EFFICACY AND CHEMOTHERAPEUTIC CONTROL OF COCCIDIOSIS IN RABBITS UNDER SMALLHOLDER PRODUCTION SYSTEMS IN CENTRAL 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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To my wife Mildred Atieno, my daughter Elsie-Favour and my mother Syprosa Ogolla,

In recognition and appreciation of the role you have played and continue to play in my life. You inspire me in your own unique ways.

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ACRONYM	DESCRIPTION	ACRONYM	DESCRIPTION
GDP	Gross Domestic Product	**Na**	Sodium
KNBS	Kenya National Bureau of Statistics	**Ca**	Calcium
MoLD	Ministry of Livestock Development	**NaCl**	Sodium Chloride
RDSF	Rabbit development stakeholders forum	**PABA**	Para-amino-benzoic acid
O.P.G	Oocyst per gram	**DNA**	Deoxyribonucleic acid
E.P.G	Eggs per gram	**EFSA**	European Food Safety Authority
FAO	Food and Agriculture Organization of United Nations	**Per Os**	administered orally
FAOSTAT	Food and Agriculture Organization Statistics	**Ad libitum**	Provided throughout
RUFORUM	Regional Universities Forum for Capacity Building in Agriculture	**MAFF**	Ministry of Agriculture, Forestry and Fishery

ACRONYM	DESCRIPTION	ACRONYM	DESCRIPTION
d.p.i	day post infection	**GPS**	Global Positioning System
r.p.m	revolutions per minute	**GIS**	Geographic Information System
SPSS	Statistical Package for Social Sciences	**SEM**	Standard Error of Mean
MS	Microsoft	**Mm**	Millimetres
ANOVA	Analysis of variance	**CW**	California white
CH	Chinchilla	**CSB**	Crossbreed
ANG	Angora	**DC**	Dutch
FG	Flemish giant	**NZW**	New Zealand White
FEL	French ear lop	**$K_2Cr_2O_7$**	Potassium dichromate

ABSTRACT

Rabbit production is a fast growing industry in Kenya. Despite this growth, knowledge on treatment and control of rabbit diseases is limited among farmers. The major disease affecting rabbits in the country is coccidiosis. Rabbit sector continues to experience huge losses in terms of morbidities and mortalities arising from coccidiosis. Currently, there are no labelled anti-coccidials for rabbits in Kenya and the ones used are labelled for poultry with unknown efficacies and safety in rabbits. The objectives of this study were: To determine the most commonly used coccidia control strategies in Nyeri and Kiambu counties; to assess efficacy of available treatment options under experimental coccidiosis, and to validate the laboratory results in natural coccidial infections in the field. A cross-sectional baseline survey involving farm visits was undertaken in the two counties to establish the commonly used coccidiosis control strategies. In each visit, a semi-structured questionnaire was administered and an observational data sheet filled. Further, faecal samples were collected in each farm to determine the prevalence and intensity of coccidial infection. Knowledge, attitude and practices on various coccidiosis control strategies were assessed. Sixty rabbits were then randomly recruited into 6 treatment groups (1A, 2B, 3C, 4D, 5E, 6F) each with 10 rabbits, in a controlled laboratory environment for safety and efficacy trials. Groups 1A and 3C served as uninfected-untreated negative control and infected-untreated positive control groups, respectively. Treatments were administered as follows in different groups; 2B was treated with amprolium hydrochloride, 4D with diclazuril, 5E with sulphachloropyrazine and 6F with trimethoprim-sulphamethoxazole combination. The following parameters were monitored: coccidian oocyst shedding, faecal scores and lesion scores in experimental cases. The drugs were then tested on naturally infected rabbits in the field. The experiment was undertaken in strict adherence to guidelines approved by University of Nairobi Ethics and Animal Use Committee. The data was analyzed to determine descriptive statistics and associations. The results

revealed that most rabbit farmers in the study area are smallholder, 53.6% of the farms having less than 10 rabbits. In most of the farms (54.6%), owners were responsible for making day to day management decisions and bulk of them (41.2%) had attained tertiary level of education. There was a strong correlation (r = 0.64) between level of education and good rabbit husbandry practices, those with tertiary education having well managed rabbitries. The most commonly kept rabbit breeds were New Zealand white (25.4%), Cross breeds (24.2%) and California white (12.9%). The overall prevalence of coccidial infection was 79.4%. The most commonly used drugs for treatment by farmers were sulphachloropyrazine (22%), trimethoprim-sulphamethoxazole (14%) and amprolium hydrochloride (9%). Majority of farmers used sulphachloropyrazine (41%) and sulphadimidine (31% for prevention of coccidiosis). Sulphachloropyrazine and diclazuril (diclosol 1%®) were effective against rabbit coccidiosis in both controlled experimental and field trials in terms of reduction of oocysts shedding, and recorded lesion scores and faecal scores approaching those of negative control group. Trimethoprim-sulphamethoxazole registered moderate to satisfactory efficacy during field trials while amprolium hydrochloride was not effective in both field and laboratory trials. This study recommends training of farmers, field extension and veterinary officers to build capacity for rabbit production in the country, including the need for prudent use of available efficacious anti-coccidials to avoid development of drug resistance. Further studies to determine if efficacies of trimethoprim-sulphamethoxazole and amprolium can be improved at higher dosages are recommended.

Key words: coccidia, anticoccidials, efficacy, *Eimeria*, rabbit

CHAPTER ONE

1.0 GENERAL INTRODUCTION AND OBJECTIVES

1.1 GENERAL INTRODUCTION

Global food economy is currently characterized by a shift of diets towards animal-based products such as meat, milk and other animal products. This has had great impact on agriculture, especially through growth of livestock production (FAO, 2015). In Kenya, agriculture is the second main pillar of the economy. Its contribution to GDP in 2016 alone was 32.6% according to World Bank data (2016). Livestock sector in the country has seen marked growth in the last few years and continues to play an important role of supporting household food and nutritional security (MoLD, 2010). According to FAO (2015), one in every nine people in the world still lack sufficient food necessary for an active and healthy life. This is in spite of steps which have been taken to avail food to the rapidly growing human population. This has necessitated a dire need to produce alternative sources of food. As such, livestock enterprises such as rabbits and poultry that are easy and relatively cheap to keep are readily adopted by small holder farmers (Oseni and Lukefahr, 2014).

Such adoption by smallholder producers, who constitute the bulk of agriculture stakeholders in the region, positions rabbit farming in emerging economies as a versatile means of making the countries food secure (Hungu *et al.*, 2013; Serem *et al.*, 2013; Okumu *et al.*, 2014). In Kenya, rabbit production has been adopted by many small scale farmers as an enterprise to complement other livestock production systems. The growth in rabbit industry has been attributed to established advantages of keeping rabbits which includes: a high reproduction rate; faster maturity; rapid growth rate; efficient land space and feed utilization; high genetic

selection potential (Oseni *et al*., 2008); limited competition with humans for similar foods; meat of high quality and; easily digestible meat with low cholesterol, low fat, high protein content and lowest calories level relative to meat from other species (Owen *et al.,* 1977; Mailafia *et al*., 2010). Rabbits also provide manure, skins, fur and are produced as laboratory animals (Oseni *et al*. 2008). Rabbit production is a fast growing livestock enterprise in the country producing about 3060 tons of meat annually (FAOSTAT, 2014). It is currently estimated that the rabbit population in Kenya is over 875,465 according to the Directorate of Livestock Research and Marketing (personal communication, 2015). However, these figures are still far below the global performance (FAOSTAT, 2014). In an effort to boost rabbit population in Kenya, National Rabbit Development Strategy and implementation framework (2013-2017) and Livestock Policy of 2008 were enacted to promote rabbit production and consumption in the country.

Despite the immense interest in rabbit production, rabbit diseases (infectious, non-infectious and parasitic) pose a major challenge among existing veterinary practices in Kenya (Borter and Mwanza, 2011). According to Okumu *et al*. (2015), ecto- and endo-parasites especially coccidiosis continue to cause huge losses in the industry. Studies in Kenya have ascribed significant economic losses in rabbit industry to coccidiosis (Hungu *et al*. 2013; Serem *et al.* 2013). A recent study in the country reported a prevalence of hepatic and intestinal coccidiosis at 11.5% and 29.5%, respectively (Okumu *et al*., 2014). Coccidiosis is a protozoal infection caused by apicomplexan parasites of the genus *Eimeria* and occurs in two forms, hepatic and intestinal, both resulting in massive economic losses (Pakandl, 2009).

Good management practices especially strict biosecurity and biosafety play a determinative role in preventing cocccidiosis (Gonzalez-Redondo *et al*., 2008; Pakandl *et al*., 2008). However, Okumu *et al*. (2014) reported that good sanitation alone does not guarantee

absence of the infection as high coccidial oocyst loads occur in relatively hygienic farms. Apart from good biosecurity and biosafety, anticoccidial drugs continue to be used globally with varied success (Coudert *et al*., 2003). Anticoccidial drugs are extensively used to control and prevent coccidiosis in poultry and this is likely to continue going forward (Chapman *et al.*, 2013). The same can be said for rabbit production especially now that attempts to develop anti-coccidial vaccine have not been fruitful as was reported by Drouet-Viard *et al.* (1997) and Pakandl (2009).

The extensive use, misuse and overuse of anti-ccocidials over the years has led to resistance, which has been recorded for most anticcocidials especially where intensive production is carried out (Chapman, 1997). Despite losses experienced by rabbit farmers from coccidiosis in Kenya, there are no specific anti-coccidials for rabbits in the Kenyan market and the ones used are labeled for poultry. Safety and efficacy of these poultry drugs in rabbits is not known. Studies done elsewhere in the world have shown that various *Eimeria* spp. are resistant to most of the anti-coccidials currently in use (Chapman, 1997). Though several *Eimeria* spp. have been isolated from rabbits in different parts of Kenya (Okumu *et al*., 2014), no study has been done to determine their sensitivity to available chemotherapeutic control options in the country.

In a previous study, Okumu *et al*. (2014) reported that some farmers that had treated their rabbits with poultry sulphonamides had tested negative for coccidial oocysts. However, there is no established scientific support of efficacy of either sulphonamides or other common coccidia control strategies farmers are currently using. Effective dosages of the available treatment options and their efficacies in rabbits need to be tested.

In this study, the efficacy of commonly used anti-coccidials [amprolium (amprolium hydrochloride®), sulphachloropyrazine (ESB3® 30%) and trimethoprim-sulphamethoxazole

(Biotrim®)] by rabbit farmers in Kenya were assessed and compared to a standard drug diclosol 1%® (diclazuril) in experimental and natural mixed *Eimeria* spp. infection. Knowledge, attitude and practices on various coccidiosis control strategies were assessed.

1.2 OBJECTIVES

1.2.1 Overall objective

To assess efficacy of available chemotherapeutic control strategies of rabbit coccidiosis in Nyeri and Kiambu counties

1.2.2 Specific objectives

1. To conduct a baseline survey on the available control strategies against coccidiosis in small-holder production systems in Nyeri and Kiambu counties.
2. To determine the comparative efficacy of commonly used off-label anticoccidial drugs and diclazuril under controlled laboratory conditions
3. To assess the efficacy of selected anticoccidial drugs on naturally infected rabbits in the field.

1.2.3 Justification

The constant increase in human population in the country has necessitated the need for production of more food in order to meet the ever growing demand. However, efforts towards increasing livestock production and farm produce has been derailed over the years by the decreasing per capita land holdings (Mailu *et al.,* 2014) and conversion of the arable lands into real estates. Currently, farmers are shifting their attention to farming practices that require less space. This has seen marked increase in the number of farmers venturing in rabbit production which is relatively cheaper and put less pressure on land (Hungu *et al.,* 2011).

Despite increasing interest in rabbit production and potential the sector has in contributing towards food security, diseases still pose a major challenge to the industry in Kenya (Borter and Mwanza, 2011). Top in this list is coccidiosis which is the major endo-parasite causing huge losses in rabbit production (Okumu *et al.,* 2015). Depending on the pathogenicity of the *Eimeria* species, losses can result from reduced growth rate and feed conversion, increased mortality and reduced immunity which predispose rabbits to secondary infections (Pakandl, 2009). Though isolation of sick rabbits, disinfection and good farm hygiene have been suggested as sufficient control method for rabbit coccidiosis in a rabbitry, use of anticoccidial drugs against coccidiosis for treatment and prevention is still common. In Kenya, there are no registered anticoccidial drugs for rabbits and those in use are adopted from the poultry industry.

These poultry anticoccidials continue to be widely used in rabbits with limited or no scientific knowledge of their efficacies. Since some of the drugs used to control chicken coccidiosis may not be effective against rabbit coccidia as was established by Pakandl (2008), their efficacies and safety need to be tested for rabbits. Knowledge on the farmer practices in treatment and control of coccidiosis is currently lacking. Furthermore, there has been a

growing concern over the farmers' contribution to development of resistance against the currently available drugs, the information on indiscriminate use of drugs by farmers can prove to be useful towards solving this menace. This study was therefore designed to establish the various coccidia control strategies used in Kenya and determine their safety and efficacies.

This will go a long way in promoting rabbit production by availing the much needed information to farmers on the best control options against coccidiosis. Since the superiority of diclazuril against rabbit coccidiosis was also shown, the study provides a good basis for its introduction in Kenya to supplement the other efficacious anticoccidials. Furthermore, the findings of this study will be of enormous importance to policy makers and interested national and international research institutions as it will provide a framework for policy formulation and further interventions that will enhance coccidiosis control strategies in rabbits and other domestic animals.

1.2.4 Hypothesis

Drugs used to treat rabbit coccidiosis in central Kenya are effective

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Rabbit production statistics

Global data on rabbit production indicate that the industry is growing (FAOSTAT, 2014). China has the highest population of rabbits in the world (235 Million (M)) followed by Uzbekistan (169.8M), Kazakhstan (78M), Italy (73.5M) Tajikistan (8.35M) among others. In Africa, Egypt is the top rabbit producer (8M) followed by Nigeria (4.151M), Algeria (1.65M), Sierra Leone (1.58M), Rwanda (0.995M), Kenya (0.872M) and Burundi (0.68M) (FAOSTAT 2014).

The current rabbit population in Kenya is estimated to be 875,465 rabbits according to Directorate of Livestock Research and Marketing (personal communication, 2015). Most of these are found in Central, Western and Rift Valley regions of Kenya (Hungu *et al*. 2013). Rabbit production in Kenya is mostly small-scale mainly for income generation and home consumption (Hungu *et al*., 2013; Serem *et al*., 2013). Hungu *et al*. (2013) attributed this to the small land space available. Out of more than 47 distinct rabbit breeds in the world, most commonly kept breeds in Kenya are the New Zealand White, California White and Crossbreeds (Serem *et al*., 2013). Others kept are local breeds like Checkered White, Kenya White, ILRI Grey, Akouti, Chinchilla, French Flop, Kenya White, and Flemish Giant (Hungu *et al.*, 2013; Okumu *et al*., 2015). Rabbit production has several advantages and qualities that confer on them a potential to bridge shortage of animal protein and also to generate income (Oseni *et al.*, 2008).

2.2 Constraints to rabbit production in Kenya

The major constraints affecting rabbit farming in Kenya have been identified as those associated with production diseases, predators like rats, deaths, shortages and poor supply of breeding stock, low supply of quality feeds, insufficient funds, limited access to technical information and lack of access to veterinary services (Hungu *et al*., 2013; Serem *et al*., 2013).

Strategies of solving these constraints were well outlined in the national rabbit development strategy and implementation framework which was published by the Kenya's Ministry of Agriculture Livestock and Fisheries in 2013. One of the strategies was the need for in-depth research on rabbit diseases and coming up with methods of their interventions. The challenges are not unique to Kenya, however, as studies done in Nigeria have identified similar constraints (Oseni *et al*., 2008; Mailafia *et al.*, 2010).

2.3 Rabbit diseases in Kenya

Several studies have identified diseases as a major constraint hindering rabbit production in Kenya (Hungu *et al*., 2013; Serem *et al*., 2013; Okumu *et al*., 2015). Amongst the parasitic diseases, mange and ear canker caused by *Sarcoptes scabie* var. *cuniculi* and *Psoroptes cuniculi*, respectively, are the frequently reported external parasites (Okumu *et al*., 2015). Other conditions affecting rabbits less frequently have been reported as abscess, pneumonia, emaciation, sore hock, snuffles, splay leg and cannibalism (Okumu *et al*., 2015**).**

On the other hand, clinical and subclinical coccidiosis of rabbits remains the major endoparasite that causes massive losses to rabbit farmers. In Kenya, high morbidity and mortality is reported despite the efforts being put to combat this disease. Coccidiosis is a readily transmissible infection through oral route caused by *Eimeria* spp. and are tissue/

organ and host specific (Georgi and Georgi, 1990). There are two forms of coccidiosis in rabbits: hepatic and intestinal coccidiosis (Pakandl, 2009). The hepatic form of coccidiosis is caused by *E. Stiedae* while intestinal coccidiosis is caused by a number of *Eimeria* spp. (Pakandl, 2009).

2.4 Life cycle of *Eimeria* species

Typical eimerian life cycle goes through three phases: sporogony (sporulation), schizogony and gametogony (Canning and Morgan, 1975). Sporogony results in the formation of the infective transmission stage called the sporulated oocyst. On the other hand, schizogony is a process of asexual reproduction also known as merogony that results in amplification of parasite numbers in the intestines and bile duct epithelium. Gametogony produces male and female gametes which, following fertilization form a zygote to become unsporulated oocysts. Sporulation involves a meiotic division followed by a mitotic division that results in the formation of four sporocysts; a mitotic division then occurs within each sporocyst to form two genetically identical haploid sporozoites (Canning and Morgan, 1975; Chapman and Jeffers, 2014). All subsequent stages in the life cycle after the meiotic division in the oocyst are haploid, thus mitotic division occurring within each schizont results in the formation of haploid merozoites which are all genetically identical and therefore represents a clonal population (Chapman and Jeffers, 2014). Genetic homogeneity is also more probable because there is a real possibility of self-fertilization following gametogony which will result in clonal offspring (Walker *et al*., 2013). Several genetic traits have been identified within species of avian coccidia, including developmental rate, enzyme variation, antigenicity and drug sensitivity (Jeffers, 1978), however, similar studies have not been done for coccidian species affecting rabbits.

Following ingestion of sporulated oocysts of *Eimeri stiediae* that causes hepatic form, sporozoites penetrate the mucosa of small intestine and pass via the mesenteric lymph nodes and hepatic portal system to the liver where they enter epithelial cells of the bile duct becoming trophozoites and then schizonts (Pakandl, 2009). The schizonts produce merozoites, but the number of asexual generations preceding gametogony is unknown (Pakandl, 2009). Oocysts pass out in the bile and appear in faeces 18 days after infection. Sporulation occurs in three days (Gardiner *et al*., 1998).

2.5 Intestinal coccidiosis

According to Coudert *et al*. (1995), Eckert *et al*. (1995) and Jithendran, (2010), intestinal coccidiosis is caused by 11 *Eimeria* species with varied pathogenicity as shown in Table 1.

Table 1. Pathogenecity of various rabbit *Eimeria* species

Species	**Pathogenicity**
E. intestinalis *E. flavescens*	Most pathogenic
E. irresidua *E. piriformis* *E. magna* *E. media*	Moderately pathogenic
E. perforans *E. neoleporis* *E. vejdovsky* *E. exigua*	slightly pathogenic
E. coecicola	Non pathogenic

Mixed infections with different eimerian species are more common according to a study by Okumu *et al*. (2014) on Kenyan rabbits. Intensely managed rabbits especially weaners (6 weeks to 5 months of age) are most susceptible and infections are usually precipitated by stress, noise, transportation or immunosuppression (Pakandl *et al*., 2006). A number of clinical signs have been shown to appear within 4 to 6 days after infection and include: rough hair coat; dullness; inappetence and/or anorexia; reduced weight gain and/or loss of weight; and dehydration (Peeters *et al*., 1984). The infected rabbits may also develop intussusception, convulsions or paralysis and death may follow within 24 hours. Death usually result from secondary bacterial infections and dehydration (Jing *et al*., 2012).

2.6 Hepatic coccidiosis

This form is caused by *E. stiedae* and results in mild to severe infections (Joyner *et al.*, 1983). Severe infections leads to economic losses arising from mortalities and organ (liver) condemnation in abattoirs. Rabbits with severe hepatic coccidiosis manifests clinical signs like depression, anorexia, brown watery diarrhea (Fig. 1), emaciation, rough hair coat, pendulous and distended abdomen, debilitation, listlessness, progressive weakness and death (Darzi *et al*., 2003). Jaundice has also been reported (Coudert *et al*., 2003). Several studies have shown that the size and weight of livers of infected animals increase due to excessive proliferation of bile duct epithelium resulting in hepatomegaly which is characteristic of this disease (Patton *et al*., 2008; Pakandl, 2009). *Eimeria stiedae* invades the epithelial cells of the bile ducts resulting in blockage of ducts that causes ascites, thus the water or pot belly symptom (Peeters *et al*., 1984). The sick rabbits may die within 10 days or eventually recover after several weeks.

At post mortem, the gross lesions seen are: hepatomegaly with irregular yellowish white nodules on the surface; thick creamy white exudates from their cut surface; and firm hepatic

parenchyma with distended gall bladder and bile ducts (Darzi *et al*., 2003). These workers further demonstrated that the peritoneal cavity may show increased quantity of dirty dull straw colored peritoneal fluid. These lesions cause disturbance of liver functions leading to decrease in α- lipoprotein, glucose and proteins; in addition to increase in bilirubin levels in blood serum (Darzi *et al*., 2003).

Conversely, moderate infections may present only with growth retardation while mild infections present with no discernible clinical signs (Patton *et al*., 2008).

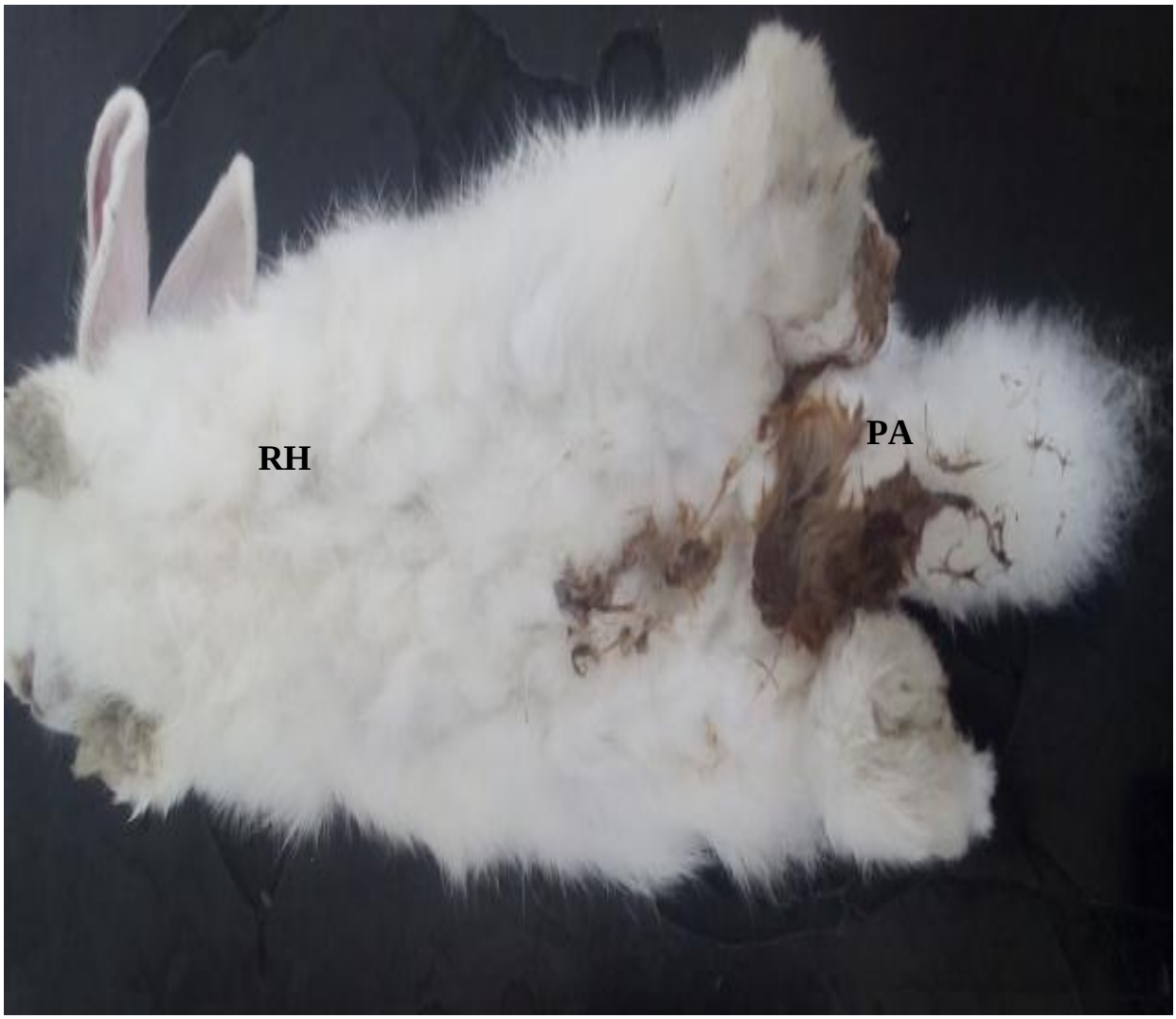


Figure 1. A rabbit with coccidiosis in Kiambu County. The rabbit had watery diarrhea that matted perineal area (PA) and rough hair coat (RH)

2.7 Available coccidiosis control strategies globally

Over the years, rabbit coccidiosis has been controlled through good husbandry and use of different curative and prophylactic anti-coccidical drugs (Kant *et al*., 2013). These drugs can either be coccidiocidal or coccidiostatic (Pakandl, 2009). Two surveys conducted between 1995 and 1999 (Chapman, 2013) and another between 2013 and 2014 (Chapman, unpublished information) showed that anticoccidials are used globally. Following extensive use of these drugs, most eimerian parasites have developed resistance especially where intensive production is carried out (Chapman *et al.,* 2013).

Consequently, to ensure effectiveness of these drugs, it is recommended that they be used prophylactically instead of therapeutically (McDougald and Reid, 1997; Coudert *et al*., 2003). Two approaches are extensively used in poultry production aimed at reducing development of resistance to anticoccidial drugs: the "shuttle" and "rotation" programs (McDougald, 1982; Pakandl *et al*., 2008). These can also be applied in the rabbit industry. Shuttle program is where different anticoccidials with varying modes of action are incorporated in different feeds fed to animals at different stages of growth; often a synthetic drug such as nicarbazin gets incorporated in the first (starter) feed followed by an ionophore (fermentative) in the second (grower) feed. In the rotation program, drugs with different modes of actions are used in successive flocks (McDougald, 1982). According to Chapman and Jeffers (2014), the principle informing these programs is that if resistance is selected during use of the first drug then it will be lost during use of the second but this is yet to be proven.

The rate at which resistance develops against some anticoccidial drugs has been documented as follows: (1) Very rapid- glycomide; (2) Rapid- quinolones; (3) Less rapid- Clopidol;

(4)Moderate-sulphonamides, robenidine, nitrofurans; (5) Slow- amprolium-; (6) Very slow- nicarbazine- and (7) Absent or very slow- monensin (McDougald and Reid, 1997).

2.8 Anticoccidial drugs commonly used globally

According to Chapman (1997), commonly used anticoccidials belong to two broad groups: ionophores and synthetic drugs produced by chemical synthesis.

2.8.1 Ionophores

These are the products of fermentation produced by *Streptomyces* and other species of fungi and are used widely as anti-coccidials. Examples are monensin, salinomycin, lasalocid, semduramicin and maduramicin (Peeters *et al*., 1984). Maduramicin and salinomycin are currently under extensive commercial use. Monensin is preferred due to its broad spectrum and because it does not develop resistance easily (McDougald and Reid, 1997). Ionophores act by facilitating movement of sodium ion into cells thus, elevating the concentration of sodium ion inside the cells. Such high intracellular sodium ion concentration inhibits some functions of the mitochondria like oxidation of substrates and hydrolysis of Adenosine triphosphate. This results in an exchange of intracellular sodium ion with extracellular calcium thus elevating calcium ion levels inside the cells resulting in cell death (McDougald and Reid, 1997).

2.8.2 Synthetic chemical anticoccidial drugs

2.8.2.1 Sulfonamides

Sulfonamides have been used therapeutically for over 5 decades (Prescott and Baggott, 1993). According to Kant *et al*. (2013), sulphonamides such as sulphaquinoxaline, sulphadimidine, sulphaguanidine, sulphadimethoxine, sulphaquinoxaline and sulphanitran

have been used in several countries to control coccidiosis. They are antimicrobials with broad spectrum of action against both Gram negative and Gram positive bacteria, and few protozoa (Prescott and Baggott, 1993). Sulfonamides are most effective against *Eimeria* spp. causing intestinal coccidiosis. They target the second generation schizonts (Coudert *et al*., 2003). Higher dosages of sulphonamides are however, required against 1st generation schizonts and sexual stages of *Eimeria* (Adams and Richard, 2001).

Sulfonamides have close structural similarity to para-amino-benzoic acid (PABA). They competitively inhibit bacterial folate synthatase resulting in failure to synthesize folic acid and therefore a number of essential metabolic reactions suffer (Adams and Richard, 2001). Sulphonamides stop proper growth of schizonts. The activity of sulfonamides is very sensitive to environment such as purulent material (Prescott and Baggott, 1993). Resistance of animal microorganisms to sulfonamides is wide because of its extensive use which reduces their efficacies. Nevertheless, they are still used widely, sometimes together with other drugs i.e., potentiated sulfonamides (Riviere *et al*., 1991). Cross-resistance between sulfonamides has also been reported (Prescott and Baggott, 1993).

Resistance of coccidia to sulfonamides has been reported in chickens, sheep and cattle, among other species. Notably, while sulfonamides may not significantly change the clinical course of already established coccidiosis, they help in decreasing the number of shed oocysts (Prescott and Baggott, 1993). In Kenya, despite their widespread usage, studies have not been conducted on their effectiveness and resistance.

2.8.2.2 Amprolium

Amprolium, a quarternized derivative of pyrimidine and a thiamine antagonist has also been used with varying success against coccidiosis (McDougald and Reid, 1997). It has a broad safety margin and targets schizonts and 1st generation trophozoites with peak activity reached

early in the 3rd day of life cycle. It has been shown to suppress the sexual stages, gametogony and sporulation of oocyst (McDougald and Reid, 1997). Amprolium can be used synergistically with ethopabate, sulphaquinoxaline and pyrimethamine to strengthen and extend its spectrum of activity. However, development of anticoccidial resistance that occurs following its prolonged usage, as has been the case in Kenya, limits its use. However, it remains one of the safest anticoccidial to be used expansively (Peeters *et al*., 1984). It is a thiamine antagonist and due to that close structural similarity, it blocks the thiamine receptors. This blockage of receptors prevents coccidia from utilizing thiamine (Peeters *et al*., 1984).

2.8.2.3 Quinolones

These are coccidiostatic targeting immature stages of *Eimeria*. Kant *et al*. (2013) established that quinolones like decoquinate, nequinate and buquinolate are efficacious against all *Eimeria* spp. of poultry but there is no data on their efficacies against rabbit coccidiosis. Most quinolones act by disrupting mitochondrial cytochrome electron transport system of coccidia with others such as decoquinate competitively inhibits DNA gyrase resulting in inhibition of DNA synthesis (McDougald and Reid, 1997). Quinolones are not readily soluble in water thus have reduced absorption and are therefore not effective in the treatment of clinical coccidiosis. These compounds may promote development of drug resistant isolates of *Eimeria* spp. as they do not completely eliminate coccidial oocysts (Kant *et al*., 2013).

2.8.2.4 Ethopabate

Ethobate is an arylamide with a single phenyl ring, and a member of monocyclic aromatics. It is mainly effective against intestinal forms of coccidia ((Maddison *et al*., 2002). This drug disrupts folate synthesis by competitively inhibiting absorption of PABA by the parasite (Adams and Richard, 2001).

2.8.2.5 Pyridinols

This class has only one member, Clopidol, which has anticoccidial properties with broad spectrum of action (Maddison *et al*., 2002). Clopidol is mainly coccidiostatic in action and targets the trophozoites or sporozoites (Peeters *et al*., 1984). Consequently, to benefit from its full anticoccidial activity, it is added to the chicken's feed when they are exposed to oocyst as the drug is not effective if given after infection has set in (Peeters and Geeroms, 1992).

2.8.2.6 Robenidine

This is a guanidine derivative with a broad spectrum coccidiocidal and coccidiostatic activities. It is majorly used as prophylactic drug against coccidiosis (Maddison and Jill, 2002). Robenidine prevents oxidative phosphorylation in first generation and second stage schizonts (Kant *et al*., 2013). Furthermore, robenidine also acts on gametocytes but is most efficacious against maturing first generation schizonts (Peeters and Geeroms, 1992).

2.8.2.7 Halofuginone

This is a derived from quinazolinone. Halofuginone is an alkaloid that was in the past isolated from *Dichroa febrifuga* plant (Adams and Richard, 2001). According to Adams and Richard (2001), halofuginone has broad-spectrum coccidiostatic and coccidiocidal activity against first and second generation schizonts. Its mechanism of action on *Eimeria* spp. is not known. It is strictly used for prophylactic purposes in young animals (McDougald and Reid, 1997).

2.8.2.8 Diclazuril

Diclazuril is another drug which has given impressive result against coccidiosis. Since 2008, diclazuril has been used as a feed additive for rabbits in France, Italy and Spain (Pakandl *et al*., 2008). In Kenya, however, this drug with proven efficacy against coccidiosis in developed countries is yet to be introduced.

Diclazuril is a chemical anticoccidial, a benzeneacetonitrile derivative which is a synthetic compound of the triazinone family. The drug acts on the intracellular developmental coccidia stages, at gametogony and schizogony phases of the life cycle. Studies have shown that diclazuril mainly interferes with the differentiation of endogenous stages in the course of parasite development. This results in widespread degeneration of gamonts and schizonts (Vanparijs *et al*., 1989b). Diclazuril exhibits very low acute toxicity and shows no evidence of genotoxicity, carcinogenicity, embryotoxicity, fetotoxicity or teratogenicity (Vanparijs *et al*., 1989a). It is a highly effective anticoccidial drug. Since the local *Eimeria* spp. isolates have not been exposed to this drug, it was used as a standard drug in our laboratory and field efficacy trials.

2.9 Ethno-veterinary treatment and use of natural alternatives against coccidiosis

Eimeria strains resistant to anticoccidials have emerged following protracted use of the available anticoccidial drugs and this has been observed in almost all the anti-coccidials currently in use as was reported by Bhat *et al*. (1996) and Quiroz-Castañeda and Dantán-González (2015). Furthermore, there has been increased pressure by consumers and government agencies of various countries advocating for the ban of use of drugs in species of animals produced for human consumption (Quiroz-Castañeda and Dantán-González, 2015). Instead, vaccines and other natural alternatives have been put forward as the best replacements particularly in European countries, Australia and USA (Quiroz-Castañeda and Dantán-González, 2015). Consequently, the development and use of other alternatives has increased tremendously over the years (Chapman *et al.,* 2013). The new alternatives that have emerged involve the use of extracts from plants, fungus and products of microorganisms (probiotics) (Chapman *et al*., 2013). Some of the compounds used are antioxidants that destroy the parasites, hence curbing infection (Karre *et al*., 2013; Masood *et al*., 2013). Since

cells are under constant threat of environmental damage and oxidative injuries induced by the cells themselves (Masood *et al*., 2013), antioxidants play a significant role in controlling and reducing oxidative injury resulting from free radicals and elevated levels of reactive oxygen species that have potential to cause cell death (Masood *et al*., 2013). In poultry production from which rabbits industry borrows a lot, antioxidants from natural sources have been used to restore oxidant: antioxidant balance thus improving the health of chickens with coccidial infection (Quiroz-Castañeda and Dantán-González, 2015). A study by Naidoo *et al*. (2008) revealed that antioxidant effects of various plant extracts exhibited the same anticoccidial properties as toltrazuril. The best sources of antioxidants are fruits and plant extracts with high levels of phenolic compounds (Masood *et al*., 2013).

Through *in-vitro* tests, essential oils derived from thyme, artemisia, clove and tea tree have been demonstrated to have the ability to disrupt the structure of oocysts therefore preventing their spread (Remmal *et al*., 2011). Even though studies are still ongoing on the mechanism of action of essential oils in destroying the oocyst which is the hardest structure of protozoa, there use will contribute immensely in tackling coccidiosis (Quiroz-Castañeda and Dantán-González, 2015).

Studies have also established that fats with high concentration of eicosapentaenoic acid, linolenic acid and docosahexaenoic acid from fish oils, flax seeds and linseed oil have a potential to reduce the severity of coccidiosis infection in poultry (Quiroz-Castañeda and Dantán-González, 2015). There are no documented studies of their effects against rabbit coccidiosis.

Another alternative approach that has received a lot of attention is the use of herbal extracts and medicinal plants. A study by Youn and Noh (2001) showed higher survival rates in birds treated with herbal extracts from *Artemisia asiatica, Ulmus macrocarpa, Torilis japonica,*

Sophora flavescens and *Pulsatilla koreana*. Similar findings have been found in other studies of herbal extracts and medicinal plants: *Artemisia sieberi* by Kheirabadi *et al*. (2014), *Moringa oleifera* by Ola-Fadunsin and Ademola (2013), *Ageratum conyzoides* by Nweze and Obiwulu (2009), *Eclipta alba* by Michels *et al*. (2011) and *Artemisia extract* by Kaboutari *et al*. (2014).

Further, promising results have been seen with immune response modulators. Studies have shown improved weight gain, reduced oocyst shedding and enhanced immunity following usage of prebiotics and probiotics (Lee *et al.*, 2008). Satisfactory results have been reported with probiotics such as *Pediococcus acidilactici* and *Saccharomyces boulardii* (Lee *et al*., 2007) and *lactobacilli* spp. (Sato *et al*., 2009).

The effects of these alternative compounds therefore range from immune stimulation, anti-inflammatory, cytoplasmic damage and antioxidant activities. Even with the remarkable results reported by studies on the alternative options, the bottom line remains that their large scale usage is yet to be adopted particularly since studies are still being conducted on how to purify and produce them in large quantities. Until that goal is met, conventional anti-coccidials will continue to be used in most parts of the world to combat coccidiosis albeit with decreasing frequency.

2.10 Vaccines

Attenuated and non-attenuated vaccines are promoted as substitute to chemotherapy in coccidiosis control in order to decimate the problem of drug resistance by pathogens and drug residues in meat (Pakandl, 2009). In the poultry industry that serves as the model of coccidosis study in rabbits, vaccination is the preferred method in prevention of coccidiosis in layer poultry stocks where it has reported varied effectiveness (Pakandl, 2009). The common commercial vaccines have live oocysts of non-attenuated and attenuated strains of *Eimeria*

spp. (Shirley *et al*., 2007). The efficacy of vaccines relies on the recycling of initially low doses of oocyst and on subsequent slow buildup of strong immunity (Innes and Vermeulen, 2006). While previously there was restricted use of live non-attenuated vaccines such as Coccivac®, Advent®, Immucox® and Inovocox® due to inherent pathogenicity of the live oocysts which necessitated their use to be accompanied by chemical treatments. Currently, they are moderately used following development of new improved methods of their administration (Shirley and Bedrnik, 1997).

On the other hand, live attenuated vaccines such as Paracox® and HatchPak CociIII® have widely been used since the possibility of disease developing is lowered by reduced proliferation of *Eimeria* spp. thus less damage to the intestines of birds (Shirley and Bedrnik, 1997). The weakening of the *Eimeria* spp. is mostly based on precociousness that involves use of population of parasites that mature early by completing life cycle sometimes up to 30 hrs earlier compared to other *Eimeria* spp. from a similar parent strain. As such, the parasites formed have weakened virulence and a marked reduction of proliferation ability (McDonald and Shirley, 2009).

These vaccines are either given in feed or drinking water at the farm or administered using spray cabinets in the hatcheries (Chapman, 2001). Some vaccines such as paracox® and coccivac® comprise of *Eimeria* spp. that were isolated before the introduction of many anticoccidial and have inherent sensitivity to these drugs (Pakandl, 2009). The seed stocks of these species have subsequently been kept in the laboratory for decades without exposure to any anticoccidial drug (Pakandl, 2009). *Eimeria* strains in the vaccines are thought to be genetically sensitive to all anticoccidials and there is a high probability that their progeny will be drug-sensitive as well. Freshly passed vaccine derived oocysts are likely to be more

infectious than older resident coccidia, thus producing many more vaccine-derived drug sensitive parasites following ingestion from the litter (McDonald and Shirley, 2009).

Similar milestones have not been reached with rabbit coccidial vaccines. The few studies carried out on rabbit vaccine development failed to yield any satisfactory results which forced most researchers to abandon this venture, particularly because of the complexity and huge financial investments that was required (Pakandl, 2009). In one of these studies, Drouet-Viard *et al.* (1997) performed vaccination trials with a precocious line of *E. magna*. Vaccination both *per os* and using spray dispersion of oocysts into nest boxes gave satisfactory results (Drouet-Viard *et al.*, 1997). However, the selection of attenuated lines is still far from development of a vaccine, including testing of its efficacy, pathogenicity and safety, registration, production and distribution to customers (Pakandl, 2009). As such, rabbit farmers around the world will continue to rely on anti-coccidial drugs for both prophylactic and chemotherapeutic purposes against coccidiosis. Therefore, there is a need to ensure that available anticoccidials have proven safety and efficacy, in order to prevent losses to farmers.

2.10.1 Efficacy trials of anti-coccidials

The only model used to develop new anticoccidial drugs has for a long time been chicken coccidia (Pakandl, 2009). Some of the drugs used to control chicken coccidiosis are, however, not effective against rabbit coccidiosis (Pakandl, 2009). Another problem that has not been addressed to date, is how to interpret the efficacy of an anticoccidial program. This is because relapse is often observed one or two weeks following even effective treatment in some cases as was noted by Vanparijs *et al*. (1989a) which makes interpretation of the results difficult. Furthermore, a life-long species-specific immunity may occur from mild infections which again complicates result interpretation as it becomes almost impossible to know whether the recovery was due to trial drugs or host immunity (Pakandl, 2009).

CHAPTER THREE

3.0 BASELINE SURVEY ON THE AVAILABLE CONTROL STRATEGIES AGAINST RABBIT COCCIDIOSIS IN SMALL-HOLDER PRODUCTION SYSTEMS IN NYERI AND KIAMBU COUNTIES

3.1 Introduction

Rabbit production is a fast growing industry in Kenya (Borter and Mwanza, 2011) especially amongst small holder farmers (Hungu *et al.,* 2013; Serem *et al.,* 2013). This growth may be attributed to increased commercialization of rabbit production for food and income generation (Serem *et al.,* 2013; Mailu *et al.,* 2014). Decrease in land sizes (Schiere, 2004) may have also contributed to farmers resorting to practices that require less space like rabbit production. These coupled with other advantages of rabbit production including; faster growth rate, high biotic potential, use of less space among others (Lebas *et al.,* 1997; Oseni *et al.,* 2008) have led to wide adoption of rabbit farming.

Despite its growth, diseases are a major challenge to rabbit production in Kenya (Borter and Mwanza, 2011). According to Okumu *et al.* (2015), ecto- and endo-parasites of rabbits especially coccidiosis are a major cause of losses in rabbit production. Coccidiosis is a ubiquitous protozoan infection of animals that significantly impairs their growth and utilization of feed (Soulsby, 2005). Eleven *Eimeria* spp. have been shown to affect rabbits with varied pathogenicity (Gardiner *et al.,* 1998). *Eimeria* spp. are highly tissue, organ and host specific (Georgi and Georgi, 1990). Even though farm hygiene is suggested as sufficient control method to coccidiosis in a rabbitry, use of anticoccidial drugs for treatment and prevention is a common practice. In Kenya there are no registered anticoccidial drugs for rabbits. The objective of this study was to carry out a baseline survey on farmer practices that

influence prevalence of coccidiosis and associated risk factors. The study also determined the options available for control of coccidiosis by rabbit farmers in Kenya.

3.2 Materials and methods

3.2.1 Study area

This study was conducted between June 2016 and November 2017 in Nyeri and Kiambu counties of central Kenya, which have established rabbit value chain in smallholder rabbit production systems (Hungu *et al*., 2013; Serem *et al.*, 2013; Okumu *et al.*, 2014). Central Kenya has farming practices with a variety of livestock and agricultural systems (Hungu *et al*., 2013). The two counties also have a variety of rabbit husbandry practices that are representative of rabbit producers in other parts of the country (Hungu *et al*., 2013; Serem *et al*., 2013; Okumu *et al*., 2014).

3.2.1.1 Kiambu County

The county is situated in central Kenya and has 12 sub-counties; Gatundu South, Gatundu North, Githunguri, Juja, Kabete, Kiambaa, Kiambu, Kikuyu, Lari, Limuru, Ruiru and Thika Town. The sampling sites are illustrated in Fig. 2 while coordinates of the study farms are as shown in Appendix 1**.** Its human population is estimated at 1,623,282 (according to 2009 census). The county covers an area of 2,543.42km^2 and enjoys a warm climate with temperatures ranging between 12˚C and 18.7˚C (Climate data, 2018).

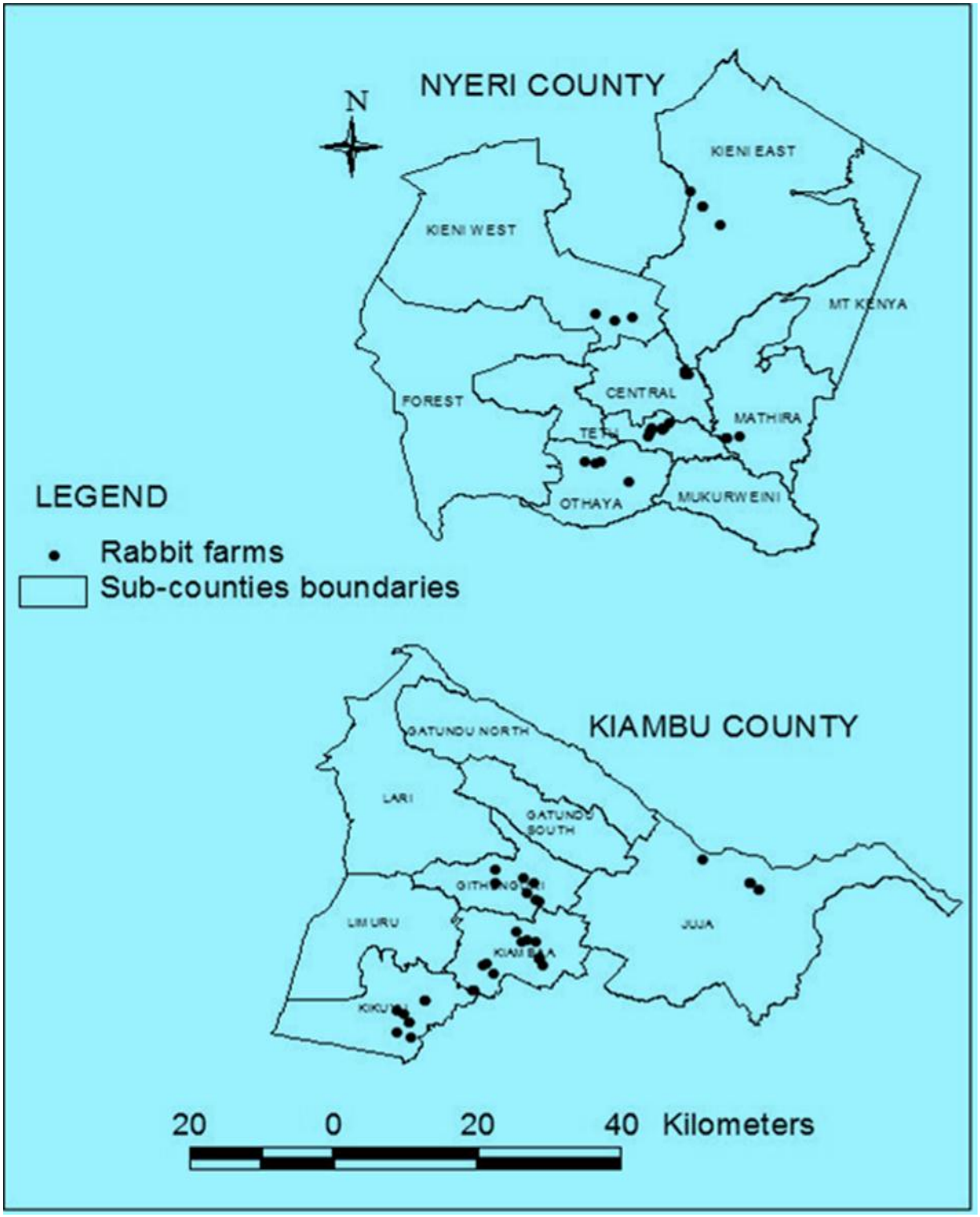


Figure 2. A map showing study area with GPS points of farms visited in Kiambu and Nyeri Counties (dark spots) as generated from Arc GIS statistical package

3.2.1.2 Nyeri County

The county is situated in the south west flank of Mt. Kenya and has 8 sub-counties: Kieni East, Kieni West, Mathira East, Mathira West, Mukurwei-ni, Nyeri Central, Othaya and Tetu. The sampling sites are shown in Fig. 2; while the coordinates of the farms are as shown in Appendix 2. The county covers an area of 3,356km^2 and has a human population of about 661,156 people (2009 census). The annual temperatures range from 12˚C in June and July which are the coldest months to 27˚C from January to March and September to October which are the hottest months. Rainfall average lies between 500mm and 1500mm during the short and long rain periods, respectively (Climate data, 2018).

3.2.2 Design of baseline survey

3.2.2.1 Selection of study farms and agro-veterinary outlets

Study farms were randomly selected from the list of rabbit farmers under the custody of livestock production officers in Kiambu and Nyeri counties, respectively. A total of 97 farms and 27 agro-veterinary outlets were visited in the two counties based on the rule of a minimum 30 respondents per county (strata) (Cohen, 1988). More questionnaires were administered due to the vastness of the counties and to more accurately represent the characteristics of the population sampled (Marcoulides, 1993).

3.2.2.2 Study scope

The study covered the following areas: general farm details, number and age groups of rabbits kept, main reasons for keeping rabbits, challenges faced, breeding practices, breeds, housing, feeding practices, feeds, hygiene, coccidia control strategies, marketing and value addition.

3.2.2.3 Selection of study rabbits

A minimum of 384 rabbits were examined from forty eight (48) randomly selected farms in each county (calculated as described by Martin *et al.* (1987) based on rabbit population and prevalence of coccidiosis in each county as established by Okumu *et al*. (2015).

$$n= Z^2X\ PQ/L^2$$

Where; n= number of rabbits to be examined

P= A priori estimate of disease prevalence (85%)

Z= the value of Z that provides 95% confidence interval (1.96)

Q= 1-P

L= desired precision (allowable error) at 0.05

Using simple random sampling method, 98 farms were chosen from the list of rabbit farmers as provided by livestock production officers in the study areas.

3.2.2.4 Questionnaire administration

A cross sectional baseline survey involving farm visits in each county was undertaken. On each visit, a semi-structured farmer's questionnaire (Appendix 4) was administered to randomly selected households who kept rabbits. The questionnaire was administered via personal interviews to either the rabbitry attendant or owner depending on the person who was more closely attending the rabbits and available between the two. A separate agro-veterinary outlet questionnaire (Appendix 5) was also administered to two randomly selected agro-veterinary outlets per sub-county. These were used to identify various rabbit management practices and coccidia control strategies used by farmers.

Questionnaires were complimented with observation data sheets (Appendix 6) that were filled after undertaking a thorough general and physical examination of rabbits and their hutches. Faecal samples were collected to identify various *Eimeria* spp. and determine coccidial infection loads in the study farms. This helped in selection of study farms with heavy coccidial loads for on-farm drug trials against coccidiosis and identification of control strategies the farmers used.

3.2.3 Clinical examination of rabbits

The rabbits were physically restrained as described by Malley (2007). Clinical examination was done for 10 randomly selected rabbits (bucks, does, weaned kits) in each farm visited. All the subjects in farms with less than 10 rabbits were examined. The parameters evaluated were body condition score, skin and hair quality, hygiene conditions and health status of the rabbits. Observations were recorded in an observation data sheet.

3.2.4 Assessment of housing hygiene

General hygiene in the rabbit hutches were assessed and graded on the basis of cleanliness of the cage floors, feeding equipment and ventilation (Gonzalez-Redondo *et al.* 2008, Okumu *et al*. 2014). Criteria for scoring is shown in Table 2:

Table 2. Hygiene grading criteria used in baseline survey

Level of hygiene		
Good	**Fair**	**Poor**
Absence of faecal matter on cage floor	Less faecal matter present on cage floor	A lot of faecal matter present on cage floor
Absence of hutch odour	Less hutch odour	A lot of hutch odour
Absence of feed on cage floor	Less feed present on cage floor	A lot of feed present on cage floor
Absence of water on cage floor	Less water present on cage floor	A lot of water present on cage floor
Absence of soiled rabbit	Slightly soiled rabbits	Many soiled rabbits

3.2.5 Laboratory analysis of samples taken during baseline survey

3.2.5.1 Faecal sample collection, handling, transportation and processing

Ten (10) samples comprising 5g of fresh faeces each were collected from cage floors and under cages in the farms visited. For rabbits that were in grouped cage(s), faeces were collected from different areas of the cage(s) as described by Cerioli *et al.* (2008). The samples were kept in corked plastic faecal pots, labeled and chilled at 4^{o}c until examined by a modified McMaster floatation technique to quantify number of coccidial oocysts per gram of faeces as described by MAFF (1986).

The numbers of coccidial oocysts within each grid of chamber were counted under a compound microscope at x10 magnification. Total number of oocysts were multiplied by 100

to give the oocyst per gram of faeces (o.p.g). Differences in morphology and colour were used to identify the helminth eggs encountered (Soulsby, 2005). Average eggs and oocysts per gram, were calculated for each farm.

3.2.5.2 Recovery and sporulation of coccidial oocysts

Faecal samples that were positive for coccidial oocysts were pooled and emulsified in a small basin into tiny particle. Saturated sodium chloride floatation fluid was prepared by dissolving 360g of NaCl in 1 liter of hot water, allowed to cool and then added into the emulsified faecal sample (Soulsby, 2005). The solution was swirled thoroughly and sieved into a separate basin. A large petri dish was partially submerged on the solution so that floating oocysts could attach onto it and allowed to stand for 30 minutes. The petri dish was lifted and adhered oocysts washed with distilled water into a separate flask. The flask was filled with distilled water and allowed to stand for 30 minutes to enable the oocysts to sediment (since they have higher density compared to distilled water).

The sediments were then harvested by pipette fillers and transferred to smaller petri dishes (4 small petri dishes (100mm x 15mm). Potassium dichromate (2.5%) was added up to 0.5 cm height of petri dishes to prevent any fungal and bacterial growth (Ryley *et al.*, 1976). The petri dishes were partially closed to let in oxygen and incubated at 27^{o}c under 60-80% humidity for seven days with on and off aeration (twice a day each for 20 minutes) to support the oocysts to sporulate. Humidity was maintained at 60-80% throughout sporulation by putting water in three separate standard-size petri dishes in the incubator. Oocyst sporulation was monitored by examining drops (0.1 ml) of the samples on a daily basis with a light microscope using oil immersion lens. An oocyst was recorded as fully sporulated when all the sporozoites within the sporocysts were completely formed. The sporulated oocysts were removed from the incubator and cleared by centrifugation at 2000 rpm for 5 minutes (Abed

and Yakoob, 2013). About half of the supernatant was discarded and the portion remaining in the centrifuge tubes transferred to a jar and mixed with equal amount of distilled water then centrifuged at 2000 rpm for 5 minutes. Oocysts were counted per 1.0 ml of the final solution after centrifugation, using the hemocytometry technique (Soulsby, 2005). The sediment having numerous sporulated oocysts was aspirated using a pippete and transferred to a separate 1 liter jar. This process was repeated 4-5 times until all the potassium dichromate were cleared.

Identification of sporulated oocysts was done based on morphological features (shapes, sizes, presence and absence of micropyle, micropyle cap, oocyst residuum, sporocyst residuum, sporozoite and its nucleus, oocyst wall and sporocyst wall, polar granules and their descriptions) according to Eckert *et al*. (1995).

3.2.5.3 Culturing of faecal sample for third stage nematode larvae

Faecal samples were examined using McMaster method to identify those that were positive for nematode eggs. Positive samples were cultured as follows. The samples were pooled per county and emulsified into tiny particles in a 150mm x 25mm petri dish (until crumbly). They were then sprinkled with water to wet them a bit. They were transferred to larger bottles until they were half full. The bottles were afterwards closed and incubated at 27^{o}C for 4-7 days with periodic aeration and sprinkling with water from a wash bottle. The L_3 larvae were then identified based on morphological features as described by Soulsby (2005).

3.2.6 Data analysis

A total of 98 farmers' questionnaires (Appendix 4) and 27 agro-veterinary outlets' questionnaires (Appendix 5) were administered and 99 observation data sheets (Appendix 6) filled. Data was entered into MS EXCEL, processed and exported to SPSS for analysis.

Descriptive statistics was used to summarize the data. Factor analysis and two- levels way analysis significant of ($p<0.05$) were used to show associations. ARC GIS was used to present farms visited during the study.

3.3 RESULTS AND DISCUSSIONS

3.3.1 Demographics of farmers and general farm details

Of the household heads, 80.6% were males and 19.4% females. Majority of rabbit farmers owned between 1-2 acres (41.5%) and less than 1 acre (39.4%) as illustrated in Table 3 below. This agrees with figures previously reported by Hungu *et al.* (2013) and *Serem et al.* (2013). Over six percent of the farmers (6.4%) owned 2-3 acres while 12.8% had more than 3 acres.

Table 3. Size of farms owned by rabbit farmers in Kiambu and Nyeri counties

Size	Frequency (n)	Percentage (%)
< 1 acre	37	39.4
1-2 acres	39	41.5
>2-3 acres	6	6.4
> 3 acres	12	12.8
Total	94	100

Rabbit farmers who owned stone walled and timber houses were 52.6% and 24.7%, respectively. This indicates that rabbit farming has been well adopted by the middle class and no longer a reserve of the low in social class. Majority of the respondents had kept rabbits for 2-5 years (30.9%) and more than 5 years (30.9%). Kiambu County had a higher proportion of

new farmers (< 6 months) venturing in rabbit production compared to Nyeri County, probably because of its proximity to an expanding market in Nairobi.

3.3.2 Main reasons for keeping rabbits

Main reasons for keeping rabbits were as a business and source of food at 72.2% and 15.5%, respectively (Fig. 3) and this was in concurrence with earlier studies by Hungu *et al.* (2013) and Serem *et al.* (2013).

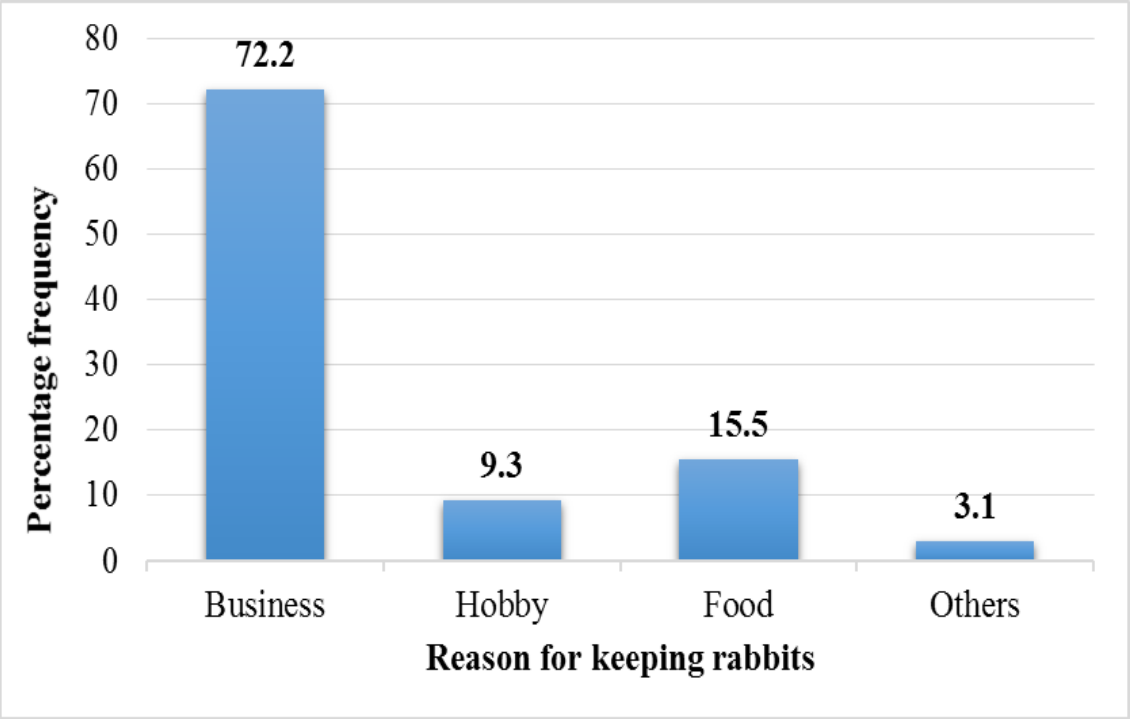


Figure 3. Main reasons given by farmers for keeping rabbits in Nyeri and Kiambu counties

This supports the importance of rabbit value chain in supplementing food and nutrition security and livelihood in the region. Other livestock kept by the farmers were mostly chicken, cattle, sheep and goats. Hungu *et al.* (2013) had attributed the popularity of chickens to the fact that they are relatively cheaper to keep and require less space. Other reasons given for keeping rabbits were for manure and urine fertilizers.

3.3.3 Number of rabbits and breeds kept

Majority of farmers (53.6%) had less than 10 rabbits and up to 11 to 20 rabbits (18.6%) (Table 4). Findings from Kenya (Hungu *et al.* 2013; Serem *et al.* (2013) and West Africa (Lukefahr and Cheeke, 1990; Lukefahr, 2007; Oseni *et al.*, 2008) also showed that rabbit production is predominantly practiced on a small scale basis in developing countries. This may be attributed to increased pressure on land with unchecked increase in human population, scanty husbandry knowledge and inadequate financial resources.

Table 4. Number of rabbits kept by farmers in Nyeri and Kiambu counties, Central Kenya

Number	Frequency (n)	Percentage (%)
1 to 10	52	53.6
11 to 20	18	18.6
21 to 30	13	13.4
31 to 40	6	6.2
>40	8	8.2
Total	97	100

New Zealand white (25.4%), cross breeds (24.2%) and California white (12.9%) were the most kept breeds (Fig. 4). This was in agreement with earlier studies by Serem *et al.* (2013) and Mailu *et al.* (2014) but differed slightly from findings by Hungu *et al*. (2013) from Kenya and Nigeria (Lukefahr *et al.,* 1995 and Oseni *et al.,* 2008) where California whites were more than crossbreeds. However, it is important to note the number of cross breeds might have increased over the years due to haphazard breeding practices and poor record keeping by farmers (Mutisya, 2014).

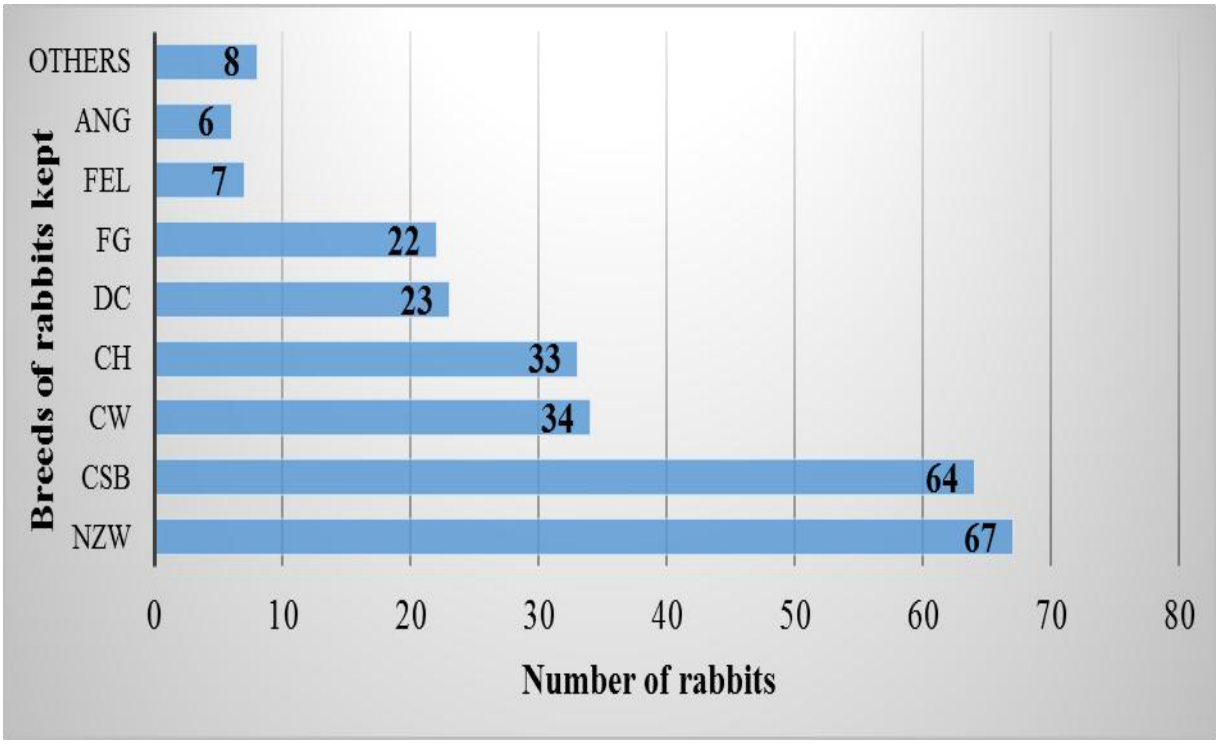


Figure 4. Breeds of rabbit farmers keep in Nyeri and Kiambu counties.

Key: CH (Chinchilla), ANG (Angora), CW (California white), CSB (Cross breed), DC (Dutch), FG (Flemish giant), FEL (French ear lop) and NZW (New Zealand white). Others were Rex, Checkered white, Kenya white and ILRI grey.

Most farms kept the following age groups of rabbit; does (35.1%), bucks (32.4%), weaners (17.6%) and kits (14.9%). The high number of does and bucks kept may be attributed to the fact that most farmers sell their rabbits for meat from 2 months and retain the adult breeding stock.

3.3.4 Source of start-up stock and breeding practices

Bulk of the farmers sourced their start-up stock of rabbits from other farmers (59.4%) and government breeding centers (14.2%) (Fig. 5). This was also the case in replacement of breeding stock, where 43.8% and 41.6% of the farmers replaced from other farmers and own stock, respectively. Similar findings were also reported by Oseni *et al.* (2008) who noted that

this practice coupled with poor record keeping encourages inbreeding and dilution of the genetic resource. This practice may contribute to spread of cocidiosis and other diseases by carriers to disease-negative farms. A greater number of the respondents replace breeding bucks and does after 1 and 2 years, respectively.

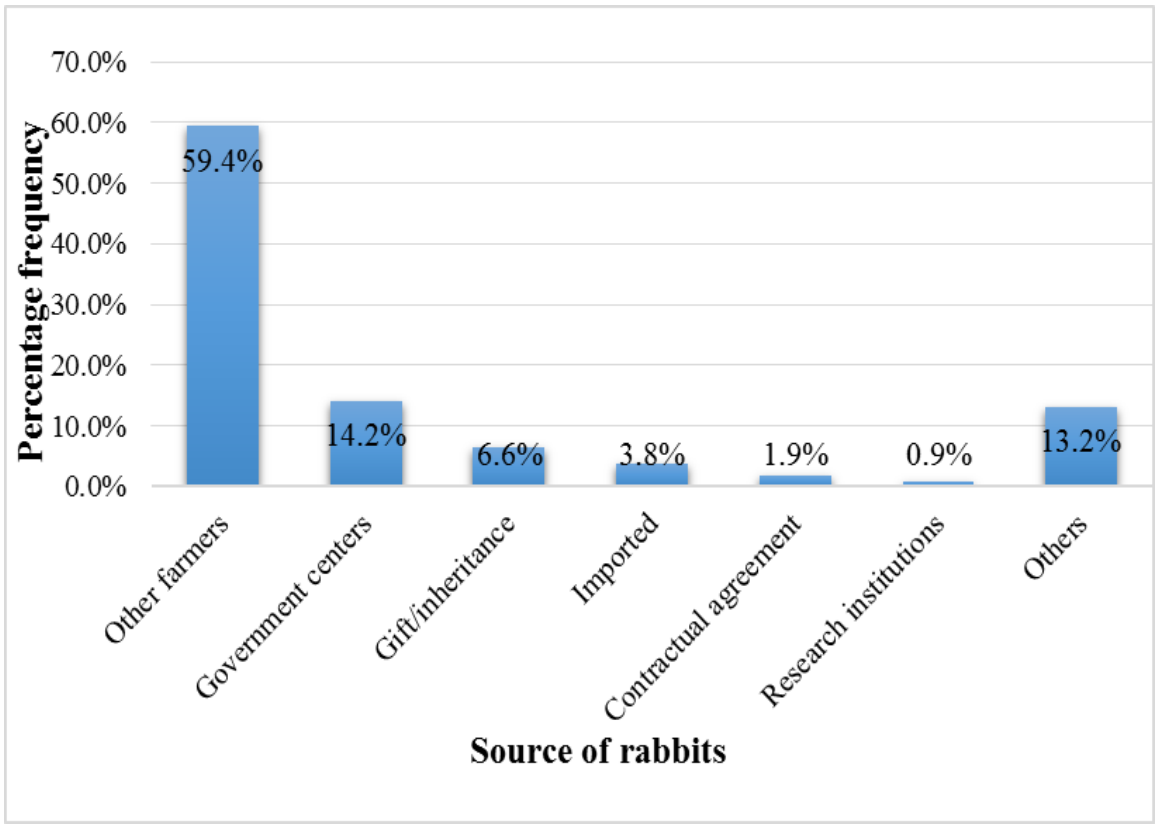


Figure 5. Source of start-up stock for rabbit farmers in Nyeri and Kiambu counties

3.3.5 Hygiene and cleaning practices

Methods of cleaning used by rabbit farmers are shown in Fig. 6. Majority (74.2%) cleaned hutches by changing beddings only. Few farmers (10.5%) cleaned with water and disinfectants. Other cleaning methods were use of disinfectants alone, putting beddings on top of feces and dusting. Since many of the hutches had wooden floor (62%), this method is not efficient and may be contributing to the high prevalence of coccidiosis in the study area. Frequencies of cleaning ranged from once per week (28.9%), daily (27.4%) after 2 weeks,

once a month, when dirty and some had never cleaned suggesting that there was insufficient knowledge on prevention and control of coccidiosis and other faecal-oral transmitted diseases.

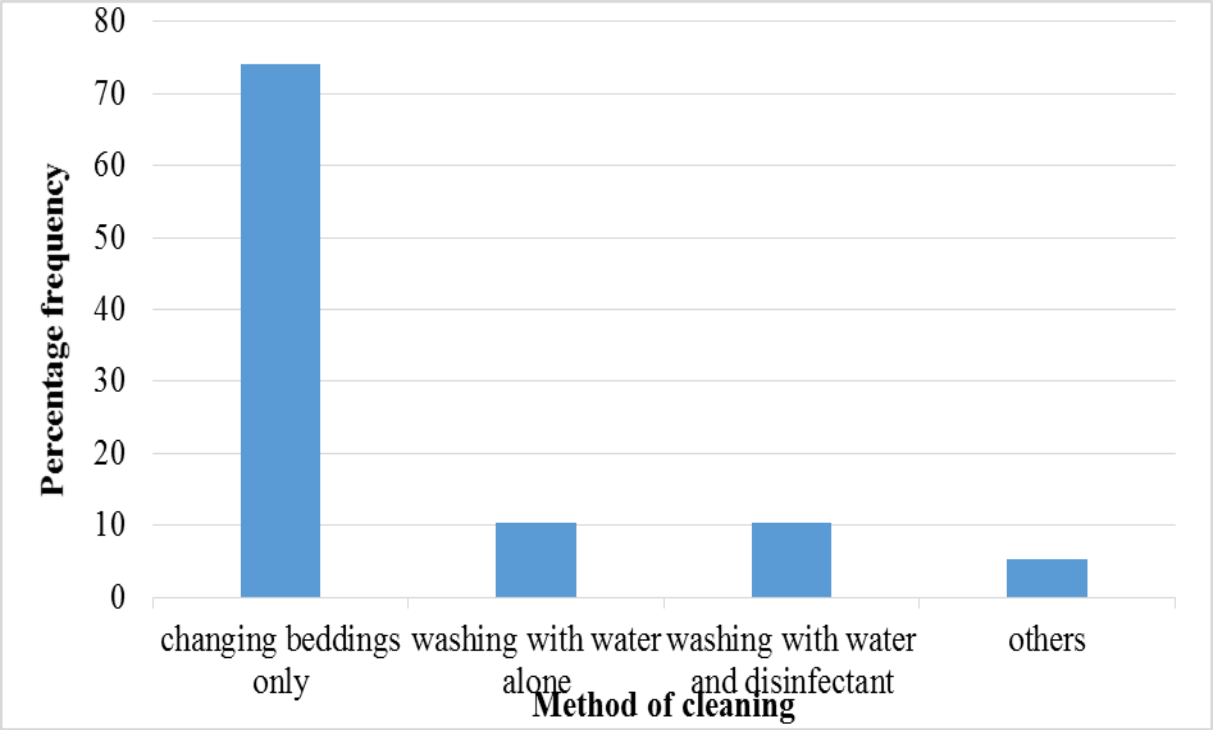


Figure 6. Methods of cleaning used by rabbit farmers to clean rabbitries in Nyeri and Kiambu counties

3.3.6 Type of cage floor

Most of the rabbit hutches had wooden floor cages (62%) and wire mesh (33%) as shown in figures 7 and 8. Wood is commonly used to build hutches in the study areas because it is readily available and relatively cheap compared to the recommended wire mesh. The same findings were reported in Cameroon (Lukefahr *et al.*, 2000), Nigeria (Oseni *et al.*, 2008) and recently in Kenya (Serem *et al.*, 2013).

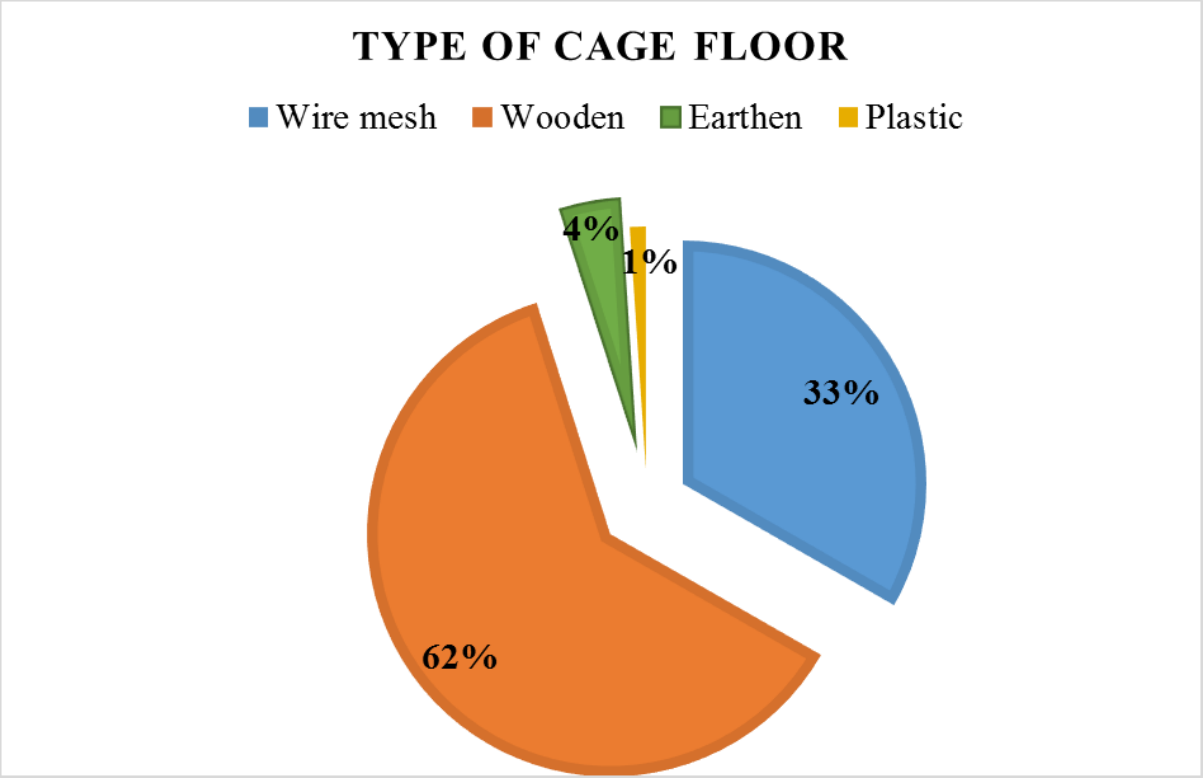


Figure 7. Percentage cage floor types of rabbit hutches in Nyeri and Kiambu counties

Figure 8. Examples of housing structures in Nyeri and Kiambu counties

The hygiene status of study farms is shown in Fig. 9. Generally, there was poor hygiene in most of the farms visited and was manifested by one or more of the following: fecal contents on cage floor (29.9%), hutch odour (6.2%), presence of feed on cage floor (36.1%), water on cage floor (9.3%) and soiled rabbits (5.2%).

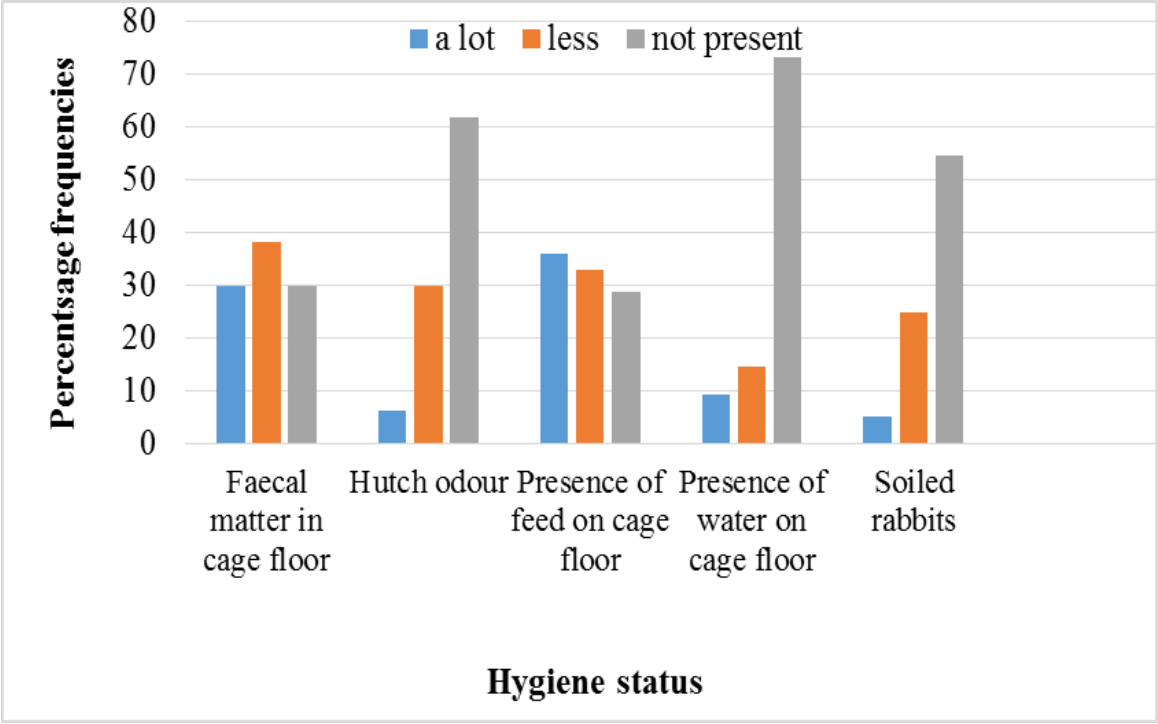


Figure 9. Clustered hygiene status in rabbit farms in Kiambu and Nyeri counties

3.3.7 Housing structures, number of tiers and location

Outdoor houses with no tier (28.2%) and 1 tier (21.4%) were the most common (Fig. 10). In most of the houses, rabbits were either caged individually (23.3%) or grouped by age (18%). Over seventeen percent (17.3%) had outdoor hutches while, 12.7% had indoor hutches with rabbits grouped by sex. Few farmers did not group their rabbits in any particular way. Previous studies by Serem *et al.* (2013) in Kenya and Oseni *et al.* (2008) in Nigeria had attributed the high number of low level tier to the small scale nature of rabbit production in

developing countries. Housing rabbits of different age groups in the same cage encourages faster spread of diseases.

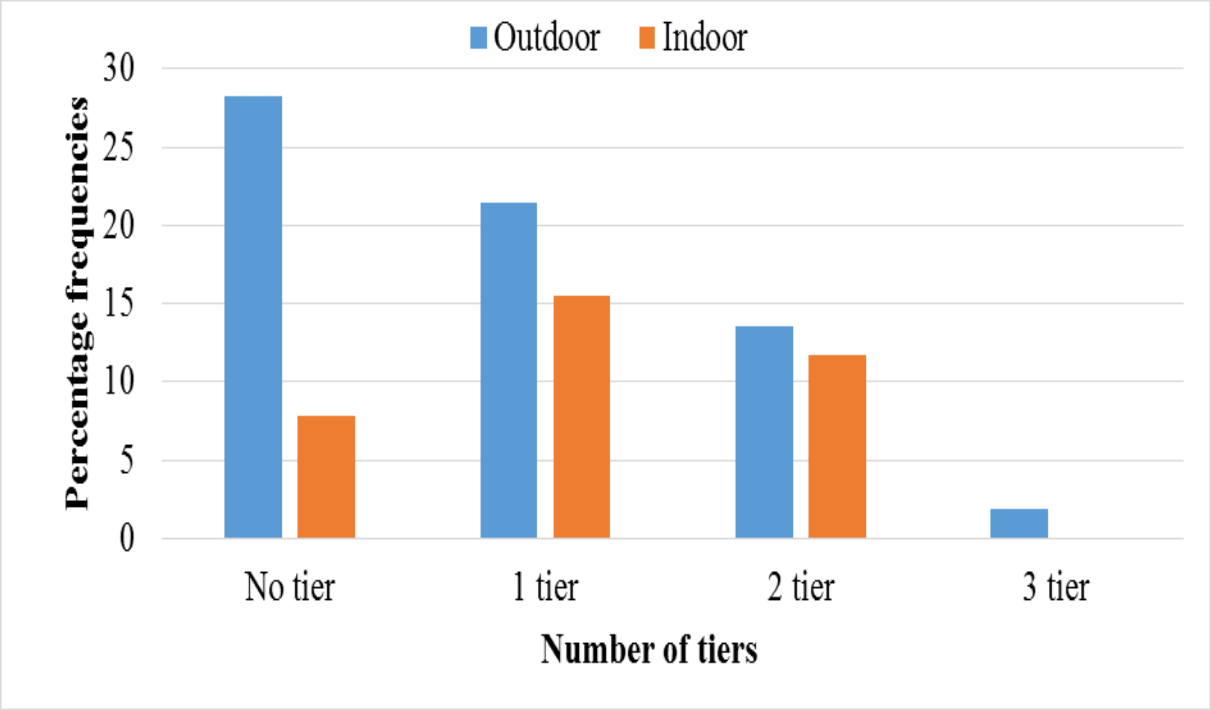


Figure 10. Location of housing structure and number of tiers

3.3.8 Feed and feeding practices

Majority of farmers (49%) reported use of forage as the only source of feed for rabbits, 42% used both forage and commercial or commercial only (9%) (Fig. 11). Farmers using commercial feeds only were from Kiambu county probably attributed to its proximity to the city compared to Nyeri county where most farmers relied on forage.

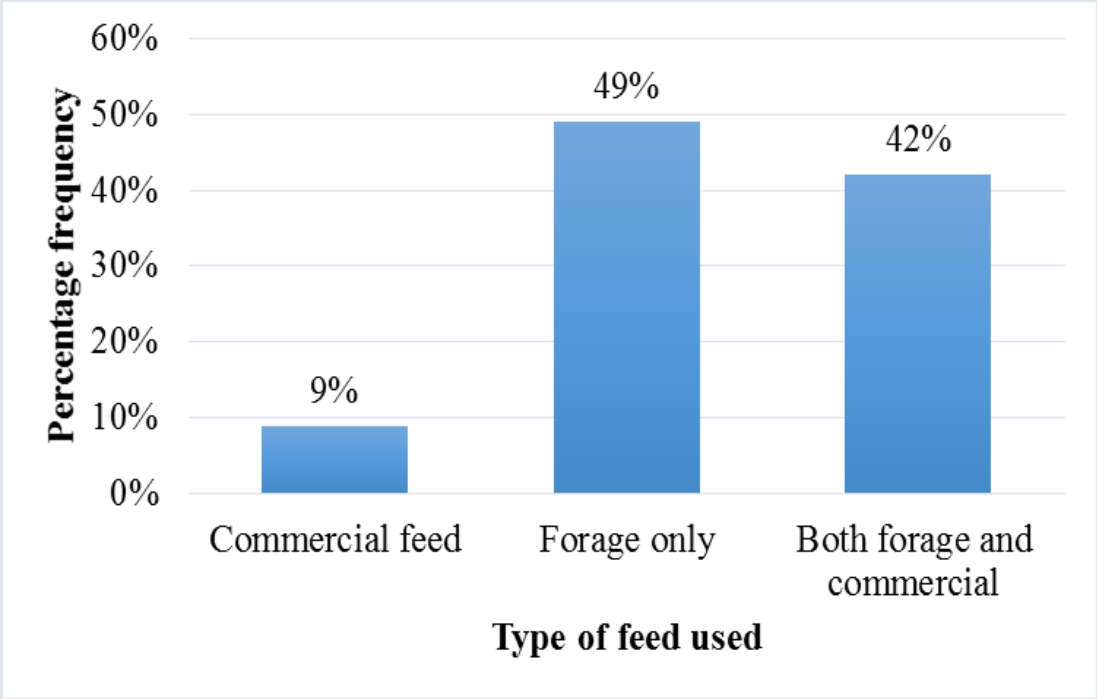


Figure 11. Different feeds used by rabbit farmers in Nyeri and Kiambu counties

Use of commercial feeds and a combination of commercial feeds and forages increased steadily with level of education as depicted in Fig. 12. This is a clear indication that management is improved with good education as was also portrayed by Serem *et al.* (2013) who showed a strong correlation between the two parameters.

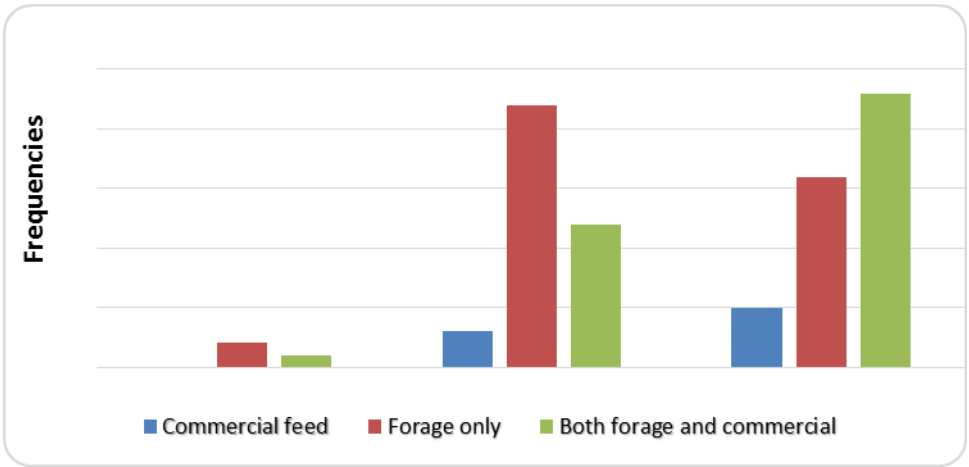


Figure 12. Type of feed used and level of education of farmers in Kiambu and Nyeri counties

Farmers associated poor quality feed such as use of cattle maize bran with bloating (27.9%), diarrhea (20.9%) and loss of appetite (11.6%) (Table 5). Other clinical signs recorded less frequently were dehydration, loss of weight, rough hair coat and lacrimation. Farmers who had suddenly changed diets for their rabbits reported clinical signs of diarrhea (29.2%), bloating (22.9%) and sudden death (22.9%).

Table 5. Clinical signs (%) that rabbit farmers associated with various feeding practices in Nyeri and Kiambu counties

Clinical signs	Sudden change of diet	Overfeed-ing	Poor quality feed	Fresh (un-wilted) forages
Diarrhoea	29.2	0.0	20.9	30.9
Bloating	22.9	57.1	27.9	32.4
Sudden death	22.9	14.3	20.9	26.5
Mucus in faeces	4.2	0.0	9.3	4.4
Lack of appetite	16.7	14.3	11.6	2.9
Stunting	2.1	0.0	7.0	2.9
Other signs	2.1	14.3	2.3	0.0

Most farmers reported to feed their rabbits on wilted forages including weeds (20%), kales (16%), cabbages, sweet potato vines both at (13%), hay (13%), grass (13%) and less frequently carrots and corn stalks (**n=413**). Feeding of un-wilted forages was associated with bloating (32.4%), diarrhea (30.9%) and sudden death (26.5%) in rabbits.

Three commonly used commercial feeds were Unga (64.3%), Sigma (15.4%) and Pembe (3.6%). Majority of the farmers associated excessive feeding of the commercial pellets with bloating (57.1%), sudden death (14.3%) and loss of appetite (14.3%) as presented in Table 5.

Out of the 27 agro-veterinary outlets interviewed, two associated use of Unga with diarrhea/mucoid feces and one associated pembe with diarrhea.

Table 6. Clinical signs in percentages (%) farmers associated with feeding commercial feeds in various age groups of rabbits

Age group	Diarrhoea	Bloating	Sudden death	Lack of appetite	Stunting	Mucoid faeces
Kits	15.2	18.8	13.6	17.9	14.3	0.0
Weaners	48.5	42.5	49.2	25.6	50.0	37.5
Growers	18.2	17.5	23.7	25.6	21.4	25.0
Pregnant doe	7.6	10.0	6.8	12.8	7.1	12.5
Lactating doe	7.6	8.8	6.8	15.4	7.1	25.0
Other ages	3.0	2.5	0.0	2.6	0.0	0.0

3.3.9 Farmer knowledge of clinical signs associated with coccidiosis

Diarrhea, distended abdomen, in appetence and sudden death were the most common clinical signs farmers associated with coccidiosis (Fig. 13). Diarrhea was frequently reported in crossbreeds (29.9%), New Zealand white (28.9%) and California white (23.7%) but this may be attributed to the high proportion of these breeds in the study area. Similar scenario was depicted for distended abdomen (New Zealand white, 25.6%; cross breeds,22.2% and California white, 18.8%), lack of appetite (New Zealand white, 21.5%; Cross breeds 17.7% and Chinchilla 15%) and for sudden death (New Zealand white, 26.9%; cross breeds, 21.7% and California white, 14.9%). Clinical signs were less frequently reported in Rex, ILRI grey, Kenya white and checkered white.

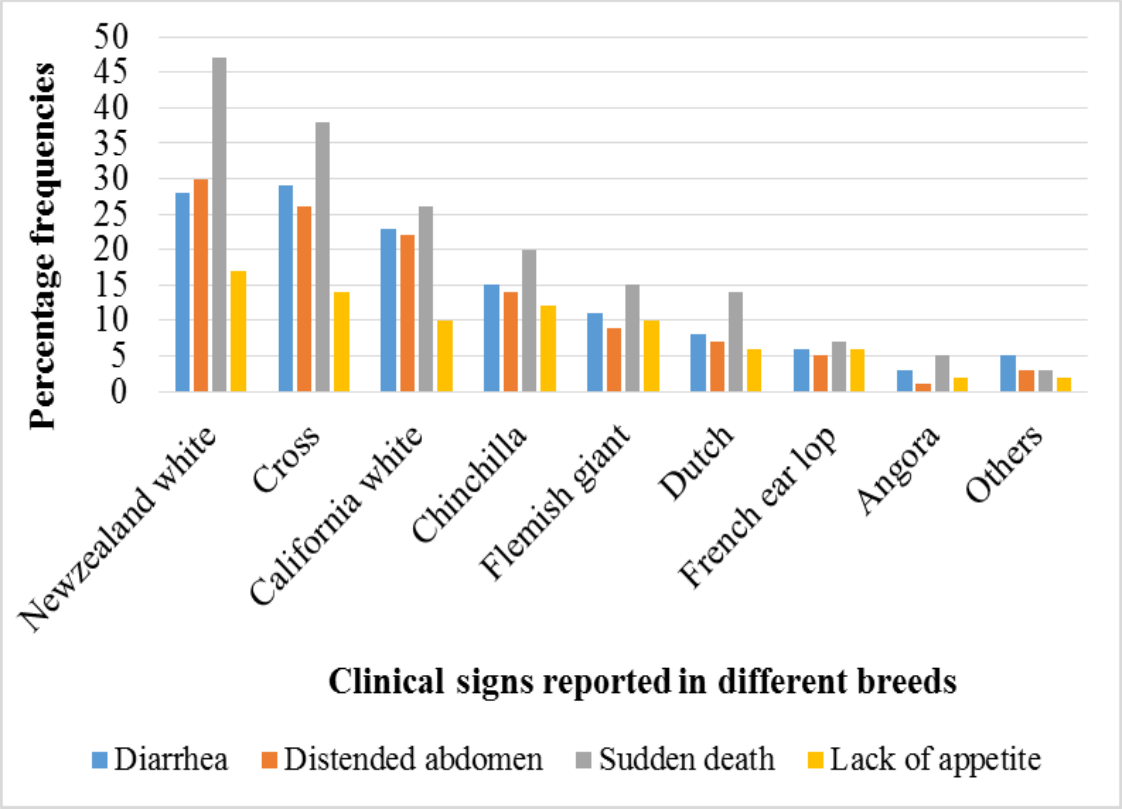


Figure 13. Clinical signs farmers associate with coccidiosis in different breeds

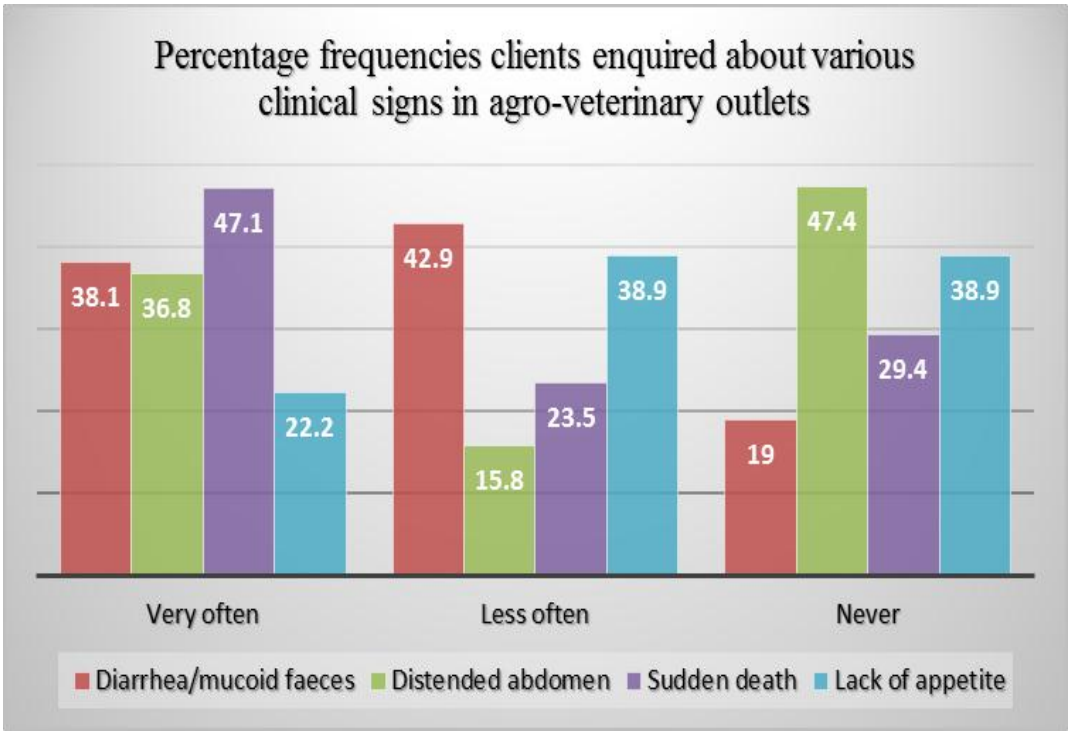


Figure 14. Clinical signs frequently reported by rabbit farmers in agro-veterinary outlets in Nyeri and Kiambu counties

3.3.10 Clinical signs reported in different age groups

Weaners had the highest number of distended abdomen (44.3%), diarrhea cases (43.2%), sudden death (39.7%) and reduced appetite (37%) compared to other age groups which reported relatively fewer clinical signs (Fig. 15).

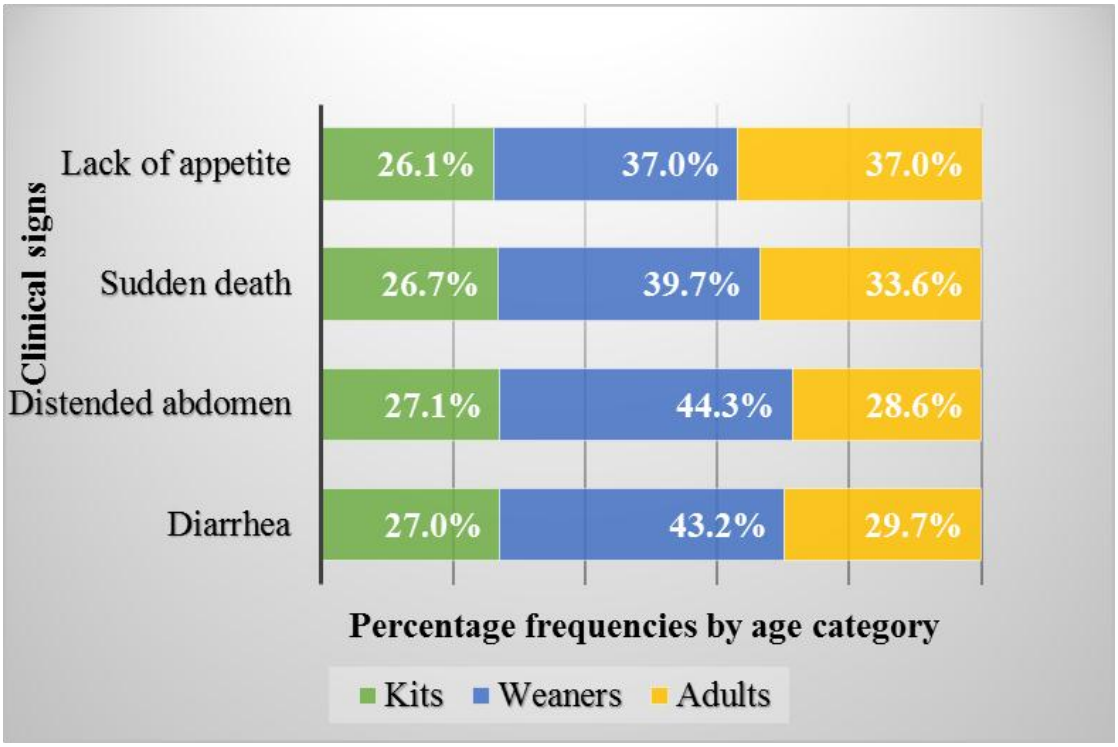


Figure 15. Clinical signs of coccidiosis reported in different age groups by rabbit farmers

3.3.11 Action taken when rabbits are sick

When rabbits are sick, majority of farmers (42%) treated sick rabbits, 33.6% called a veterinarian or animal health assistant and 9.2% seek out advice from other farmers. Those who self-treat mostly get information on drug usage from other farmers (17.5%) while, 10.3% rely on their own experience. This indiscriminate use of drugs with limited or no pharmacological knowledge contributes to development of resistance to the available

anticoccidials. Interestingly, 13.7% of the respondents reported they do nothing and let the disease take its course. Most in this group stated they were not aware rabbits are supposed to be treated when sick. As was shown by Hungu *et al.* (2013), technical information on management and control of rabbit diseases is still deficient. An earlier study done in Nakuru County had indicated that 80.1% of farmers had attended training on commercial rabbit production (Mutistya, 2014). A possible explanation for this discrepancy may be due to the erratic nature of rabbit farming as was shown by MOLD (2012) which indicated that with time experienced farmers abandon the enterprise as new ones come in.

3.3.12 Commonly used treatment and prevention strategies against coccidiosis

Sulphonamide based antibiotics sulphachloropyrazine (22%), trimethoprim/ sulphamethoxazole (14%) and amprolium (9%) were the most commonly used anticoccidials to treat clinical signs associated with coccidiosis (Table 7; Fig. 16). Also used less frequently were aminoglycoside (neomycin), sulphadimidine, tylosin and penicillins. This varied with Europe where Pakandl (2009) listed robenidine, salinomycin, diclazuril and lerbek as the commonly used anticoccidials. As opposed to Europe where prophylactic measures are emphasized, in Kenya most farmers only treat once the clinical signs set in which as Pakandl (2009) noted, is rarely successful. For prevention of coccidiosis, majority of the farmers used sulphachloropyrazine (41%), sulphadimidine (31%), trimethoprim-sulphonamide combination (18%) and neomycin (18%). Amprolium is used less frequently. All these drugs are mainly registered for use in poultry but not rabbits in Kenya. This situation strongly contrasted Europe where according to Pakandl (2009), sulphonamides are strictly used for treatment purposes in coccidiosis outbreaks. There are no registered anticoccidial vaccines in Kenya currently, but some developed from precocious lines (Drouet-Viard *et al.* 1997) are used in Europe.

Table 7. Drugs used by farmers to treat clinical signs associated with rabbit coccidiosis in Kiambu and Nyeri counties, central Kenya

Herbs, drugs and chemicals used for treatment	Clinical signs reported					
	Diarrhoea	Distended abdomen	Lack of appetite	Sudden death	Frequencies (n)	Percentages (%)
Sulphachloropyrazine	11	3	2	2	18	22
Trimethoprim-sulphamethoxazole	10	2	1	0	13	15
Amprolium	4	1	0	1	6	7
Neomycin	4	1	0	0	5	6
Sulphadimidine	4	1	0	0	5	6
Tylosin	3	0	0	1	4	5
Penicillins	1	0	0	0	1	1
Multivitamin	3	1	11	6	21	25
Liquid paraffin	3	3	0	0	6	7
Herbs(aloe vera)	2	1	0	1	4	5
Total	45	13	14	11	83	100

Some farmers also use non-conventional treatments such as liquid paraffin (14%) and herbs like, *Aloe vera* (9%) to relieve distended abdomen and diarrhea. Eighty five percent (85%) of these farmers felt liquid paraffin and herbs are effective in treatment of diarrhea and distended abdomen while 6% reported they do not work. Few farmers reported that liquid paraffin is effective in prevention of diarrhea and bloat. The use of herbal extracts (Youn and Noh, 2001) and other natural product alternatives such as fungal extracts and probiotics (Chapman *et al.* 2013) against *Eimeria* spp. have been reported in poultry.

Figure 16. Drugs commonly used by farmers to treat coccidiosis in Kiambu and Nyeri counties, central Kenya

3.3.13 Challenges faced by rabbit farmers

Farm level interviews revealed that the major challenges facing rabbit farmers could be grouped into three factors; factor 1 (marketing, diseases, cost of feed, availability of veterinary services, availability of drugs and cost of drugs), factor 2 (availability of feed and lack of breeding stock) and factor 3 (inadequate knowledge on husbandry practices) as represented in Table 8.

Table 8. Factor analysis of challenges faced by rabbit farmers

Factor	Correlation rating		
	1	2	3
Market	.814	-.457	.078
Diseases	.475	-.649	.190
Availability of feed	.357	.618	-.459
Cost of feed	.947	.044	-.256
Availability of veterinary services	.878	.066	-.207
Availability of drugs	.841	.319	.338
Cost of drugs	.859	.262	.191
Breeding stock	-.092	.766	-.299
Knowledge on husbandry	.395	.472	.625
Others	-.544	.458	.533
Extraction Method: Principal Component Analysis.			
a. 3 components extracted.			

3.3.14 Prevalence of coccidiosis and other endo-parasites

Out of 526 faecal samples collected in the two counties, 258 (49%) tested positive for coccidian parasites with oocyst per gram of feces ranging between 100 to over $12.0x10^4$. Prevalence based on farms was 79.4%. Prevalence per county were 50.4% (119 out of 236) and 47.9% (139 out of 290) for Nyeri and Kiambu counties, respectively (p=0.570). The prevalence of 79.4% is slightly lower compared to the 85.1% reported in an earlier study by

Okumu *et al.* (2014). Poor hygiene due to high number of wooden floors (Fig. 8) coupled by poor cleaning methods may be responsible for the high prevalence reported in the present study. Housing rabbits of different age groups in the same cage also contributes to spread of coccidiosis. In concurrence, studies in India and Iran have also reported high prevalence of mixed infections (Bhat *et al.*, 1996; Hamidinejat *et al.* 2010).

Eimeria species identified in decreasing order were *E. coecicola* (28%), *E. flavescens* (24%), *E. magna* (16%), *E. irresidua* (12%), *E. stiediae* (12%), *E. intestinalis* (8%) *and E. perforans* (8%) (Fig. 17). Of these, *E. intestinalis and E. flavescens* are the most pathogenic causing intestinal coccidiosis (Pakandl, 2009). In a previous study, Okumu *et al.* (2014) showed that rabbits in Kenya are mostly affected by mixed infection of *E. perforans, E. magna, E. piriformis, E. intestinalis, E. flavescens,* and *E. coecicola.* A study of intestinal coccidiosis in Italy established *E. perforans, E. exigua* and *E. magna* as the common species causing intestinal coccidiosis (Papeschi *et al.* 2013*).*

Twenty five (4.8%) and 13 (2.5%) fecal samples were positive for strongyle (A, C and D) and strongyloides (B) eggs (Fig. 19), respectively with egg counts ranging from 100 to1900 eggs per gram of feces for strongyle eggs. This was in agreement with Okumu et al. (2014) who reported very low nematode egg counts. Two samples (0.4%) and one sample (0.2%) were positive for *Giardia* cysts and tapeworm eggs respectively. The nematode larvae were identified from cultured faecal samples (Fig. 18)

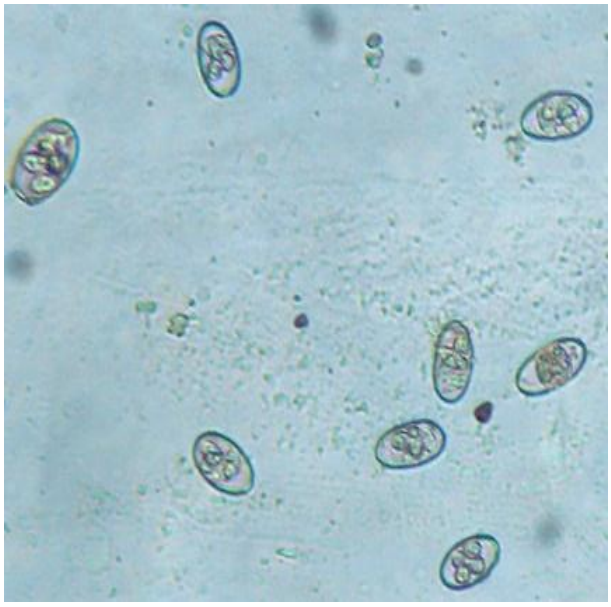


Figure 17. *Eimeria* oocysts at different stages of sporulation at x100 magnification

Figure 18. Third stage larvae from the cultured strongyle eggs at x40 magnification

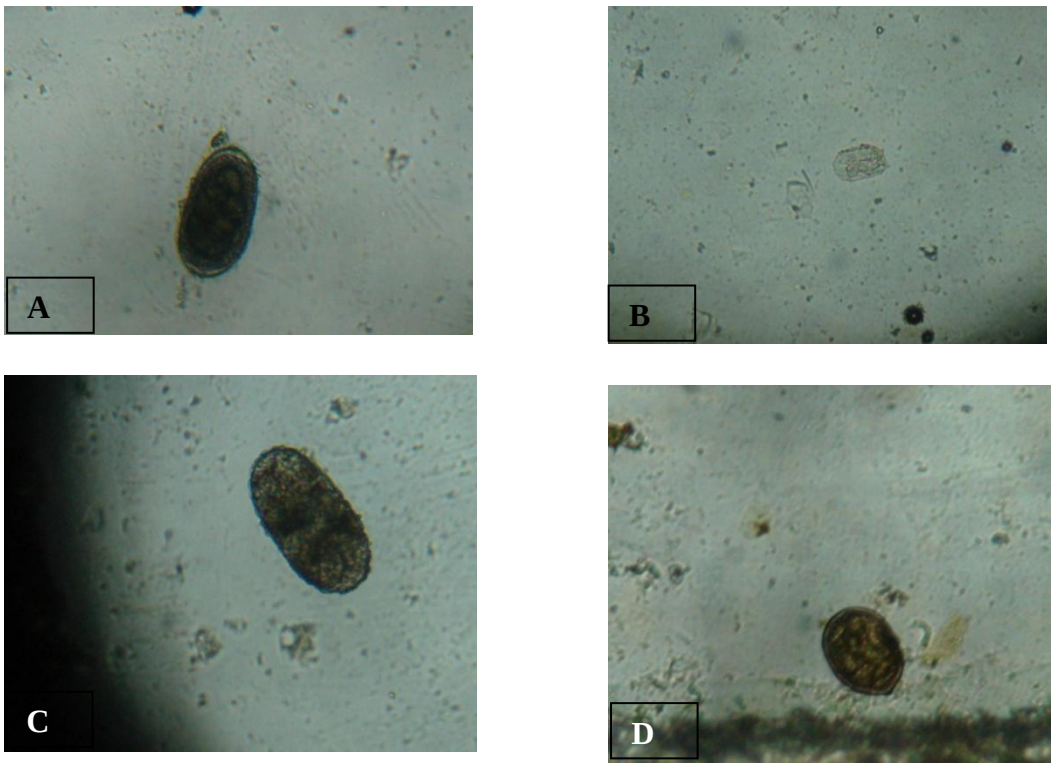


Figure 19. Helminth eggs recovered from faecal samples from Kiambu and Nyeri counties. A, C and D-strongyle eggs with varying shapes at x100 magnification. B- strongyloides egg at x100 magnification

3.4 Conclusions and recommendations

3.4.1 Conclusions

- Prevalence of coccidiosis (79.4%) in study area is as high
- Poor housing structures, inefficient and irregular cleaning methods, and lack of technical knowledge on rabbit production are the major risk factors facilitating spread of coccidiosis
- Inadequate breeding stock and poor breeding practices pose a challenge to domestic rabbit production
- Commonly used treatment options against coccidiosis are Sulphachloropyrazine (22%) and Trimethoprim-sulphamethoxazole (15%)

3.4.2 Recommendations

- The present study recommends a controlled laboratory and field study to determine the best anticoccidial amongst the above mentioned options currently in use.

CHAPTER FOUR

4.0 COMPARISON OF EFFICACY OF SELECTED LOCAL TREATMENT OPTIONS AND DICLAZURIL AGAINST COCCIDIA OF DOMESTIC RABBITS IN A RANDOMIZED CONTROLLED TRIAL

4.1 Introduction

Substantial amount of money is spent globally in treatment and control of rabbit diseases (Pakandl, 2009). The most notable of these diseases is coccidiosis which causes massive economic losses in rabbit production (Bhat *et al*., 1996). Coccidiosis results in high mortality and morbidity especially among weaner rabbits (Pakandl, 2009). Clinical signs include diarrhoea, dehydration, rough hair coat, anorexia, poor performance, and reduced productivity in domestic rabbits (*Oryctolagus cuniculus*) (Jithendran, 2010). Coccidiosis in rabbits is caused by 11 different *Eimeria* spp. (Pakandl, 2009). Two forms of coccidiosis exist in rabbits: intestinal coccidiosis where the invading agents target epithelial cells of different regions of the intestines, resulting in moderate to severe damage depending on virulence of the species (Sivajothi *et al.,* 2014), and, hepatic coccidiosis where the predilection site of the agent (*E. stiedae*) is the liver and gallbladder (Bhat *et al.,* 1996). Though most hepatic infections are mild, severe cases can result in progressive emaciation, hepatomegaly with slightly raised yellowish-white nodules on the liver, which tend to coalesce and consequently interfere with its function (Al-Mathal, 2008). Affected animals present with wasting of hindquarters and back, thirst and abdominal distension (Al-Mathal, 2008). Diarrhea and icteric mucous membranes may also occur on sick rabbits (Jithendran, 2010). Occurrence of coccidiosis in rabbitries is exacerbated by poor hygiene and high stocking densities which encourage parasite dispersal (González-Redondo *et al.*, 2008). Further, coccidia oocysts have a remarkable ability to survive in exogenous environment

making its control by common disinfectants difficult (Chapman *et al.*, 2013). Currently, several strategies are used to control and prevent coccidiosis. Proper hygiene, strict biosecurity and good husbandry practices have been shown in previous studies to play significant role in preventing entry and spread of this disease in a rabbitry (Pakandl, 2009). Despite their success in poultry industry, both live attenuated and live non-attenuated vaccines produced from precocious lines have been tried with unsatisfactory results in rabbits (Drouet-Viard *et al.*, 1997). Furthermore, the emergence of drug resistance following prolonged use and misuse of common anti-coccidial drugs has led to introduction of natural alternatives extracted from plants, fungi and other microorganisms (prebiotics and probiotics) (Quiroz-Castañeda and Dantán-González, 2015). Already published results of the first part of this study revealed that rabbit farmers in Kenya apply ethno-veterinary use of *Aloe vera* and non-conventional use of liquid paraffin in treatment of rabbit coccidiosis with varied efficacies (as shown in chapter 3). However, anticoccidials (both ionophores and synthetic chemicals) remain the mainstream agents for prevention and treatment of coccidiosis (Pakandl, 2009). The most commonly used prevention and treatment method against rabbit coccidiosis in Kenya remains the use of the synthetic chemical anticoccidial drugs labelled for poultry. Since to date there are no specific rabbit anticoccidials in Kenya, farmers have for a long time used drugs labeled for poultry in prevention and treatment of rabbit coccidiosis. This, they do using the poultry reference dosages with little or no knowledge of their safety and efficacy against the rabbit coccidian parasites. While resistance has been reported against almost all of the currently available poultry anticoccidial drugs (Chapman *et al.*, 2013), no literature exist in Kenya on their efficacies against rabbit coccidial parasites. The aim of this study was therefore to determine the efficacy of commonly used anticoccidial drugs by rabbit farmers in Kenya as was determined through a baseline survey in Chapter 3.

The three drugs were compared to a standard drug (diclazuril) that has proven efficacy elsewhere and has never been used in the country, in a controlled experimental trial.

4.2 Materials and methods

From information generated by the baseline survey (objective 1), three of the most commonly used drugs in controlling coccidiosis by farmers were identified and procured for efficacy trials under a controlled environment in the Department of Veterinary Pathology, Microbiology and Parasitology, University of Nairobi.

4.2.1 Experimental drugs

4.2.1.1 Sulphachloropyrazine (ESB_3 30%®)

Water soluble sulphachloropyrazine (ESB_3®) was obtained from the Nairobi Veterinary Centre and administered as per the manufacturer's instructions (1.5 to 2g per liter/1500ppm to 2000 ppm). This drug was administered for six days as follows: 1^{st}, 2^{nd}, 3^{th}, 5^{th}, 7^{th} and 9^{th} day.

4.2.1.2 Amprolium (Amprolium hydrochloride 20%)

Water soluble amprolium hydrochloride 20% was obtained from Nairobi Veterinary Centre and administered at 1g/liter (1000 ppm concentration) as instructed by the manufacturer. The drug was given daily for 7 days.

4.2.1.3 Trimethoprim-sulphamethoxazole (Biotrim®)

Water soluble trimethoprim-sulphamethoxazole (Biotrim®) was obtained from Nairobi Veterinary Centre and administered at 1g per liter of water (1000 ppm) for 7 continuous days, according to manufacturer's instructions.

4.2.1.4 Diclazuril (Diclosol 1%®)

Water soluble formulation of diclazuril (diclosol 1%®) was acquired from Pharmaswede Company and administered at 10 ppm in drinking water for 48 hours.

4.2.2 Experimental rabbits

A total of 65 weaner (9 weeks to 12 weeks old) rabbits of New Zealand white and California white breeds were used as experimental animals. The rabbits were obtained from the National Rabbit Breeding and Training Centre (Ngong').Weaners are the most commonly affected age group by coccidiosis as demonstrated in previous studies (Al-Mathal, 2008; González-Redondo *et al.*, 2008; Oncel *et al.*, 2011; Al- Naimi *et al.*, 2012). Faecal samples were collected from the rabbits before and after one-week acclimatization period to confirm the absence of coccidia oocysts. The rabbits were allocated to six treatment groups using a random block design. Anticoccidial-free commercial feed and water were provided to the rabbits *ad libitum*. Basic hygienic measures were maintained throughout the experiment. To prevent cross-contamination, rabbits in the negative control group were housed in the top cages. The study conformed to the recommendations of the Biosafety and Animal use Committee, Faculty of Veterinary Medicine, University of Nairobi.

4.2.3 Preparation of inoculant

The inoculant of *Eimeria* oocysts was obtained from faecal samples of naturally infected rabbits in the field. Ten (10) rabbit farms in Ngong' sub-county, Kajiado county each with at least 10 rabbits were purposively selected, visited and faecal samples collected. The samples were processed using a modified McMaster floatation technique for oocyst detection (MAFF, 1986)). Farms with positive cases of coccidiosis were identified and a second visit made to collect large quantities of faecal samples (1.5kg per farm). The samples were emulsified in

proportionate amount of floatation fluid (NaCl) then strained into 15 litre buckets and basins. To recover oocysts from the floatation fluid, large petri dishes (150 mm x 25 mm BRAND® petri dish) were placed afloat on the floatation fluids so that oocysts could stick on their submerged parts. The petri dishes were removed after 30 minutes and their submerged parts washed in distilled water into 2,000 ml measuring cylinders which were then topped up with distilled water. Oocysts were recovered through straining and sedimentation technique as described by Soulsby (2005). The recovered oocysts were then sporulated at 27^{o}C in 2.5% potassium dichromate solution for 7 days with on and off aeration as described by Ryley *et al.* (1976). Humidity was maintained at 60-80% throughout the sporulation period by placing water in two standard size (100mm x 15mm) Petri dishes full of water in the incubator. Drops (0.1 ml) of the samples were examined on daily basis with a light microscope using the oil immersion lens (X100) to record sporulation time. The sporulated oocysts were removed from the incubator and centrifuged at 1500 rpm for 10 minutes (Abed and Yakoob, 2013). About half of the supernatant was discarded and the portion remaining in the centrifuge tubes transferred to a 2-liter jar and mixed with distilled water. Distilled water was mixed with the solution containing sporulated oocysts and centrifuged at 1500 rpm for 10 minutes. The sediment having numerous sporulated oocysts was aspirated using a pippete and transferred to a different jar. This washing process was repeated 5-8 times until all potassium dichromate was cleared.

4.2.4 Quantification and identification of sporulated oocysts

The washed sporulated oocysts were counted per 1.0 ml using hemocytometry technique. The various *Eimeria* spp. in the inoculum were then identified based on morphology including size (after randomly measuring 25 oocysts in the order they were encountered with a compound microscope using a 100x oil immersion objective and an ocular micrometer)

according to Soulsby (2005). Based on counts and morphology of 25 sporulated oocyst of each species with a compound microscope under X100 oil emersion objective lens with an ocular micrometer, the inoculant dose had *E. flavescens* (20%), *E. perforans* (21%), *E. intestinalis* (9%), *E. coecicola* (4.2%), *E. media* (11.2%), *E. piriformis* (10.6%), *E. stidae* (16%) and *E. magna* (8%). The pathogenicity of inoculant was first tested in 5 pretrial rabbits to determine the optimum number of oocysts required to establish experimental rabbit coccidiosis, with a cutoff point of clinical expression of diarrhea and shedding of at least 500,000 oocysts per gram of faeces. This was established to be 120,000 oocyst per rabbit.

4.2.5 Experimental design

A total of 60 rabbits were randomly allocated into 6 treatment groups each consisting of 10 rabbits (1A, 2B, 3C, 4D, 5E, and 6F). Groups 1A and 3C served as non-infected non-treated and infected non-treated controls, respectively. Rabbits in groups 2B, 3C, 4D, 5E and 6F were challenged with 120,000 mixed *Eimeria* sporulated oocysts which were administered orally using a syringe after overnight starvation. Treatments were commenced when oocyst per gram counts reached 500,000 o.p.g and/or when clinical signs of coccidiosis were observed. Group 2B was treated with amprolium administered at 1g/liter for 7 consecutive days. Group 4D was treated with diclazuril (Diclosol 1%) at 10 ppm for 48 hours. Group 5E was treated with sulphachloropyrazine for six days as follows: 1^{st}, 2^{nd}, 3^{rd}, 5^{th}, 7^{th}, and 9^{th} at 2g/l (2000ppm). Group 6F was treated with trimethoprim-sulphamethoxazole combination administered at 1g/l (1000ppm) for 7 consecutive days. Faecal samples were collected from the 2^{nd} day post infection to day 30 post infection. Oocysts counts per gram of faeces of each treatment group was determined as described by MAFF (1986). Coccidia oocyst counts within each grid of McMaster chamber were enumerated at 10x magnification using a compound microscope.

4.2.6 Faecal scoring

A daily faecal score for each treatment group was recorded from day 2 to day 20 post infection (d.p.i). Faecal scores were determined by examining the faeces voided on a daily basis according to Ramadan *et al*. (1997) as shown in Table 9).

Table 9. Faecal scoring criteria used in the experimental efficacy trial

Faecal score	Description
1	Well-formed faeces released as pellets
2	Slightly loose faeces (Faeces had the normal pellet shape but were softer in consistency compared to normal faecal pellets)
3	Moderately loose faeces (Faeces not in pelleted form and soft in consistency)
4	Watery diarrhea, increased quantity, no blood
5	Severe diarrhea, presence of blood, markedly increased in quantity

Daily number of dead rabbits in each experimental group were recorded and survival rate determined. Weekly mean weight gain for each group was also assessed.

4.2.7 Percentage survival

Percentage survival was calculated as follows;

$$\text{Survival percentage} = \frac{\text{Number of live rabbits at end of experiment in the group}}{\text{Total number of rabbits in treatment group at start of trial}} \times 100$$

4.2.8 Post mortem and lesion scoring

In order to assess the lesion score, necropsy examination was performed on 3 randomly selected rabbits from each group at end of the experiment, in addition to those that died in the course of the experiment. The rabbits were euthanized humanely using sodium pentobarbitone (Euthatol®, Virbac AH, Inc. Texas) injection into the heart at 100mg/kg body weight for necropsy to establish the effectiveness of the anticoccidial drugs in reversing lesions on various organs and tissues. Necropsy was conducted using a protocol developed by the Department of Veterinary Pathology, Microbiology and Parasitology, University of Nairobi. The lesions were scored through gross examination of the duodenum, jejunum, ileum, caecum, colon and the liver of each rabbit. Gross lesions were scored macroscopically based on a slight modification of a scale designed by Elbahy *et al.* (2006) as shown in Table 10.

Table 10. Criteria used in macroscopic/gross lesion score during necropsy

Grade	Lesion description
0(-)	No evident lesions
1(+)	Slight hyperemia of intestinal wall, mild thickening of intestinal wall and 1-3 focal lesions in a length of 3cm of intestinal wall, slight hepatomegaly (increased by half the normal size) and 1-5 less than 1cm nodular lesions on the liver
2(++)	Moderate hyperemia of intestinal wall, mild thickening of intestinal wall, 3-6 focal lesions in 3cm length of intestinal wall, ballooning of caecum, moderate hepatomegaly (twice normal size), 6-11 raised nodular lesions 1cm in size on the liver
3(+++)	Severe congestion of intestinal wall, increased thickening of intestinal wall, ballooning of the caecum and presence of bloody caecal core, marked hepatomegaly (more than twice normal size, more than 11 raised nodular lesions 1-2 cm in size on the liver

4.2.9 Histopathology

Tissue samples were collected from the liver, duodenum, ileum, caecum and colon for histopathology and microscopic lesion scoring. Collected tissue samples were well-preserved in buffered formalin (10%) and then routinely processed according to Kiernan (1981). Histological lesions were scored according to a set criteria: marked (41-100%), moderate (21-40%), mild (11-20%) and minimal (0- 10%) by recording the nature and extent of lesion and its frequency of occurrence in randomly selected sites in the tissue (Shackfeldford *et al*., 2002). Specific intestinal and hepatic lesions scored are as shown in Appendices 11 and 12.

4.2.10 Assessment of drug efficacies

Drug efficacy was determined through faecal oocysts counts, faecal scores, lesion scores, mortality and survival rates and mean weight gains of various treatment groups. The effectiveness of test drugs was then determined by comparing above parameters for treated groups with those for positive and negative control groups.

4.2.11 Animal welfare and ethical clearance

The animals were bought from a licensed breeder and transported to University of Nairobi, Department of Veterinary Pathology, Microbiology and Parasitology where they were housed and caged separately. The rabbits were fed commercial rabbit pellets from Unga Feed Ltd and supplemented with hay. Water was provided *ad libitum*. The animals were handled humanely in accordance with the institutions animal welfare and ethics committee guidelines. All the rabbits were allowed to acclimatize for one week. The study was approved by the Institutional Animal Care and Use Committee, University of Nairobi.

4.2.12 Data analysis

The data obtained was entered in MS excel 2016 spreadsheet and cleaned. Analysis of variance was performed by one and two way ANOVA as using GenStat. Significant

differences of means of various treatment groups were illustrated by Bonferroni multiple comparison test to control overall significance levels as described in Genstat statistical analysis program (GenStat 15th Edition). The resulting data was presented as mean ± SEM (standard error of mean) and significance levels stated at $p \leq 0.05$.

4.3 RESULTS AND DISCUSSION

4.3.1 Mean faecal scores and standard error of means (SEM) from day of inoculation to day of treatment

All the 5 experimentally infected treatment groups presented clinical signs of loose faeces and diarrhea from day 6 post infection as shown in Table 11. By day 10 post inoculation, majority of rabbits had watery diarrhea that was blood stained in some while a few only released loosely formed faeces. Most of rabbits in the infected groups showed clinical signs of reduced appetite manifested by feed remaining in feeders, rough hair coats, distended and pendulous abdomen, dullness, reduced weight, matted perineal area, hepatomegaly on palpation and slight dehydration from day 6 compared to negative control group which appeared normal. There was significant difference ($p<0.05$) in faecal scores between infected group (3C) and uninfected non-treated group (1A) from day 6 post inoculation (Table 11). In an earlier experimentally induced coccidiosis study, clinical signs of bristling hair, polydipsia, loss of weight and inappetence were reported (Kulišić, 2006). Similar clinical signs were also reported by Bhat *et al*. (1996) and Al- Naimi *et al*. (2012) for hepatic coccidiosis and by Papeschi *et al*. (2013) and Oncel *et al*. (2011) for intestinal coccidiosis. In agreement with Al- Naimi *et al.* (2012), jaundice was only seen in very severe cases.

Table 11. Mean faecal scores from day of inoculation to day 11 post inoculation in rabbits on drug efficacy study for experimental coccidiosis

Group	Faecal scores on different days post infection				
	Inoculation day 0	**Day 4**	**Day 7**	**Day 10**	**Day 11**
Negative control (1A)	1.0±0.00[a]	1±0.00[a]	1.0±0.00[a]	1.17±0.17[a]	1.33±0.21[a]
Amprolium (2B)	1.0±0.00[a]	1±0.00[a]	2.0±0.37[ab]	2.83±0.31[b]	3.0±0.26[b]
Positive control (3C)	1.0±0.00[a]	1±0.00[a]	2.17±0.31[ab]	3.0±0.26[b]	3.17±0.31[b]
Diclazuril (4D)	1.0±0.00[a]	1±0.00[a]	2.33±0.33[b]	2.5±0.34[b]	2.67±0.21[b]
Sulphachloropyrazine (5E)	1.17±0.17[a]	1.17±0.17[a]	2.17±0.31[ab]	2.67±0.21[b]	2.67±0.21[b]
Trimethoprim-sulphamethoxazole (6F)	1.0±0.00[a]	1±0.00[a]	2.67±0.21[b]	2.67±0.33[b]	2.83±0.31[b]
SD	0.167	0.167	0.826	0.878	0.838
P-value	0.435	0.435	0.007	<0.001	<0.001

Values within a column without common superscript are significantly different at 0.05

Faecal score was done according to Ramadan *et al*. (1997) where a score of 1 indicated normal well-formed faecal pellets through to 5, indicating severe diarrhea with/out profuse amount of blood.

4.3.2 Mean faecal scores from day of treatment to day 20 post treatment

Diclazuril and sulphachloropyrazine showed satisfactory results 9 days after treatment in alleviation of diarrhea and promoting production of normal faecal pellets as shown in Table 12. Furthermore, diclazuril and sulphachloropyrazine treatment groups gave a significant ($p<0.05$) improvement in faecal score of 1.17±0.17 and 1.33±0.21 from 2.67±0.21 and 2.67±0.21, respectively compared to positive control group score of 3.0±0.32 at end of treatment. Diclazuril treatment group recorded a faecal score even better than that of the negative control group 1.33±0.21. This is in agreement with previous studies that also established the superior efficacy of curative diclazuril against coccidiosis in rabbits (Vereecken *et al*., 2012) and avian coccidiosis (El-Banna *et al*., 2005). Efficacy of water

soluble diclazuril against mixed *Eimeria* infection in broiler chickens was also previously demonstrated by Vanparijs *et al*. (1989b) and Conway *et al*. (2002).

No significant difference ($p>0.05$) in faecal scores was seen between amprolium and trimethoprim-sulphamethoxazole combination treatment groups relative to the positive control group. Similar results were observed on the subsequent days 13, 17and 20 post treatment as shown in Table 12.

Table 12. Faecal scores from day of treatment to day 20 post treatment of rabbits on drug efficacy trial for experimental coccidiosis

	Days post treatment					
Treatment group	**Day of treatment**	**Day 5**	**Day 9**	**Day 13**	**Day 17**	**Day 20**
Negative control 1A	1.33±0.21^{a}	1.0±0.0^{a}	1.33±0.21^{a}	1.33±0.24^{a}	1.17±0.19^{a}	1.17±0.18^{a}
Amprolium hydrochloride 2B	3.0±0.26^{b}	3.17±0.31^{c}	3.17±0.40^{b}	3.0±0.24^{bc}	2.50±0.24^{b}	2.25±0.23^{b}
Positive control 3C	3.17±0.31^{b}	3.0±0.32^{c}	3.0±0.32^{b}	3.20±0.26^{c}	2.75±0.24^{b}	3.0±0.00^{b}
Diclazuril 4D	2.67±0.21^{b}	2.17±0.40^{bc}	1.17±0.17^{a}	1.33±0.24^{a}	1.17±0.19^{a}	1.0±0.18^{a}
Sulphachloropy-razine 5E	2.67±0.21^{b}	1.83±0.31^{ab}	1.33±0.21^{a}	1.17±0.24^{a}	1.20±0.21^{a}	1.0±0.20^{a}
Trim/sulphame-thoxazole 6F	2.83±0.31^{b}	2.33±0.42^{bc}	2.0±0.37^{ab}	2.0±0.24^{ab}	2.67±0.19^{b}	2.33±0.18^{b}
SD	0.838	1.051	1.043	0.985	0.860	0.844
p-value	<0.001	0.001	<0.001	<0.001	<0.001	<0.001

Values within a column without common superscript are significantly different at 0.05

Faecal score was done according to Ramadan *et al.* (1997) with 1 indicating normal well-formed faecal pellets through 5 indicating severe diarrhea with/out profuse amount of blood.

4.3.3 Oocyst shedding from day 0 to day 10 post infection

Oocyst counts in all the treatment groups ranged from 0 to <1.0 x 10^3/g on the day of inoculation. There was no significant difference ($p>0.05$) in oocyst counts between infected groups and the uninfected negative control group from the day of inoculation to day 4 post inoculation (Table 13). Low oocyst counts in early infection were also reported by Vereecken *et al*. (2012) in an experimental infection. However, from day 6 post inoculation onwards, there was increase in oocyst shedding in infected groups compared to uninfected negative control group, which peaked between day 7 and 12 post infection. However, the positive control group demonstrated a steady increase in oocyst counts shed up to day 20 post infection after which the numbers started to decrease. This was a slight variation from results by Vereecken *et al*. (2012) who reported a gradual decrease of oocysts shed from day 15 post infection towards levels comparable with those of the negative control group in infected untreated group. Notably, oocyst counts shed by the amprolium hydrochloride treatment group presented a pattern closely similar to that of positive control group throughout the experiment.

Table 13. Summary of coccidial oocysts shed from day of inoculation to day 10 post inoculation when treatment was started in various treatment groups

Treatment group	Oocysts shed per treatment group x10^4 /gram of feces					
	Day of Inoculation	Day 2	Day 4	Day 6	Day 8	Day 10
Negative control (1A)	0.014±0.00611	0.027±0.00558[a]	0.027±0.0109	0.022±0.00401	0.021±0.00464	0.059±0.0230[a]
Amprolium (2B)	0.01±0.00342	0.09±0.0232[b]	0.09±0.0232	3.80±0.873	13.97±7.3276	19.01±9.567[ab]
Positive control (3C)	0.01±0.005	0.10±0.0239[b]	0.25±0.0239	3.82±1.468	15.63±8.791	34.93±16.280[ab]
Diclazuril (4D)	0.01±0.00987	0.04±0.00955[ab]	0.34±0.145	11.44±3.544	28.22±9.379	59.700±12.351[ab]
Sulphachloropyrazine (5E)	0.01±0.00749	0.09±0.0180[b]	0.66±0.475	12.40±9.538	56.97±38.692	149.00±110.392[ab]
Trimethoprim-sulphamethoxazole (6F)	0.01±0.0115	0.07±0.0172[ab]	0.26±0.0635	8.00±4.283	26.13±12.134	197.17±92.657[b]
p-value	0.933	0.045	0.338	0.364	0.336	0.154

Values within a column without common superscript are significantly different at 0.05

Sulphachloropyrazine and diclazuril had a significant ($p<0.05$) reduction in mean oocyst shed by day 7 post treatment at 0.83±0.401 x 10^4/g and 0.122±0.0958 x 10^4/g respectively compared to infected untreated group 170.20±68.921 x 10^4/g (Appendix 7). By day 13 post treatment, diclazuril treatment group recorded 0.00±0.00 oocyst count (Appendix 8) impressively better than even that of negative control group 0.173±0.068 x 10^4/g (3 logarithms difference lower) while sulphachloropyrazine group recorded an oocyst count of 2.03±0.829 x 104/g (about 1 logarithm higher than negative control). On day 20 post treatment when the experiment was terminated, the mean number of oocysts shed remained extremely low in the diclazuril treatment group 0.002±0.00167 x 10^4/g and sulphachloropyrazine treatment group 3.31±0.857 x 10^4/g compared to infected untreated, amprolium and trimethoprim-sulphamethoxazole treatment groups as presented in Table 14 and Appendices 7 and 8. The efficacy of sulphachloropyrazine in reduction of oocysts shed

has also been elaborated in poultry anticoccidial trials (Das *et al*., 2017). Still, superior efficacy of diclazuril in elimination of oocysts shed has been reported in several studies on rabbit coccidiosis (Vanparijs *et al*., 1989b; Vereecken *et al*., 2012) and poultry coccidiosis (El-Banna *et al*., 2005). Trimethoprim-sulphamethoxazole treatment group had a higher reduction in oocysts shed on day 7 post treatment 61.17±10.603 x 10^4/g compared to amprolium and infected untreated groups. However, the mean number of oocysts shed by the trimethoprim-sulphamethoxazole group started to rise again from day 13 post treatment and by day 20 post treatment had reached 231.67±51.43 x10^4/g. However, this was still significantly lower ($p<0.05$) compared with that of infected untreated group 737.50±213.478 x 10^4/g.

On the other hand, the number of oocysts shed by amprolium treatment group on day 7 post treatment was higher 357.67±123.451 x 10^4/g compared to that of infected untreated group 170.20±68.921 x 10^4/g though not significantly different ($p>0.05$). In this study, amprolium hydrochloride had the least efficacy compared to other test drugs. This finding agree with an earlier study by Laha *et al*. (1999) who demonstrated inability of amprolium to reverse active coccidiosis infection in rabbits and Das *et al*. (2017) who reported less than satisfactory efficacy of amprolium hydrochloride in broiler chickens. In a recent efficacy study from Ethiopia, Hunduma and Kebede (2016) also reported that amprolium was not effective in controlling coccidiosis of poultry.

Conversely, this finding slightly deviates from the moderate efficacy of amprolium hydrochloride reported by Laha *et al*. (2015) in India and El-Ghoneimy and El-Shahawy (2017) in Iran against rabbit intestinal coccidiosis. However, in the study by El-Ghoneimy and El-Shahawy (2017), they demonstrated that for best results to be achieved, it is better to concurrently use amprolium with other anticoccidials like toltrazuril. The ineffectiveness of

amprolium in this study may be attributed to development of resistance that may have arisen over the years from its extensive and indiscriminate use and misuse by the farmers as was established in the baseline survey. This inference is supported by Laha *et al*. (2015) who reported that efficacy of amprolium was region specific depending on how the drug has been used in such regions over time that may or may have not led to development of resistance.

Table 14. Oocyst counts from day 1 to day 20 post treatment of rabbits on drug efficacy trial for experimental coccidiosis

Mean oocyst shed per treatment group x 10^4/gram of feces						
Treatment group	1 day before treatment	3 days post treatment	7 days post treatment	13 days post treatment	17 days post treatment	20 days post treatment
Negative control (1A)	0.059±0.023[a]	0.093±0.022[a]	0.090±0.0304[a]	0.173±0.0679[a]	0.141±0.0396[a]	0.138±0.0383[a]
Amprolium (2B)	19.01±9.567[a]	351.00±127.691[b]	357.67±123.451[b]	416.83±129.864[a]	429.60±129.847[ab]	430.00±62.450[ab]
Positive control (3C)	34.93±16.280[a]	151.67±52.180[ab]	170.20±68.921[ab]	432.40±142.793[a]	642.40±177.504[b]	590.02±96.128[b]
Diclazuril (4D)	59.700±12.351[a]	14.198±9.178[a]	0.122±0.0958[a]	0.00±0.00[a]	0.00±0.00[a]	0.002±0.00167[a]
Sulphachloropyrazine (5E)	149.00±110.392[a]	61.91±37.202[a]	0.83±0.401[a]	2.03±0.829[a]	2.03±0.698[a]	3.31±0.857[a]
Trimethoprim - sulphamethoxazole (6F)	197.17±92.657[a]	95.08±35.184[ab]	61.17±10.603[a]	230.50±154.302[a]	358.00±163.169[ab]	231.67±51.43[a]
p-value	0.154	0.004	<0.001	0.008	<0.001	<0.001
Values within a column without common superscript are significantly different at 0.05						

4.3.4 Overall percentage reductions of oocyst shed

The trend in reduction of oocyst shed in various treatment groups is shown in Fig. 18. Substantial reduction was seen in the diclazuril and sulphachloropyrazine treatment groups relative to trimethoprim-sulphamethoxazole and amprolium treatment groups, and positive control group (Fig. 20).

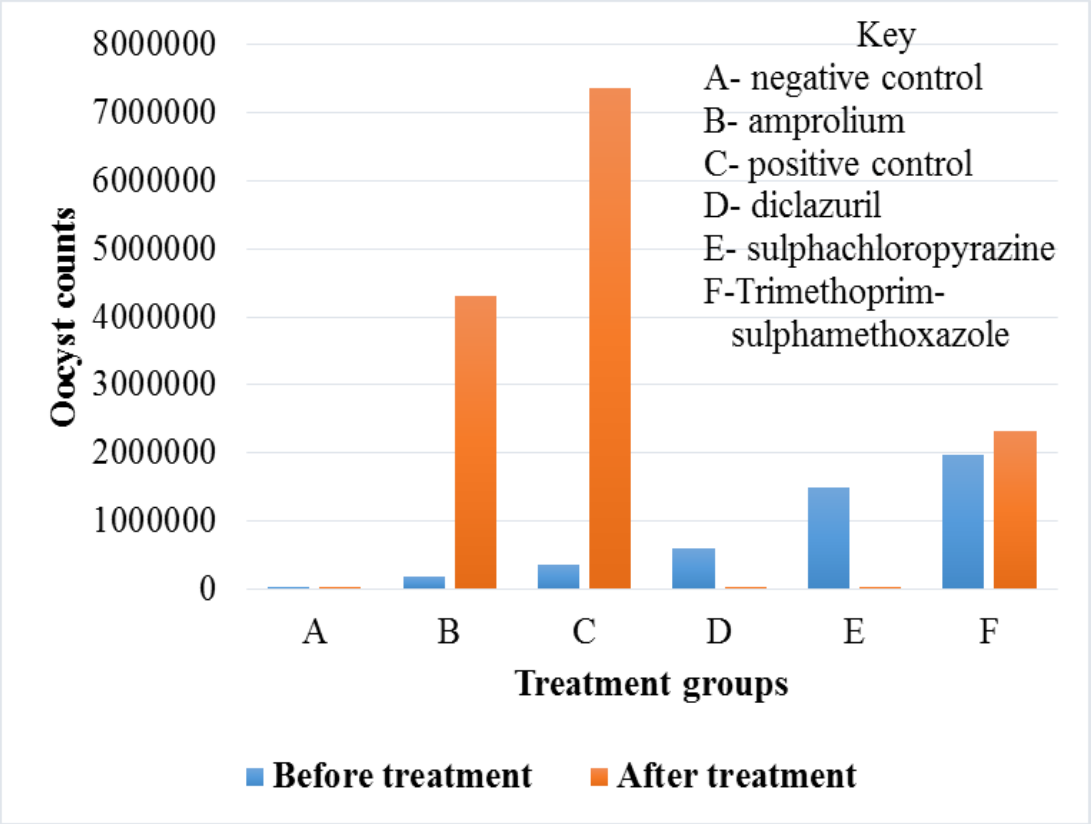


Figure 20. A bar graph showing reduction in oocysts shed before and after treatment in rabbits on drug efficacy study for experimental coccidiosis

4.3.5 *Eimeria* species identification

The inoculant used to induce experimental infection had the following *Eimeria* spp. in decreasing prevalence: *E. perforans* (21%), *E. flavescens* (20%), *E. stidae* (16%), *E. media* (11.2%), *E. piriformis* (10.6%), *E. intestinalis* (9%), *E. magna* (8%) and *E. coecicola* (4.2%). Species identification at the end of the experiment from sporulated pooled sample revealed the predominant *Eimeria* species to be *E. magna* (44.7%) and *E. stidae* (27.2%) with the rest registering lower percentages. These results agree with those of Vereecken *et al*. (2012) who reported *E. magna* as the main species that remained in rabbits after treatment with various trial drugs.

4.3.6 Liver impression smears of different treatment groups

The liver impression smears from treatment groups 2B, 3C and 6F (Fig. 21) had numerous clear fully formed coccidial oocysts mixed with few hepatobiliary parenchymal cells (Fig. 22). An ellipsoidal fully formed oocyst was the predominant developmental stage from the smears. The oocysts had a smooth, pink wall and a flat micropylar. Immature developmental stages including small microgametocytes of varied shapes within epithelial cells of the ducts (Fig. 22) and round macrogametocytes filled with uniform bluish-pink cytoplasmic granules (Fig. 22) were present in impression smears from 2B, 3C, and 6F treatment groups. Numerous clusters of cuboidal-columnar epithelial cells of the bile ducts and few inflammatory cells were also seen in these treatment groups. These results agree with the findings of Al-Rukibat *et al.* (2001) and Sivajothi *et al*. (2014) on liver impression smears of hepatic coccidiosis. On the other hand, Impression smears from sulphachloropyrazine (5E) treatment group had comparatively fewer oocysts compared with the three groups. However, smears from diclazuril and negative control groups were negative for oocysts. These results indicate that diclazuril was able to completely treat hepatic coccidiosis with sulphachloropyrazine having more than average efficacy against hepatic coccidiosis.

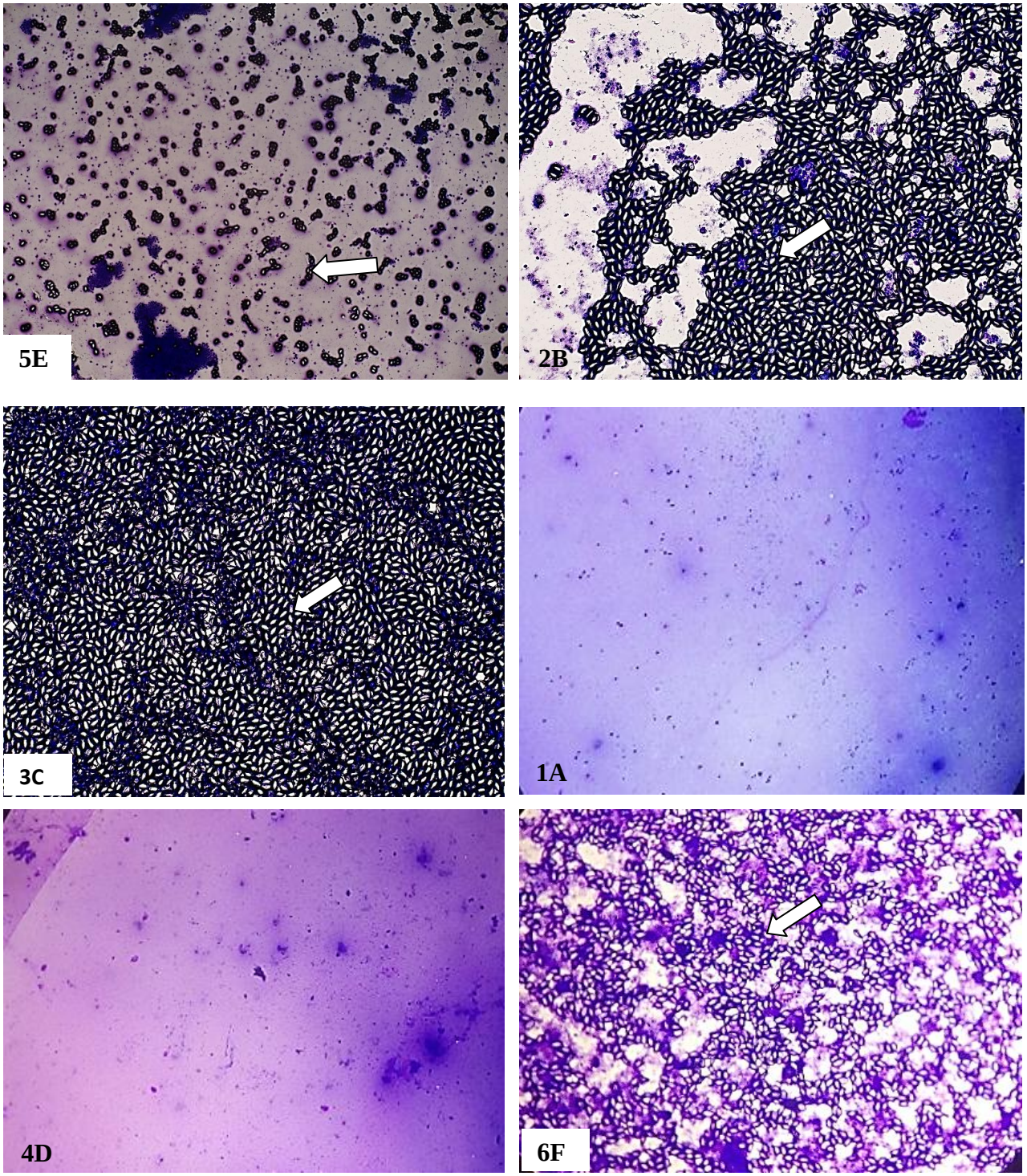


Figure 21. Gallbladder impression smears from six treatment groups. The oocysts counts did not vary much in the animals sacrificed per group. A1- liver smear from negative control group without any oocyst at x40, 2B- a smear from amprolium treatment group with numerous clear oocysts at x100 (arrow), 3C- a smear from positive control group with numerous oocyst at x40 (arrow), 4D- a smear from diclazuril treatment group without any oocyst, 5E- a smear from sulphachloropyrazine treatment group with few clear oocysts at x40 (arrow) and 6F – a smear from trimethoprim-sulphamethoxazole treatment group with numerous clear oocysts at x40 magnification (Arrow). All the slides were stained by Giemsa stain. Note that only the background is stained as the oocysts do not take the stain.

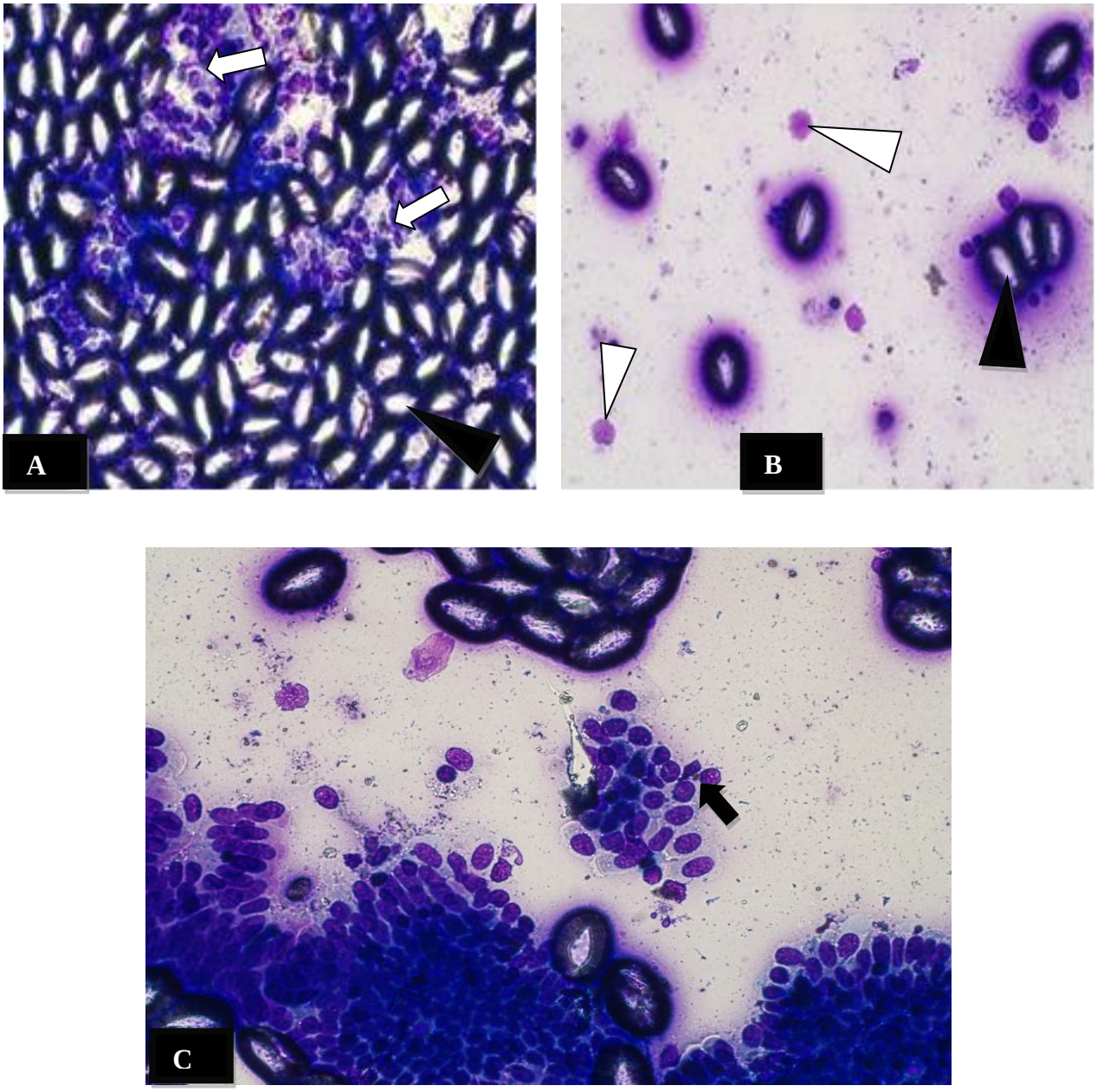


Figure 22. Impression smear characteristics of the liver. A, clear, oval to elliptical-shaped fully formed oocysts (black arrow head) and hepatobiliary parenchymal cells (white arrow) at x400, B, Macrogametocytes (white arrow head) and fully formed oocysts (black arrow head) at x400, C, cluster of billiary epithelial cells containing numerous microgametocytes (black arrow)

4.3.7 Total macroscopic mean lesion scores

Diclazuril was highly efficacious ($p<0.05$) in reduction of hepatic and intestinal lesion scores (0.33±0.33) compared to positive control group 2.67±0.33 with a lesion score difference of more than 2 logarithms. Though significantly efficacious ($p= 0.047$) compared to positive control group, sulphachloropyrazine treatment group (1.33±0.33) had some mild lesions compared to negative control group as depicted in Table 15. Strikingly, there was no significant difference ($p<0.05$) in lesion scores recorded for amprolium, trimethoprim-sulphamethoxazole and positive control treatment groups. Macroscopic intestinal lesions were relatively less severe in comparison to hepatic lesions. The intestinal lesions ranged from severe congestion, mild haemorhages in the lumen, hyperemia of intestinal mucosa, ballooning of caecum, edema of intestinal mucosa in 2B, 3C and 6F groups to fairly normal intestines in 1A, 4D and 5E treatment groups (Figs. 28 and 29). The raised nodular lesions observed on the liver were absent in the intestines. A study by Oncel *et al.* (2011) of intestinal coccidiosis reported macroscopic lesions of distended, hyperemic and oedematous intestines. Moreover, a direct relationship was observed between the severity of lesions at post mortem and evident clinical signs seen before the rabbits were sacrificed with the asymptomatic rabbits presenting with moderate to minor lesions. This is in agreement with a study by Darzi *et al.* (2007) which reported severe lesions at necropsy in rabbits which presented overt clinical signs of listlessness, lack of appetite, debility, diarrhea, jaundice and distended abdomen.

Table 15. Total mean lesion scores in six treatment groups in rabbits on drug efficacy trial for experimental coccidiosis

Treatment group	Animal 1	Animal 2	Animal 3	Mean	Sem
Negative control 1A	0.00	0.00	0.00	0.00[a]	0.00
Amprolium 2B	3.00	2.00	3.00	2.67[b]	0.33
Positive control 3C	2.00	3.00	3.00	2.67[b]	0.33
Diclazuril 4D	1.00	0.00	0.00	0.33[a]	0.33
Sulphachloropyrazine 5E	2.00	1.00	1.00	1.00[ab]	0.33
Trimethoprim-sulphamethoxazole 6F	3.00	3.00	2.00	2.67[b]	0.33

p-value < 0.001

Values within a column without a common superscript are significantly different at 0.05

Lesion score was done according to modified Elbahy *et al.* (2006) with 0 indicating absence of any evident lesion through 3 indicating severe intestinal and hepatic lesions

4.3.8 Hepatic lesions in various treatment groups

Figures 23, 24, 25, 26 and 27 below show the effectiveness of various drugs in reversing hepatic lesions 20 days post treatment. There was evident congestion, hepatomegaly (almost 3 times the normal size) and increased dark straw colored peritoneal fluid in trimethoprim-sulphamethoxazole (6F), amprolium hydrochloride (2B) and positive control (3C) treatment groups. Additionally, livers from the above three treatment groups had raised yellowish-white multi-nodular lesions 0.5mm-2 cm in diameter covering the entire liver surface and its parenchyma (Fig. 23). The gallbladder was markedly distended and contained thick yellowish-white contents whose consistency ranged from free flowing greenish content to firm cheesy material (Fig. 24). There were fibrin strands on the surfaces of the livers with numerous necrotic spots. On incision, the liver parenchyma from these treatment groups were firmer compared to those of negative control group that had soft consistency. These results concur with an earlier study by Al- Naimi *et al.* (2012) who attributed the firmness to post necrotic scarring of liver parenchyma as a result of injuries arising from excessively proliferated bile ducts. The results also agree with the description of hepatic coccidiosis by Darzi *et al.* (2007) and Abbas (2009).

Sulphachloropyrazine group (5E) had mild to moderate hepatomegaly (between half to twice the normal size), slightly raised nodular lesions (1mm - 1 cm in diameter) with mostly white contents, slightly - moderately distended gall bladder with greenish-yellow contents. Livers from the group treated with diclazuril (4D) did not present with significant gross lesions relative to the negative control group (1A) apart from the few fibrotic areas. Efficacy of diclazuril in reverting hepatic and intestinal coccidiosis has been demonstrated in other studies (Vanparijs *et al.* 1989a) and recently (Vereecken *et al.*, 2012) which the present study agrees with.

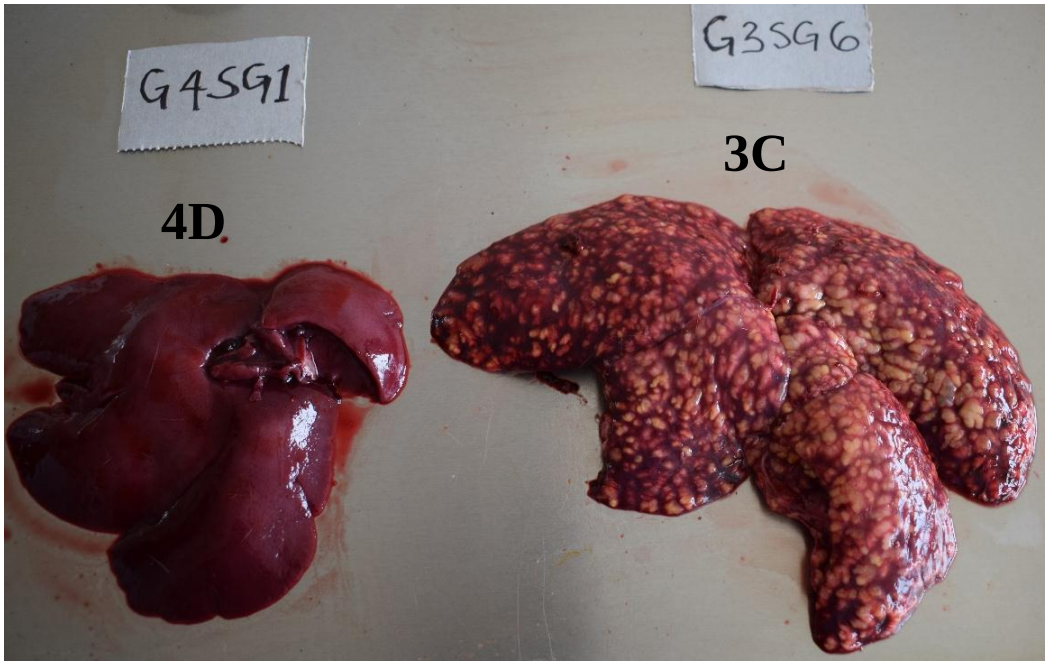


Figure 23. Normative liver from diclazuril treatment group (4D) and one with marked hepatomegaly from coccidia-infected but not treated group (3C) 30 days post infection with yellowish-grey nodules (arrow)

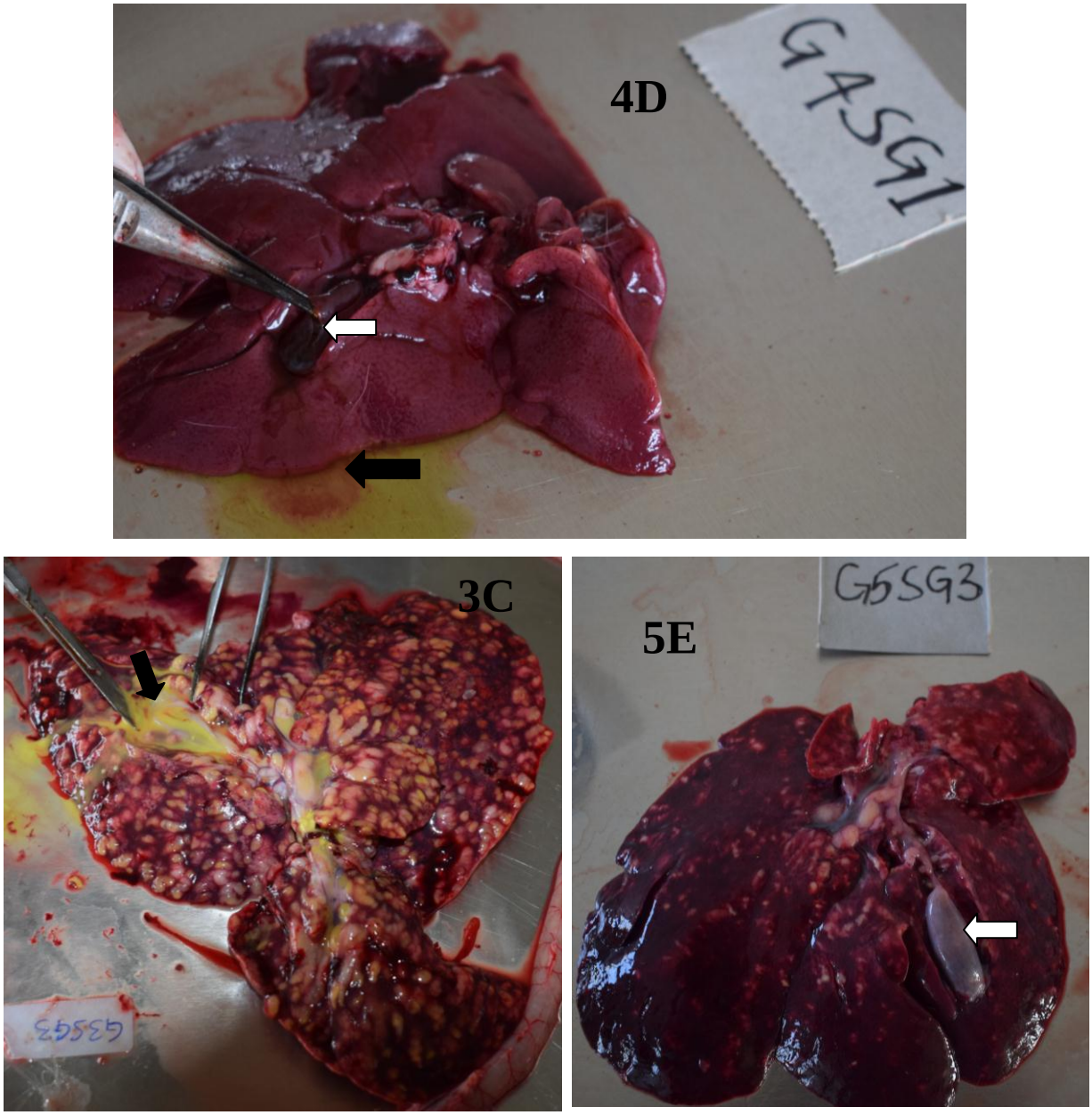
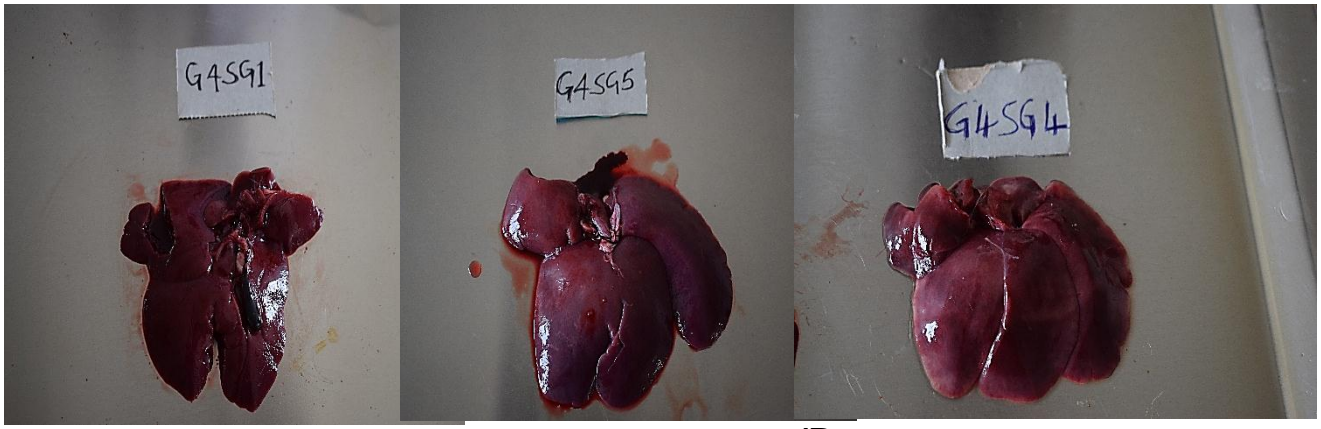
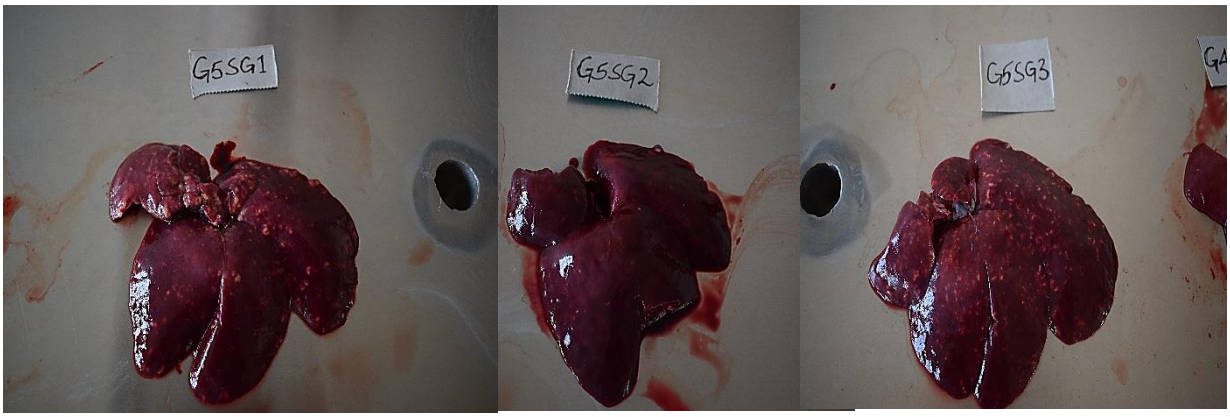


Figure 24. Hepatic lesions at the end of the efficacy trial: 4D-greenish-yellow contents from a normative gallbladder from diclazuril treatment group (black arrow), and gallbladder with normal dark appearance (white arrow). 3C- thick whitish-yellow contents from incised gallbladder with numerous multinodular lesions from coccidia-infected untreated group. 5E- slightly distended gallbladder dark in appearance from sulphachloropyrazine treatment group

6F **6F** **6F**



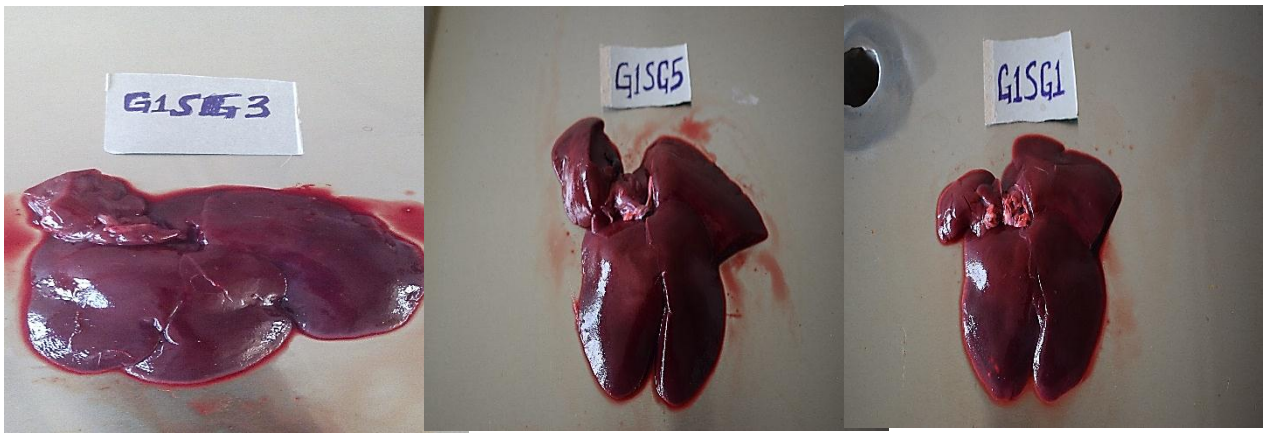
4D **4D** **4D**



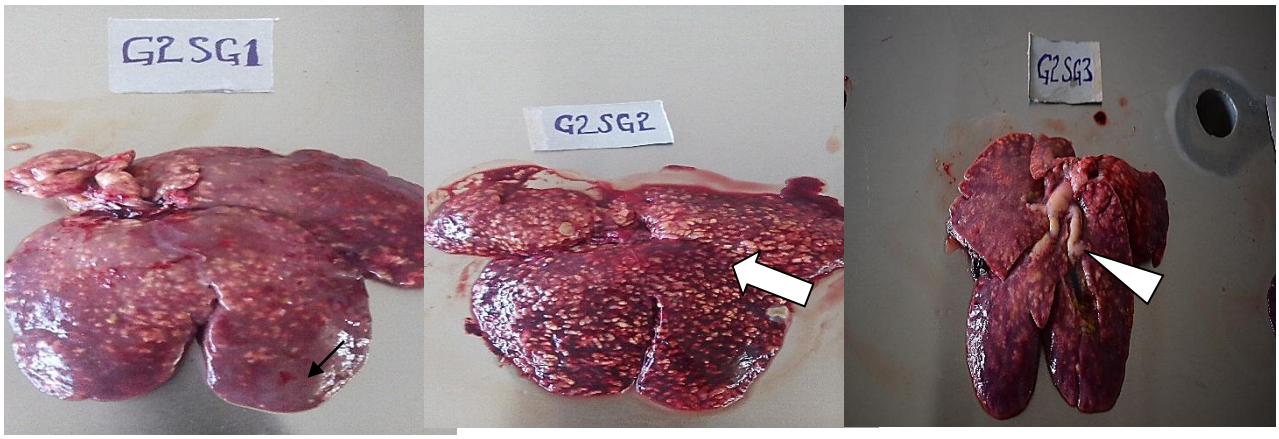
5E **5E** **5E**

Figure 25. Hepatic lesions in various treatment groups at experiment termination, 6F-enlarged livers with multinodular whitish-yellow coccidian lesions from trimethoprim-sulphamethoxazole treatment group, 4D-fairly normative livers from diclazuril treatment group and 5E- slightly enlarged livers with tiny whitish-yellow fibrotic spots from sulphachloropyrazine treatment group

3C 3C 3C



1A 1A 1A



2B 2B 2B

Figure 26. 3C- markedly enlarged livers with raised multinodular whitish-yellow lesions from positive control group, 1A- normal-looking livers from negative control group and 2B-enlarged livers with fibrin strands (small arrow) from amprolium treatment group; note the hepatic multinodular lesions (arrow) and the markedly distended bile duct (arrow head)

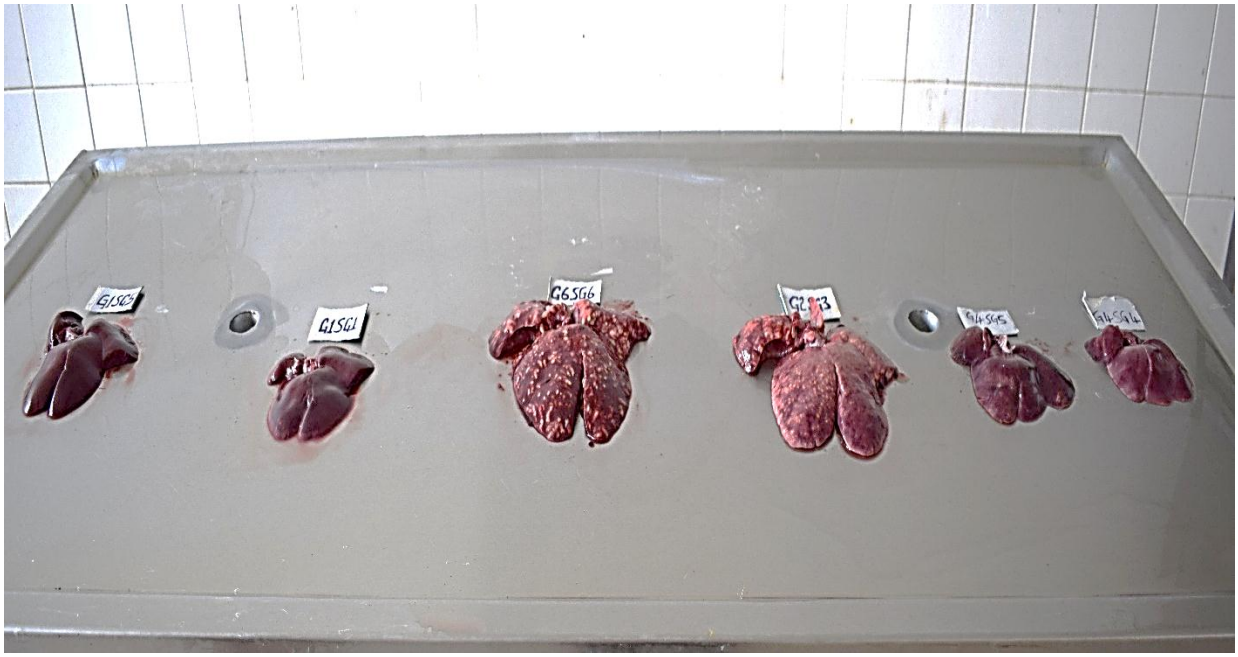


Figure 27. Showing livers from five treatment groups. From left to right: Negative control, Negative control, trimethoprim-sulphamethoxazole, amprolium, sulphachloropyrazine and diclazuril. Note the marked hepatomegaly and multinodular lesions in the amprolium and trimethoprim-sulphamethoxazole treatment groups

4.3.9 Macroscopic intestinal lesions

The intestinal lesions ranged from severe congestions, mild haemorhages in the lumen, ballooning of caecum, edema of intestinal mucosa in 2B, 3C and 6F groups to fairly normal intestines in 1A, 4D and 5E treatment groups (Figures 28 and 29). The raised nodular lesions observed on the liver were absent in the intestines.

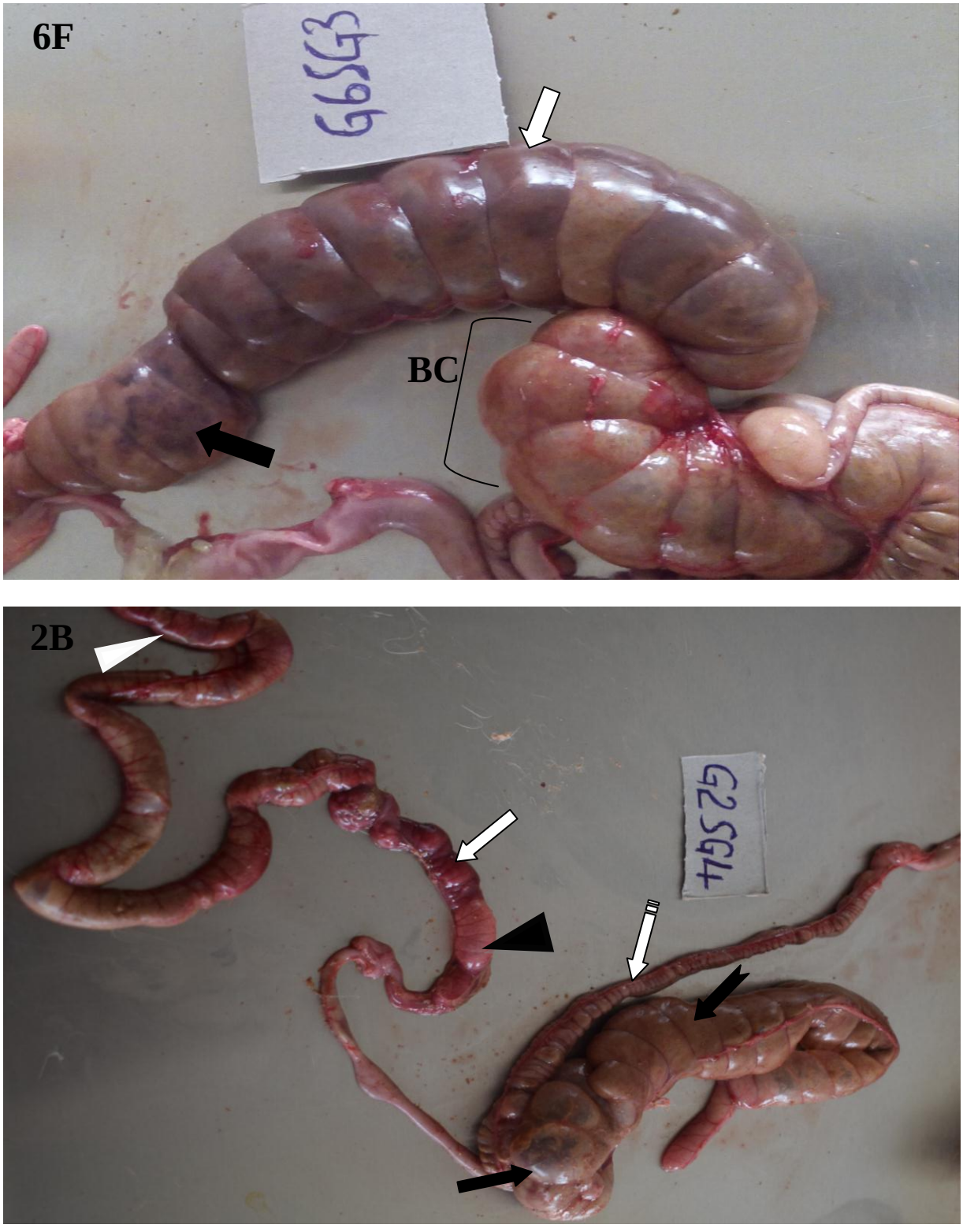


Figure 18. Gross intestinal coccidian lesions from trimethoprim-sulphamethoxazole treatment group (6F) showing marked congestion (white arrow), necrotized caecal parts (black arrow) and ballooned section of the caecum and, amprolium treatment group (2B) showing ballooned section of the ileum (black arrow head) and caecum (black arrow), extensive congestion and hyperemia of the ileum (white arrow), jejunum (white arrow head) colon (white arrow with broken ends) caecum (black arrow with curved end)

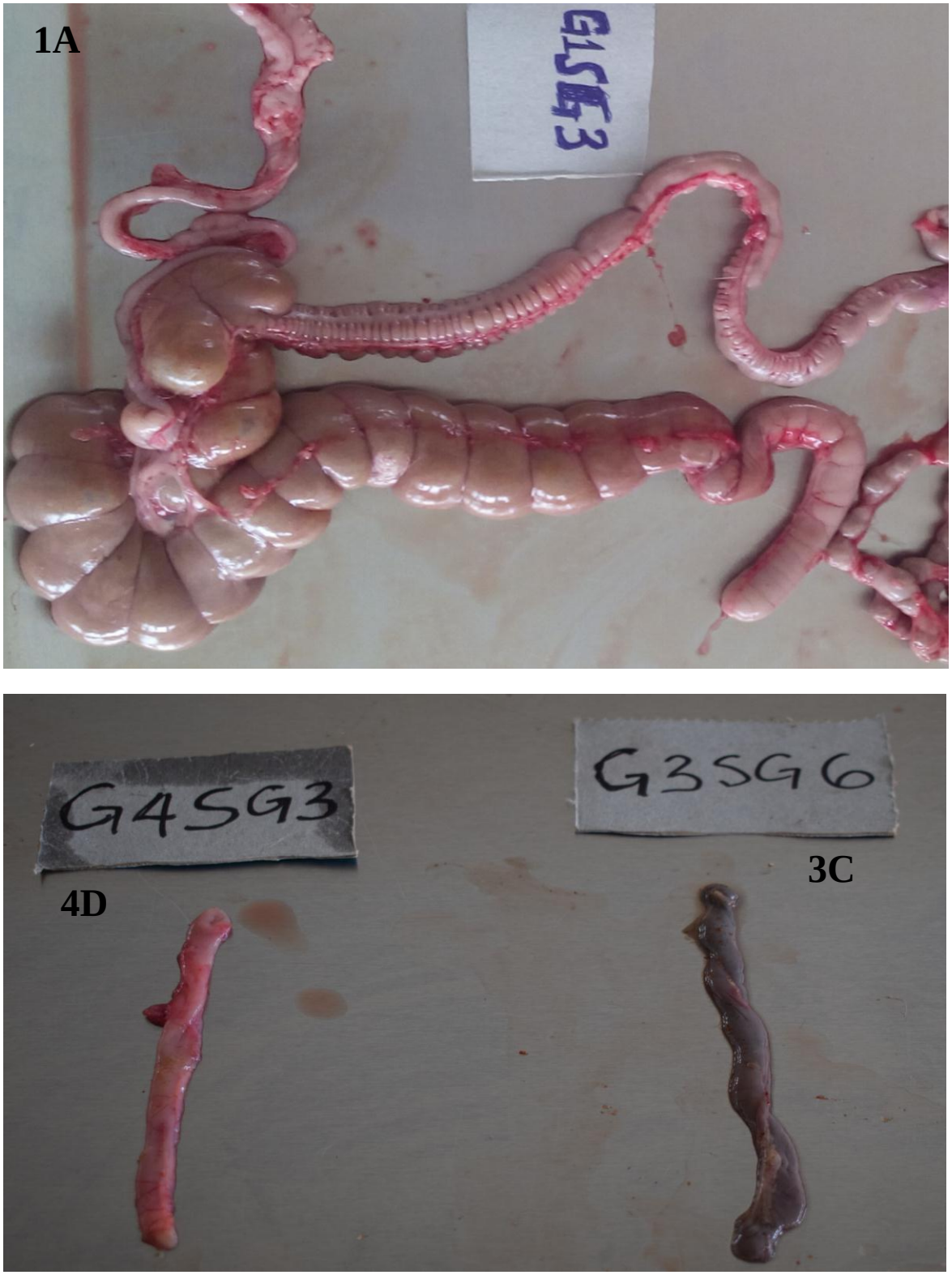


Figure 19. Gross intestinal coccidian lesions: 3C- a healthy pink caecum and colon from negative control group, 4D- duodenal section from diclazuril treatment group with normative pink colour compared with the highly congested and necrotic duodenal section from positive control group (3C)

4.3.10 Total microscopic mean lesion scores

Microscopic examination of the intestines revealed severe desquamation of epithelium (Fig. 30A), marked hyperplasia of submucosal goblet cells (Fig. 30C and D) and different developmental stages of *Eimeria* spp. (Fig. 30E and F) in treatment groups 2B, 3C, 5E and 6F. Mean lesion scores are summarized in Table 16. Other lesions observed less frequently but not scored were congestion, capillary haemorrhages and dilation of blood vessels, red blood cells within the lumen of the intestines, fusion of villi, precipitates of protein in gut lumen, oedema of muscularis and necrosis of enterocytes.

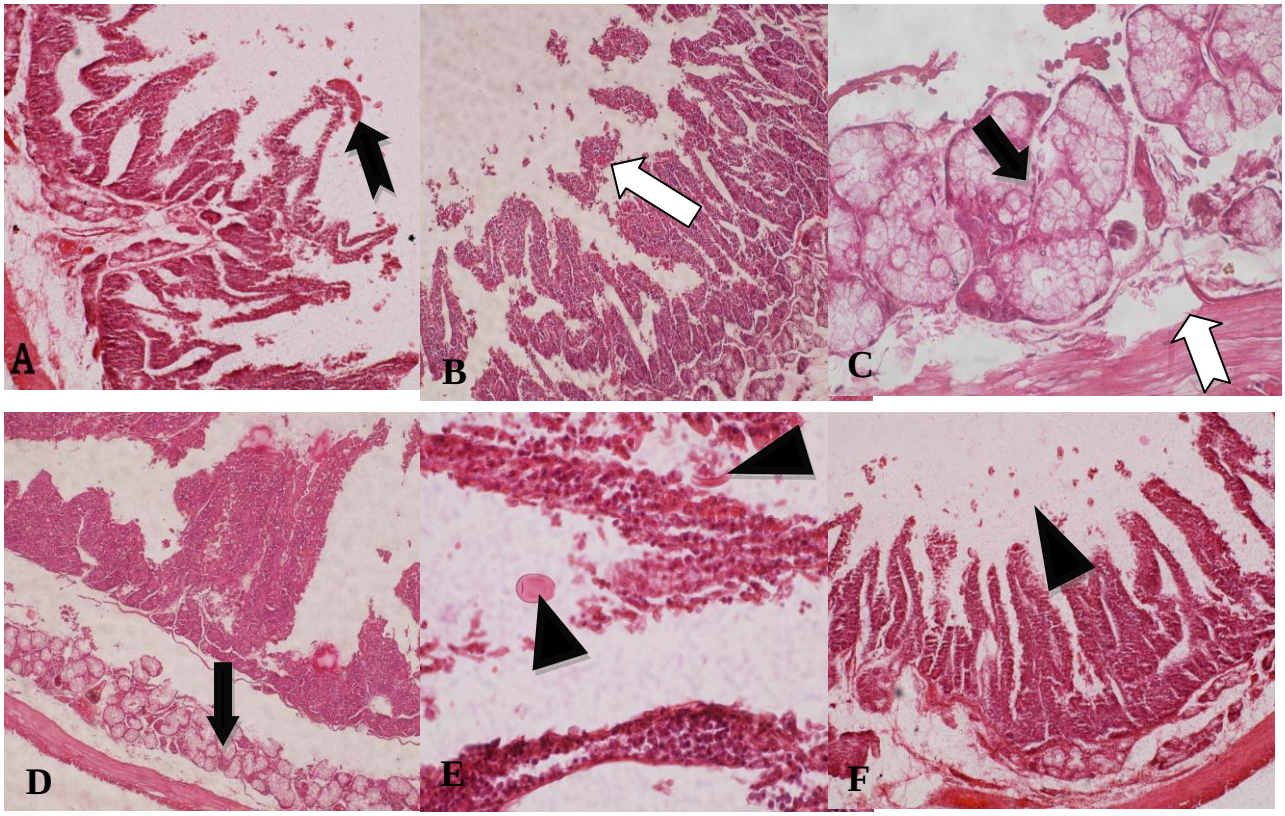


Figure 20. Microscopic characteristics of intestinal coccidian lesions after staining with Hematoxylin and Eosin. A, desquamated intestinal epithelium. B, disintegration of intestinal mucosa x400 (amprolium group). C, hyperplasia of goblet cells x630 (trim-sulphamethoxazole group). D, hyperplasia of goblet cells within the submucosa x40. E, oocysts within the intestinal lumen x400. F, several oocysts within the intestinal lumen x40

Table 16. Intestinal rabbit coccidiosis lesion scores in various treatment groups

Treatment group	Caeco-colon lesions			Duodenal lesion scores		
	Epithelial desquamation	Hyperplasia of goblet cells	Eimeria stages in intestinal tissue and lumen	Epithelial desquamation	Hyperplasia of goblet cells	Eimeria stages in intestinal tissue and lumen
Negative control (1A)	1.33±0.33[a]	2.67±0.88[a]	1.00±0.00a	2.33±0.67ab	3.67±0.33[a]	1.00±0.00[a]
Diclazuril (4B)	1.67±0.67[ab]	2.00±0.58[a]	1.00±.00[a]	1.67±0.33[a]	2.67±0.33[a]	1.00±0.00[a]
Sulphachloropyarazine (5E)	3.00±0.58abc	3.33±0.33[a]	3.00±0.58[b]	3.00±0.00[ab]	4.00±0.00[a]	3.33±0.33[b]
Amprolium (2B)	3.67±0.33[bc]	4.00±0.00[a]	3.67±0.33[b]	4.00±0.00[b]	3.67±0.33[a]	4.00±0.00[b]
Trimethoprim-sulphamethoxazole (6F)	4.00±0.00[c]	4.00±0.00[a]	4.00±0.00[b]	4.00±0.00[b]	4.00±0.00[a]	4.00±0.00[b]
Positive control (3C)	4.00±0.00[c]	4.00±0.00[a]	4.00±0.00[b]	3.67±0.33[b]	3.67±0.33[a]	4.00±0.00[b]
P value	0.001	0.035	<0.001	0.001	0.044	<0.001
Values within a column without a common superscript are significantly different at 0.05						

Table 17. Jejuno-ileal lesion scores in various treatment groups

Jejunum-ileum lesion scores			
Treatment group	**Epithelial desquamation**	**Hyperplasia of goblet cells**	***Eimeria* spp. developmental stages in intestinal tissue and lumen**
Negative control	3.0±0.00[a]	2.67±0.67[a]	1.00±0.00[a]
Diclazuril	2.67±0.33[a]	3.67±0.33[a]	1.00±0.00[a]
Sulphachloropyrazine	3.00±0.00[a]	3.00±0.00[a]	2.33±0.88[ab]
Amprolium	4.00±0.00[b]	4.00±0.00[a]	4.00±0.00[b]
Trimethoprim-sulphamethoxazole	4.00±0.00[b]	3.00±0.58[a]	4.00±0.00[b]
Positive control	4.00±0.00[b]	4.00±0.00[a]	4.00±0.00[b]
P-value	<0.001	0.119	<0.001

Values within a column without a common superscript are significantly different at 0.05

Hepatic lesions observed at histopathology included marked fibrosis and hyperplasia of the peribiliary/periductal parts (Fig. 31A & B) with mononuclear cell infiltration (Fig. 31E) and formation of new ductules around the ducts (Fig. 31D). There were severe distention of the bile duct accompanied by flattening and desquamation of their duct epithelium, hyperplasia of peribiliary with varied developmental stages of *Eimeria* (thin walled ovoid oocysts, macrogametocytes and microgametocytes) spp. Atrophy and necrotic degenerative changes of the hepatocytes and multiple coalescing lesions were observed in treatment groups 2B, 3C and 6F as shown in Figure 31. The enlarged bile ducts were lined by pronounced columnar epithelial cells that formed several papillary fronds which extended to the lumen of the duct (Fig. 32A). Observed less frequently were congested and dilated blood vessels, haemorrhages in the liver parenchyma, and precipitation of protein in duct lumen. There were areas with

mononuclear inflammatory cell infiltration, fibrosis and oocyst granulomas. The bile ducts lumen were filled and distended with almost mature *Eimeria* oocysts which resulted in pressure atrophy on neighbouring hepatocytes (Fig. 32E). These lesions were severe in groups treated with amprolium, trimethoprim-sulphamethoxazole and infected-untreated control group with only few seen in diclazuril and sulphachloropyrazine treatment groups but were absent in the negative control group (1A). Mean lesion scores are summarized in Table 18.

These results agrees with hepatic lesions described by Al-Naimi *et al.* (2012) who attributed hyperplasia of bile duct epithelium to the multiplication of *Eimeria* spp. parasites in the epithelium predilection site of the parasite. Studies have shown that disruption of the continuity of bile ductile epithelium often results in formation of occyst granuloma as the oocysts acts like foreign bodies (Al-Naimi *et al*., 2012; Sivajothi *et al.,* 2016). Other lesions observed in previous studies include deposition of bile pigment, obstructive jaundice in hepatic parenchyma and sinusoid dilatation (Mehmoud and Ibrahim, 1989; Sanyal and Shama, 1990; Singla *et al*., 2000).

Table 18. Hepatic coccidiosis lesion scores in various treatment groups

Treatment group	Hepatic Lesion Score					
	Epithelial desquamation	Duct distension	Eimeria stages in liver tissues	Hepatocyte necrosis	Periductal/ peribiliary fibrosis	Multiple coalescing lesions
Negative control	1.33±0.33[a]	1.67±0.33[a]	1.00±0.00[a]	1.00±0.00[a]	1.00±0.00	1.00±0.00[a]
Diclazuril	1.00±0.00[a]	1.33±0.33[a]	1.00±0.00[a]	1.00±0.00[a]	1.00±0.00	1.00±0.00[a]
Sulphachloropyrazine	3.33±0.67[b]	2.33±0.33[a]	3.67±0.33[b]	2.67±0.33[b]	2.00±0.00	1.67±0.33[a]
Amprolium	4.00±0.00[b]	4.00±0.00[b]	4.00±0.00[b]	4.00±0.00[c]	4.00±0.00	4.00±0.00[b]
Trimethoprim-sulphamethoxazole	4.00±0.00[b]	4.00±0.00[b]	4.00±0.00[b]	4.00±0.00[c]	4.00±0.00	4.00±0.00[b]
Positive control	4.00±0.00[b]	4.00±0.00[b]	4.00±0.00[b]	4.00±0.00[c]	4.00±0.00	3.67±0.33[b]
P-value	<0.001	<0.001	<0.001	<0.001	-	<0.001
Figures within a column without common superscript are significantly different at 0.05						

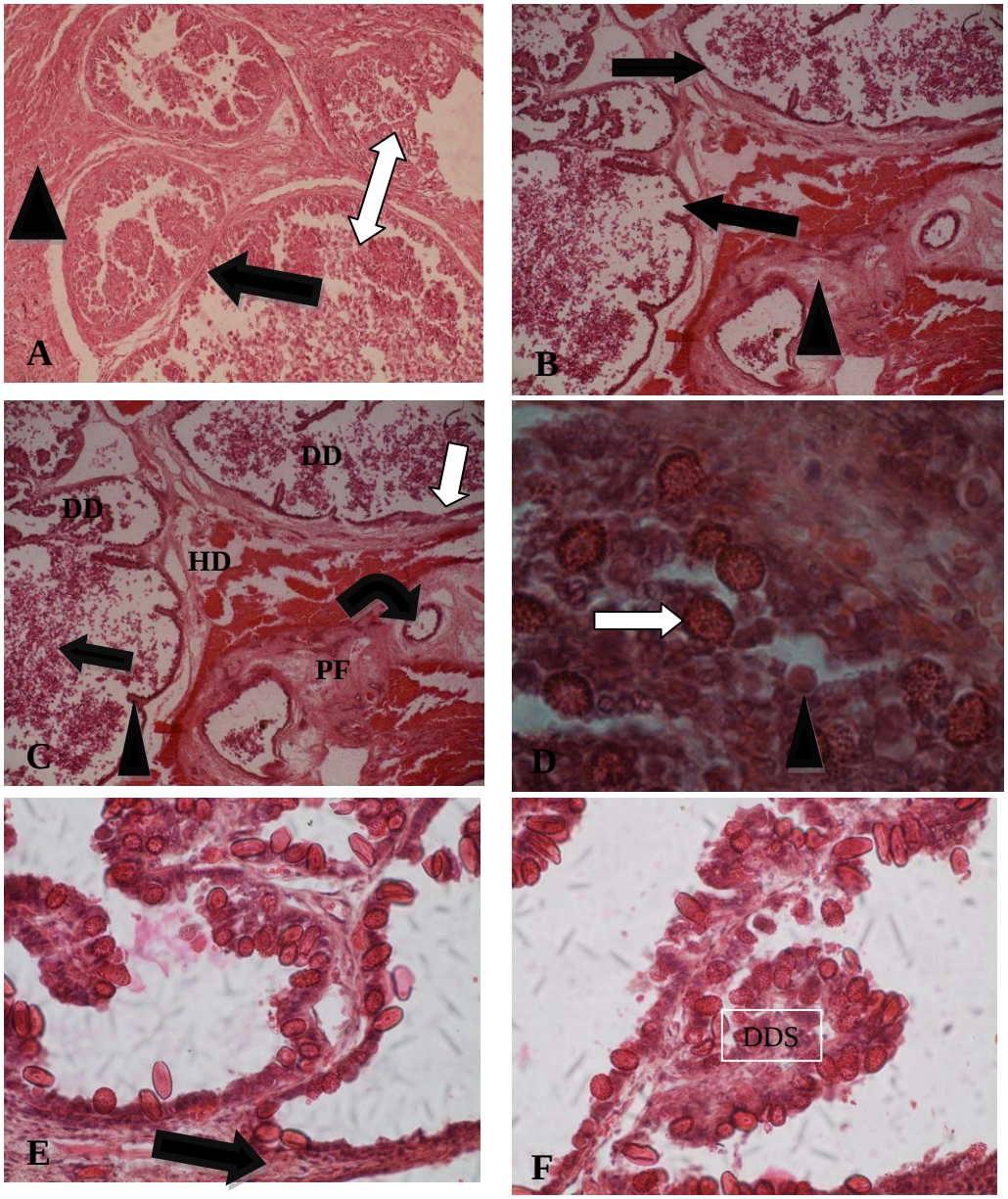


Figure 21. Microscopic characteristic of hepatic lesions stained with Hematoxylin and Eosin. A, distended bile duct containing numerous oocysts in the lumen (Double-headed white arrow), marked periductal/peribiliary fibrosis (Black arrow head) and desquamated duct epithelium (arrow) x40 from a rabbit treated with amprolium. B, Severe desquamation of duct epithelium (Arrow), and periductal fibrosis (arrow head) x100 in an infected-untreated rabbit. C, Distended ducts (DD) having flattened epithelium with minimal (arrow head) to no projections into the lumen (white arrow) filled with oocysts (black arrow), peribiliary fibrosis (PF), formation of new ductules (Bent arrow) and an area of hepatocyte necrosis and degeneration (HD) x100 in a rabbit from trim-sulphamethoxazole group. D, oval to circular large macrogametocytes within duct epithelium (White arrow), a round small microgametocyte (arrow head) at x1000 in a rabbit from amprolium treatment group. E, Different developmental stages of *Eimeria* within the duct epithelium with infiltration of inflammatory cells (Arrow) x400 in a rabbit from amprolium group. F, different developmental stages of *Eimeria* with varied shapes and sizes (DDS) x400 from trim-sulphamethoxazole treatment group.

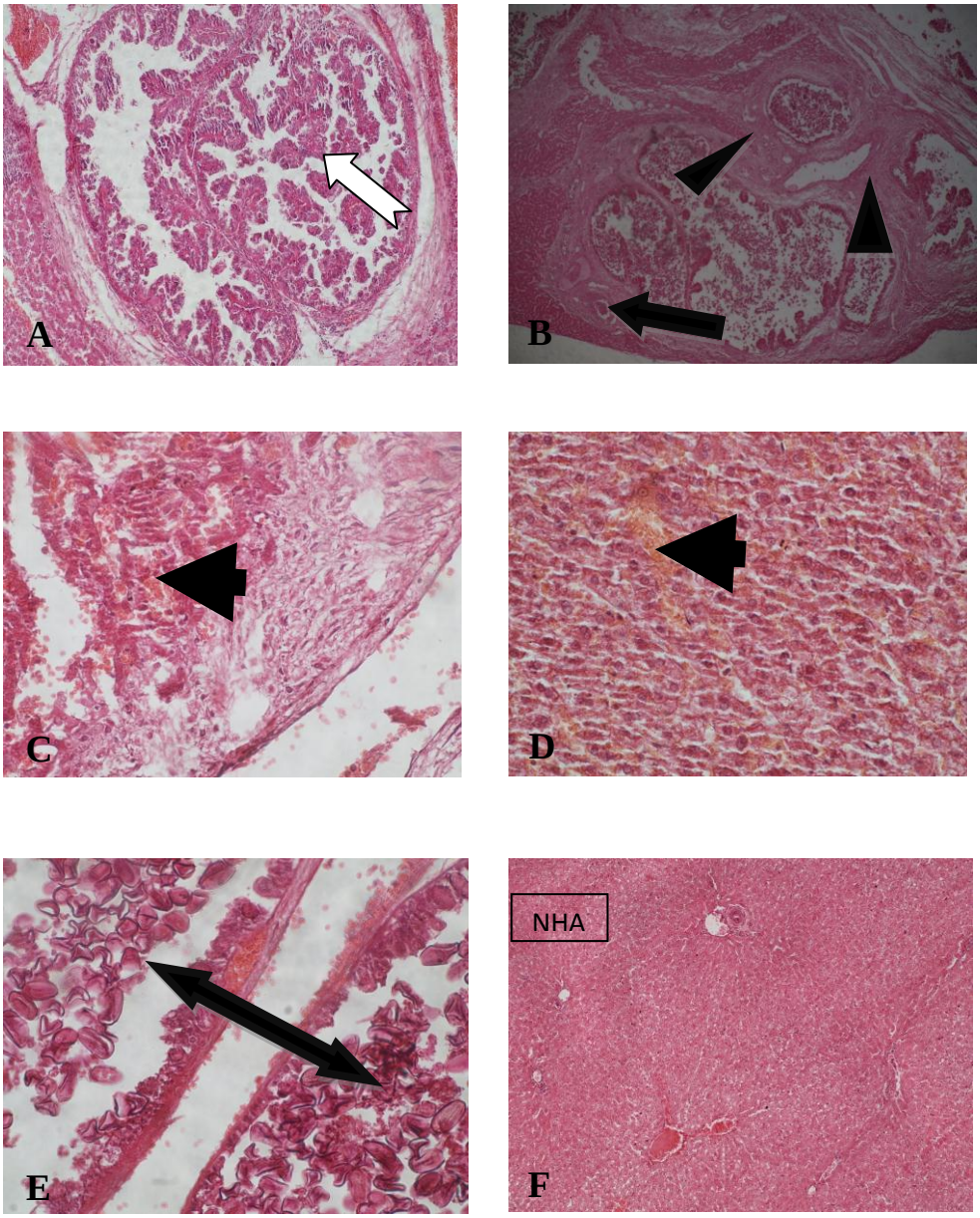


Figure 22. Microscopic hepatic lesions stained with Hematoxylin and Eosin. A, Numerous papillary branches in the bile duct arising from extensive proliferation of columnar epithelial cells of the biriary system x 40 (white arrow) in a rabbit from trim-sulphamethoxazole group. B, Congestion (arrow), peribiliary fibrosis (arrow heads) x100 in rabbit from sulphachloropyrazine group. C, Bile pigmentation x630 (black arrow) (trimethoprim-sulphamethoxazole group). D, Bile pigmentation within the hepatic parenchyma x400 (trim-sulphamethoxazole group). E, Numerous mature oocysts in the lumen of two ducts with desquamated epithelium (double-headed arrow) x400 (amprolium group). F, Liver with relatively normal hepatic architecture (NHA) from diclazuril treatment group x40.

4.3.11 Mortality and survival rates

The mortality and survival rates in six treatment groups are as shown in Table 19. Highest mortality rate (60%) directly attributable to coccidiosis was recorded in the amprolium treatment group and was slightly higher than that recorded by the positive control group (50%). The lowest mortality rates from coccidiosis were reported in the sulphachloropyrazine and diclazuril treatment groups both at 20%. The single mortality reported in the negative control group was confirmed through necropsy not to have resulted from coccidiosis.

Table 19. Mortality and survival rates in six treatment groups

Treatment group	Number at beginning	Number at end	Number dead	Percentage survival (%)
Negative control-1A	10	9	1	90
Amprolium hydrochloride-2B	10	4	6	40
Positive control-3C	10	5	5	50
Diclazuril-4D	10	8	2	80
Sulphachloropyrazine-5E	10	8	2	80
Trimethoprim sulphamethoxazole-6F	10	6	4	60

N/B Two rabbits in diclazril treatment group died on day 10 before initiation of treatment on day 11

4.3.12 Average weights and weight changes

Rabbits recruited for this study all had weights around 820g at start of the study. The highest significant ($p<0.05$) mean weight gain (38%) at the end of the experiment was seen in the negative control group. Diclazuril (17%) and suphachloropyrazine (12.35%) treatment groups, also recorded significantly ($p<0.05$) increased weight gains. Trimethoprim-sulphamethoxazole treatment group recorded the highest mean weight loss of -13.17% followed by amprolium and positive control groups at -3.7% and -1.21% respectively as shown in table 20. The mean weight in the six treatment groups were significantly different at $\alpha= 0.05$.

Table 20. Average weights and mean weight gains of rabbits under various treatments for coccidiosis

Treatment group	Weight at beginning	Weight at the end	Mean weight gain/loss	%weight gain
Negative control (1A)	830.00±17.00	1116.67±65.62[b]	315.00	38.0
Amprolium (2B)	815.00±28.92	825.00±32.27	-30.00	-3.70
Positive control (3C)	825.00±30.96	750±70.71[c]	-10.00	-1.21
Diclazuril (4D)	825.00±34.36	1031.25±44.26	140.00	17.0
Sulphachloropyrazine (5E)	810.00±31.45	931.25±72.54	100.00	12.35
Trimethoprim – sulphamethoxazole (6F)	835.00±31.67	741.67±37.45[ac]	-110.0	-13.17
SD	89.947	207.762	195.68	
p-value	>0.05	<0.05	<0.05	
Values within a column without common superscript are significantly different at 0.05				

4.4 Conclusions

- The controlled laboratory experimental trials demonstrated the superior efficacy of diclazuril in treating rabbit coccidiosis as it completely eliminated *Eimeria* spp. in all experimental rabbits

- Suphachloropyrazine showed a satisfactory efficacy against mixed *Eimeria* infections and recorded lesion scores, faecal scores and oocyst counts at levels approaching those of negative control group after treatment

- Trimethoprim-sulphamethoxazole combination recorded less than satisfactory efficacy against coccidiosis at recommended poultry reference dosages.

- Amprolium was not efficacious against intestinal and hepatic coccidiosis

CHAPTER FIVE

5.0 COMPARATIVE EFFICACY OF SELECTED DRUGS USED TO TREAT NATURAL RABBIT COCCIDIAL INFECTIONS AT FARM LEVEL

5.1 Introduction

Control of rabbit coccidiosis is still a major challenge in Kenya (Hungu *et al.*, 2013; Serem *et al.*, 2013). Huge economic losses are incurred by farmers arising from this disease (Mailu *et al.*, 2014). The problem is further compounded by lack of specific anticoccidial drugs for rabbits in Kenya. The few drugs used against rabbit coccidiosis in the country are labelled for poultry. As at now, no studies have been conducted to test the efficacy and safety of these poultry products in rabbits. The purpose of this field study was to validate results obtained in a controlled laboratory efficacy trial (chapter 4) in rabbits naturally infected with coccidiosis under field conditions. Sulphachloropyrazine, trimethoprim-sulphamethoxazole, amprolium hydrochloride and diclazuril were tested in a field trial.

5.2 Materials and methods

5.2.1 Selection of rabbit farms for field validation

Rabbit farms were randomly sampled in Kiambu County for the field validation study. A preliminary faecal sampling was taken in the farms to check for clinical coccidial infections. Farms that tested positive for coccidiosis and met the inclusion criteria were recruited for the study and rabbits randomly allocated to various treatment groups.

5.2.2 Inclusion criteria

Any rabbit that had ≥ 500,000 oocysts per gram of faeces or < 500,000 oocysts per gram of faeces but presenting with clinical signs of coccidiosis (diarrhea, in-appetence, dehydration, rough hair coat, mortalities in weaners) was recruited into the study.

5.2.3 Study design

A total of 10 farms with confirmed clinical cases of rabbit coccidiosis were recruited for the field study with a consent from farm owners. Rabbits were randomly allocated into four treatment groups: F1, F2, F3 and F4. Each treatment group had 90 rabbits with clinical coccidiosis. Each treatment group was further subdivided into 18 sub-treatment groups cumulatively clustered in cohorts of 5 rabbits, on a case by case basis as encountered in different farms, in order to mimic the varied environmental factors that occur in natural setting. This gave a total of 18 replications. Group F1 received diclazuril (diclosol 1%) at 10 ppm for 48 hours while group F2 was given sulphachloropyrazine at 2g per liter (2000 ppm) on days 1, 2, 3, 5, 7 and 9. Group F3 received trimethoprim/sulphamethoxazole combination at 1g per liter (1000 ppm) administered daily for 7 days and finally, group F4 was put under amprolium hydrochloride (20%) treatment at 1g per liter (1000 ppm) for 7 consecutive days. All the drugs were administered in drinking water and were changed every morning with freshly reconstituted solutions. Oocyst counts were pooled for each sub-treatment group and mean oocyst counts per treatment group determined after every two days up to day 20 post treatment. Throughout the experiment, rabbit farmers were involved in administration of treatments, monitoring progress and determination of recovered cases to mimic field situation.

5.2.4 Assessment of drug efficacies

Efficacy of drugs was based on reduction of faecal oocyst counts and reversal of clinical signs of coccidiosis.

5.2.5 Animal welfare

The animals in this study were handled humanely in accordance with the University of Nairobi Animal Welfare and Ethics Committee guidelines.

5.2.6 Data analysis

The data obtained was entered in MS excel 2016 spreadsheet and cleaned. Analysis of variance was performed by one and two way ANOVA using GenStat. Significant differences of the means of various treatment groups were illustrated by Bonferroni multiple comparison test to control overall significance levels as described in Genstat statistical analysis program (GenStat 15th Edition). The resulting data was presented as mean ± SEM and significance levels stated at $p \leq 0.05$.

5.3 RESULTS AND DISCUSSION

Table 21 summarizes the effects of respective treatments on oocyst shedding with time. In this field trial, diclazuril and sulphachloropyrazine were efficacious against coccidiosis as indicated by reduction in oocysts shed from 473.44±176.01 x 10^3 and 280.33±44.67 x 10^3 on day of treatment to 0.00±0.00 and 0.44±0.14 x 10^3 o.p.g, respectively by day 16 post treatment. Trimethoprim-sulphamethoxazole combination had moderate to satisfactory efficacy manifested by reduction in oocyst shed from 266.78±37.03 x 10^3 to 0.75±0.11 x 10^3. Amprolium hydrochloride was not able to control clinical coccidiosis in the field as shown in Table 21 and Figure 33.

Table 21. Oocyst counts from day of treatment to day 20 post treatment of rabbits

Treatment Group	Oocysts shed per treatment group x 10^3/gram of feces					
	1st day of treatment	Day 2 of treatment	Day 6 of treatment	D ay 10 after treatment	Day 16 after treatment	Day 20 after treatment
Diclazuril (F1)	473.44±176.01[a]	506.44±187.63[a]	1.13±0.73[a]	0.13±0.10[a]	0.04±0.03[a]	0.00±0.00[a]
Sulphachloropyrazine (F2)	280.33±44.67[a]	300.50±52.94[a]	15.54±3.96[a]	1.07±0.22[a]	0.59±0.14[a]	0.44±0.14[a]
Trimethoprim/ sulphamethoxazole (F3)	266.78±37.03[a]	235.72±31.68[a]	40.34±9.80[a]	1.36±0.31[a]	0.75±0.11[a]	0.91±0.11[a]
Amprolium (F4)	454.06±93.93[a]	513.50±115.82[a]	318.43±72.94[b]	188.31±45.86[b]	232.47±61.97[b]	258.92±70.15[b]
p-value	0.345	0.212	<0.001	<0.001	<0.001	<0.001
Values within a column with different superscript letters are significantly different at 0.05						

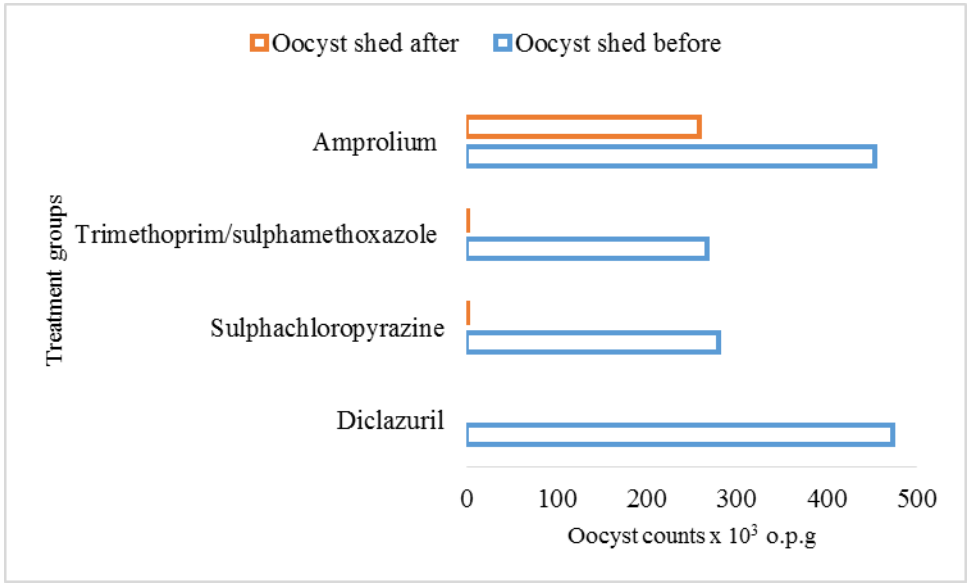


Figure 23. Bar graph showing the reduction in oocysts counts in the various coccidial infection test groups during the field trial

5.4 Conclusions

- Diclazuril recorded the highest efficacy as it completely eliminated *Eimeria* spp. in all infected rabbits

- Efficacy of suphachloropyrazine in the field trials was slightly better than during laboratory trials since oocyst counts after treatment were markedly lower with some sub-groups recording negative results for *Eimeria* spp.

- Trimethoprim-sulphamethoxazole efficacy was moderate to satisfactory in field validation trials at the recommended poultry dosages

- Amprolium was not effective against intestinal and hepatic coccidiosis in the field trials

CHAPTER SIX

6.0 GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

6.1 General discussions

In the first objective, a baseline survey was conducted on available coccidial control strategies in Kiambu and Nyeri counties, Kenya. This involved administration of two sets of questionnaires: one for farmers and another for agro-veterinary outlets. Faecal samples were also collected at this stage to assess the prevalence and intensity of coccidial infection in the two study counties. The purpose of the baseline survey was to establish anticoccidials used by farmers from which three most commonly used drugs were selected for laboratory (objective 2) and field (objective 3) efficacy trials, respectively. In order to successfully undertake the baseline survey, all sub-counties with established rabbit production were visited in the two counties which resulted in a total of 97 farmers and 27 agro-veterinary outlets being visited (Fig. 2). The reason for visiting the agro-veterinary outlets was to corroborate information the farmers gave as they acquired most of their medications from these outlets. This information provided a better perspective of rabbit coccidiosis control strategies used in the two counties. The baseline survey, however, failed to capture reasons that informed farmers' choice for various anticoccidial drugs that they used. My conjecture is that anticoccidials farmers settled for had more to do with availability and affordability of the drugs rather than their perceived effectiveness. An earlier study in the country reported that production costs influence most of the farmers' decisions and that they are likely to go for items that are less costly regardless of their value (Mailu *et al.,* 2014).

In the second objective, laboratory efficacy trials of selected anticoccidial drugs was undertaken. In this study, conventional rabbits were used as low levels of oocysts were

reported in all the treatment groups including negative control group before induction of experimental infections. The conventional rabbits were advantageous to this study as opposed to laboratory raised coccidia-free rabbits since they provided a better simulator of field coccidia pathogenesis. The low levels of oocyst counts in the negative control group provided a good indicator of the level of oocysts the rabbits can accommodate without coming down with clinical disease. The conventional rabbits also enabled assessment of inoculant dose likely to introduce a clinical case regardless of their natural immunity. Such low levels have been shown to be useful to rabbit health as they help the host to develop immunity against infective *Eimeria* spp. (Pakandl, 2009). Furthermore, continuous exposure to low levels of *Eimeria* spp. is the principle behind the development of *Eimeria* vaccines (Drouet-Viard *et al*., 1997). It was not possible to introduce a uniform infection in all experimental rabbits due to variations in body physiology including individual immunity levels. Nevertheless, such inherent variations were accounted for in four ways; one, rabbits were randomly allocated to various treatment groups before induction of artificial infection to eliminate any bias. Two, a uniform homogenous inoculant was used to introduce infection in all infected groups. Three, the criteria for determining clinical cases before commencement of treatment was uniform for all treatment groups, and lastly, judgement about response to treatments was based on a uniform criteria. This is in agreement with several other experimental studies (Percy and Barthold, 1993; Coudert *et al*., 2003; Kulišić, 2006; Vereecken *et al*., 2012). It is important to note that uniform infections are not possible even under natural conditions as we saw during the field trials. Efficacies of amprolium hydrochloride, trimethoprim-sulphamethoxazole and sulphachloropyrazine were all tested at the poultry reference dosages of 1000ppm, 1000ppm and 2000ppm, respectively. Only sulphachloropyrazine reported satisfactory efficacy against both intestinal and hepatic coccidiosis in the controlled experimental infections. It would have been better to conduct

titrations on trimethoprim-sulphamethoxazole and amprolium hydrochloride to assess if they can have better efficacies at higher levels, but such titrations were not conducted in this study. Further performance at such higher dosage levels above the manufacturer's recommendation should be matched with subsequent safety studies which was beyond the scope of this study. The laboratory study was terminated on day 20 post treatment (30 days post infection) at which point the test drugs should have controlled coccidial infection and reversed most of the associated lesions. This was based on the manufacturers' instructions on test drugs' labels and on studies by Pakandl (2009) and Vereecken *et al.* (2012). These workers reported that faecal oocyst counts, lesion and faecal scores in the positive control group begin to reduce to levels comparable to the negative control group after 15 days post infection following develoment of natural immunity. In this study, identification of various *eimeria* spp. at different stages of treatment as was reported by Vereecken *et al.* (2012) was not done even though this would have been useful in showing the gradual actions of the drugs on the different *Eimeria* spp. Instead, *Eimeria* spp. were identified at two stages in this study; at inoculation stage to quantify the percentages of the various *Eimeria* spp. in the inoculant and at termination of the experiment (day 20 post treatment). This still gave a good representation of action of drugs on *Eimeria* spp. in the amprolium, trimethoprim-sulphamethoxazole and sulphachloropyrazine treatment groups where there was no complete elimination of oocysts and the widespread effect of diclazuril in all *Eimeria* spp. causing complete elimination of oocycts at end of treatment (Appendix 8). Diclazuril and sulphachloropyrazine further recorded the lowest microscopic intestinal and hepatic lesion scores which demonstrated their efficacy.

Finally, in the field validation trial, a total of 7 farms were recruited in the study (GPS coordinates shown in Appendix 10). Inclusion criteria into the treatment groups was based on presentation of clinical signs associated with coccidiosis and oocyst counts. This is because

oocyst counts alone is not a good indicator of clinical disease since sheding depends on the area of the intestine affected. Infections targeting lower intestine like those caused by *E. coecicola* and *E. piriformis* are likely to shed more oocysts even though they are less pathogenic (Abbas, 2009; Pakandl, 2009). Diclazuril recorded similar results in natural infections and laboratory trial since it completely eliminated oocysts in all infected rabbits. Field performance of sulphachloropyazine was slighlty better than the laboratory results as oocysts were completely eliminated in some of the rabbits. No mortality was recorded in both diclazuril and sulphachloropyrazine treatment groups. Similarly, trimethoprim/sulphamethoxazole combination recorded moderate to satisfactory results in terms of oocyst reductions in natural infections than those of the controlled laboratory trials. The improved performance of sulphachloropyrazine and trimethoprim-sulphamethoxazole in the field may be attributed to the fact that most infections in the field were of intestinal coccidiosis and were comparatively less severe compared to the experimental infections. It is important to note that, the intestinal lesion scores were also comparatively less severe after treatment with test drugs during the laboratory trials. An inference can be made that these drugs are somewhat less efficacious against active hepatic coccidial infection. There was no noticeable difference between the laboratory and field performance of amprolium as its performace was less than satisfactory in both trials.

6.2 Conclusions

i. The farm prevalence of coccidiosis (79.4%) is high

ii. Major risk factors associated with the high prevalence of coccidiosis were poor housing structures, inefficient and irregular cleaning methods, and lack of technical knowledge on rabbit production

iii. Commonly used treatment options of coccidiosis in rabbits are Sulphachloropyrazine (22%), sulphamethoxazole-trimethoprim combination (15%)

iv. Of these drugs, sulphachloropyrazine was the most efficacious against coccciciosis in our experimental study followed by trimethoprim-sulphametoxazole while amprolium hydrochloride was not effective

v. The efficacy of diclazuril (standard drug) was superior relative to other test drugs

6.3 Recommendations

i. Training of farmers to adopt present findings to improve on best rabbit management practices (feeding, housing and breeding) that will promote rabbit production in Kenya

ii. Training and capacity building for field extension officers and veterinarians to adopt present findings in promoting good rabbit production prudent use of anticoccidials

iii. That in order of priority, trimethoprim-sulphamethoxazole should be the first line drug against natural coccidiosis and sulphachloropyrazine should only be used in cases where the former has not worked

iv. Develop policy brief from present results and share with the Directorate of Veterinary Services to consider registering other rabbit anticoccidial products like diclazuril which was superior in this study to supplement the few efficacious anti-coccidials in the country.

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8.0 Appendices

Appendix 1. Global positioning system coordinates of farms visited in Kiambu County during baseline survey to collect information on farmer practices and coccidiosis control strategies

Latitude	Longitude	Latitude2	Longitude2
36.833	-1.975	36.805	-1.162
36.804	-1.137	36.790	-1.155
36.779	-1.749	36.801	-1.162
36.777	-1.193	36.815	-1.155
36.751	-1.214	36.839	-1.930
36.759	-1.216	36.859	-1.100
36.750	-1.180	36.849	-1.775
36.692	-1.228	36.838	-1.768
36.656	-1.269	36.867	-1.103
36.674	-1.275	36.775	-1.183
36.671	-1.256	36.782	-1.192
36.656	-1.240	36.751	-1.213
37.378	-1.430	36.749	-1.211
37.775	-0.999	36.749	-1.212
37.108	-1.833	36.701	-1.223
36.839	-1.180	36.710	-1.227
36.829	-1.156	36.708	-1.231
36.819	-1.149	36.712	-1.212
36.812	-1.151	36.716	-1.214
36.828	-1.955	36.733	-1.219
36.813	-1.685	36.747	-1.224
36.827	-1.742	36.982	-0.944
36.764	-1.181	37.685	-1.073
36.802	-1.163	37.107	-1.606
37.103	-1.540		

Appendix 2. Global positioning system coordinates of farms visited in Nyeri County during baseline survey to collect information on farmer practices and coccidiosis control strategies

Latitude	Longitude	Latitude2	Longitude2
37.662	-0.513	36.987	-0.480
37.618	-0.512	36.989	-0.479
36.989	-0.495	36.904	-0.525
36.916	-0.570	36.909	-0.522
36.940	-0.538	37.087	-0.568
36.936	-0.516	37.099	-0.580
37.494	-0.556	37.115	-0.933
37.467	-0.568	37.128	-0.802
37.600	-0.561	36.929	-0.434
37.170	-0.514	37.156	-0.401
37.162	-0.515	37.668	-0.493
37.162	-0.512	37.678	-0.492
37.853	-0.354	37.841	-0.491
37.896	-0.352	37.384	-0.225
37.935	-0.351	37.592	-0.215
37.308	-0.859	37.592	-0.215
37.341	-0.765	37.188	-0.409
37.178	-0.243	37.160	-0.404
36.997	-0.455	37.160	-0.408
36.942	-0.429	36.892	-0.300
36.950	-0.424	36.899	-0.301
36.950	-0.335	36.314	-0.303
36.928	-0.339	36.891	-0.524
36.904	-0.331	36.995	-0.472

Appendix 3. Global positioning system coordinates of agro-veterinary outlets visited in Kiambu and Nyeri during baseline survey to collect information on coccidiosis control strategies

Latitude	Longitude	latitude	longitude
37˚5.777'	1˚4.391'	37˚05.695'	00˚26.873'
36˚49.733'	1˚9.38'	37˚01.372'	00˚10.188'
37˚8.295'	1˚9.086'	37˚07.595'	00˚28.973'
36˚51.230'	1˚5.617'	37˚09.358'	00˚24.039'
36˚46.713'	1˚3.376'	37˚10.365'	00˚22.029'
36˚45.339'	1˚10.503'	37˚09.364'	00˚24.034'
36˚48.295'	1˚9.086'	37˚09.365'	00˚24.029'
36˚46.309'	1˚11.312'	36˚56.975'	00˚32.930'
36˚42.780'	1˚13.174'	36˚56.929'	00˚32.934'
36˚39.846'	1˚14.668'	36˚59.064'	00˚28.933'
36˚45.339'	1˚10.503'	36˚58.408'	00˚29.044'
36˚59.935'	00˚27.554'	36˚58.485'	00˚29.034'
36˚54.283'	00˚19.662'	37˚05.610'	00˚21.009'
37˚05.610'	00˚21.015'		

Appendix 4. Farmers questionnaire used in baseline survey to assess the farmer practices and control strategies of rabbit coccidiosis

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Date of interview .....…… Tel. No. ..... Code .....

QUESTIONNAIRE FOR RABBIT FARMERS ON ASSESSMENT OF EFFICACY OF COMMONLY USED DRUGS IN THE CONTROL OF COCCIDIOSIS AND ECTOPARASITISM OF DOMESTIC RABBITS IN SMALLHOLDER PRODUCTION SYSTEMS IN KENYA

A) Background information

1) County ---------- Sub-county .....Ward ----------
---Village.....

2) GPS READING: Eastings……. Northings ……. Elevations -------
---

3) Acreage of the farm ……..

4) Note the type of farmers house (tick appropriately)

(1) Stone (2) Timber (3) Mud (4) Iron sheets (4) Others (specify)……..

A. Biodata of owner

1. Name of household head ……

2. Age of household head?

   [1] 21-30 years [2] 31-40 years [3] 41-50 years [4] > 50years

3. Gender of household head? [1] Male [2] Female

4. Main occupation of household head:

   (1) Farming (2) Business (3) Salaried employee (4) Other …… (Specify)

5. Education level of household head…….....

   [1] No formal education [2] Primary level [3] Secondary level [4] Tertiary level

5) Name of respondent: ……

6) Relationship of interviewee to household head.....

   (1) Owner (2) Spouse (3) Daughter (4) Son (5) Worker (6) Other Specify…….

7) Who is responsible for the day to day management decisions of the farm?

   1) Owner (2) Spouse (3) daughter (4) Son (5) Worker (6) Other Specify……

8) What is the education level of the person responsible for day to day management decisions?

(1) No formal education (2) Primary level (3) Secondary level (4) Tertiary level

A. Management

1. Number of rabbits kept currently ……..……

2. Age groups of rabbits kept currently……..(tick as appropriate)

Age	Kits (< 1 month)	Weaners (1-4months)	Bucks (males > 4months old)	Does (> 4 months old)

3. Breeds kept? (Tick appropriately)……..

Breed	NZW	CW	FG	CH	FLP	DU	ANG	Cross breeds	Others (specify)

4. How long have rabbits been kept on the farm?

[1] <6 months [2] 6 month-2yrs [3] >2yrs- 5yrs [4] Others specify…….....

5. What is the main reason for keeping rabbit? Tick one ……..**...**

[1] Business [2] Hobby [3] Food [4] Others specify……

6. Where did you source your first stock? .....

Source of first batch	Other farmers	Government	Research institutions	Imported	contractual agreement (specify group)	Gift /inheritance	Others (Specify)

7. What is the main source of your breeding stock in the farm? (Tick as appropriate)

Source of stock	Own stock	Other farmers	Government farms	Research institutions	Imported	contractual agreement (specify group)	Gift /inheritance	Others (Specify)

8. After how long do you change your breeding stock for BUCKS?

[1] After 1yrs [2] After 2 Yrs [3] After >5 Yrs

9. After how long do you change your breeding stock for DOES?

[1] After 1yr [2] After 2 Yrs [3] After >5 Yrs

10. What other animals/ livestock do you keep in the farm? (Tick appropriately)

Animals kept	Cattle	Sheep and goats	Chicken	cats	dogs	Others *specify*
No.						

11. How do you clean rabbit houses?

[1] Changing beddings only [2] Washing with water alone [3] Washing with water and disinfectant [4] Other (specify)……

12. How frequently do you clean rabbit houses?

[1] Daily [2] Once a Week [3] Every 2 weeks [4] Others (specify)…….

13. Rate the challenges you face as a rabbit farmer? Tick appropriately.

	Tick appropriately		
Challenges faced	**Major**	**Minor**	**Not**
[1] Marketing			
[2] Diseases			
[3] Availability of feed			
[4] Cost of feed			
[5] Availability of veterinary services			
[6] Availability of drugs			
[7] Cost of drugs			
[8] Breeding stock			
[9] Knowledge on husbandry			
[10] Others (Specify)			

14. Note the type of housing used in the farm and tick appropriately. How are the rabbits housed in the farm?

Housing type	**Housing system**			
	[1] No grouping	[2] Individual cages	[3] grouped by age	[4] grouped by sex
Indoor				
Outdoor				

15. Note the type of housing structures in the farm and tick appropriately the No. of tiers in each structure.

No. of housing tiers	Housing type	
	Indoor	Outdoor
[0] No tier		
[1] 1		
[2] 2		
[3] 3		
[4] 4		
[5] >4 specify		

16. Note the floor type in the farm and tick appropriately

[1] Wire mesh [2] wooden [3] Earthen [4] Others *please specify*

……

17. Observe the hygiene status of rabbit housing and *tick appropriately*

Hygiene	tick appropriately		
	A lot	**Less**	**Not present**
[1] Fecal matter in cage floor			
[2] Hatch odour			
[3] Presence of feed on cage floor			
[4] Presence of water on cage floor			
[5] Soiled rabbits			

B. Ecto-parasites, coccidiosis and mucoid enteropathy

18. Please note the breeds CURRENTLY KEPT by the farmer and then ask the following question(s) to fill in the table below as necessary. Has the breed ever shown the following clinical signs in the last six (6) months?

Yes = 1, No = 0 N/A=9

Symptom/breed	NZW □	CW □	FG □	CH □	FEL □	DU □	ANG □	Other Specify	Cross Specify
Scratching/loss of hair									
Wounds on the skin									
Crust /dandruffs									
Head tilting									
Presence of parasites on the skin (specify)									
Diarrhea/ mucus in feces									
Distended abdomen									
Sudden death									
Lack of appetite									
Others specify									

19. Which age groups are frequently affected by symptoms below?

Symptoms	**Kits**	**Weaners**	**Adults**
Scratching/loss of hair			
Wounds on the skin			
Loss of weight			
Head tilting			
Presence of parasites on the skin (specify)			
Diarrhea/mucus in feces			
Distended abdomen			
Sudden death			
Lack of appetite			
Other specify			

20. What do you do when your rabbits are sick? Tick as appropriate

[1] Call a vet/ paravet [2] Self-treat [3] Do nothing [4] Advice from others [5] Others specify……

21. If self-treat, what do you use? Indicate the trade name if possible.

Symptoms	Antibiotics	Acaricides/ insectides	Mineral oil/liquid paraffin	Withdraw/change feed	Multi vit	herbs	Injection	Other (specify)
Scratching/ loss of hair								
Wounds on the skin								
Loss of weight								
Head tilting								
Presence of parasites on the skin								
Diarrhea/mucus in feces								
Distended abdomen								
Sudden death								
Lack of appetite								
Other specify								

22. How frequently do you apply the following to treat external parasites?

Method of control	**Frequency of application**					
	Once	Daily	Weekly	Every two weeks	Monthly	Others (specify)
Acaricide / Insect ides (specify)						
Mineral oils (specify)						
Liquid paraffin						
Injection						
Others (specify)						

23. How frequently do you apply the following to prevent external parasites in the farm?

Method of control	**Frequency of application**					
	Once	Daily	Weekly	Every two weeks	Monthly	Others (specify)
Acaricide / Insect ides (specify)						
Paraffin and oils (specify)						
Mineral oils						
Injection						
Controlling rodents						
Others (specify)						

24. Who informs you on how to apply the following regimes to treat and control external parasites? Do the regimes work?

Regimes	**Source of information** [1] Manufacturer's Instructions [2] Vet Advice [3] Advice from Agrovet [4] Advice from other farmers [5] Own experience) (Insert the appropriate code)	**Do the regimes work?** [1]Yes [2] No [3] Not always (Insert the appropriate code)	
		Treatment	**Control**
Acaricide / Insecticides (specify)			
Paraffin and oils (specify)			
Mineral oils			
Injection			
Controlling rodents			
Others (specify)			
Paraffin and oils (specify)			

25. For the regimes that do not work, what do you do?

[1] Increase the dose [2] Increase frequency of application [3] Dilute [4] Others *Specify* ……..

E) Coccidiosis and mucoid enteropathy

26. What type of rabbit feed do you use? .....

(1) Commercial (2) Forage Only (3) Both (4) Others

27. What commercial rabbit feeds do you use?

Commercial feed	**Tick appropriately**
[1] Unga	
[2] Pembe	
[3] Isinya	
[4] Naku modern	
[5] Sigma	
[6] Pwani	
[7] Royal	
[8] Belfas	
[9] Don't know	
[10] Other (specify)	

28. Have you associated any of these symptoms with the following feeds? Tick appropriately

FEED	Unga	Pembe	Isinya	Nak.	Sigma	Pwani	Royal	Belfas	Forages	Other
[1] Diarrhea										
[2] Mucus in feces										
[3] Bloating										
[4] Sudden death										
[5] Lack of appetite										
[6] Stunting										
[7]Other specify										

29. Of clinical signs identified in (**28)** above, do you associate them with the following feeding practices. Tick appropriately

Clinical signs	Sudden change in diet	Overfeeding	Poor quality feed	Fresh forages (not wilted)	Others specify
[1] Diarrhea					
[2]Mucus in feces					
[3] Bloating					
[4]Sudden death					
[5] Lack of appetite					
[6] Stunting					
[7] Other specify					

30. Which forages do you feed the rabbits? How do you feed them? Tick as appropriate

Type of Forage	**Fresh**	**Wilted**
[1] Kales		
[2] Cabbages		
[3] Weeds		
[4] Carrots		
[5] Corn stalks		
[6] Grass		
[7] Hay		
[8]Sweet potato vines		
[9] Other specify		

31. Do you associate commercial feeds and or forages with occurrence of any of the following symptoms in the age groups of rabbits shown below? tick appropriately

AGE	kits<4 wks	Weaners> 4wks	Growers >12wks	Pregnant doe	Lactating doe	Other specify
[1] Diarrhea						
[2] Mucus in feces						
[3] Bloating						
[4] Sudden death						
[5] Lack of appetite						
[6] Stunting						
[7] Other(specify)						

Which of the following do you use to prevent the listed *clinical signs*? Tick as appropriate against all applicable methods. If possible give trade names.

PRACTICES	Antibiotics	Multi vitamins	Herbs	Not changing feed	Vet visits	Nothing	Others
[1] Diarrhea							
[2] Mucus in feces							
[3] Bloating							
[4]Sudden death							
[5] Lack of appetite							
[6] Stunting							
[7]Other(specify)							

32. Rate the importance of the following animal management methods in rabbit production?

Management practice	**Tick appropriately**		
	Very important	**Moderately important**	**Not important**
[1] Proper selection of breeding stock			
[2] Timely feeding			
[3] Quality feeds			
[4] Providing kindling nest boxes			
[5] Timely breeding			
[6] Separating sexes at time of weaning			
[7] Removing the doe to another cage when weaning instead of kits			
[8] Good housing/cages			
[9] Provision of clean water with clean watering equipment			
[10] Using clean feeders raised above the floor (crocks or cans)			

D) Identification of actual value chain actors

33. Are you organized in groups with other farmers? 1) Yes___ 2) No___.

34. If the answer is yes, what are the main reason for being grouped

Reasons		Tick appropriately		
		Very important	Moderately important	Not important
1	For marketing			
2	For trainings on rabbit keeping			
3	For finance			
4	Social benefits e.g., welfare			

35. In relation to rabbit keeping and marketing, which information do the following resources provide you with? Tick all that apply

	Feeding	Breeding	Housing	Disease management	Rabbit meat products	Marketing:	Other information specify
County extension officers							
NGOs/private agents							
Internet							
Radio							
TV							
Newspaper							
Mobile phone							
Agricultural shows/field days							
Neighbors and family							
Banks/credit institutions							
Rabbit farmers association							

36. Which rabbit products do you sell? At how much? Indicate the cost

Product	Tick appropriately	Cost per Unit	Unit
Adult rabbits			
Young rabbits: kits/ weaners			
Rabbit meat			
Rabbit urine			
Rabbit manure			
Rabbit fur			
Rabbit skin			
Other product e.g., sausages, samosas, cooked meat			

37. Indicate the challenges that should be addressed in the rabbit value chain with regard to the following.

Production	**Processing**	**Marketing**
1.	1.	1.
2.	2.	2.
3.	3.	3.
4.	4.	4.

38. Do you have a production/market contract with any organization/ farmer? 1) Yes___ 2) No___.

39. If yes, name the organization/s you have contracts with ……..., ……., ……..., ……..,…… ……..

40. What costs do you incur on commercial feeds for your rabbits?

Item	**Amount of feed given/per day/per animal**	**Cost of feed per Kg**
Commercial feed given to weaners		
Commercial feed given to adult rabbits		

41. Which other feeds do you use (e.g. weeds, grass, hay, kitchen waste, any other)

	Do you buy/pay for the feed product? Yes or No	**At what cost per week**
Weeds		
Grass		
Hay		
Kitchen waste		
Any other		

42. In the table below give the age and weight that your rabbits are ready for market?

Product	**Market weight in kg**	**Age in months**
Rabbit for meat		
Weaners		
Breeding does		
Breeding bucks		

43. What other cost do you incur under the following categories?

Cost items	**Specify where appropriate**	**Approximate cost**	**How frequently paid**
Feed supplements			
Labour			
Paid extension			
Training cost			
Marketing			
Medication			
Others ….specify			

.....Thank You.....

Appendix 5. Questionnaire administered to agro-veterinary outlets during the survey

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COLLEGE OF AGRICULTURE AND VETERINARY SCIENCE

Date of interview ……. Telephone No.. .. ..… Code …….

QUESTIONNAIRE TO AGROVET OWNERS ON ASSESSMENT OF THE EFFICACY OF COMMONLY USED DRUGS IN THE CONTROL OF COCCIDIOSIS AND ECTOPARASITISM OF DOMESTIC RABBITS IN SMALLHOLDER PRODUCTION SYSTEMS IN KENYA

NOTE: Information given will be treated with full confidentiality

C. Background information

9) County ---------- Sub-county .....Ward ----------

Village..... Shopping center ……..

10) GPS READING: Eastings…….Northings ……. Elevations -------

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11) Name of Agrovet ……

12) Main occupation of the owner of Agrovet:

[1] Farming [2] Business [3] Salaried employee [4] Other …… (specify)

13) Are you the owner of Agrovet? Yes [1] No [2]

14) Education level of the owner of Agrovet …….....

[1] No formal education [2] Primary level [3] Secondary level [4] Tertiary level

15) Age of the owner of Agrovet?

[1] 18-30 years [2] 31-40 years [3] 41-50 years [4] > 50years

16) Gender of owner of Agrovet? [1] Male [2] Female

17) Name of respondent: ……

18) What is the education level of respondent?

[1] No formal education [2] Primary level [3] Secondary level [4] Tertiary level

19) Gender of the respondent..... Male [ ] Female [ ]

20) Has the respondent attended training in animal health? [1] Yes [2] No

21) If yes, what is the highest level attained? .....

[1] Certificate [2] Diploma [3] Higher diploma [4] Degree

[5] Postgraduate

22) How long ago did you attain the level of training above? ……..

[1] <1 year [2] 1-5 Years [3]6-10 years [4] > 10years

23) How long have you worked in the Agrovet?

[1] <6 months [2] 6 month-2yrs [3] >2yrs- 5yrs [4] >6 years

B. Information on drugs

24) What are the sources of your veterinary drugs and feeds? (Tick as appropriate)

Source of stock	Direct import	Wholesalers/Distributors	Government institutions	Research institutions	Others (Specify)
Drugs					
Feeds					

25) Do you stock specific drugs for treatment of rabbit diseases?

[1] Yes [2] No

26) How frequently do you get clients enquiring about drugs for treatment of rabbits?

[1] Never [2] Every week [3] Every month [4] Every 6 months

[5] Every year

27) What do you do when a client reports a case of sick rabbits? Tick as appropriate

[1] Call a Vet/ Paravet [2] Prescribe a drug [3] Visit the farm and treat

[4] Others …… (specify)

28) Rate the frequency that clients have reported any of the following symptoms in rabbits in the last six (6) months? (Tick as appropriately).

Symptoms	**Very often**	**Less often**	**Never**
Scratching			
Loss of hair			
Wounds on the skin			
Loss of weight			
Head tilting			
Presence of parasites on the skin			
Diarrhea /mucus in feces			
Distended abdomen			
Sudden death			
Lack of appetite			
Other specify			

29) From the options below, what would you advise your clients to use in treatment of the following clinical signs? Give trade names where applicable.

Clinical signs	Antibiotics	Acaricides / Insecticides	Mineral oil	Withdraw/change feed	Multivit	Injection	Other(specify)
Scratching							
Loss of hair							
Wounds on the skin							
Diarrhea /mucus in feces							
Head tilting							
Presence of parasites							

on the skin							
Diarrhea							
Distended abdomen							
Sudden death							
Lack of appetite							
Mucus in feces							
Loss of weight							
Other specify							

30) What options do you have for control of external parasites in rabbit farms? Give trade names if possible?

Method of control	**Trade names**
Acaricide / Insecticides (specify)	
Mineral oils (specify)	
Injection	
Others (specify)	

31) How frequently do you advise farmers to apply control methods named below?

Method of control	**Frequency of application**					
	Once	Daily	Weekly	Every two weeks	Monthly	Others (specify)
Acaricide / Insecticides (specify)						
Mineral oils (specify)						
Injection						
Others (specify)						

32) What informs you to advise farmers on regimes used for treatment and control? Do the regimes work?

(Insert the appropriate code)

Regimes	**Source of information** [1]Manufacturer's Instructions [2] Vet Advice [3] Advice from other agrovet [4]Feedback from farmers [5] Own experience	**Do regimes work?** [1] Yes [2] No [3] Not always	
		Treatment	**Control**
Acaricide / insecticides (specify)			
Paraffin and oils (specify)			
Mineral oils			
Injection			
Controlling rodents			
Others (specify)			
Paraffin and oils (specify)			

33) If no what do you advice the farmers?

[1] Increase dose [2] Increase frequency of application [3] Others specify

34) Which options do you have to prevent occurrence of the following sickness/symptoms? Give trade names as appropriate

PRACTICES	Antibiotics	Multi vit.	Herbs	Do not change feed	Vet visits	Nothing	Others
[1]Diarrhea/mucus in feces							
[2] Bloating							
[3] Sudden death							
[4]Lack of appetite							
[5] Stunting							
[6]Other(specify)							

35) Over the last 6 months, what commercial rabbit feeds have you stocked in your Agrovet? Tick appropriately

Unga	Pembe	Isinya	Nak.	Sigma	Pwani	Royal	Belfas	Hay	Others (specify)

36) Has your client associated commercial feed with any of the following clinical signs?

Tick appropriately (If YES)

Clinical signs	**Unga**	**Pembe**	**Isinya**	**Nak**	**Sigma**	**Pwani**	**Royal**	**Belfas**	**Hay**	**Other**
[1] Diarrhea										
[2] Mucoid feces										
[3] Bloating										
[4] Sudden death										
[5] In appetence										
[6] Unthriftiness										

.....**Thank You.....**

Appendix 6. Observation data sheet used in baseline survey

OBSERVATION DATA SHEET

Examine rabbits from at least ten percent of the hutches and record the clinical observations encountered in rabbit farm

Name of household..... Farm questionnaire number.....

County ---------- Sub-county .....Ward ---------- Village.....

GPS READING: Eastings…….Northings ……. Elevations ----------

Rabbit health

Number of rabbits examined

Observations	Remarks (insert numbers affected as appropriate)
Body condition score	Good [ ] Fair [ ] Poor [ ]
Demeanor	Active [ ] Dull [ ]
Movement	Dragging [ ] Paralyzed [ ]
Posture	Tilting of head [ ] Sore hocks [ ]
Dental status	Long incisors [ ] Normal length incisors [ ]
Body surface	Swelling [ ] Nodules [ ] Abscess [ ] Erosions [ ]
Fur coat	Rough [ ] Smooth [ ] Alopecic [ ]
Presence of parasites	Ticks [ ] Mites [ ] Lice [ ] Fleas [ ]

Itching/scratching	Present [ ] Absent [ ]
Ear scabs	Present [ ] Absent [ ]
Skin crusts/scabs	Present [ ] Absent [ ]
Eye discharges	Present [ ] Absent [ ]
Diarrhea/soiled perineum	Yes [ ] No [ ]
Mucoid feces	Yes [ ] No [ ]
Stunting	Yes [ ] No [ ]
Distended abdomen/bloat	Yes [ ] No [ ]

Hygiene and environment

Hygiene	**Remarks (tick appropriately)**		
	Plenty	**Less**	**Not present**
[1] Fecal matter in cage floor			
[2] Hatch odour			
[3] Presence of feed on cage floor			
[4] Presence of water on cage floor			
[5] Soiled rabbits			

Housing	Remarks (insert numbers as appropriate)
Housing type	Indoor [ ] Outdoor [ ]
Cage floor	Wire mesh [ ] Wood [ ] Ground [ ]
Cage density	Crowded [ ] Not crowded [ ]
Housing structure	New [ ] Old [ ] Poorly maintained [ ]
Housing hygiene	good [ ] poor [ ]

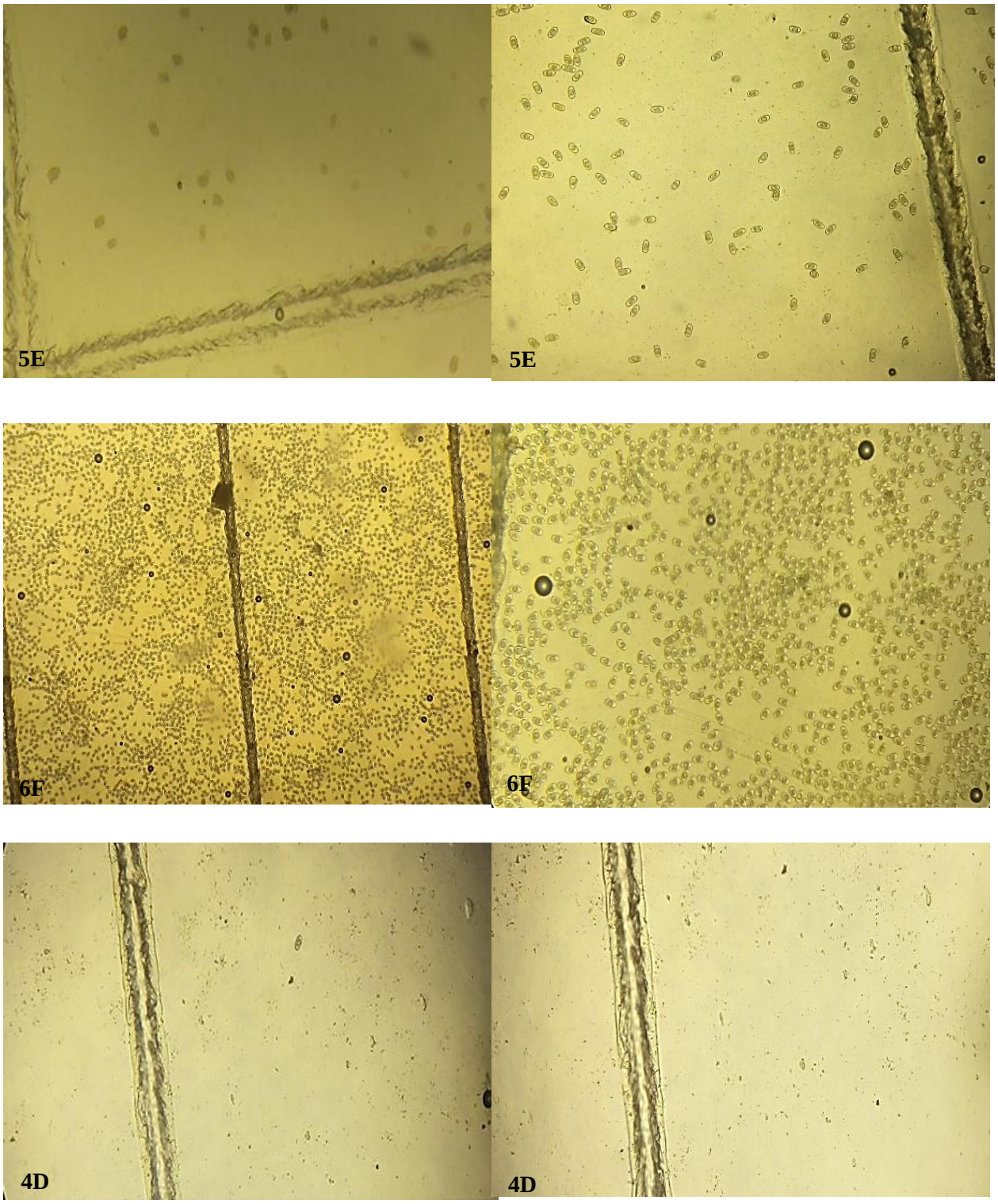
Breeds kept in the farm (observe/ask and insert numbers)

Breed	NZW	CW	FG	CH	FLP	DU	ANG	Cross breeds	Others (specify)
No.									

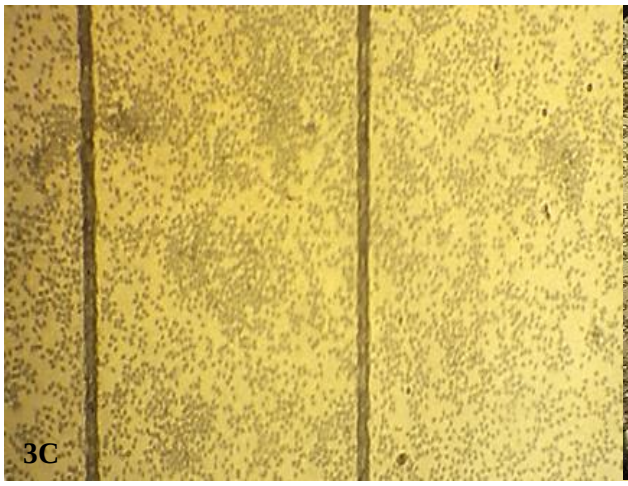
Clinical cases and sample collection

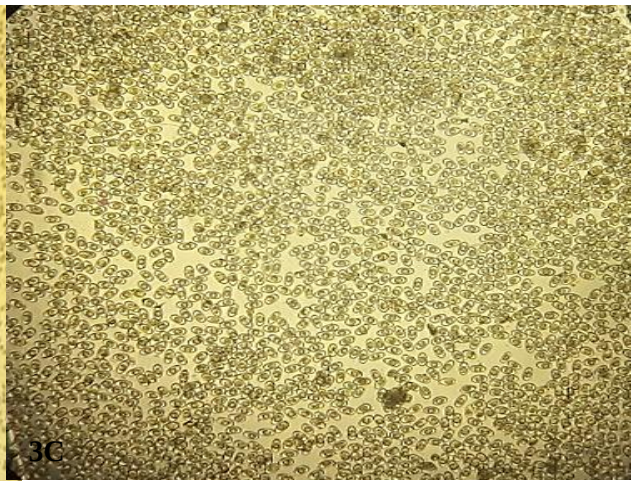
Characteristics	**Faecal samples**			**Skin scrapping/ear scabs/ parasites**		
Age	Weaners cages	Adults cages	Mixed cages	Weaners cages	Adults cages	Mixed cages
Sex						
Breed						
Sample 1						
2						
3						
4						
5						
6						
7						
8						
9						
10						
Clinical signs						
Treatment given						
Confirmed diagnosis (to be filled in the lab)						

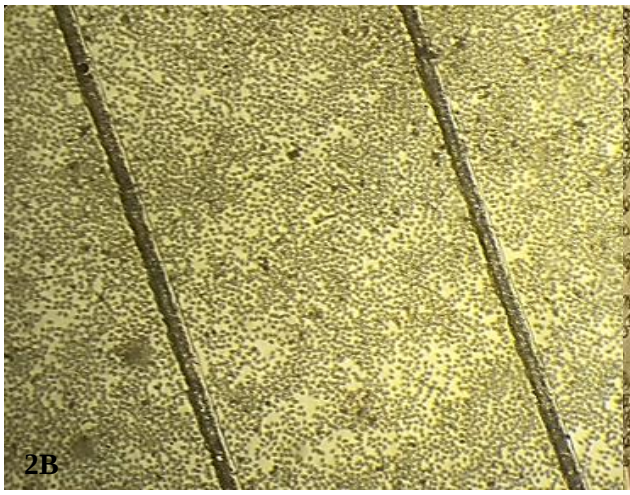
Appendix 7. Oocysts shed by various treatment groups on day 20 post treatment sulphachloropyrazine (5E), trimethoprim-sulphamethoxazole (6F) and diclazuril (4D) treatment groups on day 20 post-treatment in the laboratory trial

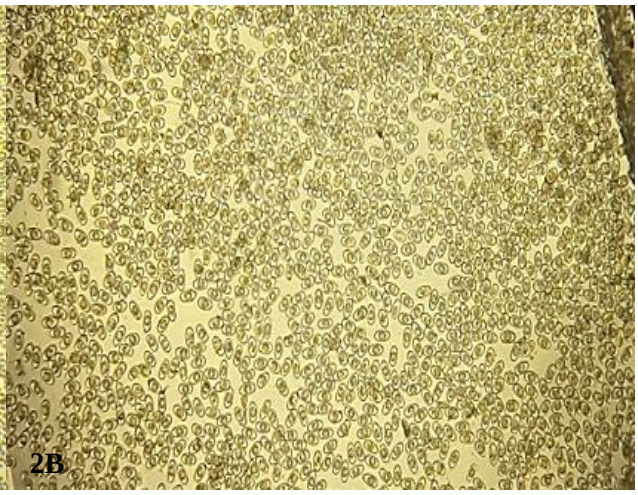


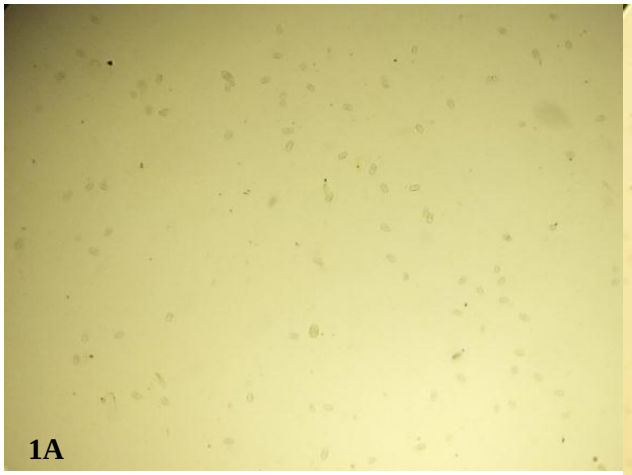
Appendix 8. Oocysts shed by infected untreated (3C), amprolium (2B) and negative control groups on day 20 post-treatment during laboratory trial

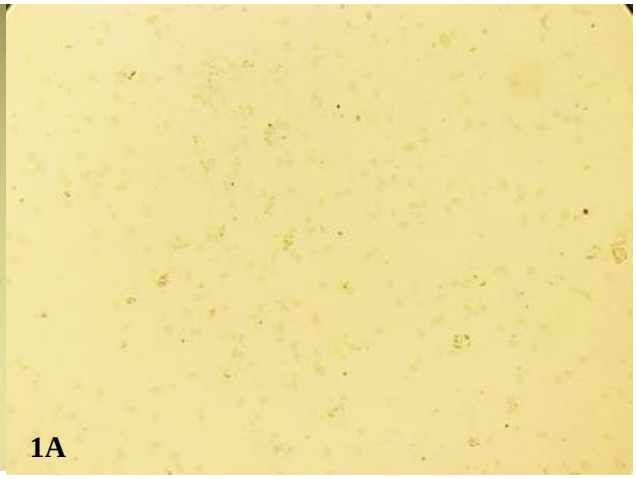












Appendix 9. Percentage reduction in oocyst counts after treatment for 3 weeks using the test drugs

Groups	Oocyst shed before treatment	Oocyst shed after treatment	Reduction	% reduction
Negative control 1A	591.6667	1316.5	-724.8333	-123%
Amprolium 2B	190,100	4,300,000	-4,109,900	-2162%
Positive control 3C	349,333	7,375,000	-7,025,667	-2011%
Diclazuril 4D	597,000	17	596,983	99.9%
Sulphachloropyrazine 5E	1,490,000	33,100	1,456,900	98%
Trimethoprim/ sulphamethoxazole 6F	1,971,667	2,316,667	-345,000	-17%

Appendix 10. Global positioning system coordinates of farms visited during the field trials in Kiambu, Karen and Ngong' areas

FARMER'S NAME	NO. OF RABBITS RECRUITED	LOCATION	LATITUDE	LONGITUDE	ELEVATION
LEAH KIMANI	45	Karen	36.716	1.321	1861
MR. NJERI	20	Ngong'	36.674	1.371	1852
ST. JOSEPH KAFASSO	90	Kamiti	36.891	1.176	1589
MRS. CHARITY IRUNGU	40	Kamiti	36.898	1.177	1580
MR. GEORGE OCHIENG'	50	Kamiti	36.897	1.176	1580
MWANGAZA INSTITUTE	25	Mwangaza	36.722	1.366	1870
NGONG' BREEDING CENTRE	90	Ngong'	36.769	1.543	1600

Appendix 11. Lesion scoring criteria used and specific liver and intestinal lesions scored in experimental efficacy trial

Grade/ Score	**Grade description**	**Histological change**	**Focal, multifocal**			**diffusely distributed lesions**	
			various *Eimeria* stages in lamina propia and enterocyte	**various *Eimeria* stages in liver ducts**	**Multifocal lesions coalescing in liver architecture**	**Desquamated epithelium/ enterocytes**	**Desquamated duct epithelium**
1	Minimal	Minor, small and infrequent lesions	<10% of tissue involved	<10% of tissue involved	<10% of the tissue is involved	<10% of tissue is involved	<10% of tissue is involved
2	Mild	Noticeable lesion but not a prominent feature of the tissue	11-20% of the tissue involved	11-20% of the tissue involved	11-20% of tissue involved	11-20% of tissue involved	11-20% of tissue involved
3	Moderate	Lesion is a prominent feature of the tissue	21-40% of tissue section involved	21-40% of tissue section involved	21-40% of tissue section involved	21-40% of tissue section involved	21-40% of tissue section involved
4	Marked	Lesion is an overwhelming feature of the tissue	41-100% of tissue section involved	41-100% of tissue section involved	41-100% of tissue section involved	41-40% of tissue section involved	41-40% of tissue section involved

Appendix 12. Lesion scoring criteria used and specific liver lesions scored in experimental efficacy trial continued

Grade/ Score	Grade description	Hyperplastic			Hepatocyte necrosis
		Hyperplasia of goblet cells in submucosa	Peribiliary/ Periductal fibrosis	Distension of duct lumen	
Grade 1	Minimal	<10% increase in volume	<10% of peribiliary fibrotic	<10% increase in diameter	<10% atrophy of hepatocytes
Grade 2	Mild	11-20% increase in volume	11-20% of peribiliary fibrotic	11-20% increase in diameter	11-20% atrophy of hepatocytes
Grade 3	Moderate	21-40% increase in volume	21-40% of peribiliary fibrotic	21-40% increase in diameter	21-40% atrophy of hepatocytes
Grade 4	Marked	41-100% increase in volume	41-100% of peribiliary fibrotic	41-100% increase in diameter	41-100% atrophy of hepatocytes