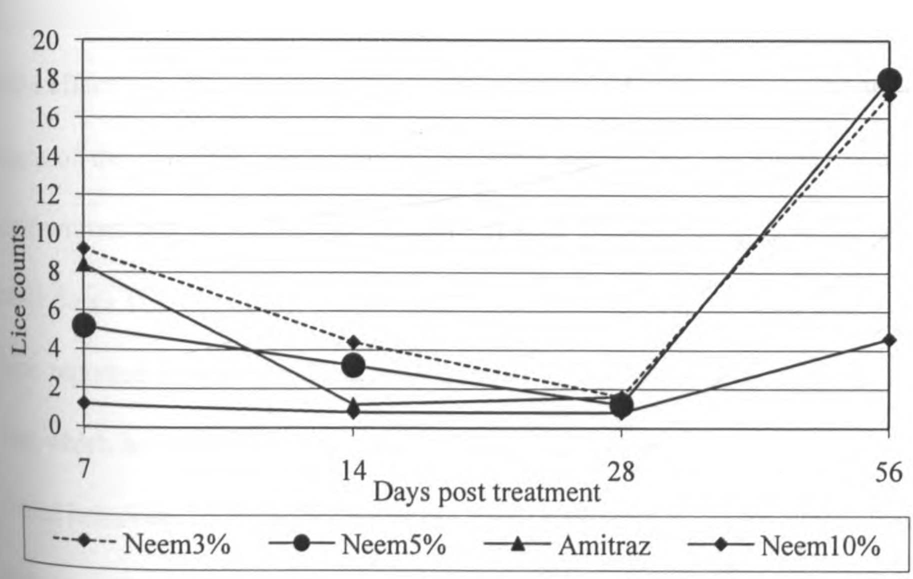


Fig 6.14. **Percentage mortality in *Haematopinus suis* exposed to various concentrations of levamisole**

6.3.4 Evaluation of efficacy of herbal remedies on *H. suis* infestation in pigs

The effect of neem oil and amitraz on *H. suis* counts in pigs is shown in Fig 6.15. At Day 0, the lice count in all the pigs was heavy (>100) and it was not possible to count the numerous lice. Upon treatment, the lice counts declined (to levels of +1 i.e. 1-10) consistently ($p<0.0001$) in all the treatment groups up to 28 days post treatment. In descending order, the highest efficacy was noted in the 10% neem oil, 5% neem oil, amitraz and 3% neem oil. The only significant ($p=0.046$) difference was that between the mean counts in the 10% neem oil and 3% neem oil. However, at 56 days post treatment, the mean counts for the pigs sprayed with 10% neem oil, 5% neem oil and 3% neem oil were 4.6 (+1), 18 (+2) and 17.2 (+2) respectively. For amitraz, at 56 days post treatment, three out of 5 pigs had a heavy lice infestation (+3). The infested untreated control group had heavy infestation (+3) throughout the study.



NB: Control group had heavy infestation (not enumerated) of lice throughout the study.

Pigs in Amitraz group had heavy lice infestation on day 56

Fig 6.15. Effect of neem oil and amitraz on *Haematopinus suis* in pigs

In all the groups, there was an increase in weight for the pigs. The mean weight increase for the 3% neem oil, 5% neem oil, 10% neem oil, amitraz, and control group at 56 days post treatment respectively was 9.2 kg, 9.1kg, 9.3kg, 8.1kg, and 7.9kg. The increments were not significantly related to the groups ($p<0.05$). The mean daily weight gain ranged from 141 to 164g/pig/day.

6.4 DISCUSSION

Overall, the plant products used in the current study had both *in vitro* and *in vivo* lethal effects against a gastro-intestinal nematode *Oesophagostomum* spp and the ectoparasite *H. suis*. At *in vitro* level, the current study showed that the pawpaw products (latex and papain) had effects against the 3 key lifecycle stages *Oesophagostomum* spp, that is eggs, larvae and adult worms, indicating their potential as anthelmintics. Generally, papain and papaya latex were the most effective products against *Oesophagostomum* spp, the most lethal effect being on egg hatching and the survival of adult worms. By affecting these two stages of the parasites, the products can affect the normal physiology and anatomy of the adult worms and also affect the viability of eggs excreted by pigs to the environment. The lethal doses (LD_{50}) against adult worms were however higher than that of levamisole and those reported for benzimidazoles (Varady *et al*., 1996). Papain is an extract from papaya latex which has been shown to be efficacious against nematodes such as *Ascaris suum*, and several helminths of mice (Satrija *et al*., 1994; Stepek *et al*., 2005; 2006; 2007). The current study reports for the first time the effect of papaya latex and papain on *Oesophagostomum* spp. The LD_{50} of papain and crude papaya latex against motility of the mice nematode *H. bakeri* was reported as 7.5 and 12.5μM (Behnke *et al*., 2008), values which are lower than those reported in this study. The variation could be related to other factors including amount of active ingredients in the products used as well as host and parasite differences. On the other hand, the pawpaw seeds and the water extract from seeds had moderate effect on egg hatching and had minimal effects on larval development and adult worms. This shows that there were some water soluble active principles in pawpaw seeds. Alcohol extracts of *C. papaya* seeds were also shown to affect the egg hatching, larval migration and adult worm motility of *H. contortus* and *T. colubriformis* by Houzangbe-Adote *et al.*, (2005). For *H. contortus* and *T. colubriformis*, the percent immobile adult worms (at 24 hours) at 2500µg/ml concentration of the pawpaw extract were 100% and 38.1% respectively (Houzangbe-Adote *et al.*, 2005). In contrast, the current study reported the LD_{50} for aqueous extract and seed powder against adult worms of *Oesophagostomum* spp as 2,572µg/ml and 4,743µg/ml, respectively.

The pawpaw latex and papain appeared to have similar effects on the adult worms, mainly affecting the cuticle of the adult worms. The observed lesions included loss of normal architecture of the cuticle, formation of crinkled ridges, bursting and erosion of the cervical vesicle. At higher concentrations (>15mg/ml), the products caused the worms to disintegrate. The cuticle mainly consists of collagen-like proteins (mainly at the medial and basal layers) cross-linked by disulphide bonds (Behnke *et al.*, 2008) and it is possible that the proteinases could be targeting these bonds, resulting in weakening of the cuticle mainly the basal and medial layers. The role of protease is also critical during molting, where an exsheathment protease is responsible for dissolution of hypodermis and basal layers of cuticle (Wharton, 1991). During the current study it was observed that on exposure to the papain and pawpaw latex, the outermost layer of the worms' cuticle crinkled and the ridges (crêtes) lost their rigidity. The combination of eroded cuticle and internal high hydrostatic pressure within the pseudocoelomic cavity caused the worms to burst open carrying externally sections of internal organs. The lack of toxicity of pawpaw latex on the pigs could be due to low concentration used and lack of the targeted proteins in the gastrointestinal tract mucosa.

In the field based study, the products were tested against *Oesophagostomum* spp since it was the commonest nematode in the sampled pigs. All the administered products had varied effects on the fecal egg counts. That levamisole is highly effective on pig nematodes was observed where the drug caused 100% reduction in worm counts 7days post treatment, although at day 14 and 28, the reduction was less. During the earlier farm survey (Chapter 4), Levamisole (Wormicid® tablets, Cosmos Ltd, Kenya) was the commonest conventional drug used by the pig farmers in the district, possibly because it is cheap and readily available. The less than 90% efficacy of levamisole at days 14 and 28 implies either an occurrence of anthelmintic resistance or under strength drug, which should be further investigated. The occurrence of *Oesophagostomum* spp resistant to levamisole and the use of substandard anthelmintics have been reported to occur in Kenya (Kagira *et al.*, 2003).

As in the *in vitro* tests, the *in vivo* studies revealed that papain and papaya latex were the most effective pawpaw products, causing an EPG reduction of 70.3% and 65.7%, respectively at day 28 post treatment. There was sustained reduction between days 7 and

14 post treatment. It is important to note that pigs infected with *Oesophagostomum* spp do not develop immunity against this worm, and thus reduction of worm burden due to development of immunity was not suspected. In the current study, the latex and papain were administered at 1 mg/kg and thus it is possible that higher doses will cause a higher reduction in worm burdens. However, it should be noted that paw paw latex might not affect developmental stages of the *Oesophagostomum* spp, which are located within the mucosa, and this may be responsible for increase in EPG at 56 days post treatment. This shows the need for multiple treatments. Similarly, mucosal stages of *H. bakeri* and *T. muris* were not affected by pawpaw latex (Behnke *et al*., 2008). This may be a useful property; where treatment with latex will be stage-specific damaging only the adult worms and leaving out the developmental stages which can repopulate the gut subsequently. As suggested by van Wyk, (2001), this may slow any development of anthelmintic resistance, although this would mean that treatments would have to be repeated frequently where exposure to infective stages is high. Alternatively, the animals can be regularly fed supplements with raw pawpaws.

Pawpaw seed powder was least in causing the fecal egg count reduction (36.1% at day 7, 52.8% at day 14, 41.7% at day 28 and 27.1% at day 56). It is important to note that a substantial number of farmers in Busia District used pawpaw seed to deworm their pigs as was earlier reported in this study (see Chapter 3). The farmers had indicated that they used a handful of seeds which they fed to their pigs. Since a handful will vary from individual to individual administering the seeds, the variation of amount given by farmers would be wide. However, quantitatively, the amount of seeds given in this study

(>30g/pig) appeared far above that given by farmers, and thus the claimed efficacies might not be justifiable. Reports of the effect of pawpaw seeds on gastrointestinal nematodes are scarce. Fajimi *et al.*, (2002) reported that dried pawpaw seeds administered at 50mg/kg, twice a week for 6 weeks (total dose =600mg/kg) to sheep, caused the EPGs of *Oesophagostomum* spp, *Trichuris*, and *Trichostrongylus* spp to reduce by 87%, 95% and 93%, respectively. Papaya seeds also caused 80% reduction in fecal egg counts in sheep (Hounzangbe-Adotte *et al.*, 2001) while an infusion of papaya seeds administered for 3 consecutive days also showed high efficacy (96%-100%) against *Aspiculuris tetraptera* and *Hymnolepsis nana* infections in mice (Satrija *et al.*, 2001). The mode of administration, quality of seeds used, geographical differences of cultivated pawpaw, host and parasite differences could have influenced the variation between these studies. Future experiments should evaluate the higher doses of pawpaw seeds as well their extracts (eg., alcohol) to determine their efficacies against the nematodes of pigs.

At *in vitro* level, neem oil was effective against the larvae and adult worms at concentrations above 6.25%. At *in vivo*, neem oil had maximum efficacy (50%) at day 56 post treatment. The effect of neem oil on animal nematodes has not been earlier reported in published literature. Other products of neem plants have been shown to have some efficacy against a number of livestock nematodes (Ahmed, 1994; Chandrawathani *et al.*, 2006). In sheep, neem kernel powder was shown to cause a decrease in nematode eggs and an increase in body weight (Ahmed, 1994). Chandrawathani *et al.*, (2006) reported that *ad libitum* feeding of fresh neem leaves produced 82% reduction in worm eggs, while a study by Githiori (2004) did not reveal any significant reduction by neem extracts on helminth infections in mice and sheep. The higher level of Azidachtrin A concentration in neem oil (~25mg/100g) compared to only ~0.59/100g (Sindaram, 1996) in leaves could partly explain the variation in the effect of neem oil on nematodes. In the current study, the neem oil appeared to form droplets along the cuticle lining, thereby disrupting the normal structure of the cuticle. It has been reported that apart from effects of azadirachtin, clarified hydrophobic extracts of neem oil can possibly cause death of parasites through disruption of the outer membrane/cuticles and/or interference with respiration. Further studies on the effect of higher doses of neem oil on pig nematodes should be evaluated.

In spite of the decline in EPG counts in the treated groups, the weight changes in the different groups were not significantly different from that of controls, although the mean weight in the latter group was the lowest. It is possible that the period of monitoring was not long enough to allow the weight gain differences to be significant. Further, the weight gains of all the groups were quite low considering the pigs used were growers. The daily weight gain (DWG) ranging between 111-145g /day was low compared to other studies where pig growers are expected to have DWG of more than 300g/day (Nguyen *et al.*, 1997). This could be explained by the fact that pigs in our study were crossbreeds with poor productive potential and were mainly grazers which were not fed substantial supplements. In Vietnam, the mean daily live weight gain of pigs under the traditional feeding system was observed to be low (202- 230 g/day) but was significantly increased between 363-and 366 g/day by giving the protein supplement (Nguyen *et al.*, 1997). Although the pigs in the current study were kept in conditions which were comparable, the weight changes should be held with caution since it could not be ascertained that they were given a similar diet.

The pigs administered with the various plant products did not manifest any clinical sign that could be associated with toxicity. Others have shown that high doses of papaya latex (8mg/kg) produce diarrhea and constipation in pigs (Satrija *et al.*, 1994) and mice (Satrija *et al.*, 1995). Purified enzymes from pawpaw have also been observed to have low toxicity to humans, thus chymopapain is a licensed drug for treatment of inter-vertebral disc disease in human beings, while proteases have been used as meat tenderizers (Behnke *et al.*, 2008).

At *in vitro* levels, neem oil was found to be very effective against *H. suis*. When subjected to neem oil at concentration of 25-100%, all the lice were killed on contact with the oil. At lower concentrations (<25%), neem oil effect was both time and concentration dependent. Within a period of 10 minutes, all the concentrations of neem oil above 3.125% were able to kill more than 50% of the lice. There was no other product which was able to kill lice within such a short period, showing that neem oil has a superior knock down effect on *H. suis* and is pediculicidal. The observed rapid effect of neem oil was surprising because it has been previously assumed that the effect of neem on insects is protracted (Habluetzel *et al.*, 2007). Recently, a neem based shampoo was shown to be highly active *in vitro* against head lice in humans (Heukelbach *et al.*, 2006) where all lice were killed within 5 minutes, which is similar to the current study. However, a neem extract killed head lice slowly *in vitro* in a filter paper test (Morsy *et al.*, 2000). Further, a commercial neem seed extract (Praneem® ,New Chem Laboratories Pty, USA) was observed to have less than 10% effect on head louse (*Pediculus humanus*) after exposure for 3 hours (Heukelbach *et al.*, 2008). The variation in the outcome of the tests emanates from the varieties of neem extracts used in each study as well as the type of test used (Heukelbach *et al.*, 2006).

Similar to the *in vitro* data, neem oil at concentrations between 3-10% was effective in controlling the lice in pigs. It is important to note that lice infestation in the pigs was quite heavy at the start of experiment, and in the control group, the lice densities remained high throughout the experiment. The effect of neem oil was time and dose dependent with maximum activity observed in the 10% group and at 28 dpi. Doramectin and ivermectin at 300μg/kg were previously found to have an efficacy of between 80-100% against *H. suis* (Stewart *et al.*, 1981). In other studies, Azadachtrin A was able to significantly reduce biting lice densities in sheep and goats infected with *D. ovis* and *D. limbata* (Heath *et al.*, 1995, Habluetzel *et al.*, 2007). A reduction in louse densities of 76-96% was observed from weeks 2 to 18 after treatment with Neem Azal® solution containing azadachtrin at a concentration of 650ppm (Habluetzel *et al.*, 2007). In sheep, lice reduction of between 85-99% from week 2 to 7 was observed when they were treated with neem extract (1000ppm, fortnightly) (Heath *et al.*, 1995). Although azadachtrin has rapid decay due to photosensitivity (Heath et al., 1995), its strong lipophilicity might protect it from oxidation, leading to long lasting effect. The pig skin has high fat content which may also help in retaining the lipophilic product. Due to the possible effect of neem oil on sarcoptic mange and gastrointestinal nematodes, it would be important to evaluate the possibility of using this oil in control of pig parasites. However, these studies should be repeated on wider scale using higher number of pigs in variety of production systems.

Although neem oil contains at least 35 biologically active compounds, Azadirachtin A is the most active compound (Mitchell *et al.*, 1997). Most of its activity has been evaluated in insects, where it mainly affects feeding and reproduction (Habluetzel *et al.*, 2007). At high concentration, the rapid knockdown effect of neem oil used in this study could be due to direct effect of neem compounds on critical survival organs in the lice. Other studies have shown the presence of hydrophobic substances in neem oil, which cause respiratory failure and lethal desiccation through loss of cyclic gas exchange in insects and arthropods (Handule *et al.*, 2002).

At *in vitro* level, 5mg/ml of amitraz was only able to kill 50% of the lice in 20 minutes. Nevertheless, the same concentration of amitraz caused a 100% mortality of lice within 24 hours, while lower concentrations caused relatively minimal effect on the lice. The lengthy killing time has also been observed when head lice (*H. capitis*) from humans were exposed to permethrin leading to a mortality of 20% at 5 min, 50% at 15 minutes, and 74% after 3 hours (Heukelbach *et al.*, 2006). In the current study, Amitraz was also able to effectively control lice in infested pigs, such that at 28 days post treatment, the mean lice count per pig was 1.6. However, there was a rise in lice counts at 56 days post treatment, and the source could have been from the environment. This shows there is a need for a second treatment, nearly two months after the first treatment. In a previous study conducted in housed pigs in USA, amitraz was able to provide a 100% control of *H. suis* when a second treatment was instituted at 14 days (Williams and Gaafar, 1988). Amitraz has a broad-spectrum activity against a variety of livestock parasites including ticks, lice, and mites (Harrison and Palmer, 1980; Wabacha *et al.*, 2006) and in Busia 24% of pig farmers use this ectoparasiticide.

The pawpaw products, latex and papain, were also tested for their *in vitro* effects against *H. suis*. Both products were effective in killing this louse and this indicates that cysteine protease has detrimental effect to *H. suis*. As observed for the gastrointestinal nematodes, cysteine protease could digest the cuticle of *H. suis* leading to its mortality. In this study, the examination of the cuticle under the microscope was not undertaken and thus it was not possible to confirm the cuticular effects of the pawpaw products. Future experiments should be undertaken to determine the effects of these products on lice as well as their *in vivo* efficacies. Literature is scarce on the effects of pawpaw latex and papain on ectoparasites of animals. However, it is noteworthy that cysteine proteases, from pawpaw and fig tree respectively, have some insecticidal properties which provide the plants with a general defense mechanism against herbivorous insects (Kotaro *et al.*, 2004). Further, extracts from the seeds and stem bark of the North American pawpaw plant (*Asimina triloba*) have acetogenins which are active against various insects (Ratnayake *et al.*, 1993). Thus, it is possible that pawpaw extracts could have similar effects on lice. In humans, a shampoo made from extracts from pawpaw leaves (0.5%), thymol (1%) and tea tree oil (0.5%) effectively (100%) controlled head lice in 16 children in USA (McCage *et al.*, 2002).

The effect of Levamisole against *H. suis* was also tested in the current study. At a concentration of 7.5 and 3.75mg/ml, the drug was able to cause a mortality of above 50%, 24 hours after incubation. There are no reported cases of *in vitro* tests of levamisole against *H. suis*. However, levamisole was shown to have some pediculicidal activity against head louse, where an efficacy of 85% was observed in human patients orally administered Levamisole at 3.5mg/kg daily for 10 days (Namazi, 2001). The drug levamisole is widely used against gastrointestinal nematodes where it inhibits the cholinergic synapses in their nervous systems and neuromuscular junctions (Namazi, 2001). A similar mode of action could be responsible for the effect of levamisole against lice (which have numerous cholinergic synapses), since other cholinesterase inhibitors such as malathion and carbaryl have been used extensively as pediculicides. At *in vivo* level, pigs treated with levamisole at 7.5mg/kg did not have any changes on the lice densities. It is possible that the amount of levamisole after single administration of drug was not enough to affect the numerous lice in pigs. In future, a longer regimen and higher dose of levamisole should be evaluated.

The treatments of lice did not significantly affect the weight changes of the pigs, however, the lowest weight gain was registered in the control group. As indicated above, this could be attributed to short period of monitoring as well as poor feeding of the pigs in the study area. However, it has been shown that average weight gains are poor indicator of cure of *H. suis* infestation in pigs (Davis and Williams 1986).

CHAPTER SEVEN: GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

7.1 General discussion

This study was undertaken in Busia District and aimed at characterizing the pig production and trade system and determining the epidemiology of pig parasites and efficacy of pawpaw products and neem oil against *Oesophagostomum* spp and *Haematopinus suis* infections in pigs. In an effort to achieve these objectives, a number of studies were conducted.

A comprehensive questionnaire study revealed that the pig production system in the district can be described as free-range, small scale, and low-input with an income generation objective. The production system had two main groups of farmers: a few breeders who kept breeding sows and then sold piglets to a second category who then fattened the weaners for sale. The study further revealed that the farmers did not utilize improved breeds and sourced foundation stock from fellow farmers; this may give rise to inbreeding and associated low productivity. Housing was rarely provided and pigs were fed locally available feeds. These characteristics of the pig production system in Busia District are common in resource poor pig production systems in many parts of the world as reported by Permin *et al.* (1999), Lemke and Zarate (2007) and Nsoso *et al.* (2006). The main production constraints included diseases and high cost of commercial feeds. The farmers identified the main disease constraints as African Swine Fever and parasitic infections. These diseases are common in free-range smallholder pig production systems in developing countries (de Fredrick and Osborne, 1977; More *et al.*, 1999). The observed poor husbandry skills and constraints mainly emanated from lack of extension information which was common in the study area. The Kenya government and relevant NGOs should consider offering affordable animal health and production services to these farmers.

The slaughter-slab survey revealed that the number of pigs slaughtered in the district was only second to that of cattle. Most butchers had operated the butchery business for long periods, and the average net income per annum for each butcher was Ksh 62,784 (897 USD). There were several constraints to the pig trade including conflicts with regulatory authorities especially during transportation of pigs and inadequate number of slaughter-pigs. Similar constraints have been observed in countries like Nigeria (Ajala and Adesehinwa, 2007). To address these constraints, the role of government and other extension service providers will be important.

The prevalence and intensity of parasites was investigated in slaughtered pigs at the slaughter slabs level and live pigs at farm level. The study revealed that the pigs were infected with various gastrointestinal nematodes including *Oesophagostomum* spp, *Strongyloides ransomi*, *Ascaris suum*, *Trichuris suis*, *Physocephalus sexalatus* and *Globocephalus urosulubatus* and the lungworm *Metastrongylus* spp. This study provides the first report of the occurrence of *Metastrongylus* spp, and *G. urosulubatus* in Kenya. In individual slaughtered pigs, the prevalence of pigs excreting *Oesophagostomum* spp (100%) and *A. suum* (19%) was higher than proportion of pigs having nodular lesions (?%) and liver spots (43%). However, the prevalence of pigs excreting *Metastrongylus* spp eggs (54%) was lower than those having lungworm lesions (97%). The variations can be attributed to resolution of the lesions, sex ratio imbalance, life cycle, and clearance of infection by immune response as reported by Bernado *et al.*, (1990). The pathology observed in in pigs infected with metastrongylosis can seriously constrain the health and productivity of the pigs.

The study has for the first time comprehensively determined the relationship between prevalence of parasites and host/environmental factors in pigs kept in a free range production system in Kenya. The prevalence of nematodes was significantly ($p<0.05$) related to previous history of deworming (*A. suum*) and provision of night housing (mixed species, *Metastrongylus* spp and *S. ransomi*). The farmers should be encouraged to undertake regular deworming and housing of pigs, as these activities can significantly interrupt the life cycle of the nematodes. The prevalence of nematodes was significantly associated with age (*Oesophagostomum* spp, *A. suum*, *S. ransomi* and *T. suis*) and thus, *Oesophagostomum* spp was more common in adults possibly due to limited immunity which the nodular worm stimulates in the host (Roepstorff and Nansen, 1994) and as such closer attention should be targeted towards the sows which are the main source of *Oesophagostomum* infection in a herd. On the other hand *Metastrongylus* spp and *S. ransomi* were more common in piglets probably due to related development of immunity in adult animals (Roepstorff and Nansen, 1994). The high prevalence of *S. ransomi* in piglets could be due to trans-colostrol transmission (Nansen and Roepstorff, 1999). Deworming of sows before farrowing can therefore effectively reduce infection in piglets.

The prevalence of nematodes was also significantly associated with division of sampling and subsequently the amount of rainfall. Thus the prevalences of *Oesophagostomum* spp, *A. suum* and *Metastrongylus* spp were higher in pigs from divisions with higher amount of rainfall such as Township and Nambale. As reported by others (Roepstorff and Nansen, 1994), moist warm environment enhances the development and transmission of these nematodes which could account for the higher prevalence in divisions with higher amounts of rainfall. On the other hand, *S. ransomi* was more common in the drier Budalangi Division, which could indicate that the parasite can thrive well in areas where there are high temperatures and relatively low rainfall. Most nematode parasites were reported to occur in higher prevalence in male than female pigs, possibly due to animal specific behaviour and hormonal influences (Studnitz *et al.*, 2003, Roepstorff and Nansen, 1994). There is also a possibility that farmers may give more attention and treatment to the females than males so as to increase the reproductive potential of the females.

Prevalence of *Taenia solium* cysticercosis was recorded at 4% using an antigen ELISA in a farm survey. The prevalence recorded was within the range reported from other studies in western Kenya (Githigia *et al.*, 2006; Mutua *et al.*, 2007) but lower than those reported elsewhere in Tanzania and Uganda (Ngowi *et al.*, 2004). Lack of latrines was the only significantly risk factor associated with the prevalence of porcine cysticercosis. Other risk factors for occurrence of cysticercosis which were found in some farms included home slaughter, lack of meat inspection and history of human taeniosis infection. Some of these features were also observed amongst the butchers and have been reported to be common in pig rearing areas in other developing countries (Ngowi *et al.*, 2004). Public health education and evaluation of the occurrence of human form of cysticercosis should be undertaken in the study area.

The gastrointestinal protozoan parasites observed in this study includes coccidia, *Balantidium coli*, *Tritrichomonas suis* and *Entamoeba* spp. Apart from coccidia, the other three parasites have not previously been reported in pigs in Kenya. The prevalence and intensity of coccidia was high, possibly due to high humidity conditions in the district. Occurrence of the species of *Eimeria* observed in this study including *E. debliecki* and *E. scabra* can be detrimental to pigs (Hill *et al.*, 1985). *Balantidium coli* occurred in higher prevalences in wetter divisions such as Township, while Coccidia, *Tt suis* and *Entamoeba* spp occurred in higher prevalences in drier divisions such as Budalang'i. Although *B. coli*, *Tt. suis* and *Entamoeba* spp are natural inhabitants of swine intestines, they can be of zoonotic significance (Barnish and Ashford, 1989; Dwyer, 2006). Future studies should investigate zoonotic significance of these parasites in Busia District.

The ectoparasites found in pigs in the study included *H. suis*, *S. scabiei*, and *Ixodid* ticks and *T. penetrans* (jiggers). Apart from *S. scabiei*, the other ectoparasites have not previously been reported in Kenyan pigs. The prevalence of clinical mange was related to the age of pigs being highest in adults but lowest in piglets, which is similar to that reported in other studies (Alonso *et al.*, 1998). The high prevalences of *H. suis* in outdoor pigs is associated with pasturing and keeping of pigs in unhygienic conditions (Damriyasa *et al.*, 2004) and in the current study it was associated with age (more in sows), lack of spraying and housing. Lack of housing was associated with higher prevalence of ticks showing that where pigs kept in free-range conditions, tick transmission is common (Holness, 1999). The control of these ectoparasites should also be emphasized in free-range pigs, as they are transmitters of several livestock diseases including eperythrozonosis and babesiosis (Permin *et al.*, 1999). The only haemoparasite in the study was trypanosome infecting only one pig in each survey. Low prevalence of trypanosomes in Busia District has been reported by other authors (Angus, 1996; Ngayo *et al.*, 2005).

The efficacy of pawpaw and neem products against the *Oesophagostomum* spp and *H. suis* in pigs was determined at both the *in vitro* (laboratory) and *in vivo* (field) levels. This is the first time such a study has been undertaken in Kenya. The *in vitro* assays revealed that papain and papaya latex were the most effective products against *Oesophagostomum* spp, the most lethal effect being prevention of eggs of the parasites from hatching and reduction of adult worms' survival. The study also demonstrates for the first time that the pawpaw latex and papain affected the cuticle of adult *Oesophagostomum* spp worms leading to loss of normal architecture of the cuticle, formation of crinkled ridges, bursting and erosion of the papillae. The *in vivo* studies in pigs showed that papain and papaya latex were the most effective pawpaw products. In previous studies, crude papaya latex was shown to be efficacious against *A. suum* and worms in rodents (Satrija *et al.*, 1994, Stepek *et al.*, 2007). Although a substantial number of farmers in the district indicated that they used pawpaw seeds to treat their pigs, pawpaw seed powder in the current study had least effect on the EPGs. It is thus possible that the amounts given by farmers have minimal effects on worms. Future studies in this area should evaluate the effectiveness of higher doses of the pawpaw products.

Neem oil caused 100% mortalities (100%) of *H. suis in vitro* when used at concentrations above 25%. This is the first report on the lethal effects of neem oil on *H. suis*, as previous studies have mainly documented the negative effect on reproduction of other types of lice in livestock (Mitchell *et al.*, 1997). In the field study, neem oil at a concentration of between 3 and 10% was found to be effective in controlling the *H. suis* in pigs, although an increase in *H. suis* counts at 56 days post treatment revealed a need for repeat treatment. The multiple effect of neem oil on ectoparasites and GIT nematodes makes it a good candidate for use in the control of parasites in pigs. High efficacy of conventional drugs, levamisole and amitraz in the control of GIT nematodes and *H. suis*, respectively was observed in this study. These drugs were used by a substantial number of farmers, although the high costs can reduce their usage among resource poor small scale farmers.

7.2 Conclusions

The following conclusions were made from this study:

- The pig production system in Busia District can be characterized as free-range, small -scale, and low-input but geared towards generation of income.
- Poor husbandry skills and risk factors for zoonoses occur in the farms
- According to the farmers, the important constraints to production included diseases, high cost of feeds, neighbourhood conflicts, unreliable markets and lack of breeding animals.

- The main disease constraints as described by farmers are African Swine Fever (ASF), helminth infections, respiratory problems, pediculosis and mange.
- The constraints to pig trade in the district include conflicts with regulatory authorities, poor transporting methods for pigs and inadequate number of slaughter-pigs
- The nematodes parasites infecting the pigs include *Oesophagostomum* spp, *Strongyloides ransomi*, *Ascaris suum*, *Trichuris suis*, *Metastrongylus* spp, *Physocephalus sexalatus* and *Globocephalus urosulubatus*. *Metastrongylus* spp and *Globocephalus urosulubatus* are reported for the first time in pigs in Kenya.
- The prevalence of nematodes in pigs is clearly associated with division of origin of the pigs, host (age, sex) and management of pigs present at farm level.
- The prevalence of porcine cysticercosis was characterized using highly sensitive antigen ELISA and was shown to be significantly associated with lack of latrines at homestead level
- The gastrointestinal protozoan parasites observed in this study include coccidia, *B. coli*, *Tt. suis* and *Entamoeba* spp. This is the first report on the occurrence of *B. coli*, *Tt. suis* and *Entamoeba* spp in Kenya.
- The prevalence of gastrointestinal protozoan parasites was associated with division of origin of the pigs and host factors
- The ectoparasites found in pigs included *H. suis*, *S. scabiei*, *Ixodid* ticks and *T. penetrans*. This is the first report on the occurrence of *H. suis*, *Ixodid* ticks and *T. penetrans* in pigs in Kenya.

- The prevalence of *H. suis*, *S. scabiei* and *Ixodid* ticks was high and was associated with division of origin, management of the pigs and host factors
- The results of this study demonstrate for the first time that pawpaw and neem oil products are effective against *Oesophagostomum* spp and *H. suis*. The study also demonstrates for the first time that the effects of pawpaw latex on *Oesophagostomum* spp can be attributed to adverse destruction of the parasite's cuticle.

7.3 Recommendations

- Majority of observed constraints at the farm level can be addressed through provision of affordable extension services to the farmers.
- Future research and development approaches should focus on the integration of small-holders farmers from Western Kenya into the country's market chains and also breed improvement. The role of gender in management, control of pig diseases and marketing needs to further studied.
- Longitudinal epidemiological studies needs to be undertaken with a view of further elucidating the role of seasons in influencing the occurrence of pig parasites and possible interventions.
- The presence of risk factors for zoonoses as well as the occurrence of cysticercosis in the study area calls for public health education targeting all stakeholders. The prevalence of *Taenia solium* taeniosis/cysticercosis needs to be investigated in the human population.

- Further studies on the pawpaw and neem products including evaluation of the possible toxic effects on the pigs, dosage formulation and effectiveness of the products in integrated control programmes for pig parasites are recommended

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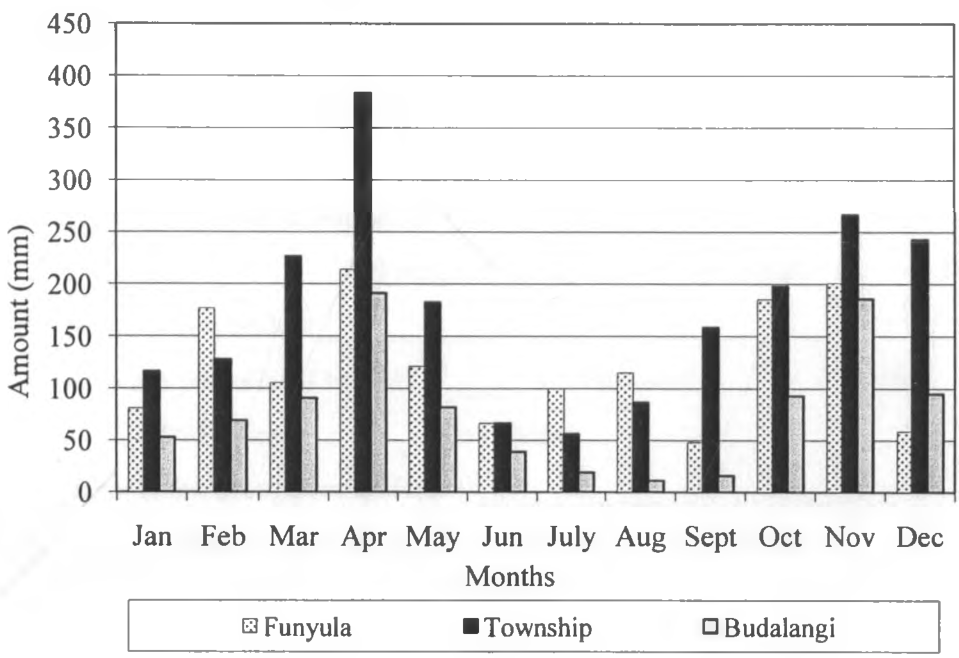
APPENDICES

APPENDIX I. RAINFALL DISTRIBUTION IN THREE DIVISIONS IN BUSIA DISTRICT IN 2006



APPENDIX II. QUESTIONNAIRE USED IN THE CROSS-SECTIONAL STUDIES (SOCIO-ECONOMIC AND FARM SURVEY)

Name of Interviewer____

1. General details

Division ____________________Location ______________________________
Sublocation____________________Village________________________________
GPS reading____________________

2. Household information

a) Name of household Head (HH) ______________________________________

b) Gender of HH__________ Age of HH_________________

c) What is the level of education level of HH _____________

1= None, 2=Primary school, 3=High school, 4=Tertiary

d) Name of respondent_______________________________

e) How are you related to HH?_______ 1 = Husband, 2= wife,

3= Sibling, 4= Others

f) What is the occupation of HH? __________________________

3. Questions related to pig production and farm characteristics

a) For how long have you been keeping pigs?____________________

☐< 1 year ☐>1 year and < 5 years, ☐ > 5 years

b) Where did you buy or acquire the current pigs?

☐ = From other farmers ☐ = FTC ☐ = Raised in the farm ☐ = other ____

c) What is the predominant colour of your pigs?____________________

☐=White, ☐=Black ☐= Black and white, ☐=Others (specify)

d) What is the size of your herd?________________________

e) What is the type of your operation in the last three years______ ?

☐= Breed piglets for selling to other farmers, ☐=Piglets for fattening ☐= Piglets for creating a breeding herd, ☐= Others (specify)___________

f) What other livestock do you keep in your farm?________(number)

g) What is the acreage of your farm?______________ acres

4. Management practices at farm level

j) How do you rear your pigs during the crop season?__________________

☐= Confined, ☐=Free range, ☐=Tethering, ☐= Mixed (explain)_______

k) How do you rear your pigs during the dry season?_______________

☐= Confined, ☐=Free range, ☐=Tethering, ☐= Mixed

l) Who takes care of the pigs (feeding, giving water)?

☐= Father, ☐=Mother, ☐=Children, ☐=Other

m) What type of housing do you provide for your pigs?__________

☐= Indoors with concrete, ☐= Indoors with earthen floor, ☐= None

☐ = mix with other livestock ☐ Others (specify)________

n) What is the nature of the floor?

1= Earthen, 2= Concrete, 3= Others (specify)_______________

o) Where do you get advice on pig husbandry?

1= Veterinary extension officers, 2= Other farmers 3=Over the counter sales people

p) What type of food do you give to the pigs?_____________________

q) What is the source of water for the pigs?

☐= Take them to the local river, ☐= Drink water at home ☐= Others___

r) At what age do you wean the pigs?---------------------days/months

s) Do you castrate your pigs? _______

t) If yes, at what age do you castrate your pigs)_________

5. Productivity constraints

a) Which of the following production constraints are found in your farm?

Constraint	Tick
Feed	
Labour	
Disease	
Marketing	
Water	
Credit	
Breeding	
Any others	

b) What are the five most common diseases that affect your pigs?

Disease 1	Disease 2	Disease 3	Disease 4	Disease 5

c) How significant is intestinal worms infection to your herd?

☐= mild significance, ☐= Large significance, ☐= Don't Know

d) What clinical signs do pigs show when they are infected with intestinal worms?

☐= Don't know, ☐= Distended stomach, ☐= Poor growth/ body condition,

☐=others__________

e) Where do you get advice on pig keeping?

☐ = Other farmers, ☐ = extension officers ☐ = Others__________

f) Do you treat pigs for worms?

☐= Yes, ☐= No

g) If yes, which drugs are used?

☐= Piperazine, ☐Levamisole, ☐= Bezimidazoles, ☐= Others

g) What governs you in choosing a given anthelmintic?____________________

☐=Price, ☐=Activity through experience,

☐=Is orally given, ☐=Veterinarian's advice,

☐=Over the counter advice, 5= Others: specify

g) How often do you deworm the animals?______________

☐= Every one month ☐= Every 1-2 months ☐= Once every 3 months ☐=Once every months

h) How significant is the impact of ectoparasites in your herd?_____________

☐= No significance, ☐= Mild significance, ☐= Large significance,

☐= Don't Know

i) Do you treat pigs for ectoparasites – (Lice and mange)?_____________________

☐= Yes, 2= No

j) If yes, which drugs do you use?_____________

☐= Amitraz, ☐=Ivermectin, ☐= Others ____

k) How often do you spray/treat pigs for ectoparasites?______________

☐=Every week, ☐= Every 2 weeks, ☐= 2 weeks ☐=Others______

6. Questions related to marketing

a) Where do you buy your replacement stock?______________

☐=Other farmers, ☐=Others (specify)____________________

b) Where do you sell your pigs?______________________

☐=Farmers choice company, ☐=Local butcheries, ☐=Slaughter at home,

☐=Others (specify)____________

c) When selling pigs for slaughter, what do you consider?

☐=Live body weight, ☐=Age ☐=Other

d) At what age of pig do you sell_______________Months

e) Do you have market problems for your pigs?______________________

1= Yes, 2= No

g) Which marketing problems do you encounter?____________________

☐= Lack of market, ☐= Poor prices, ☐=Others__________

h) What price do you usually sell your pigs when they are ready to be slaughtered?

i) What price do you usually sell your piglets (aged 4 months or less) ___________

7. Questions related to zoonoses transmission and risk factors

a) Where do you usually get your water for domestic use?:

☐= River, ☐=Bore-hole, ☐= Well, ☐ =Other (please specify) __________

b) Do you boil your drinking water?________________________________

☐= Always , ☐= Sometimes, ☐=Never

c) How often do you eat pork?______________________________

☐= At least once a month, ☐= Less than once a month but at least once a year

☐= Less than once a year, ☐= Never

d) How often do you eat beef?______________________________

☐= At least once a month, ☐= Less than once a month but at least once a year

☐= Less than once a year, ☐= Never

e) Do you have a pit latrine at home?

☐= Yes, ☐= No

f) Do all adults use the latrine for long calls? ☐= Always , ☐= Sometimes,

☐=Never

g) At what age do the children start using the latrines ___________ and where do defaecate before _______________

h) Where do adults and children defaecate, if not using latrines?___________

i) How often do you slaughter pigs at home?____________________________

☐= At least once a month, ☐= Less than once a month but at least once a year,

☐= Less than once a year, ☐= Never, ☐= Can not remember, do not know

j) If ever, how often is the meat inspected by a qualified inspector?

☐= Always ☐= Sometimes, ☐= Never, ☐= Can not remember

k) Were you ever told that your pigs were infected with cysts (cysticercosis) [description of cysts to be made to the farmer, local animal health workers to be consulted if possible]?

☐= Yes , ☐= No

l) If **Yes above,** when were you told that your pigs were infected with cysts (cysticercosis)? ☐= In the past year, ☐= One (1) to five (5) years ago, ☐= More than five years ago ☐= Never told, ☐= Can not remember

m) When that happened, was the meat eaten?

☐= Yes, ☐= No, ☐=Can not remember

n) If the infected meat was sold, what price was quoted ________________

o) Have you ever heard of tapeworm infection in humans?

☐=Yes ☐=No

p) How did you learn about it?

☐=Infected family member, ☐= Infected villager/neighbour

☐= School going children ☐=Other (Specify) _________

q) How was the tapeworm infection manifesting?

☐= They saw it in their faeces ☐= They had diarrhea, ☐= They had fever

☐= Other (Specify) ______________

r) How does a person get tapeworm infection? ________________

☐= They do not wash their hands before eating ☐= They eat undercooked pig meat, ☐= They are in contact with an infected person ☐= Other (Specify) _

The following two items should be completed for ALL respondents after direct observation of latrine.

s) Presence and type of latrine (**to be assessed by direct observation)** :____

☐= Absent ☐= Present and completely enclosed ☐= Present but without a door ☐= Shallow pit (easily accessible to roaming pigs)

t) Is there evidence of recent use of the latrine (by anyone):_____

☐= Yes ☐= No

APPENDIX III. ENZYME-LINKED-IMMUNOSORBENT ASSAY FOR THE DETECTION OF CIRCULATING *T. SOLIUM* CYSTICERCI ANTIGENS (AG-ELISA) IN SERUM

The serum samples are examined for presence of *T. solium* cysticercal antigens using a monoclonal antigen-based double sandwich Ag-ELISA. The monoclonal antibodies (MoAb) used were monoclonal antibody B158C11A10 as first MoAb and a biotinylated MoAb B60H8A4 used as detector antibody (second MoAb).

The sera were pre-treated in order to remove non-specific immune-complexes to increase the specificity and sensitivity of the assay. The serum samples were pre-treated by mixing an equal volume of serum and 5% trichloroacetic acid (TCA) (Sigma, Chemical Co.). For the negative control sera, 75 µl of serum was used while 150 µl of serum was used for the pre-treatment of positive control and the test sera. These mixtures of sera and 5% TCA solution were then incubated for 20 min at room temperature. After incubation, the mixture was centrifuged at 12,000 rpm for 9 min and the supernatant of the same volume of the added sera removed and aliquoted into microtitre tubes. The pH of the collected supernatant was then raised by adding an equal volume of 75 µl sodium carbonate/bicarbonate buffer (0.610 M) at pH 10.0 (neutralisation buffer) to the supernatant of the negative control sera and 150 µl neutralisation buffer to the supernatant of positive control and the test sera. 100 µl of this mixture at final serum dilution of 1: 4 is then used in the Ag-ELISA protocol.

In the Ag-ELISA protocol, the plate was coated with 100 µl of MoAb B158C11A10 diluted at 5 µg/ml in carbonate buffer (0.06 M, pH 9.6) and incubated at 37°C on a shaker for 30 min. After coating, the plate was washed once with PBS–T20 and drained by beating the plate vigorously on blotting paper. Blocking to avoid non-specific reactive sites was done by adding 150 μl per well of PBS-T20/1% NBCS (block solution) and then the plate incubated on a shaker for 15 min at 37°C. Thereafter, the plate was drained and without washing, 100 μl of pre-treated sera at a dilution of 1/4 was added and plate incubated at 37°C on a shaker for 15 min. The plate was then drained and washed five times. 100 μl of biotinylated MoAb B60H8A4 diluted at 1.25 μg/ml in PBS–T20/1%NBCS was added and the plate incubated at 37°C on a shaker for 15 min. The plate was drained and washed five times with PBS–T20 as above. 100 μl of streptavidin-horseradish peroxidase (Jackson Immunoresearch Lab, Inc.) diluted at 1/10,000 in PBS–T20/1%NBCS was added to act as conjugate after which the plate was incubated on a shaker at 37°C for 15 min. One tablet of the chromogen/substrate, orthophenylene diamine (OPD) (SIGMA, #P-8412) is added to 180 ml of distilled water. Then 100 μl of this solution was added to the wells and incubation done at room temperature for 15 min in the dark without shaking. To stop the reaction, 50 μl of 4N H_2SO_4 was added to each well. The plate was then read using an ELISA reader (Labsystem Multiskan RC) at 492 nm.

APPENDIX IV. ADULT WORM MORTALITY ASSAY (AWMA)

After collection from the slaughterhouse, the adult *Oesophagostomum* were then washed and kept in phosphate buffered saline (PBS). The assay was performed in 5cm diameter petridish where 8-10 worms were then placed in petridishes filled with 4 ml of the various plant products and levamisole. The pawpaw aqueous extract and powder were prepared in concentrations of 30, 15, 7.5, 3 and 1.75mg/ml. The pawpaw latex and papain were prepared in concentrations of 10, 1, 0.5, 0.25 and 0.125mg/ml. A PBS alone petridishes acted as control group. Levamisole Hcl diluted in PBS at the concentrations of 0.5, 0.25, 0.125, 0.0625 and 0.03125mg/ml was used as positive control. After 24 hours the extracts were washed away and the parasites re-suspended in PBS for 30 minutes for possible recovery of parasite motility. Finally, the number of motile (alive) and immotile (dead) worms was be counted under the dissecting microscope and recorded for each concentration. Death of worms was ascertained by absence of worm motility after observation for a period of 5 minutes. A mortality index was calculated as the number of dead worms divided by the total number of worms per petri dish. The worms' surface cuticles were also examined under the microscope, morphological changes described, and photography of the worms undertaken.

APPENDIX V. *IN VITRO* EFFICACIES (%) OF VARIOUS PRODUCTS AGAINST *H. SUIS*

Drug	Dosage	Time post exposure (minutes)							
		5	10	20	40	60	90	120	1440 (24hrs)
Neem	100%	100	100	100	100	100	100	100	100
	50	100	100	100	100	100	100	100	100
	25	100	100	100	100	100	100	100	100
	12.5	90	90	90	90	100	100	100	100
	6.25	68	68	68	80	80	80	80	93
	3.125	50	52	68	74	74	74	74	86
	0.156	20	20	25	25	15	28	28	30
Amitraz	5mg	38	50	50	63	63	63	75	100
	2.5	44	56	56	56	56	56	66	80
	1.25	0	20	20	20	20	30	30	40
	0.625	0	0	0	0	0	22	26	30
Latex	30mg/ml	57	60	60	60	63	64	64	76
	15	30	30	45	45	45	45	45	55
	7.5	10	15	24	24	24	34	38	40
	3.75	9	11	23	22	22	23	23	23
Papain	30mg/ml	45	52	54	54	56	60	64	83
	15	25	32	50	50	53	53	56	64
	7.5	18	18	26	27	27	31	42	50
	3.75	3	6	6	11	60	16	16	17
Levamisole	7.5	40	42	53	53	63	63	71	83
	3.75	40	40	47	47	53	53	63	70
	1.875	20	25	25	25	25	38	38	40
	0.9	0	0	0	8	25	25	28	33