A STUDY OF FACTORS ASSOCIATED WITH THE PREVALENCE OF COCCIDIA INFECTION IN CATTLE AND ITS SPATIAL EPIDEMIOLOGY IN BUSIA, BUNGOMA AND SIAYA COUNTIES, KENYA.

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DECLARATION

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

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ABSTRACT

Coccidiosis is a protozoan infection that affects most domestic species: canine, feline, equine, porcine, **bovine,** ovine, lagomorphs and avian. The overall objective of this study was to determine the prevalence and spatial distribution of coccidia infection in cattle, and associated factors in different production systems in Busia, Bungoma and Siaya Counties Kenya. Specific objectives were to determine the prevalence and spatial distribution of coccidia infection in cattle as well as assess the factors associated with the infection in these Counties Kenya.

The three Counties covered by the study were purposively selected for this study on prevalence of coccidia infection in cattle and associated factors in 'unorganised' production systems given that studies done in Kenya have concentrated on zero grazing and dairy production units. Also, being that this was part of a larger project in these areas (People Animals and their Zoonoses –PAZ); the three Counties were logistically favourable. The non-animal factors considered included: season, rainfall, housing, geographical location hygiene, nutrition, activity of the animals, and keeping of various species and veterinary attention. Animal related factors considered were: breed, age, gender, and physiological status (pregnant/lactating), body condition, and other infections.

Households were then selected randomly using ArcMap 2.0 software to generate random points (and a back-up for each random point) within the study area. From the random point, the closest homestead within 300 m was selected for sampling. Spatial distribution of coccidian infection in these Counties was done using QGIS 2.0.1 software for mapping.

Data on nutrition, seasons, housing, disease occurrence, veterinary attention, herd profile and use of the animals were obtained using questionnaire interviews. From the clinical examination, physiological status (lactating, pregnant), concurrent infections and presence of

other endoparasites and ectoparasites were ruled in or out. Faecal samples (at least 5 gm) were collected directly from the rectum via digital extraction using lubricated gloves. Samples were analysed using McMaster and Kato Katz techniques. R Statistical software was used for univariate and multivariate logistic regression analysis of prevalence of coccidiosis in relation to various factors at p=0.1 and p=0.05 respectively.

A total of 983 cattle were sampled from 416 households in all 3 Counties. Of this study population, 66% were female; about 273 cows had calved before in their life time at the time of study, 42.6% of these had calved within one year from the time of study. Prevalence of coccidia infection obtained using McMaster technique was 32.76% while Kato Katz technique indicted a prevalence of 8.75%. Given that these tests used different principles, the agreement was very weak with a calculated Kappa of 0.272 and a standard error of 0.029 (p=0.05).

All significant variables at p=0.1 were then modelled in logistic regression model at p=0.05 with a backward elimination approach. These variables included: age, sex/gender, prophylactic treatment, regular vet visits, ploughing, treatment, lice and tick infestation. Only age was found to be statistically significant in explaining coccidia infection in cattle. Older animals were 0.71 times less likely to suffer from coccidiosis relative to younger animals.

Clustering of bovine coccidia infection in space was evident with the resulting heat map indicating hotspots in South of Busia and North of Busia. Relationship between these clusters and geographical features revealed increased disease occurrence observed along river basins.

This study showed that coccidia infection in cattle is prevalent in Busia, Bungoma and Siaya Counties at 32.76%. However coccidiosis in these three Counties presents in the subclinical form and thus no characteristic /overt clinical signs. Age was negatively associated with disease, a higher prevalence Oocysts per Gram count (OPGs) was observed in younger

animals. Breed, level of activity, use of bulls for breeding, physiological status and concurrent infections were not significantly associated with infection at p=0.05.

Good medical attention should be given to the calves and animals less than 30 months of age. Since the husbandry methods currently practised do not predispose cattle to coccidia infection, introduction of more intense farming methods may be accommodated only if high levels of hygiene are observed. Clustering of coccidia infection in cattle in Busia, Bungoma and Siaya Counties around wet lands and rivers indicated that common watering points pose a risk as disease foci and therefore farmers should be advised to water their animals from the homesteads or use clean water for their animals.

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The results described in this thesis are part of a large study on People and Their Zoonoses (PAZ) being carried out by International Institute of Livestock Research (ILRI). I therefore wish to thank ILRI for giving me the opportunity to be part of this great team. Not forgetting the great technical and logistical support accorded to me during my work. It went a great deal towards the success of this research.

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I deeply thank the farmers and household member of the households that took part in this study. Without you and your good will, this work would have been impossible. Thank you very much. Also the team of enumerators and community based animal health assistants who worked and walked with me in the difficult terrain to collect this data, much gratitude goes to you.

In conclusion, my deepest gratitude to my family and close friends for their prayers and priceless support offered to me during this period.

In all things, to God be the glory,

Great things He has done.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

CHAPTER 1.0 INTRODUCTION

Coccidiosis is protozoan infection that affects most domestic species: canine, feline, equine, porcine, **bovine,** ovine, lagomorphs and avian. It is a serious condition with adverse effects on general health of various domestic animals. Infection is characterised by acute invasion and destruction of intestinal mucosa, diarrhoea, fever, anorexia, weight loss, emaciation and sometimes death (Coetzer and Justin, 2004). Although Eimeria and Isospora are the most commonly known genera, other protozoan genera can be involved resulting in: cryptosporidiosis, sarcocystosis, and toxoplasmosis (The Merck Manual, 2005). Coccidiosis generally has host species specificity and cross infection between hosts has been documented as impossible (Quigley, 2001).

Bovine coccidiosis has a worldwide distribution (Coetzer and Justin, 2004). The most common pathogenic species are *E. bovis, E. zuernii* .The disease mainly affects calves less than six months old and is associated with intensive management systems where hygiene is not well observed. In the temperate countries, outbreaks of this disease have also been associated with cold seasons and this is attributed to the stress caused to the animals (Maas, 2007). Other stressors such as weaning and crowding also predispose animals to outbreaks of the disease (Rodríguez, 1996).

Munyua and Ngotho (1990) estimated the prevalence of bovine coccidiosis in Kenya at 67.4% .In general, diarrhoea has been documented as one of the leading causes of mortality in calves in Kenya (Gitau *et al*., 1994).

Coccidiosis results in losses due to weight loss, stunted growth reduced appetite and even death as well as treatment costs. The disease therefore reduces the productive potential of these animals and the benefits accrued by the owners (Kristijanson *et al*., 2004).

This study was carried out as a part of the "People, Animals and their Zoonoses"(PAZ) project, a research project carried out jointly by the University of Edinburgh, UK and the International livestock Research Institute(ILRI) to investigate the epidemiology of zoonotic diseases in western Kenya since 2010 (Doble and Fevre , 2010). The study area for the PAZ project was Busia, Bungoma and Siaya Counties in western Kenya. The general objective of this study was to determine the prevalence, spatial distribution and factors associated with the prevalence of coccidia infection in cattle in these Counties (factors considered included all factors documented to be associated with bovine coccidiosis in other parts of the world).

1.1 Objectives of the study

1.1.1 Overall objective

1. The overall objective of this study was to determine the prevalence and spatial distribution of coccidia infection in cattle, and associated factors in different production systems in Busia, Bungoma and Siaya Counties in Kenya.

1.1.2 Specific objectives

1. To determine the prevalence and spatial distribution of coccidia infection in cattle in Busia, Bungoma and Siaya Counties, Kenya.

2. To assess the factors associated with coccidia infection in cattle in Busia, Bungoma and Siaya Counties, Kenya.

1.2 Justification

Previous studies on prevalence of coccidia infection in cattle in Kenya have documented bovine coccidiosis in organised production systems-zero grazing units and dairy farms (Waruiru *et al.*, 2000). The three Counties covered by the study were moreover purposively

selected with regards to accessibility and logistical constraints. Availability of laboratory facilities in Busia was an added advantage for easy sample analysis while the three Counties allowed for inclusion of various husbandry systems. Given that bovine coccidiosis is a disease that may often go unnoticed yet can result in heavy losses in the cattle industry, identification of relevant risk factors is necessary to avert the heavy losses in extensive and semi-intensive production systems (unorganised production systems).

1.3 Research questions

1. Are the non-animal factors: season, rainfall, housing, geographical location hygiene, nutrition, activity of the animals, and keeping of various species and veterinary attention significant risk factors for coccidia infection in cattle in Busia, Bungoma and Siaya Counties, Kenya?

2. Are animal factors: breed, age, gender, and physiological status (pregnant/lactating), body condition and other infections significantly associated with coccidia infection in cattle in Busia, Bungoma and Siaya Counties, Kenya?

CHAPTER 2.0 LITERATURE REVIEW

2.1.0 General information

2.1.1 Etiology of coccidiosis

The order Coccidia belongs to the phylum Apicomplexa and contains numerous species that are parasitic to both vertebrates and highly organised invertebrates. Organisms in this order are obligate intracellular and affect epithelial cells of the gastrointestinal system including associated glands as well as other visceral organs e.g. liver and the kidney (Radostits *et al*., 2000).

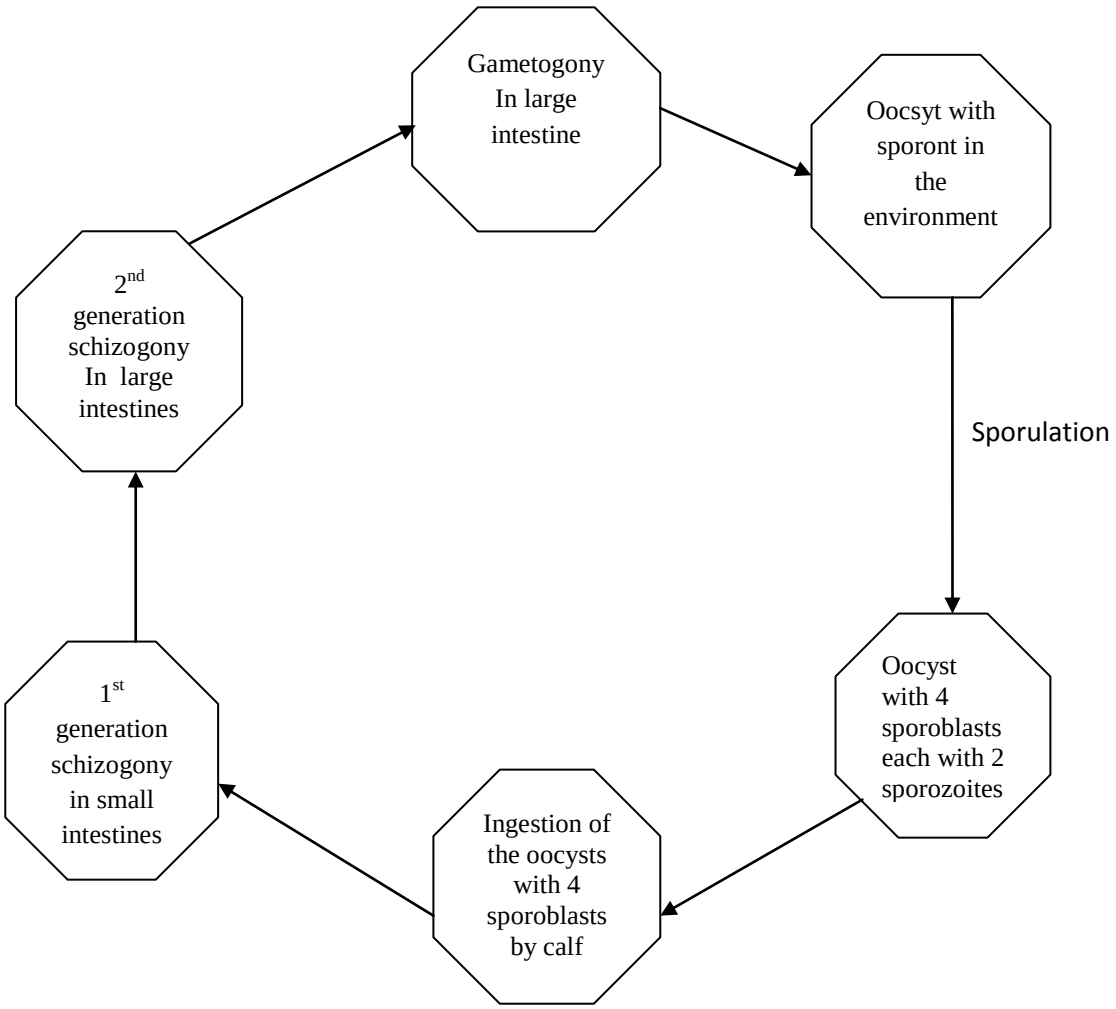
The species of this order that cause clinical disease belong to the suborder Eimeridea and family Eimeriidae. However the most pathogenic species of this suborder belong to only two genera: Eimeria and Isospora (The Merck Manual, 2005). These require a single host to complete their lifecycles. About 11 species of Eimeria have been identified and documented to cause disease in cattle: *E. bovis, E. zuernii, E. auburnensis, E. ellipsoidalis, E. canadensis, E. alabamensis, E. subpherica, E. bukidnonensis, E. wyomingensis, E. cylindrica, E. pellita and E. subsphaerica.*

The lifecycle is direct and transmission is faecal-oral, from contaminated water and feed. Inactive oocysts enter the environment from faeces from an infected animal. In the environment with humidity, warmth and oxygen, the oocysts sporulate and become infective. During this sporulation, the cytoplasm decreases and sporozoites develop in the oocysts transforming into sporocysts. The Eimeria species has 4 sporocysts each containing two sporozoites each containing four sporozoites (Coetzer and Justin, 2004).

A healthy animal ingests the sporulated oocyst; the oocyst wall disintegrates releasing the sporozoites into the intestinal lumen. The sporozoites then invade the intestinal mucosa cells

and develop into a schizont which rupture the cell to release the merozoites that invade new cells and repeats the process. After several asexual reproductive cycles, the merozoites transform into either macro gametes (females) of micro gametes (males). Then fertilization occurs resulting in oocysts which are then passed in faeces to the environment (Coetzer and Justin, 2004).

Life cycle of *E. bovis* (Dwight, 1995).



Studies on causes of calve mortality in dairy production in Kenya have been largely attributed to gastrointestinal parasites and diarrhoea (Gitau *et al*., 1994). From literature review, most studies have concentred in more organised dairy production systems (Munyua and Ngotho, 1990; Gitau *et al*., 1994; Waruiru *et al*., 2000). Production systems in Busia, Bungoma and Siaya Counties were mainly non-organised cattle production systems (semi-intensive and extensive) (Omore et at., 1999). In an attempt to alleviate poverty in western Kenya various programs have been initiated to adopt a cow for each household (Kristjanson *et al*., 2004). Various studies have highlighted different factors associated with cattle suffering from coccidiosis (Radostits *et al*., 2000; Munyua and Ngotho, 1990; Waruiru *et al.*, 2000) which were considered in this study.

2.1.2 Economic importance of bovine coccidiosis

Although the economic impact of coccidiosis has not been accurately quantified, coccidiosis can be considered sufficiently important economically in calves. Derickson, (2000) estimated the losses at more than $400 million annually in lost profits due to reduced feed efficiency, slower weight gain, and increased susceptibility to other diseases. In a more recent study, the economic loss from coccidiosis is estimated at about $100 million each year (Maas, 2007). According to Derickson, (2000) 95% of all losses are due to subclinical coccidiosis. Coccidiosis in bovine species despite not having direct negative effects may manifest losses such as the reduction in the animals' health status; weight loss hence reduced productivity (Mass, 2007; Lassen, 2009). Mortality from coccidiosis is usually associated with severe diarrhea, which causes loss of electrolytes and dehydration (Quigley, 2001) as well as secondary infections.

2.1.3 Epidemiology of bovine coccidiosis

The two main species that cause clinical disease in bovine are *E. zuernii* and *E. bovis.* There are various factors (environmental and host) that influence the occurrence of coccidiosis. These include, age of individual host, climate and farm management practices (Rodríguez, 1996). The management practices include overstocking in pasture, overcrowding in indoor stalls, poor sanitation and hygiene. Most outbreaks occur following weaning (Radostits *et al*., 2000). Though most outbreaks are recorded in cold and wet seasons, coccidiosis can be severe in dry years suggesting that immunosuppressive effect of weaning and dietary stress is a major precipitating factor (Radostits *et al*., 2000). Coccidia infect all breeds of cattle and although the disease is seen more commonly in calves four to-twelve months of age, it may occur in yearlings and adults (Kennedy, 2000). The morbidity is relatively high although clinical disease is as low as 10 to 15%. However in case of an outbreak however this infection rate can go up to 80%. Generally low mortality rates are experienced with exception of cases of winter coccidiosis accompanied by nervous signs (Radostits *et al*., 2000). In North America, the disease occurs most commonly in beef feedlots after weaning and at autum. Disease outbreaks may also occur in prolonged cold seasons and mid-winter. In Northwestern and Midwestern parts of America, disease prevalence is highest in summer, fall and spring (Radostits *et al*., 2000).

Multiple infections by more than one species have a 95% prevalence rate in the USA with most infections being attributed to the occurrence of both *E. zuernii* and *E. bovis* (Radostits *et al*., 2000). *E. alabamensis* is however responsible for outbreaks of diarrhea in a number of the European countries (Urquhart *et al*., 1996).

Eimeria zuernii is the most common cause of red diarrhea (hemorrhagic diarrhea) in cattle in Europe and is also known as winter coccidiosis (Soulsby, 1968; Urquhart *et al.,* 1996). However despite being the principle coccidian parasite in Europe it is spread worldwide (Urquhart *et al.,* 1996). It has a peripatent period that ranges from 15 to 17 days. Its patent period ranges from 5 to 12 days depending on infecting dose and attacks the caecum and colon in heavy infestation resulting in bloody diarrhea and tenesmus. During this phase, it produces small spherical oocysts 16 micrometers in diameter (Radostits *et al*., 2000; Urquhart *et al*., 1996). The other species of coccidia do not contribute greatly towards the disease incidence and prevalence in Europe .The life cycle of *E. zuernii* is similar to that of *E. bovis.*

Eimeria bovis is the most prevalent species in North America. It chiefly affects the colon and caecum resulting in severe enteritis and diarrhea in heavy infestations (Urquhart *et al*., 1996). The peripatent period ranges between 15 and 20 days and a patent period of 5 to 12 days depending on the infecting dose. During this phase it produces large oocysts that are egg shaped measuring 28 by 20 micrometers (Urquhart *et al*., 1996). Schizonts of *E. bovis* may be found in the central lacteals of villi.

Many studies have been done in various parts of the world including Africa aimed at establishing the prevalence of this disease. In Kenya, the prevalence of bovine coccidiosis was estimated at 67.4 % in unselected animals in Kabete area (Munyua and Ngotho, 1990). The most prevalent species was *E. bovis* at 79.0% followed by *E. zuernii* at 60.2%. Other species in Kenya included: *E. ellipsoidalis, E. cylindrica, E. auburnensis, E. alabamensis, E. subspherica* and *E. wyomingensis.* From this study Munyua and Ngotho (1990) confirmed that age and seasonal variation had an influence on the intensity of infection.

In another study carried out in central Kenya alongside helminth study in dairy cattle, Waruiru *et al*., (2000) estimated the prevalence of coccidioisis at 30.9%. From this study, about eight species of Eimeria were identified. The most prevalent of these were *E. bovis* and *E. zuernii*. Season, age and farm had significant influence on the prevalence of Eimeria.

Chibunda *et al*., (1997) concluded that coccidiosis was common in Morogoro, Tanzania. In this study, the prevalence of bovine coccidiosis was estimated at 56% in calves between 12 days and 4 months. Calves less than 12 days had no infection while those more than 4 months had a low prevalence rate. The species identified included: *Eimeria bovis*, *Eimeria zuemii*, *Eimeria ellipsoidalis*, *Eimeria cylindrica*, *Eimeria auburnensis*, *Eimeria alabamensis* and *Eimeria subspherica* in order of prevalence. Mixed infections were common involving two or three species.

In Ethiopia, Dawid *et al*., (2012) reported that younger aged calves and poor hygienic status of the farms were strongly associated with infection of coccidiosis in dairy farms. However in another study, Alemayehu *et al*., (2013), agreed that age was a significant factor but breed, body condition, sex, and management system were not significantly associated with the disease.

In a study done in Zimbabwe to assess the effects of region, age, sex and season on gastrointestinal parasites, Pfukenyi *et al*., (2007) reported that age, pregnancy, lactation, high rainfall, and rainy season were significantly associated with high OPG counts of coccidia parasites in cattle.

In a study done in South Africa, Matjila and Penzhorn, (2002) reported that the prevalence of coccidiosis was highest in the dairy farms in Pienaars River at 52%. There were various Eimeria species identified from the three localities. Most prevalent species in all three

localities was *E. zuernii* and *E. bovis*. Prevalence of *E. ellipsoidalis* was noted in only one of the localities (Mallesons) while the other two were prevalent in Kaalplaas and Pienaars River. Oocysts per Gram (OPGs) of feces as high as 2000 were recorded in calves; adults had relatively low OPGs.

In a study done in Mexico, Rodriguez *et al*., (1996) concluded that the main factors affecting the prevalence of *Eimeria* in calves were high rainfall, large herd size and rainy season of the year. In the same study Rodriguez *et al.*, (1996) concluded that of these factors only the herd size and rainy season influenced the excretion and spread of Eimeria. Nine species of *Eimeria* were identified in faeces of *Bos indicus* growing calves in Yucatan, Mexico. These were: *E. bovis, E. auburnensis, E. ellipsoidalis, E. canadensis, E. zuernii, E. alabamensis,* E. *cylindrical, E. subpherica, E. bukidnonensis.*

In Japan, Oda and Nishida (1990) reported that the prevalence of coccidia in two week old dairy and beef herds was 59%. The highest prevalence was in animals between six and 11 months. This prevalence decreased to 25% in animals 24 months old and above. Eleven species of Eimeria and one of Isospora were identified in Japan. These included: *E. bovis, E. zuernii, E. wyomingensis, E. bukidnonesis, E. subspherica*, and *Isospora* species in the order of frequency.

The prevalence of coccidiosis in farms in China was found to be 47.1% with the highest prevalence being observed in calves less than 4 months old and least in animals more than 12 months old (Dong *et al*., 2012). In this study, Oocysts per Gram of feces (OPG) levels decreased with age from calves, weaners, and adults. Multiple infections with 2- 8 species was common.

In another study done on Dutch farms, a total of 12 species of Eimeria were identified all with varying prevalence. Cornelissen *et al*., (1995) established that prevalence of Eimeria varied significantly with age. The overall prevalence was highest in calves at 46% with the highest OPG levels followed by yearlings (43%) and the older cows (16%).

Mitchell *et al*., (2012) identified age, water hygiene and keeping of other animal species with cattle as factors significantly influencing the prevalence of coccidiosis in farms in Wales, England. From this study, good water hygiene, and farms that kept both cattle and sheep in the same premises had lower prevalence of coccidiosis.

Güleğen and Okursoy (2000) concurred with Cornelissen *et al*., (1995) that the prevalence and OPG levels of Eimeria differed with age of the host. In Turkey, *E. bovis* (28.5%) was the most prevalent species followed by *E. auburnensis* (17.2%) while *E. zuernii* came fourth (12.4%). The least prevalent was *E. bukidnonensis* (0.5%). Multiple infections were also confirmed involving up to six species in one host.

In Germany, the prevalence of coccidiosis in dairy farms was 95.4 % (Bangoura *et al.*, 2011). The main pathogens were *E. bovis* and *E. zuernii* with the latter being more prevalent of the two. *E. zuernii* had a higher OPG (2,950) while *E. bovis* had OPG of 700, with the higher OPGs being recorded in calves.

Similarly, in Toba-Tek Singh County in Pakistan, the prevalence of coccidiosis in bovines was estimated 47.09% (Rehman *et al*., 2011) and the two most prevalent species were *E. bovis* and *E. zuernii*. *E. cylindrica* was least prevalent of all six species identified. However, prevalence was higher in the ground fed and pond watered calves compared to other management regimes. This study concluded that the husbandry practices influence occurrence of disease.

In Poland, Klockiewicz *et al*., (2007) refers that the *Eimeria* spp. were very common pathogens in cattle farms. The highly pathogenic *Eimeria (E. bovis and E. zuernii)* occurred more frequently in big rather than in small farms.

2.2.0 Clinical signs of bovine coccidiosis.

The severity of the disease depends on several factors including the number of oocysts ingested, the species of coccidia present and the age of the host (Kennedy, 2000; Maas, 2007). After infection, the incubation period is about 17-30 days (Radostits *et al*., 2000). The patient may present with mild fever in early stages though seldom. More often the temperature is normal or subnormal. The most common presentation is either chronic or subclinical (The Merck Manual, 2005; Maas, 2007). Cattle infected with a few oocysts are only mildly affected. Under crowded conditions, large numbers of oocysts are ingested causing severe or fatal infections, particularly in calves. Acute coccidiosis is accompanied by hemorrhagic diarrhea and a large number of oocysts in the feces (Soulsby, 1968; Kennedy, 2000; The Merck Manual, 2005; Maas, 2007).

Calves are usually the most affected of all age groups and show the clinical form. They appear unthrifty, perineum stained with feces, watery feces sometimes with blood (Radostits *et al*., 2000; Coetzer and Justin, 2004; Fitzpatrick, 2006; Maas, 2007).Severely infected animals present with thin bloody diarrhea, which may persist for about one week, or merely thin feces with shreds of intestinal epithelium and mucus and eventually anemia may develop (Coetzer and Justin, 2004). Dehydration, weight loss, depression, anorexia, straining after defecation and occasionally death may occur (Soulsby, 1968; Kennedy, 2000; Maas, 2007). Mortality is however acute as a result of the infection or later due to secondary complications (The Merck Manual, 2005). Less severe infections in which the animal survives and develops

resistance, may nevertheless affect the growth and health of an animal thus the animal remains stunted (Kennedy, 2000; The Merck Manual, 2005; Maas, 2007).

Coccidia normally have self-limiting infections and spontaneous recovery without specific treatment is common when the multiplication stage of coccidia has passed (Soulsby, 1968; Kennedy, 2000; Coetzer and Justin, 2004; Fitzpatrick, 2006). Cattle that recover from coccidiosis usually acquire a carrier status and continue to shed oocysts in the environments resulting in spread of the disease (Soulsby, 1968; Kennedy, 2000; Fitzpatrick, 2006). However there is no cross immunity and thus another species of Eimeria can still cause disease in these animals (Radostits *et al*., 2000). Nervous coccidiosis develops in some calves with acute intestinal coccidiosis (Kennedy, 2000).This condition is highly fatal (80-90%) within 24 hours of presentation of the first signs which include: muscle tremors, hyperesthesia, and clonic-tonic convulsions with ventral flexion of the head and neck and nystagmus (Radostits *et al*., 2000; The Merck Manual, 2005).

2.3.0 Diagnosis of bovine coccidiosis

Coccidiosis results in diarrhea due to intestinal irritation and mucosal damage. Non-hemorrhagic diarrhea in calves is thus clinically non-specific. Severe dysentery may be noticed days before any oocysts are isolated since shed of oocyst begins two to four days after start of diarrhea (Radostits *et al*., 2000). This form of diarrhea can be attributed to enteritis as a result of various gastrointestinal pathogens. However, presence of blood or shreds of mucosa (fibrin) in feces is a more specific indicator of pathogenic *Eimeria* species (Soulsby, 1968; Lassen, 2009).Like helminths, shedding of oocysts in feces is not a constant finding and thus fecal analysis may not be a definitive test for infection. Therefore results from fecal analysis- high OPG- should be related to the presenting clinical signs (Soulsby, 1968; Coetzer and Justin, 2004; The Merck Manual, 2005). Although the presence of oocysts

alone does not indicate conclusive diagnosis of an active infection, a count of more than 5000 OPG is considered significant. However, this may vary with species of coccidia and environment since the OPG may be less than 5000 or more than 10,000 which is most commonly recorded in out breaks (Radostits *et al*., 2000; Fitzpatrick, 2006).

In per acute cases especially resulting from *E. zuernii,* oocysts shed may be few but marked pathological effects are produced by the developmental stages of prior to shedding of oocysts. Identification of the particular species involved is done microscopically according to shape and size of oocysts. Most common shapes are spherical, ovoid or ellipsoidal and the common sizes range from 15- 50 micrometers (Urquhart *et al*., 1996).

At postmortem, the affected mucus membranes appear thickened, edematous and covered with mucofibrinous coat. Congestion, hemorrhagic enteritis, thickening of mucosa of the caecum, colon, rectum and distal ileum are also present. Small white like cysts may be visible on the tips of villi. Ulceration may also appear especially in severe cases. *E. bovis* causes diphtheric typhilitis and colitis in small and large intestines (Radostits *et al*., 2000; Coetzer and Justin, 2004). Histologically, denudation of the epithelium, merozoites in some cells, and developmental stages in smears of mucosa or intestinal content may be observed (Radostits *et al*., 2000).

2.4.0 Diagnostic methods for coccidiosis

Qualitative fecal examination combined with microscopic examination is the most commonly used procedure in the laboratory. These include:

The simple / direct smear method which involves preparation of fecal sample and making a smear on a glass microscope slide which is examined under the microscope (Kaufmann, 1996). The use of a cover slip improves the optics, subdues eddy currents and prevents

soiling of the objective lens. The use of saline instead of water is preferred as it prevents lysis of fragile trophozoites of protozoa (Dwight, 1995). Low efficiency is a major limitation of this procedure. McMaster technique (Levecke *et al*., 2011**)** is one of the methods of analysis that is highly reliable and simple to use. It is thus commonly used in labs and analysis. It involves determining the number of nematode eggs and protozoan oocysts per gram of feces in order to estimate the level of infection in an animal. This technique exploits the principle of floatation as the oocysts are suspended in a fluid with a higher density than water. The advantage of this method is that it is quick as the eggs/cysts are floated free of debris before counting. The disadvantage however is that you must use a special counting chamber (Nolan *et al.*, 2006).

Katz direct examination is a modified thick smear technique adapted for use in field studies (Endriss *et al*., 2005). It has been observed that the Kato-Katz method more sensitive than the thick smear technique for diagnosing helminthes and is reliable, practical for the quantitative diagnosis of *Schistosome* infections (Santos *et al.*, 2005).

Sedimentation technique is similar to direct smears but more sensitive, this method demonstrates objects too heavy and delicate to concentrate with floatation method. It is more appropriate for trematodes and acanthocephalan eggs and amoebas. It is however less sensitive than floatation for coccidian oocysts and in order to replicate similar success as floatation method, it is necessary to examine at least half of the sediment microscopically (Dwight, 1995).

Where more distinct conclusions are needed, the oocysts can be cultured to allow for sporulation and hence more specific identification (Dwight, 1995).

2.5.0 Treatment and control of bovine coccidiosis

In survivors, the disease is self-limiting and clinical signs subside once multiplication stage has passed. Drugs used are however most effective during this phase as they act on the early schizonts.

For treatment, various coccidiostats are effective. These include: Sulphadimidine 140mg/kg orally for 4 days, Nitrofurazone 15mg/kg for 7 days or 0.04% in feed for 7 days or 0.0133% in water for 7 days, Amplorium 10mg/kg daily for 5 days, Monensis 2mg/kg for 20 days from day of inoculation (Radostits *et al*., 2000; Coetzer and Justin, 2004; Maas, 1996, 2007).

These drugs have also been used for prophylaxis to reduce the prevalence of coccidia. Sulphadimidine 35mg/kg for 15 days, Nitrofurazone 33mg/kg for 2 weeks, Amplorium 5mg/kg for 21days, Monensis 20 mg/kg feed, Lasalocid 0.5- 1 mg/kg per day in feed for up to 6 weeks, Decoquinate 0.5mg/kg in feed for at least 28 days, and Nitrofurazone 10-20mg/kg daily for five days (Radostits *et al*., 2000; Coetzer and Justin, 2004; Maas, 1996, 2007).

Though coccidiostats can be given for prophylaxis at the suspected first exposure, hygiene, optimum stocking rate and avoidance of fecal contamination to feed or water cannot be substituted. Moreover frequent pasture rotation if practiced assists in control of the lifecycle (Fitzpatrick, 2006). Induced immunity can also be achieved by regulated exposure of the calves to contaminated feed without clinical disease. However young susceptible animals should be kept in clean dry stalls and fed in clean troughs with uncontaminated feed and water and stress associated factors should be minimized (Coetzer and Justin, 2004). In areas with heavy infestations, soil and bedding fumigation with formaldehyde or disinfection with 1.25% sodium hypochlorite or 0.5% cresol are inexpensive effective methods of destroying oocysts (Coetzer and Justin, 2004).

CHAPTER 3.0 MATERIALS AND METHODS

3.1 Selection of study area

The study area was selected purposively to be Busia, Bungoma and Siaya Counties in Western Kenya (Figure 3.1) which were also areas mapped out by the umbrella PAZ project. Previous studies have been done in zero grazing units and dairy production systems (organised production systems); however, these Counties practised unorganised production and thus were of interest to study coccidia infection in cattle. Busia County borders Lake Victoria to the Southwest, Republic of Uganda to the West, North and Northeast, Bungoma and Kakamega Counties to the East, Siaya County to the Southeast and South. At coordinates 0.4333° N, 34.1500° E, Busia County covers an area of about 1695 Km^2, has an average ambient temperature of 22^0C and annual rainfall ranges between 750 mm-1800 mm. The main economic practices in Busia County include: tourism, agriculture, and trade, fishing and commercial business. Main agricultural products include: livestock and livestock products, millet, cassava, maize beans, sweet potatoes and rice.

Bungoma County borders Busia to the East, bordering Uganda. It spans an area of 3,030 Km^2 and has two rainy seasons with rainfall per annum ranging from 1200 mm – 1800 mm ambient temperatures range from 15 – 30^oC. The main economic practice is agriculture and the major crops are maize and sugar cane. However the beef and dairy industry thrives well in this environment. Other agricultural products include: coffee, milk, sweet potatoes, bananas and tobacco.

Siaya County is located at longitude 33° 58’ East and 34° 33’ West, is one of the Counties in the former Nyanza Province bordering Busia County to the South and Southeast. Siaya County covers an area of approximately 2,530 Km^2 and experiences rainfall ranging from

1,170 mm and 1,450 mm per annum. Ambient temperatures range from 15^{o}C – 30^{o}C and the main economic include subsistence farming, livestock keeping, fishing and rice farming.

Livestock production systems in these three Counties are mainly 'unorganised' (semi-intensive and extensive) (Omore *et al*., 1999). The three Counties covered by the study were purposively selected with regards to accessibility and logistical constraints. The cattle production systems and their relationships with various disease morbidities and mortalities have not been explored in well-designed epidemiological studies. Availability of laboratory facilities in Busia was an added advantage for easy sample analysis while the three Counties allowed for inclusion of various husbandry systems.

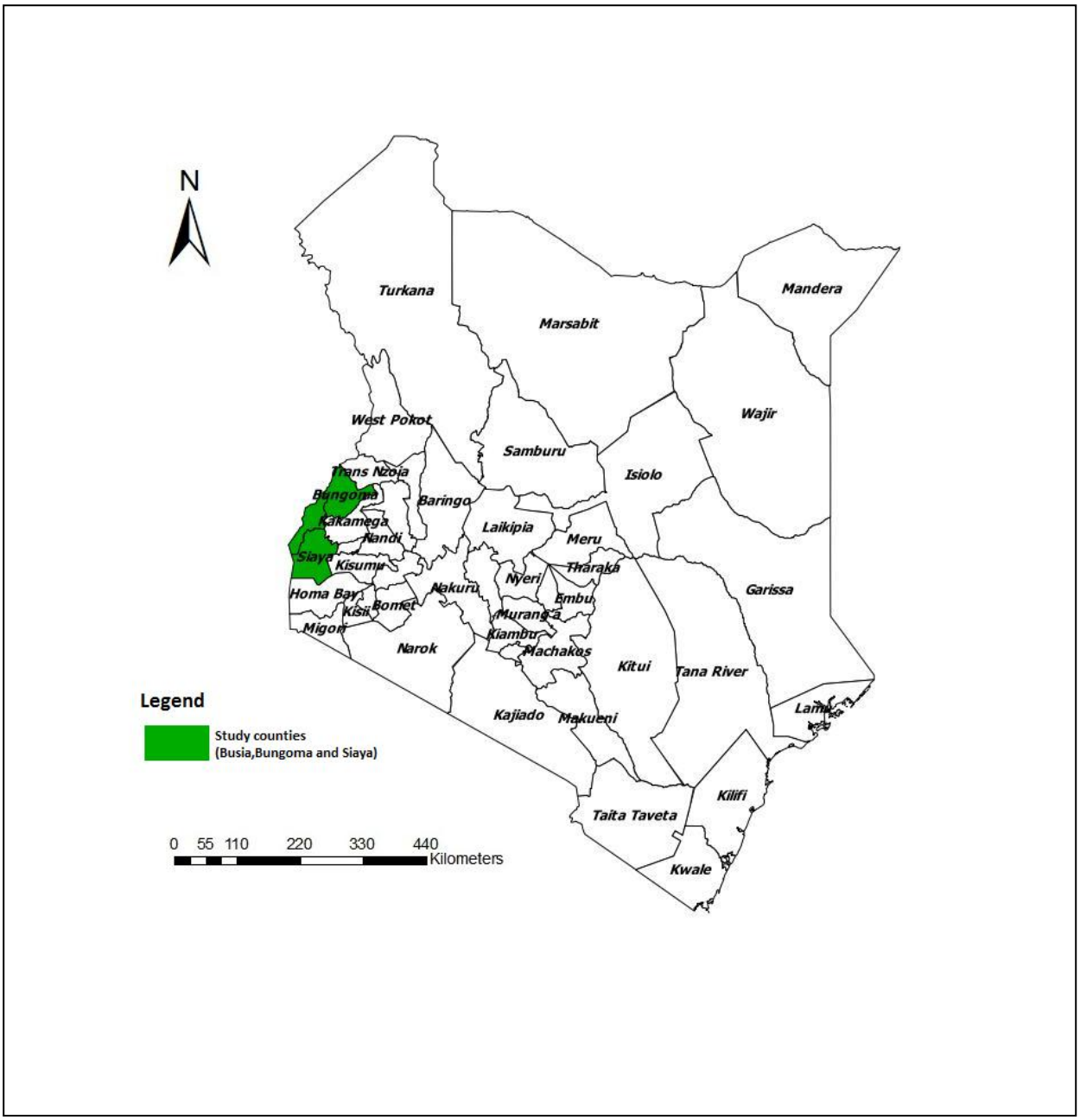


Figure 3.1: Map of Kenya highlighting area covered by this study in Busia, Bungoma and Siaya Counties in western Kenya. (Generated from ArcMap 2.0.)

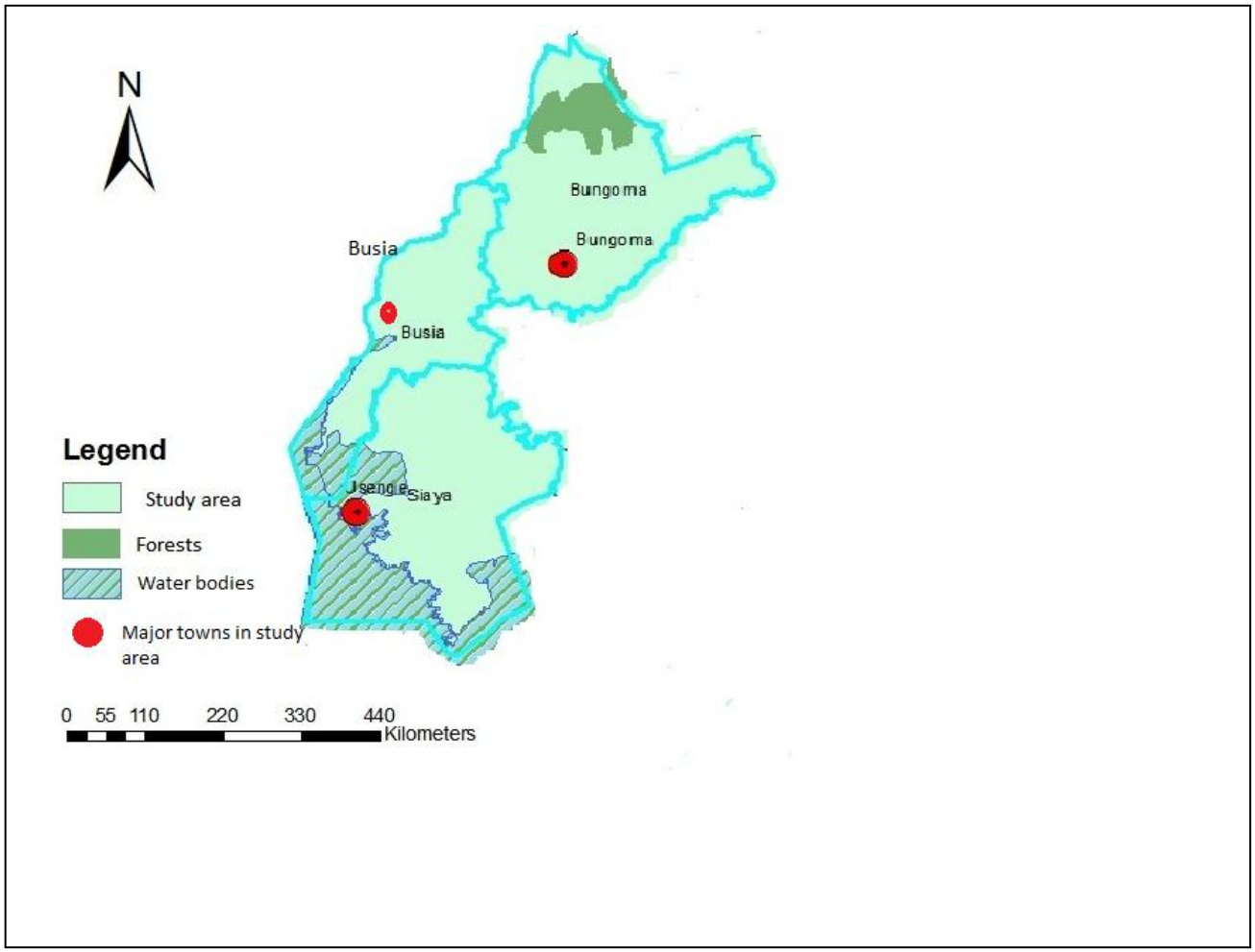


Figure 3.2 Detailed map outlining some features in relation to study area.

3.2 Study design

The area covered by the study was selected purposively and was marked as shown in Figure 3.1. Households were then selected randomly using ArcMap 2.0 software to generate random points (and a back-up for each random point) within the study area. These random points were entered into a GPS (Garmin Etrex) which was used to locate the random point. From the

random point, the closest homestead within 300 m was selected for sampling. The first step entailed seeking consent from the respective area chief and the households. In a case where there was no homestead within 300 m or if the homestead was reluctant to participate, then the back-up point was traced and the process repeated. All cattle owned by the household were sampled.

The sample size was calculated using the formula described by Dohoo *et al.,* (2003),

$$n = \frac{Z_{0.05}^2 * p\ q}{L^2}$$

(L= 0.05 margin of error, p= 0.67 which is the highest prevalence recorded by other studies in Kenya – Munyua and Ngotho, 1990, q= 1-p = 0.33, $Z_{0.05}$ is the normal deviate from the mean in Z distribution =1.96). The calculated sample was at least 340 animals.

To correct for clustering, the formula by Dohoo *et al.,* (2003),

n'= n(1+ ϱ (m-1)) the calculated sample was 442 animals. (n =380, ϱ=0.3 which is the intra class correlation documented for Eimeria in similar studies by (Otte and Gumm, 1997) and m=2 given that most of the households had 1-2 animals per house hold.) However the total sample population in this study was 983 cattle.

3.3 Data collection

In the company of the Community Based Animal Health Assistants (CBAHA) - who served as interpreters, a close ended questionnaire (Appendix I) was administered to the most responsible family member of selected and consenting households.

From Section I of the questionnaires, data on nutrition, seasons, housing, disease occurrence, veterinary attention, herd profile and use of the animals were obtained. A unique barcode number was then allocated to the household questionnaire for identification in the given random point and geographical coordinates recorded from Global Positioning Systems (GPS) receiver.

The interview was then followed by general and physical clinical examination of all cattle present was done followed by collection of faecal samples from the rectum of the cattle using a lubricated glove.

Each animal in the particular household was examined to ascertain the body condition and coat appearance. Physical clinical examination was also done which included taking of vital parameters as well as faecal samples at the end of the examination. The findings from the examination were entered in Section II of the questionnaire (Appendix I) and a unique barcode assigned to the form. From the clinical examination, physiological status (lactating, pregnant), concurrent infections and presence other endoparasites and ectoparasites was determined. Faecal samples (at least 5 grams) were collected directly from the rectum via digital extraction using lubricated gloves. The faecal samples were stored in sealed plastic bags and labelled with the barcode corresponding with the individual animal form. The samples were then transported to IDEAL field based laboratory in Busia a cool box packed with ice in Busia. On arrival at the laboratory, the samples were booked- in by affixing a copy of the barcode on the laboratory analysis form (Appendix III) and stored at 4^0C while awaiting coprological analysis. The samples were analysed using McMaster and Kato Katz techniques to allow identification of any other possible parasitic infestations in the study population.

Plate 3.0: Questionnaire administration to the most responsible member of the selected household.

Plate 3.1: Animal health assistant taking rectal temperature from a restrained animal in the household.

3.4 Laboratory analysis of fecal samples

Two main procedures were used for coproparasitological analysis; McMaster technique as documented by (Nolan *et al*., 2006) was modified whereby, 42 ml of tap water was added to 3 grams of fecal samples in a plastic beaker and homogenized by stirring until the sample and water mixture was completely homogenized. The homogenate was filtered and centrifuged then the sediment re-suspended in concentrated sodium chloride solution. The sediment was thoroughly mixed with the sodium chloride solution before filling the chambers of McMaster slide and oocysts within the etched grids of the McMaster slide counted under microscope at x10 and multiplied by 100 and results recorded in the form shown in Appendix III. Further details on the modified procedure were as indicated Appendix II.

For the Kato-Katz technique, the kit used was that of a 50 mg template; where the template was placed on a microscope slide and completely filled with sample feces, which was then covered with pre-soaked cellophane strip. To facilitate clearing of the slide, the slide was then placed in an incubator at 40^0C for 3-5 minutes. The slide was then pressed on a tile before being mounted on a microscope for observation and the number of oocysts counted and thereafter multiplied by 20. The detailed procedure was followed as described by Endriss *et al*., (2005). Results were recorded in a form as shown in Appendix III.

3.5 Data handling and management

All geo-referenced households and individual animals sampled were matched using the barcode numbers, to the corresponding laboratory results and questionnaire responses. The data was then entered into MS Access 2010.

3.6 Data analysis

The data was first transferred from Ms Access to R 2.0 Statistical software for analysis. Prevalence of coccidia infection in cattle in Busia, Bungoma and Siaya was obtained from

both the descriptive analysis of the questionnaire responses and further statistical analysis of laboratory results using R Statistical software. An individual animal was considered positive if Eimeria oocysts were present in fecal samples using the McMaster technique. Kato Katz technique although used to check for Eimeria, was purposed for identification of other parasitic infections e.g., Shistosomiasis. Mean Oocysts Per Gram counts and household demographics were calculated in form of proportions while tests for correlation, odds ratios and test for agreement were used to ascertain statistical significance of the factors analyzed. Spatial distribution of coccidia infection in cattle in these Counties was done using QGIS 2.0.1 software for mapping. From this analysis a geographical view of the infection in relation to various geographical features (rivers, wetlands, and individually owned farms) was presented in a point map. QGIS is Geographical Information Systems (GIS) software that allows the representation of data in table form on preexisting maps. Area maps were obtained from pre-existing Government of Kenya and ILRI databases.

Clustering of coccidia infection in cattle in the area was evaluated using kernel plots and heat-maps created using QGIS to depict disease intensity per km^2. Heat maps were generated at 6.5km bandwidths and 500m grid cells (Pfeiffer *et al*., 2008).

R Statistical software was used for univariate and multivariate logistic regression analysis to determine the association between the prevalence of coccidiosis and various factors: breed, level of activity, age, gender, physiological status (lactating, pregnant), management (husbandry method, intervention in disease) and concurrent infections/disease conditions. Logistic regression model was used to model coccidiosis as outcome at p=0.1 for univariate and p=0.05 for backward elimination and age, gender, level of activity, physiological status (lactating, pregnant), herd size, management (husbandry method, grazing in different seasons and intervention in disease), concurrent infections/disease conditions as explanatory

variables. Only variables that were significant in explaining prevalence of coccidiosis at p=0.1 were modeled in the multivariate regression at p=0.05.

$\hat{y}$=β0+ β1(ploughing) + β2 (mastitis)+ β3(age)+ β4(lice) + β5 (lactating)+ β6 (pregnant) + β7(sex)+ β8(treatment)+ β9(type of treatment)+ β10 (Amblyomma tick infestation).

CHAPTER 4.0 RESULTS

4.1 Herd demographics and profiles.

A total of 983 cattle were sampled from 416 households during this study in the three Counties. The study population consisted of 66% (649/983) females and 34% (334/983) males. The response rate of my questionnaires in this study was 87.9%. The sample population was composed of 249 animals less than 15 months old, 112 (11.4%) of the population was between 31 and 42 months old while the age 32.65% of study population was unknown. The results of the various factors are summarised as herd and management/ husbandry factors as shown in tables 4.1 and 4.2 below.

Table 4.1 indicates that only 273(42.06%) of the females in the study population had calved at least once in their lifetime at the time of the study. Of these, 128 had calved within the last one year and 57 of the females (8.8%) were in the early stage of pregnancy. Only 2.3 % of the cows had a history of abortion. The reproductive history of about 16% of the cows was unknown at the time of study 50.1% of cows that had a history of calving were lactating. Of all male animals owned by these households, about 100 (29.9%) of them were used for breeding, while the rest were used for cart pulling and ploughing.

Table 4.1 Herd profile and characterisation of the households sampled in Busia, Bungoma and Siaya Counties as at December 2013.

Animal factor	Category	Number (Percentage)
Age	2 weeks-15 months	249 (25.3)
	15 – 18 months	106 (10.8)
	18-24 months	111 (11.3)
	25-30 months	85 (8.6)
	31-42 months	112 (11.4)
	Unknown	320 (32.6)
Reproductive status	Calved before	273 (42.1)
	Never calved before	360 (55.5)
	Calved 12 months ago	106 (39.0)
	Calved within last 30 days	27 (9.7)
	Calved within the last 7 days	4 (1.4)
	Calved within the last year	128 (46.9)
	Calving date unknown	8 (2.9)
	Unknown	16 (2.5)
Stage of pregnancy	Early < 3 months	57 (8.8)
	Mid (3-6)	46 (7.1)
History of abortion	Yes	15 (2.3)
	No	634 (97.7)

Table 4.2 Breed, husbandry and management practices for cattle in households sampled in Busia, Bungoma and Siaya Counties as at December 2013.

Variable	Categories	n=983 (Percentage)
Breed	Zebu	328 (33.4)
	Shorthorn and zebu crosses	433 (44.1)
	Exotic and local cross breeds	52 (5.2)
	Exotic	15 (1.5)
	Shorthorn	155 (15.8)
Source of animal	Bred within the household	529 (54.0)
	Purchased from market	338 (34.0)
	From another homestead in this village	59 (6.0)
	From a homestead in another village	48 (5.0)
	Other	9 (1.0)
Husbandry in dry season	Tethered	672 (68.4)
	Herded with animals from other homesteads	168 (17.1)
	Herded with animals only from this homestead	115 (11.7)
	Free grazed	8 (0.8)
	Zero grazed	6 (0.6)
	Grazed with other herds	5 (0.5)
	Other	9 (0.9)
Husbandry during rainy season	Tethered	685 (69.7)
	Herded with animals from other homesteads	157 (16.0)
	Herded with animals from this homestead	133 (13.5)
	Free grazed	11 (1.1)
	Zero grazed	6 (0.6)
	Other	11 (1.1)

Of the total study population (n=983), most commonly kept breeds were indigenous and their crosses with up to 44.04% of zebu-shorthorn crosses and 33.4% of pure zebu cattle (n=983). Exotic cattle in this area contributed to only 1.5% of breeds being sampled. More than half (54%) were bred within the individual households and only 34% of these animals were introduced into the households from neighbouring markets (Table 4.2). During the dry season, 68% of the animals were tethered on a movable peg (Plate 4.0) and moved from one spot to another, 17% were herded with other animals, and 15% were herded by single households. About 70% of the cattle were tethered in wet season, 16% herded with other animals while only 0.6% of all animals in the study population were zero grazed during both wet and dry seasons. This difference in management systems was not significantly different with t= (-15.351) at p=0.05.

Plate 4.0: An animal grazing in the field and tethered to a movable peg so that it is moved from place to place.

Various clinical signs were observed in the study population (Table 4.3a & b). Twenty three (2.3%) of animals were dull and listless, 12% had diarrhoea but only 4 animals (0.41%) had haemorrhagic diarrhoea. 19.5% had a rough hair coat, 25.6% had mild dehydration levels while 1.1 % was severely dehydrated and 10% were anorexic while only 4% appeared to have lost weight. Some of the animals however showed a multiplicity of these clinical signs as shown in Table 4.3 a & b. Concurrent infections were assessed by presence or absence of other clinical signs; 19% and 34.2% of sampled animals had parotid and pre-scapular lymph nodes enlarged indicating active disease or recovering status.

Animal in these Counties suffered from heavy infestation of ectoparasites (mainly ticks).The most prevalent tick species were *R. appendiculatus* and other *Rhipicephalaus* ticks with 844(85.9%) having a moderate infestation and 4.4% with severe infestation of and *Rhipicephalus* ticks. Fleas, lice and mite infestations (scabies) were also present but with no severe infestations.

Table 4.3 a Clinical indicators of disease as observed in cattle in sampled households in Busia, Bungoma and Siaya Counties as at December 2013.

Clinical sign	Category of clinical signs	n=983(Percentage)
Respiration rhythm	Normal	970 (98.6)
	Exaggerated effort	6 (0.6)
	Fast	7 (0.7)
Fecal consistency	Normal	941 (95.7)
	Hard	26 (2.6)
	Diarrheic	12 (1.2)
	Haemorrhagic	4 (0.4)
Skin elasticity	Normal	723 (73.6)
	Mildly poor	249 (25.6)
	Very poor	11 (1.1)
Protid lymph nodes	Normal	730 (74.3)
	Enlarged Unilaterally	64 (6.5)
	Enlarged bilaterally	187 (19.0)
	Abscessed unilaterally	2 (0.2)
Prescapular lymph nodes	Normal	477 (48.5)
	Enlarged Unilaterally	168 (17.1)
	Enlarged bilaterally	336 (34.2)
	Abscessed unilaterally	2 (0.2)
Presence of ocular discharge	Yes	809 (82.0)
	No	174 (18.0)
Character of ocular discharge	Mild serous unilateral	65 (6.6)
	Mild serous bilateral	90 (9.2)
	Moderate mucoid unilateral	15 (1.5)
	Moderate mucoid bilateral	1 (0.1)
	Severe serous unilateral	2 (0.2)
Presence of nasal discharge	Yes	656 (67.0)
	No	327 (33.0)
Character of nasal discharge	Mild serous unilateral	39 (4.0)
	Mild serous bilateral	230 (23.4)
	Moderate mucoid unilateral	56 (5.7)
	Moderate mucoid bilateral	2(0.2)
	Severe serous unilateral	1 (0.1)

Table 4.3b Presence of ectoparasites infections as observed in cattle in sampled households in Busia, Bungoma and Siaya Counties as at December 2013.

Variable	Category	n=983 (Percentage)
Ectoparasites		
Adult *Rhipicephalus appendiculatus*	Present	844 (85.9)
	Severe	43 (4.4)
	None	96 (9.8)
Adult Amblyomma	Present	595 (61.0)
	Severe	10 (1.0)
	None	378 (38.0)
Adult Boophillus	Present	62 (6.3)
	None	921 (93.7)
Lice	Present	32 (3.3)
	Severe	4 (0.4)
	None	947 (96.3)
Scabies	Present	4 (0.4)
	None	979 (99.6)
Fleas	Present	13 (1.3)
	Severe	1 (0.1)
	None	969 (98.6)

Figure 4.0 shows the types of veterinary products administered to cattle in the three Counties. About 179/416 (43%) of the households did not know what medication was being administered to their animals. Of the animals treated, 68/683 (7%) had been treated with antibiotics (other than tetracycline) and another 68/683 (7%) with drench; 59 animals (6%) had been treated for tick-borne infections, 39(4%) had oxytetracycline injections administered to them while 334 (34%) of the study population had been treated with an unknown drug. Most of these animals 128/983 (13%) were handled by private vets, 68/983 (7%) by animal health assistant, 49 (5%) by other individual people and only39/983 (4%) by government vets. The most commonly reported acaricide in use was amitraz used on 482 animals (49%), 315/983 (32%) had been sprayed with unknown substances while 68/983 (7%) had been sprayed with deltamethrin. For households that identified animal ailments and

sought intervention, more than half of these animals were reported to be treated for acute cases (51%), 7% were treated for gastrointestinal parasite and only 5% of the veterinary visits were aimed at advising the households on good husbandry and production techniques.

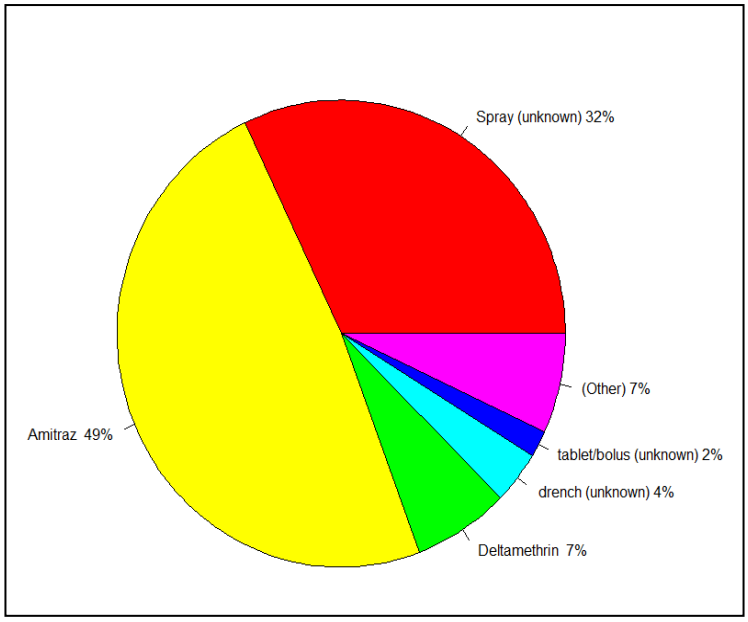


Figure 4.0 Type of veterinary products administered to cattle for ectoparasites control in Busia, Bungoma and Siaya Counties as at December 2013.

4.2 Prevalence of coccidia infection in cattle in Busia, Bungoma and Siaya Counties as at December 2013.

There was a significant difference in the prevalence of coccidiosis across the Counties from the two methods used for coproparasitological analysis at p=0.05. Prevalence obtained from McMaster Technique was 32.76% while Kato Katz technique indicted a prevalence of 8.75%. The average OPG was 327 and 1447 Oocysts per Gram Count according to Kato Katz and McMaster Techniques respectively. Given the difference in the tests used, there was very weak agreement between these two tests with a calculated Kappa of 0.272 and a standard error of 0.029.

4.3 Animal factors associated with presence of coccidia infection in cattle in Busia, Bungoma and Siaya Counties as at December 2013.

The results of univariate logistic regression for animal factors (age, breed, and sex) in relation to the presence of coccidia oocysts as a disease outcome showed that only age and sex were significantly associated with coccidia infection at p=0.1. All levels of age by dentition were significant factors associated with high prevalence of coccidiosis while the females significantly associated with high prevalence of disease. Bulls used for breeding, animals in the second trimester and lactating animals were at a lower risk of disease at p=0.1.

Table 4.4 Logistic regression of individual animal attributes explaining prevalence of coccidia infection in cattle in Busia, Bungoma and Siaya Counties as at December 2013.

Variable		Coefficient	z-value	p-value
Age	Intercept	0.2640	1.945	0.0517
	age_dentition2	-0.5051	-2.080	0.0375
	age_dentition3	-0.6279	-2.569	0.0102
	age_dentition4	-0.8802	-3.204	0.0013
	age_dentition5	-1.4003	-5.322	<0.000
	age_dentition6	-1.7119	-8.630	<0.000
Sex	Intercept	-0.3689	-3.234	0.0012
	cattle_sex2	-0.3775	-2.624	0.0086
Physiological status	Intercept	-0.32603	-4.120	<0.0000
	last_calving2	-1.15558	-2.303	0.0213
	last_calving3	-1.16345	-4.768	<0.0000
	last_calving4	-1.22625	-4.521	<0.0000
Lactation status	Intercept	-0.42056	-5.516	<0.0000
	currently_lactating1	-1.03743	-5.245	<0.0000
	providing_homestead_milk1	-1.03574	-5.061	<0.0000
Pregnancy	Intercept	-0.56202	-7.730	<0.0000
	currently_pregnat1	-0.49577	-2.038	0.0415
	stage_pregnacy2	-0.73727	-1.925	0.0542
Breeding	Intercept	-0.58315	-8.252	<0.0000
	breeding_bull1	-0.64063	-1.747	0.0806

Key:

Age_ dentition 1=2weeks-15 months Age_dentition 2=15-18 months Age_dentition3=18-24months
Age_dentition 4=25-30 months Age_dentition 5=31-42 months Age_dentition 6=unknown
Cattle _sex 2=female Cattle_sex 1= male Currently lactating1=Yes Currently lactating 2= No
Providing_homestead_milk 1= Yes Providing_homestead_milk 1= No Currently_pregnant1=Yes
Currently_pregnant1=No Stage_pregnacncy2=3-6months Stage_pregnacncy1=0-3months
Stage_pregnacncy3=6-9 months Breeding bull 1= Yes Breeding bull 0=No

4.4 Effect of management/husbandry methods on prevalence of coccidia infection in cattle in Busia, Bungoma and Siaya Counties as at December 2013.

Veterinary attention and use of boluses for prophylaxis against infections were significantly associated with lower prevalence of coccidiosis at p=0.1 in a univariate logistic regression analysis. Other management factors that were also significantly associated with prevalence at p=0.1 were regular visits by veterinarians, visits aimed at controlling helminths and related gastrointestinal parasites, source of the veterinarian –animal health assistants- associated with lower risk of disease while high level of work/activity the animal was exposed to (eg. Ploughing) was associated with higher prevalence of disease.

Table 4.5 Logistic regression of husbandry factors associated with prevalence of coccidia infection in cattle in Busia, Bungoma and Siaya Counties as at December 2013.

Variable		Coefficient	z-value	p-value
Treatment	Intercept	-0.2989	-2.136	0.0327
	prophylactic_treatment1	-0.4092	-2.539	0.0111
Type of treatment	Intercept	-0.52390	-6.269	0.3630
	treatment1	-0.27035	-1.805	0.0711
Vet attention	Intercept	-0.56847	-7.505	<0.0000
	vet_visit_reason6	-1.30333	-1.707	0.0877
Activity	Intercept	-0.56517	-7.920	<0.0000
	Ploughing2	-0.72760	-2.347	0.0189

Key:

Prophylactic_treatment1=Yes Prophylactic_treatment0 =No Treatment 1= Tablet/bolus Treatment 1= Tablet/bolus Treatment 2=Drench Treatment 3= Spray Treatment 4= Pour on Treatment 5= Dip Vet_visit_reason 6= helminth control Vet_visit_reason 5= Failure to thrive Vet_visit_reason 4= Advice on production Vet_visit_reason 3= Dystocia Vet_visit_reason 2=Acute sickness Vet_visit_reason 1=Acute injury Ploughing 2= Yes Ploughing 1= No

4.5 Concurrent infections associated with coccidia infection in cattle in Busia, Bungoma and Siaya Counties as at December 2013.

In a univariate logistic regression model at p=0.1, staring/rough hair coat, moderate fever infestation by lice, and increased respiration rate were clinical signs significantly associated with higher prevalence of coccidia infection in cattle. Only mastitis among the concurrent infections was significantly associated with a higher risk of coccidia infection in cattle at p<0.1.

Table 4.6 Logistic regression of concurrent infections explaining prevalence of coccidia infection in cattle in Busia, Bungoma and Siaya Counties as at December 2013.

Variable		Coefficient	z-value	p-value
Ticks	Intercept	-0.47184	-4.318	<0.000
	amblyoma1	-0.23495	-1.655	0.0978
Lice	Intercept	-0.64255	-9.069	<0.000
	lice1	0.99085	2.583	0.0098
Mastitis	Intercept	-0.71886	-8.168	<0.000
	Mastitis1	0.33478	2.305	0.0211

Key:

Amblyoma 1= Present Amblyoma 2= Severe infestation Mastitis 1= Yes Mastitis 0= No
lice1= presence of lice lice2= Severe infestation of lice

All significant variables at p=0.1 were then modelled in multivariate logistic regression model at p=0.05 with a backward elimination approach. These variables included: age, sex/gender, prophylactic treatment, regular vet visits, ploughing, and treatment, lice and Amblyomma tick infestation.

The final most parsimonious model obtained was:

$$\hat{y} = 0.0517 - 0.33487 \text{ (age)}.$$

Older animals were 0.71 times less likely to suffer from coccidiosis relative to younger animals.

4.6 Spatial prevalence of coccidia infection in cattle in Busia, Bungoma and Siaya Counties in Kenya as at December 2013.

Households in which animals were positive for coccidiosis were mapped on the study area. Figure 4.1a outlines the herds that tested positive for bovine coccidiosis in the study area while Figure 4.1 b show the contrast between coccidiosis positive and negative households. There was evidence of disease clustering in space as shown in the heat map (Figure 4.2) which indicated highest prevalence of coccidiosis in the Southern area of Busia County. These clusters were analysed against geographical features in the area and revealed that coccidia positive herds were mainly found along rivers and river basins (Figure 4.3).

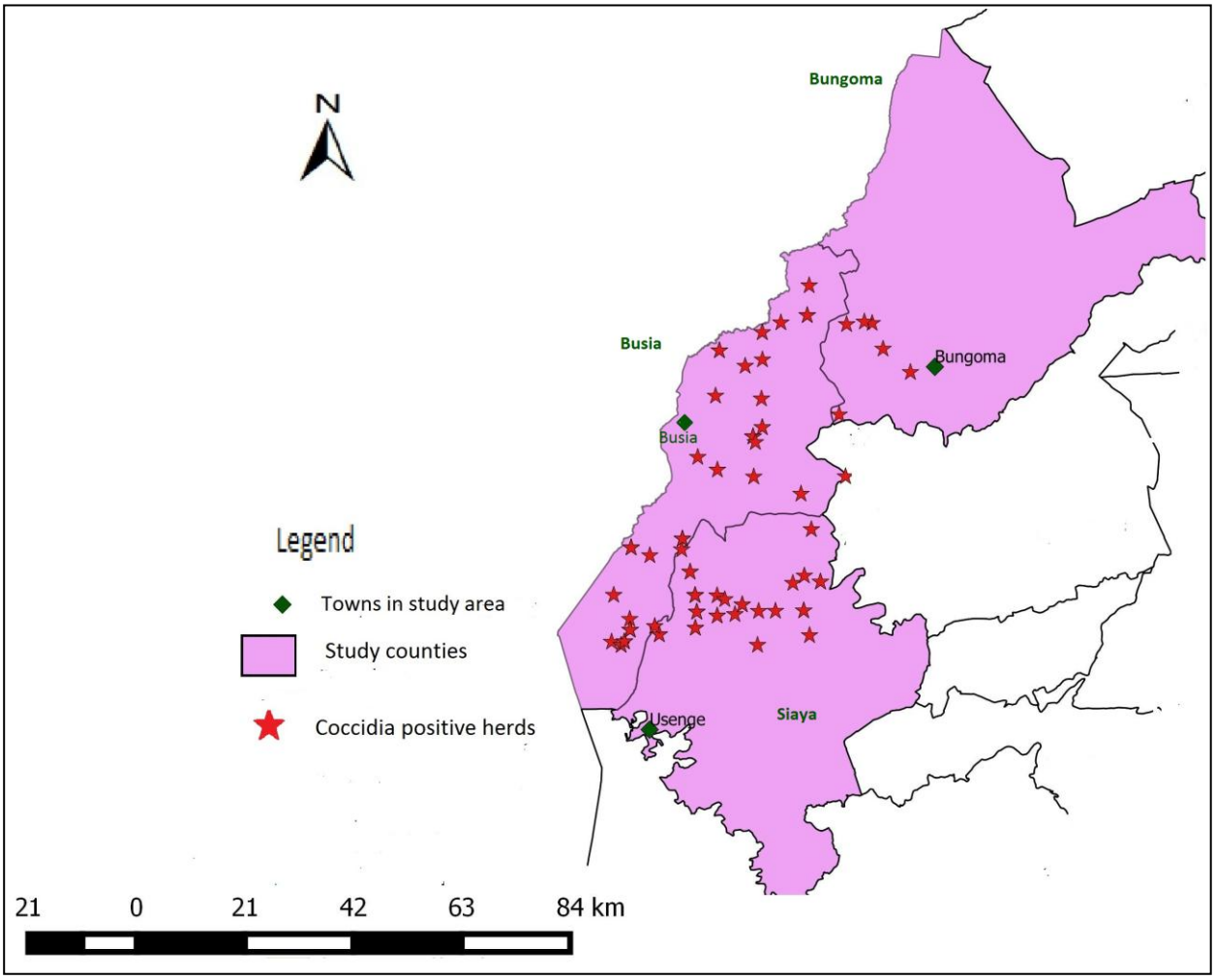


Figure 4.1a: Spatial distribution of coccidia infection in cattle herds in Busia, Bungoma and Siaya Counties as at December 2013.

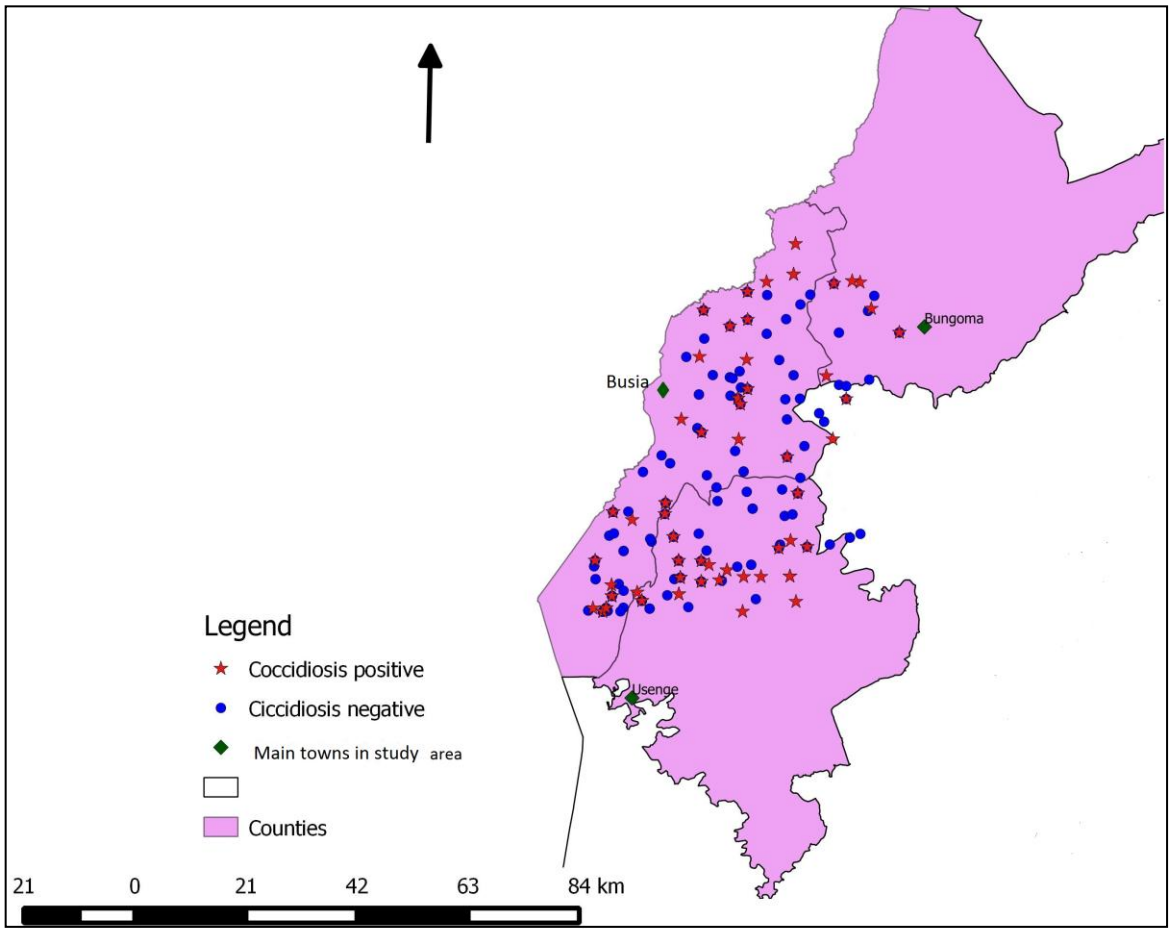


Figure 4.1b: Map showing distribution of coccidia positive and negative herds in Busia, Bungoma and Siaya Counties as at December 2013.

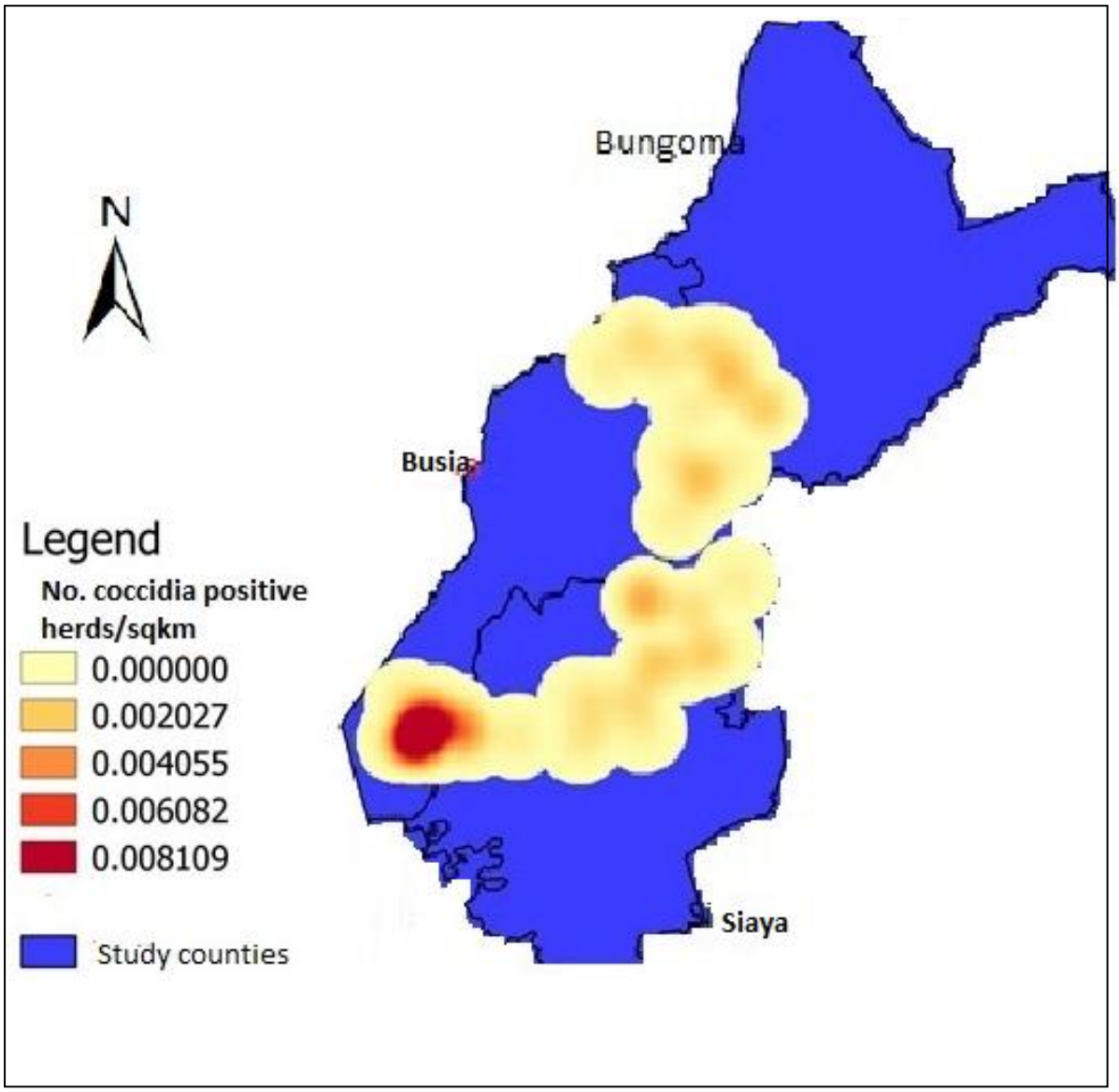


Figure 4.2 Heat map showing intensity of coccidia infection in cattle herds in Busia, Bungoma and Siaya Counties as December 2013.

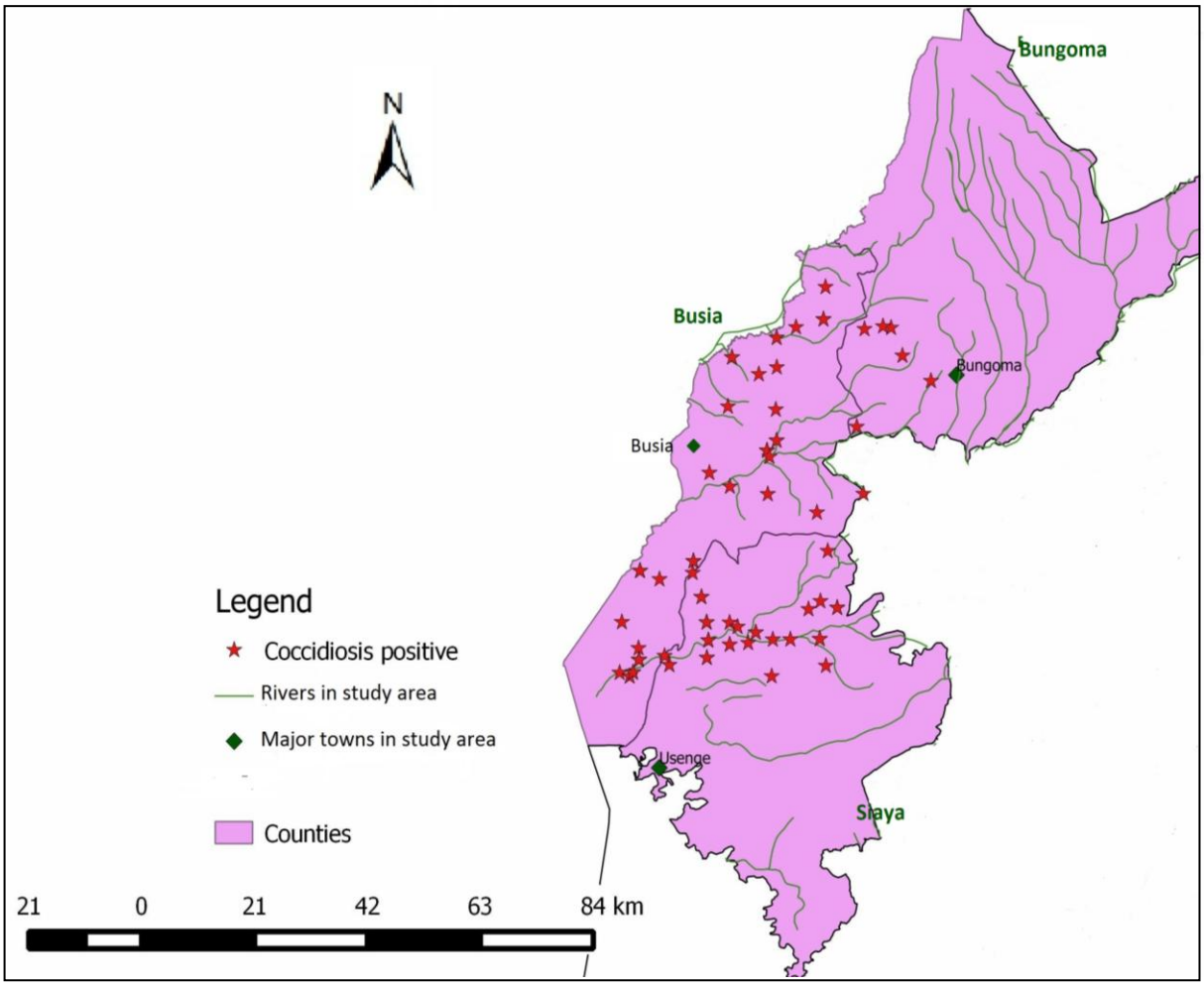


Figure 4.3 Prevalence of coccidia infection in cattle herds in relation to river basins in Busia, Bungoma and Siaya Counties as at December 2013.

CHAPTER 5.0 DISCUSSION

This study focused on occurrence of coccidian infection by Eimeria species and associated factors: individual, management and geographical in Busia, Bungoma and Siaya Counties. Although coccidia infection in cattle has been documented in various studies as being a potentially fatal disease, in this study, no fatalities were directly associated with the disease and thus mortality attributed to coccidia infection in cattle was not quantified. Most of the households kept female animals in anticipation of increasing their numbers from calves born. Moreover, various NGOs in the area have advocated for adoption of a cow per household as poverty mitigation project hence the higher female population in the study (Kristjanson, 2004).

The highest proportion of the study population from this study was indigenous cattle breeds and their crosses followed by exotic breeds. This farming pattern was influenced by the many reasons why animals were kept as reported by these households as was observed. Some kept them for work such as ploughing and cart pulling, manure and milk for the households hence the low fertility (calving rates and intervals) and the production indices did not appear as of concerns to the farmers. These findings were contrary to those documented in other smallholder practises in central Kenya (Bebe *et al*., 2003). Exotic breeds in Kenya are known to be less tolerant to harsh climate and low level of management practices. Although most households in these Counties understood the genetic superiority of exotic breeds, most kept indigenous breeds despite the low productivity and few crosses of exotic-indigenous animals. This finding was in agreement with that of Omore *et al*., (1999).

Husbandry methods in these Counties did not differ between wet and dry seasons. Most of the households practiced semi-intensive and extensive methods of management livestock keeping practices. These findings were similar to those documented by Omore *et al*., (1999).

Crop farming appeared to be the more valued economic activity in these Counties, although more subsistent than economic given that most households preferred to tether their animals as they till their farms (personal observation).

Based on the results of this study, animals shedding oocysts did not present with pathognomonic clinical signs of coccidiosis. This finding concurred with studies by Cornelissen *et al.,* (1995) in Dutch dairy farms and Farkas *et al.,* (2007) in Hungarian dairy farms who concluded that the prevalence of coccidia oocysts was influenced by age and management although no clinical cases were observed in the study populations. In these two studies, shedding of oocysts (presence of oocysts in fecal samples) was the criterion used to rule if a case as positive for coccidia infection in cattle.

The results showed that there was a difference in results between McMaster and Kato Katz technique used in this study. This was attributed to the difference in sensitivity of the techniques as documented by Levecke *et al*., (2011), who concluded that McMaster was a more viable technique to establish gastrointestinal parasites in the field. However another study by Levecke *et al*., (2012) concluded that McMaster technique was biased and influenced by the number of oocysts. However it was more accurate compared to the rest of the tests viable for use in the field. On the other hand, Tarafder *et al*., (2010) concluded that although Kato Katz was a fairly accurate technique in coprological analysis, the sensitivity and specificity however decreased with the length of time between collection and analysis of sample. In this study McMaster technique was thus preferred as a more reliable technique for reporting prevalence.

The prevalence (32.7%); was lower than what has been documented in other studies in similarly "un-organized" production systems Pandit, (2009) recorded a prevalence of 75.8% in calves in unorganised production systems in Kashmir valley in India. This relatively low

prevalence from this study can be attributed to outdoor husbandry management widely practised and therefore direct exposure of the environment to sunlight has been documented in other studies to significantly reduce the survival of oocyst in the environment (McAllister, 2007). Various other factors are known to influence the prevalence of coccidia infection in cattle. In this study, age of the animal was found to be of the only influence in coccidiosis prevalence. Age was inversely correlated with disease as a higher prevalence (OPGs) was observed in younger animals. This finding concurs with (Dong *et al.*, 2012) in his study in dairy farms in China. The risk of infection of coccidiosis decreases with age as the immunity of the individual aging animal increases. According to the results of this study, husbandry methods did not significantly affect the risk level of coccidia infection in cattle. These findings were contrary to the findings by Cornelissen *et al.*, (1995); Dawid *et al.*, (2012) who concluded that husbandry systems-housing- significantly influenced prevalence of disease. Husbandry in either wet or dry season did not differ significantly between the farms in Busia, Bungoma and Siaya Counties was not a significant risk factor for disease. Zero grazing units and intensive farming systems were very uncommon in these households. Most animals were tethered and exposed to the same environmental conditions in Busia, Bungoma and Siaya Counties. Bangoura *et al*., (2011); Alemayehu *et al.*, (2013) agreed with these findings in a study done in Germany where the management system was not correlated with prevalence of coccidia infection in cattle although the floor type of the housing used for the cattle (floors with litter) was significantly associated with higher prevalence of coccidia infection . In these "unstructured production systems" this type of floor type was not used and most animals were not housed but rather tethered under a shade.

Stressful factors in general increase the risk of infection and hence associated significantly with the prevalence of bovine coccidiosis (Munyua and Ngotho, 1990; McAlister, 2007). Considering heavy workload or degree/intensity of activity as a stress factor, its effects were

included in this study. The use of animals for ploughing and cart pulling is a source of stress and was expected to significantly influence the prevalence of coccidiosis. However level of activity was not significantly associated with prevalence of coccidiosis in these areas. The immunity of indigenous breeds is documented to be superior to that of exotic breeds in tropical climates (Perry *et al.*, 2002; Bebe *et al*., 2003). On the contrary the results according to this study breed did not appear to significantly influence prevalence of coccidiosis in Busia, Bungoma and Siaya Counties. Lactation and pregnancy are considered to be stressful in animals in most intensive farming practices. The physiological status (pregnancy, lactation status) was not significantly associated with disease prevalence contrary to the findings by (Pfukenyi *et al.*, 2007). In this case however, the indigenous breeds (which comprise the larger proportion of the study population), are known to be more tolerant and thus responded better to the various stressors. Moreover, the husbandry methods employed were largely semi intensive thus reducing stress on the animals hence not being associated with disease occurrence.Concurrent infections in these animals were not significantly associated with prevalence of coccidiosis at p=0.05.

Regular veterinary visits and helminth control also had no statistically significant effect on the disease prevalence at p=0.05. These findings were contrary to the associations identified by Lassen, (2009) who concluded that the mucosal damage of the digestive system created a favourable environment for the persistence of giardia, cryptosporidium among other gastrointestinal infections. Moreover, given that clinical presentation was not very distinct in coccidiosis most animals treated or attended to by the veterinarian was for advice on production and ectoparasite control and also helminth control. Drugs used for these activities (although largely unidentified by the farmers) were not likely to be coccidiostats and thus did not affect the protozoan parasites in the digestive system.

Coccidia infection in cattle in Busia, Bungoma and Siaya Counties was observed to cluster in space. Infection was observed to cluster around river basins. Various studies (Pfukenyi *et al.*, 2007; Munyua and Ngotho,1990 ; Rodríguez-Vivas, 1996) have associated wet season with bovine coccidiosis and thus a risk factor of disease. However in this study, the climatic conditions of the 3 Counties are generally similar and season had no significant effect on management and hence not associated with the prevalence of coccidiosis.

However, there was spatial association between disease and river basins indicating that wet environments are a risk factor for the prevalence of coccidia infection in cattle. According to Coetzer and Justin, (2004) warm humid environments are favourable for the propagation of the parasite. In the favourable environments, schizogony and sporogony occur in warm, humid environment with adequate oxygen. Dry cold environments result in death of most oocysts in the environment. Since transmission is fecal-oral, contaminated water presents a common point for disease transmission and propagation as these points are used as communal watering grounds.

CHAPTER 6.0 CONCLUSIONS AND RECOMMENDATIONS

6.1 CONCLUSIONS

- Coccidia infection in cattle is present in Busia, Bungoma and Siaya Counties. However coccidiosis in these three Counties presents in the subclinical form and thus no characteristic /overt clinical signs.

- Breed predisposition to coccidiosis is not significantly evident in cattle kept in Busia, Bungoma and Siaya Counties.

- The risk of infection with coccidia decreases with increase in age of the cattle in Busia, Bungoma and Siaya Counties.

- The husbandry methods (open tethering) currently practised does not predispose animals to coccidiosis and should be maintained.

- Coccidia infection in cattle in Busia, Bungoma and Siaya Counties is clustered around wet lands and rives and thus common watering points pose a risk as disease foci and transmission areas.

- Regular farm visits for helminth and ectoparasites control was not associated with coccidia infection in cattle in Busia, Bungoma and Siaya Counties.

6.2 RECOMMENDATIONS

- Animals with subclinical coccidiosis act as reservoir for the disease constantly shedding oocysts and thus intense disease management mechanisms should be implemented.

- Households should be advised to gradually upgrade their indigenous breeds of cattle to increase productivity.

- Better medical attention should also be given to the calves and animals under 30 months of age.

- Introduction of more intense farming methods may be accommodated only if high levels of hygiene are observed and maintained.

- Farmers should be advised to water their animals from the homesteads or use clean water for their animals.

- Farmers should be encouraged to include coccidiostats in their endoparasite control measures.

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CHAPTER 8.0 APPENDICES

APPENDIX I: Cattle form and Questionnaire

Date _____ **Time _____**

Homestead Barcode _____ **Animal Barcode_____**

Please mark where appropriate and explain in field 'other'.

SECTION I

1. What breed is this cow? Shorthorn ☐ Zebu ☐ Shorthorn-Zebu ☐
   Grade ☐ Local x Exotic ☐ Ankole ☐ Other: ☐
2. Is this animal used for ploughing? Yes ☐ No ☐ Not Known ☐
3. What age is this animal? (Months) _____ If Unknown Age type 000
4. What gender is this animal? Male ☐ Female ☐
5. Has this animal had any calves? Yes ☐ No ☐ Unknown ☐
6. How many times has this animal calved? ☐
7. When did this animal last calve? Within the last 7 days ☐ Within the last 30 days ☐
   Within the last year ☐ Over 12 months ago ☐ Unknown ☐
8. Is this animal currently lactating? Yes ☐ No ☐ Unknown ☐
9. Is this animal pregnant at the moment? Yes ☐ No ☐ Unknown ☐
10. What stage of pregnancy is this animal? Early (<3 months) ☐ Mid (3-6 months) ☐
    Late (>6 months) ☐ Unknown ☐
11. How was this animal kept during the last dry season? Zero grazed ☐
    Herded with only your own animals ☐ Herded with animals from other herds ☐
    Tethered ☐ Other ☐
12. How this was animal kept during the last wet season? Zero Grazed ☐ Tethered ☐
    Herded with only own animals ☐ Herded with other peoples animals ☐
    Free Grazing ☐ Other ☐
13. In the last 12 months has this animal experienced any periods of ill health?
    Yes ☐ No ☐ Unknown ☐
14. What signs did you observe? Diarrhoea ☐ Hard faeces ☐ Fever ☐
    Staring Coat ☐ Lack of appetite ☐ Weight loss ☐ Other ☐
15. In response to this period of ill health, did you treat the animal with anything?
    Yes ☐ No ☐ Don't Know ☐

16. What treatment did you give this animal? Drench ☐ Tablet/bolus ☐

Pour-on Spray ☐ Dip ☐ Injection ☐

17. Who administered this treatment? Myself ☐ Another member of this homestead ☐
Government Vet ☐ Private Vet ☐ Animal Health Assistant ☐

18. In the last 12 months has this animal received ANY other treatment/prophylactic treatment?

Yes ☐ No ☐ Unknown ☐

19. What treatment was this? Tablet/bolus ☐ Drench ☐ Spray ☐
Pour-on ☐ Dip ☐

20. Who administered this treatment? Myself ☐ Another member of this homestead ☐
Government Vet ☐ Private Vet ☐ Animal Health Assistant ☐ Other ☐

21. Has this animal received any more treatments in the last 12 months?

Yes ☐ No ☐ Unknown ☐

22. If another reason is given for treatment with this drug please write it here?_____

23. In the past 12 months has this animal been visited by ANY animal health professional?

Yes ☐ No ☐ Unknown ☐

24. Who visited this animal? Government Vet ☐ Private Vet ☐ Animal Health Assistant ☐
Other ☐ Unknown ☐

25. Why did they visit this animal? Acute Injury ☐ Acute Sickness ☐ Dystocia ☐

To give advice on production ☐ Failure to thrive ☐ Worms ☐

26. For what other reason did they visit this animal? _____

SECTION II

27. Examine the animal from a distance: What is the animals' demeanor?

Normal Dull ☐ Unresponsive ☐ Not Evaluated ☐

28. Observe the animal at rest: What is the respiration pattern? Normal ☐

Exaggerated effort ☐ Fast respiration ☐ Not Evaluated ☐

29. Evaluate the hair coat : Normal Staring ☐ Abnormal ☐ Other ☐
Not Evaluated ☐

30. Observe and palpate the animal; what is the body condition score? 1 2 ☐ 3 ☐ 4 ☐
5 ☐ 6 ☐ ☐

31. Rectal temperature (in degrees C) _____

☐ ☐

32. Faecal consistancy : Normal ☐ Hard ☐ Diarrhetic ☐ Haemorrhagic ☐
Not Evaluated ☐

33. Skin Elasticity : Normal ☐ Mildly poor ☐ Very poor ☐ Not Evaluated ☐

34. Evaluated the Parotid Lymph Nodes: Normal ☐ Enlarged Unilaterally ☐
Enlarged Bilaterally ☐ Abcessed Unilaterally ☐ Abcessed Bilaterally ☐
Not Evaluated ☐

35. Evaluate the Suprascapular Lymph Nodes : Normal ☐ Enlarged Unilaterally ☐
Enlarged Bilaterally ☐ Abcessed Unilaterally ☐ Abcessed Bilaterally ☐
Not Evaluated ☐

36. Is there any nasal discharge? Yes ☐ No ☐ Not Evaluated ☐

37. What is the character of the nasal discharge? Mild serous unilateral ☐ Mild serous bilateral ☐
Moderate serous unilateral ☐ Moderate serous bilateral ☐
Severe serous unilateral ☐ Severe serous ☐

38. Is there any ocular discharge? Yes ☐ No ☐ Not Evaluated ☐

39. What is the character of the ocular discharge? Mild serous unilateral ☐ Mild serous bilateral ☐
Moderate serous unilateral ☐ Moderate serous bilateral ☐
Severe serous unilateral ☐ Severe serous bilateral ☐

40. Is there any genital discharge? Yes ☐ No ☐ Not Evaluated ☐

41. What is the character of the genital discharge? Mild serous unilateral ☐ Mild serous bilateral ☐
Moderate serous unilateral ☐ Moderate serous bilateral ☐
Severe serous unilateral ☐ Severe serous ☐

42. Age by dentition: 2 weeks-15months ☐ 15-18 months ☐ 18-24 months ☐
25-30 months ☐ 31-42 months ☐ Unknown ☐

43. Are any of the following tick species present?

Adult *Rhipicephalus* &appendiculatus: Severe ☐ Present ☐ None ☐
Not Evaluated ☐

Adult Amblyomma : Present ☐ Severe ☐ None ☐ Not Evaluated ☐

Adult Boophillus : Present ☐ Severe ☐ None ☐ Not Evaluated ☐

44. Are any of the following ectoparasites present?

Lice Present ☐ Severe ☐ None ☐ Not Evaluated ☐

Scabies Present ☐ Severe ☐ None ☐ Not Evaluated ☐

Fleas Present ☐ Severe ☐ None ☐ Not Evaluated ☐

45. Is there evidence of mastitis? Male animal ☐ Yes ☐ No ☐ Not Evaluated ☐

46. Girth Circumference (cm) _____

APPENDIX II: McMaster Technique – Cattle

PROCEDURE:
Provided 30th November 2009 by George Omondi as protocol followed by IDEAL

1. Take a rectal sample (ideally 5g minimum) into a suitable container (plastic bag, disposable glove, plastic pot).

2. Weigh 3g sample into a plastic beaker.

3. Add 42 ml tap water.

4. Homogenise until feaces is well mixed and /or pellets are completely broken up.

5. Pass through a tea strainer, collecting filtrate in a suitable container.

6. Mix filtrate and pour immediately into a centrifuge tube, leaving a small space at the top of the tube to facilitate mixing.

7. Centrifuge at 15 rpm for five minute.

8. Pour off supernatant and re-suspend pellet using a vortex mixer.

9. Add concentrated NaCl to original level tube.

10. Mix by inverting six times, allowing bubble to reach the bottom of tube each time.

11. Immediately fill the chamber(s) of a McMaster slide using a disposable pipette.
N.B It is important that the pipette is filled from the middle of the tube as soon as step 10 is completed and that the contents of the pipette are transferred to the McMaster slide immediately otherwise will already have started to float. Only the required amount should be filled into the pipette so that the entire sub-sample is placed in the McMaster slide.

12. Allow the slide to stand for two minutes to ensure that all the eggs have floated.

13. Using a dissecting microscope at x400 and a hand-held tally counter, count total number of eggs on the etched area of the slide. If no eggs are apparent in the etched area, the surrounding area should be examined.

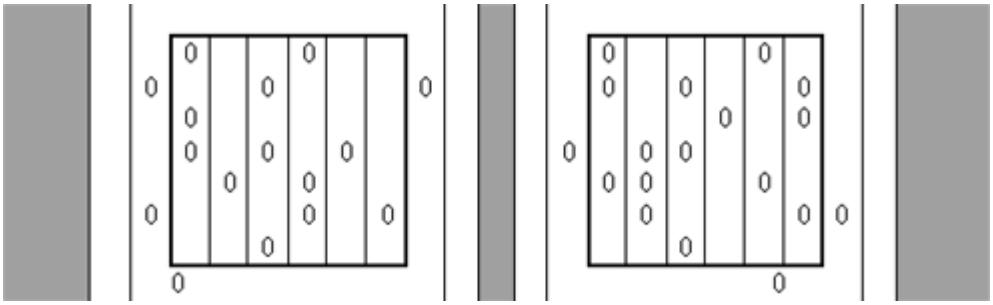
14. The number of eggs counted is multiplied by 100 for a single chamber McMaster slide (or a single chambered of a double chambered slide) or multiplied by 50 if both sides of a double chamber slide have been counted. If no eggs are observed in the etched area but eggs are present in the surrounding area, they should be recorded as an egg count of <50 or <100 depending on weather two or one chamber were counted.

15. Eggs should be differentiated and counted where possible: i.e. numbers should be recorded for trichostrongyle, Nematodirus spp, Toxocara, Trichuris, Capillaria, Strongyloides, Moniezia and Coccidia oocysts.

16. Results should be immediately entered in a lab book.

Microscopy Examination:

The number of eggs per gram can be calculated as follows:
Count the number of eggs within the grid of each chamber, ignoring those outside the squares.
Multiply the total by 50 – this gives the eggs per gram of faeces (e.p.g.)



For example: 12 eggs seen in chamber 1 and 15 eggs seen in chamber 2 = (12 +15) x 50 = 1350 e.p.g.

Do not delay reading the count beyond the recommended time as the flotation fluid may distort or destroy delicate eggs. Only process a few samples at a time and keep the filtrate in the second container in the refrigerator (4°C).

APPENDIX III: Faecal sample analysis form for cattle in Western Kenya.

Geo. Code	**Farm Bar code No.**	**Sample Bar code No.**	**Overall OPG**