RISK ASSESSMENT OF CYSTIC HYDATIDOSIS IN CATTLE SLAUGHTERED AT BUSIA TOWN ABATTOIRS -KENYA

BY

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DECLARATION

Declaration by the Candidate

This Thesis is my original work and has not been presented for a degree in any other University. No part of this thesis may be reproduced without the prior written permission of the author and/or Moi University.

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DEDICATION

This work is dedicated to my mother Elizabeth Ogutu, my wife Dr. Ruth Akinyi, my daughter Paula Achien'g Ogutu, my sons Paul Omondi Ogutu and Henry Joash Ogutu Junior and all my siblings Eunice Omwandho, Joseph Ogutu, Meresa Makoyo, Judith Ogutu and Emmaculate Ogutu. You were always available for me with constant encouragements throughout this journey.

ABSTRACT

Introduction: Hydatidosis, a re-emerging parasitic zoonosis caused by larval stage of *Echinococcus* is endemic in South America, Asia and East Africa including Kenya. Globally, hydatidosis causes economic losses of more than three billion United States Dollars (USD) annually and in Kenya, losses are more than 240,000 USD. Known risk factors for transmission of *E. granulosus* include allowing dogs to roam freely, feeding dogs on infested viscera, slaughtering animals at home which lead to improper disposal of infested organs and carcasses, drinking non-boiled water, eating raw vegetables, failing to wash hands before meals, presence of wild carnivores near homesteads, low knowledge, attitude (KAP) and poor practices and uncontrolled movement of livestock from endemic to non-endemic areas. Busia offers livestock market for Kenya and Uganda.

Objectives: The study estimated prevalence, identified possible risk of CH to Busia, and assessed KAP among cattle owners, traders and abattoir workers.

Methods: A cross sectional study was conducted on cattle slaughtered in two Busia town abattoirs between May and June 2018. In-person interviews were done using a structured questionnaire to assess KAP of participants on hydatidosis and establish origin of each slaughtered animal. Routine meat inspection was done to determine CH infestation status of carcasses. Whole cysts were removed and put in labelled and zipped polythene bags for confirmation using polymerase chain reaction (PCR). Data were analyzed using Microsoft Excel and Epi info to calculate proportions, 95% confidence intervals and do logistic regressions for associated factors respectively. We used bivariate and logistic regression to examine factors associated with knowledge on CH among study participants.

Results: A total of 302 carcasses; 222 (73.51%) males and 80 (26.49%) females were inspected and 310 questionnaires administered to participants. Nine (2.98%, 95% CI: 1.46-5.78) carcasses were positive for hydatidosis; eight (88.89%) carcasses were female and five of the nine positive cases (55.56%) had multiple organ infestations. Main infested organs were liver (n=7) and lung (n=4). Total samples collected were 14, of which 13 (92.86%) were positive on PCR test. All the positive carcasses were from West Pokot County which was a major risk. Male participants were 260 (83.87%, 95% CI: 79.19 – 87.69); median age was 41 years (range = 21-69). Participants with adequate knowledge were 40 (12.90%) and with good attitude were 123 (39.68%). Dog keepers were 221 (71.99%, 95% CI: 66.55 – 76.87) of which 83 (37.56%, 95% CI: 28.33 – 48.52) improperly disposed of dog faeces. Home slaughtering was practiced by 196 (63.23%, 95% CI: 58.78-69.80); 115 (58.67%, 95% CI: 51.44-65.64) were not inspected and 85 (43.37%, 95% CI: 36.32-50.62) of raw organs fed to dogs.

Conclusions: The study reported a prevalence of 2.98% for CH in Busia, however all cases were imported from West Pokot. The imported cattle from West Pokot via cross county trade were a major risk factor for introducing the parasite to Busia. Furthermore, communities in Busia are unfamiliar with CH and engage in practices that may increase their risk of infestation.

Recommendations: To reduce the risk of introducing the parasite into Busia, proper meat inspection should be done and infested organs or carcasses be condemned and properly disposed of as animals from endemic areas are screened for CH before being allowed for slaughter in Busia County. Busia communities need public health education to improve their KAP on CH and also to practice responsible dog ownership. Future studies can focus on prevalence of CH in humans and dogs in Busia.

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DEFINITION OF TERMS

When reading this thesis, the following are the meaning of the words used.

Assessment is the act of judging or deciding the amount, value, quality, or importance of something.

Attitude is having an opinion, thinking or feeling about something or someone whose risk is already known.

Butchery is a place or shop where meat is cut and prepared for sale.

Control is to reduce the incidence or severity of something, especially a disease in a population.

Economic losses are losses due to condemned organs, carcasses, reproduction wastages, reduced hides value and quality, reduced milk production, losses due to resources spent on treatment and patient maintenance and also losses due to reduced quality of human life which affects human labor with regard to hydatidosis.

Flayer is a person who removes or strips off the skin or hides of slaughtered animals in the abattoir.

Hot spot is a region which is endemic for cystic hydatidosis and shares socio-economic interactions with Busia through livestock trade or movement hence pose a risk of transmission of the disease to Busia.

Hydatid Cyst is the larval cyst of a tapeworm (genus *Echinococcus*) occurring as a fluid-filled sac having a distinct membrane and developing in an organ or muscle of the body which may be fertile or infertile containing daughter cysts in which scolices develop.

Knowledge is the state of having facts, information, skills or being aware of something through experience or association.

Meat trader is a person who dresses meat for market or sale.

Practice is to perform or exercise a skill repeatedly or regularly in order to improve or maintain something or a condition.

Prevention includes all measures that limit the progression of disease at any stage of its course.

Risk is the probability of an event to occur during a specified period of time.

Surveillance is the ongoing, systematic collection, recording, analysis, interpretation and dissemination of data on disease and disease trends in a population for use in the control of a disease.

ABBREVIATIONS AND ACRONYMS

AH	Alveolar Hydatidosis
CDC	Centres for Disease Control and Prevention
CDVS	Director of Veterinary Services
CH	Cystic Hydatidosis
CI	Confidence Interval
DALY	Disability-Adjusted Life Year
DNA	Deoxyribonucleic Acid
ELISA	Enzyme Linked Immuno-sorbent Assay
GDP	Growth Domestic Product
ILRI	International Livestock Research Institute
IREC	Institutional Research and Ethics Committee
KAP	Knowledge, Attitude, Practice
KNBS	Kenya National Bureau of Statistics
LAMP	Loop Mediated Isothermal Amplification
MALFD	Ministry of Agriculture, Livestock and Fisheries Development
MoH	Ministry of Health
PAIR	Puncture-Aspirate-Inject-Re-aspirate
PCR	Polymerase Chain Reaction
PHE	Public Health Education
PTA	Percutaneous Thermal Ablation
USA	United States of America
USD	United States Dollar

UV	Ultra Violet
WHO	World Health Organization
$	United States Dollar

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CHAPTER ONE

INTRODUCTION

1.1 Background

Hydatidosis is a neglected parasitic zoonotic disease caused by larval stage of *Echinococcus* species, also known as dog tape worm. Its life cycle involves carnivores (dogs), livestock and humans (Grosso *et al.,* 2012; Dinkel *et al.,* 2004). There are four species of Echinococcus, but only two species are of public health importance (Pedro 2015). These two species include; *Echinococcus granulosus* (Thatcher and Sousa, 1966; Boubaker *et al*., 2013) which causes cystic hydatidosis (CH) and commonly occurs in tropical regions, *E. multilocularis,* (Jabbar *et al*., 2011; Tackmann *et al*., 1998) which causes alveolar hydatidosis (AH) and occurs in the temperate regions. These species have a wide range of intermediate hosts, but *E. granulosus* affects ungulates as its prime intermediate host hence the significance in livestock. *E. granulosus* has ten genotypes represented as (G1-G10), (Alvarez *et al*., 2014). Three genotypes (G1-G3) have been found in China, (Yan *et al*., 2013; Bart *et al*., 2006). Different strains infests different host species (Maillard *et al*., 2011; Wahlers *et al*., 2012; WHO report, 2011; Httner & Romig, 2009; Dinkel *et al*., 2004). Other species of *Echinococcus* include *E. vogeli,* (Rausch *et al*., 1981) and *E. oligarthrus,* (Patkowski *et al*., 2017).

To design an effective control program for cystic hydatidosis, detailed epidemiological data that identify risk factors for the spread of the disease are required. Furthermore, information on the knowledge, attitude and practices (KAP) of the livestock value chain actors is needed since this influences their level of participation in any intervention programs that may be instituted.

1.2 Distribution of the Disease

Hydatidosis has been reported in all the countries in Europe except Ireland, Iceland and Denmark. It is endemic in the Mediterranean areas and Eastern Europe such as Bulgaria, (Grosso *et al*., 2012). In Asia the parasite is endemic in China and re-emerging in the former Soviet Republics, (Elzein *et al*., 2016). It is also found throughout the Indian Subcontinent and the Middle East. In south eastern Australia, infection prevalence in wild dogs populations may reach 100% with worm burdens in excess of 100,000 worms (Jenkins and Morris, 2003). In Africa, *E. granulosus* is a particular problem in Tunisia, Morocco, Libya and Algeria and it is of specific concern in Kenya, Turkana (Macpherson *et al.,* June 2002).

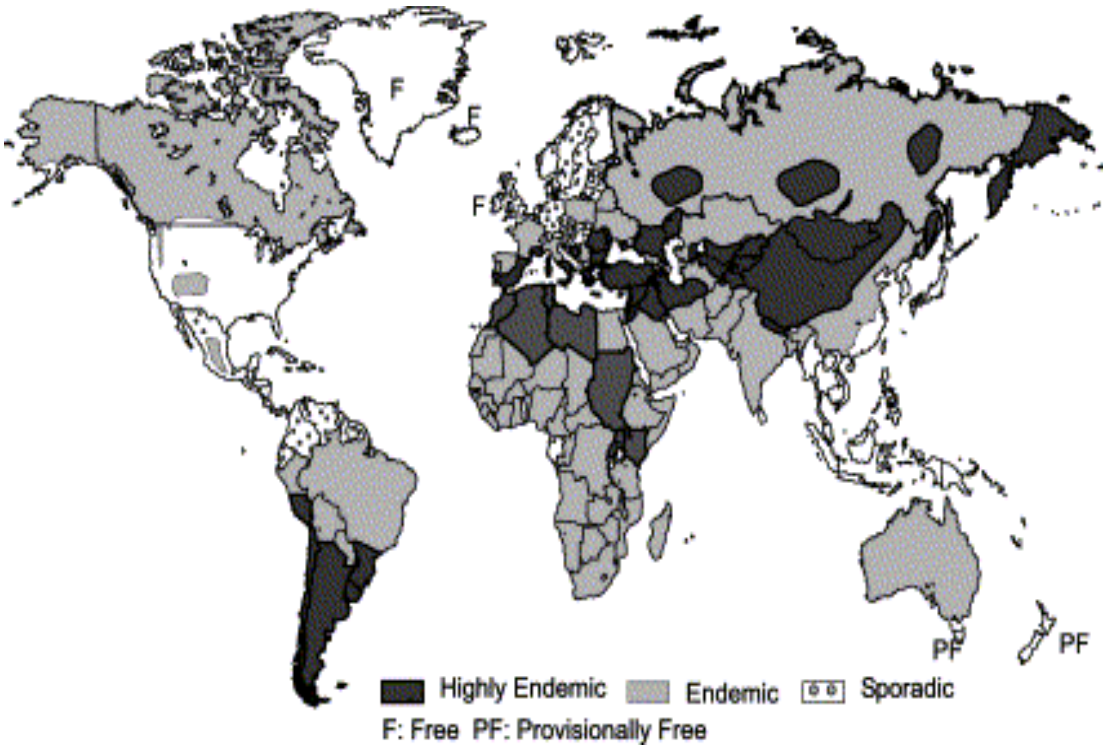

Fig.1.1: Geographical distribution of the zoonotic strains of *E. granulosus*. Adapted from *Eckert et al.*, 2000 and Eckert *et al.*, 2001.
Source: Institute für Parasitologie, Universität Zürich.

1.3 Health and Economic losses

1.3.1 The burden of human hydatid disease

Human CH infestation ranges from less than 1 person per 100,000 to more than 200 persons per 100,000 in certain rural populations like in Tibet where there is close contact between humans and domestic dogs, (Berbri *et al*., 2015). Worldwide, there may be an excess of one million people living with hydatid disease at any one time, (WHO report, 2011). More than half of these people experience serious clinical syndromes which are life-threatening if left untreated. Even with treatment, people often face reduced quality of life (WHO report, 2011). For CH, there is an average of 2.2% post-operative death rates for surgical patients and about 6.5% of the cases always relapsing after intervention that requires prolonged recovery times. Estimates suggest that CH results in the loss of at least one million disability life adjusted years (DALYs) annually. Alveolar hydatidosis results in the loss of about 650 000 DALYs annually, (WHO report, 2011). Total cost for treating the current infested population is estimated at U.S. $1,507,224 (95% CI = U.S.$ 525,737–2,496,698), with a per capita lifetime cost of U.S.$23.94 (95% CI = U.S.$8.30–39.38) and an annual cost of U.S.$32,788 (95% CI = U.S.$11,120–54,215), equating to a loss of approximately 0.2% of per capita GDP each year (WHO report, 2011). So far there is no clear data on the hydatidosis burden in humans in Kenya much as a lot of studies have been done in humans in Turkana and some parts of Maasailand (Njoroge *et al*., 2002).

1.3.2 Livestock hydatid disease burden

Depending on the species involved, effects of *E. granulosus* on livestock production include death, reduction in carcass weight, decrease in hide value, decrease in milk production, reduced fertliity and condemnations of infested carcasses (Gebremichael *et al*., 2013; Budke *et al*., 2005).

Estimated livestock related losses in studies done in Australia and Ethiopia, with 95% CIs, associated with *E. granulosus* infestations showed that infested livers alone equated to U.S. $185,635 (CI = U.S. $167,793–205,389) per year. Annual animal losses due to hydatidosis were USA $218,676 (CI = U.S.$189,850–247,871), when only liver-associated losses in livestock are assumed. This equates to approximately U.S.$3.47 per person annually or 1.4% of *per capita* GDP. The infestation of the livers and lungs account for almost 90% of organ cases however, the disease can infest any organ in the body, (Mirzaei *et al*., 2015).

A survey done in slaughter houses in Dodoma, Tanzania in December 2013 indicated that a total of 9015 (10.5%) lungs, 6276 (7.3%) intestines, 5402 (6.3%) livers, 3291 (3.8%) kidneys and 41 (0.05%) carcasses were condemned. Pulmonary emphysema (3.4%), fasciolosis (4.5%), pimply gut (5.7%), kidney congenital cysts (1.9%) and hydatidosis (3.1%) were major causes of organ condemnations leading to economic losses estimated at $9,892. Condemnations of organs and carcasses represent a vital loss of meat and revenue which have negative effects on the livestock industry. The estimated economic losses justify reasons for appropriate surveillance and disease control programs (Tembo *et al*., 2013).

1.4 Problem Statement

The impact of CH among pastoral communities has long been recognized all over the world. The disease is a public health problem affecting around two to three million people worldwide in extensive livestock farming areas (WHO report, 2011). It was responsible for animal deaths in 2012 which contributed to economic losses estimated at three billion dollars ($) in intervention costs, livestock organ condemnations and reduced livestock productivity (Dawit *et al*., 2013).

In Kenya, especially among the Turkana, Pokot and Maasai communities, the prevalence of *E. granulosus* is estimated as 3.6% to 25.8% and infests goats, sheep and cattle. A study conducted in slaughtered animals in three divisions of northern Turkana on a total of 5752 goats, 588 sheep and 381 cattle, recorded a prevalence of 19.4% in cattle, 3.6% in sheep and 4.5% in goats (Njoroge *et al*., 2002). Another study conducted in slaughter slabs in Narok recorded a prevalence of 25.8 % in cattle (151/587), 16.5 % in sheep (71/430) and 10.8 % in goats (21/194), which showed a significant increase compared to surveys done in the past (Addy, 2012). In this study, majority of cysts were reported to occur in the liver (56 % in cattle, 70 % in sheep and 65 % in goats). The differences in prevalence rates in different study areas have been attributed to differences in livestock stocking density, environmental conditions and cross-border migration of livestock, some of which are infested with hydatidosis. Cross border movements of infested livestock is suspected to be contributing to the spread of this parasite (Odero, 2015). This study was conducted to identify if such free movements of livestock from perceived endemic areas could contribute to the introduction of the parasite into Busia County.

1.5 Study Justification

Busia provides market for livestock from endemic areas like Turkana, Maasailand, Pokot and Uganda through trade. It is feared that the parasite may be disseminated along livestock marketing chains hence putting Busia at risk of infestation (Odero, 2015). According to Animal Disease Act CAP 364 of the laws of Kenya, animal movements across borders is restricted by issuance of movement permits and no objection certificates, but this restriction is sometimes not obeyed by traders and value chain actors in livestock trade industry. This leads to spread of diseases to regions where such diseases have never been reported. Spread of such diseases leads to economic losses to

traders and farmers due to deaths and condemnations of infested or infected organs. The migration of pastoralists during drought also makes it difficult to control movement of livestock from one region to another in Kenya.

There are few or no published studies on prevalence of CH in Busia, risk factors for spreading hydatidosis and level of KAP on hydatidosis in cattle among livestock value chain actors in Busia, though these are very important strategies in controlling the disease.

1.6 Use of Study Results

The study provided baseline data on hydatidosis for future studies in Busia. The results are relevant to guide the Ministry of Health (MOH), department of public health and Ministry of Agriculture, Livestock and Fisheries (MALF) to recommend for information sharing between human and animal handlers both at farm and abattoir levels to improve case management, treatment and eliminate sources of infections. The results may be used to help veterinary authorities to develop control strategies in livestock and create awareness and formulate policy to help in control and prevention of this disease.

1.7 Research Questions

How does cross county trade on livestock contribute to the spread of CH between Busia and other perceived endemic areas?

1.8 Objectives

1.8.1 Broad objective

To conduct risk assessment of CH among cattle slaughtered at Busia Town abattoirs.

1.8.2 Specific objectives

1. To estimate the prevalence of cystic hydatidosis among cattle slaughtered at Busia Town abattoirs.
2. To determine risks factors for introducing CH to Busia Town.
3. To assess the KAP of beef value chain actors in Busia Town abattoirs on CH.

CHAPTER TWO

REVIEW OF LITERATURE

2.1 Description of the Parasite

Echinococcus belongs to the family of *Taeniidae* consisting of two genera; *Echinococcus,* and *Taenia,* (Nakao *et al*., 2010). Adult *Echinococcus* is rarely more than 7mm long and usually has not more than six segments. It has flat segmented body with anterior scolex and posterior strobila. Adults are hermaphroditic, lacking gut and all metabolic interchanges occur across the syncytial outer covering known as the tegument (Thompson and Mcmanus, 2012).

2.1.1 Classification of *Echinococcus*

Table 2.1: Classification of *Echinococcus*

Classification level	Nomenclature
Kingdom	*Animalia*
Phylum	*Platyhelminthes*
Class	*Cestodae*
Order	*Cyclophyllidea*
Family	*Taeniidae*
Genus	*Echinococcus*
Species	*E. granulosus, E. multilocularis, E. oligarthrus* and *E. vogeli*

Source: The Taxonomicom (Last updated: 07.04.2012) (Date accessed: 17.06.2016)

2.2 Life cycle and Transmission cycle of *Echinococcus*

Understanding the etiology of diseases demands appreciation of the life cycle of the etiological agents and their transmission routes. Comprehensive understanding of the

sylvatic and domestic cycles is pivotal to the diagnosis, control and treatment of the parasites and the diseases they cause. Studying the life cycle of parasites with different manifestations in different classes of hosts and whose life cycles are enhanced by transmission cycle is of scientific importance. *Echinococcus* species is perpetuated in a life cycle requiring two groups of mammals in a prey-predator relationship to complete a cycle as shown in Figure 2.1 (Nakao *et al*., 2010). Stage one of the disease is a sylvatic stage involving carnivores like dogs (definitive hosts) which harbor the hermaphroditic adult parasites in the intestines while herbivores and omnivores play the intermediate hosts. The definitive host passes on the parasite to intermediate host by releasing the infective larvae in their feces (stage 2) into the environment. Intermediate hosts like livestock and accidental or aberrant hosts like human get infested with the larvae via oral route during feeding. After ingestion by a suitable intermediate host, the egg hatches in the intestine and releases an oncosphere that gets attached to the intestinal mucosa (stage 3) (Nakao *et al*., 2010).

The oncospheres penetrate the intestinal wall and enters the portal blood/lymph where they are transported passively throughout the body to major filtering organs mainly liver and lung. After localizing in an organ, the parasite develops into larval hydatid cyst (stage 4) as unilocular fluid-filled bladder (Zanco 2003). These consist of two parasite-derived layers; an inner nucleated multipotential germinal layer and an outer acellular laminated layer surrounded by a host-produced fibrous capsule. The hydatid cyst at this stage may contain numerous tiny tapeworm heads called protoscolices or brood capsules filling the cyst interior. Brood capsules or protoscolices evaginate from the germinal membrane. They increase in number over time via asexual/clonal reproduction. Human activities and behavior, politics and the presence of wildlife

reservoirs influence transmission of *Echinococcus*, particularly within the domestic situation.

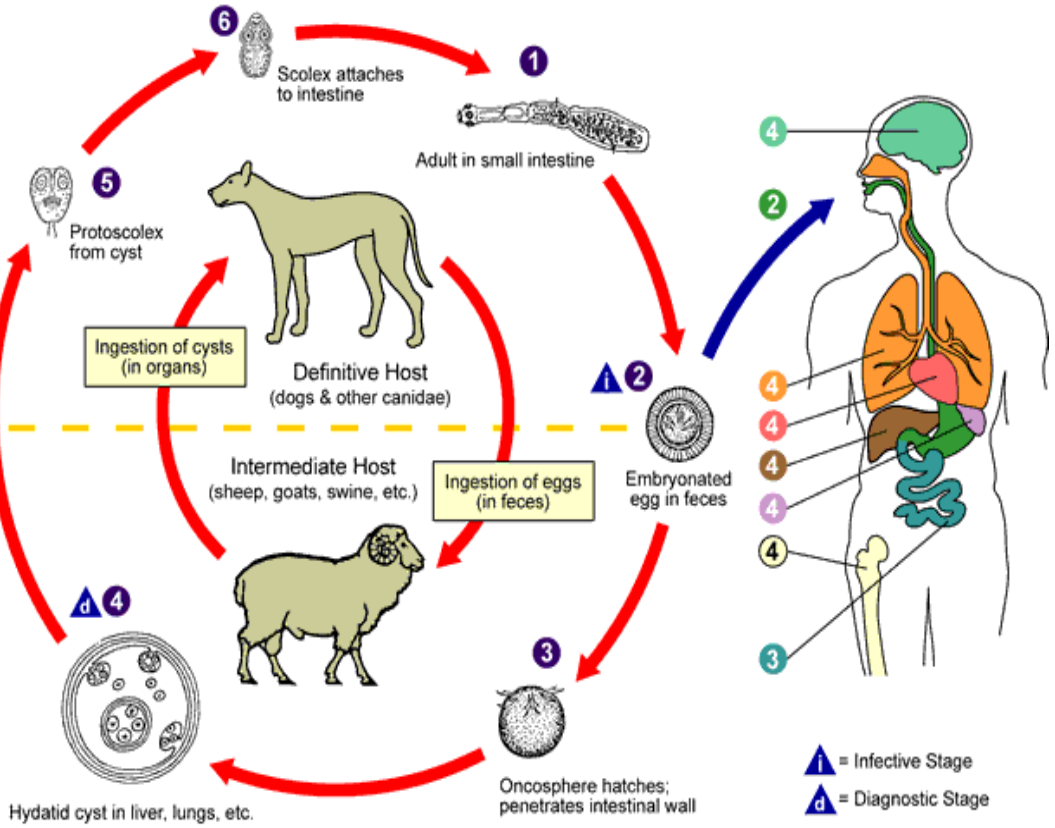

Figure 2.1. Life cycle and cycle of transmission of *Echinococcus* species
Source: (http//www.dpd.cdc.gov/dpdx) [Last updated: 20.08.2012] [Date accessed: 16.08.2018]

The growth rate of cysts is highly variable and may depend on strain differences (Eckert *et al*., 2001); however they all share the unique hermaphroditic and clonal reproduction systems (Casulli *et al*., 2012). The larvae of *Echinococcus granulosus* enlarge in size in connection with the asexual reproduction of scolices in the bladder-like cyst. Despite the primary infestation route, a secondary echinococcosis can occur within an intermediate host. Secondary infestation is caused by spontaneous trauma or during medical interventions where the larval tissue proliferates after being spread from the primary site of the metacestode (Thompson *et al*., 2001). The parasite's life cycle is completed when the intermediate host dies and a carnivore consumes the organs

containing parasite cysts. The ingested scolices (stage 5 and 6) attach to the intestinal mucosa and develop into egg-producing adult tapeworms consisting of a chain of proglottids with genital organs. Proglottids and eggs released from the adult worm initiate new life and transmission cycles. In many cases, the definitive host does not suffer adverse effects, even with a relatively heavy parasite burden. Current studies show that in ruminants, there is significant difference found in prevalence rates of the various ruminants, and also between the sexes. Male ruminants are less likely to be infested than female animals and the infestation rates of different ruminants increase significantly with age, ($p<0.05$), (Mirzaei *et al*., 2015; Assefa *et al*., 2015).

2.3 Signs and Symptoms of Cystic Hydatidosis

Human infestation with cystic hydatidosis leads to the development of one or many cysts located mainly in the liver and lungs, and sometimes in the heart, bones, kidneys, spleen, muscles, central nervous system and eyes, (Imad 2014). The asymptomatic incubation period of the disease can last many years until hydatid cysts grow to an extent that presents clinical signs. Non-specific signs include anorexia, weight loss and weakness. Other signs of the disease depend on the location and size of the cyst(s) and the pressure it exerts on the surrounding tissues. Abdominal pain, nausea and vomiting are commonly seen when hydatids occur in the liver. If the lung is affected, clinical signs include chest pain, shortness of breath and chronic cough (Imad 2014; Derbel *et al*., 2012).

2.4 Diagnosis of Cystic Hydatidosis

The best means to diagnose hydatidosis in definitive hosts is the demonstration of the adult worm in the intestine at post mortem or in the mucus after a diagnostic test (arecoline purgation), or finding the proglotids (tapeworm segments) in faeces,

(Schwarz *et al*., 2017). In intermediate hosts, cysts are often detected during meat inspection or by ultrasound examination but specificity of imaging is poor (<40%) (Macpherson, 1983). Serological tests for cystic hydatidosis in cattle, sheep and pigs are not used routinely because of variable sensitivity and specificity (Ammann *et al*., 2004).

A single-tube multiplex PCR assay has been established to differentiate the *Echinococcus species* responsible for infestations in intermediate and definitive hosts. Primers specific for *E. granulosus* and *E. multilocularis* are designed based on sequences of the mitochondrial dehydrogenase subunits. This multiplex PCR accurately detects *Echinococcus* DNA without generating nonspecific reaction products. Specificity of the multiplex PCR is 100% when evaluated using DNA isolated from other cestodes (Han *et al*., 2019). However, when using Echinococcus eggs obtained from fecal samples of infected dogs, the sensitivity of the multiplex PCR is low (<40%), (Boubaker *et al*., 2013). The major advantage of this multiplex-PCR method is that it does not involve a second step of purification resulting in a simpler and more economical method, (Petrigh and Fugassa, 2013). The positivity rates obtained for the field-collected fecal samples are higher using loop-mediated isothermal amplification (LAMP) than PCR and copro-ELISA. Compared with the conventional PCR, the LAMP assay provides 88.8% specificity and 100% sensitivity, (Ni *et al*., 2014).

The higher sensitivity of the LAMP method has also been shown by the fact that it could detect the presence of laboratory challenges of dog infestations with *E. granulosus* four days earlier than the multiplex PCR method (Ni *et al*., 2014). The overall sensitivity and specificity of diagnosis by ELISA for cystic echinococcosis are

78.3% (47/60), and 98.3% (59/60), respectively (Ni *et al*., 2014). The cross reaction with sera of alveolar echinococcosis is 40.5% (15/37), (Jalousian *et al*., 2017). Another diagnostic test used in dog faeces is fecal floatation which has a sensitivity and specificity of 78 % (25/32) and 93 % (215/229) as was revealed in a study in Moroto and Bukedea districts in Uganda, (Oba *et al*., 2016).

2.4.1 Polymerase Chain Reaction

Polymerase chain reaction is a method developed by Mullis in 1983 to analyze a short sequence of DNA or RNA even in samples containing only minute quantities of DNA or RNA, (Boubaker *et al*., 2014). It is a simple, yet elegant, enzymatic assay, allowing for the amplification of a specific DNA fragment from a complex pool of DNA. Dr. Kary Mullis, who discovered the PCR assay, stated it "lets you pick the piece of DNA you're interested in and have as much of it as you want" (Mullis, 1990).

Polymerase chain reaction-based strategies have propelled huge scientific endeavors such as the Human Genome Project. The technique is currently widely used by clinicians and researchers to diagnose diseases, clone and sequence genes, and carry out sophisticated quantitative and genomic studies in a rapid and very sensitive manner. One of the most important medical applications of the classical PCR method is the detection of pathogens. In addition, the PCR assay is used in forensic medicine to identify criminals. Due to its widespread use, it is important to understand the basic principles of PCR and how its use can be modified to provide for sophisticated analysis of genes and the genome. PCR can be performed using source DNA from a variety of tissues and organisms, including peripheral blood, skin, hair, saliva, and microbes. In this test, only trace amounts of DNA are needed for PCR to generate enough copies to be analyzed using conventional laboratory methods, (Garibyan and Avashia, 2013).

For this reason, PCR is a sensitive assay. Each PCR assay requires the presence of template DNA, primers, nucleotides, and DNA polymerase. The DNA polymerase is the key enzyme that links individual nucleotides together to form the PCR product. The nucleotides include the four bases – adenine, thymine, cytosine, and guanine (A, T, C, G) – that are found in DNA. These act as the building blocks that are used by the DNA polymerase to create the resultant PCR product. The primers in the reaction specify the exact DNA product to be amplified. The primers are short DNA fragments with a defined sequence complementary to the target DNA that is to be detected and amplified. These serve as an extension point for the DNA polymerase to build on. The above mentioned components are mixed in a vacutainer and then placed in a machine that allows repeated cycles of DNA amplification to occur in three basic steps. The machine is essentially a thermal cycler. It has a thermal block with holes, into which the test tubes or plates holding the PCR reaction mixture are inserted. The machine raises and lowers the temperature of the block in discrete, precise and pre-programmed steps (Weier and Gray, 1988).

The initial step is the denaturation or separation of the two strands of DNA molecule. This is accomplished by heating the starting material to temperatures of about 95°C for one minute. Each strand is a template on which a new strand is built. In the second step, the annealing step, the temperature is reduced to about 55ºC for forty five seconds so that the primers can anneal to the template. In the third step, the extension step, the temperature is raised to 72ºC for two minutes and the DNA polymerase begins adding nucleotides onto the ends of the annealed primers. At the end of the cycle, which lasts about five minutes, the temperature is raised and the process begins again. The number

of copies doubles after each cycle. Normally 25 to 30 cycles produce sufficient amount of DNA, (Garibyan and Avashia, 2013).

2.5 Surveillance of Cystic Hydatidosis

Surveillance of cystic hydatidosis in animals is complex because the infestation is asymptomatic in livestock and dogs. Surveillance is so far not recognized or prioritized by communities or local veterinary service authorities. Therefore infected animals are normally noticed during meat inspection (after slaughtering) or during post mortem (Macpherson, 1983). Modern techniques can be used for surveys; the copro-antigen ELISA to detect *E. granulosus* in dog populations and ultrasonography in combination with 21 serology for mass diagnosis of CH in humans (Craig *et al*., 2016; Schantz, 2005; Christofi *et al*., 2002; Ricciardi and Ndao, 2015).

2.6 Risk of spreading Cystic Hydatidosis in Kenya

The non-pastoral communities in most parts of the world are unfamiliar with the clinical manifestation and economic impact of cystic hydatidosis. This is due to poor knowledge, low level of awareness regarding the prevention measures and risk practices on meat hygiene and dog management that could contribute to spread and persistence of the disease in the environment (Khan *et al*., 2018a; Othieno *et al*., 2018).

In Kenya, interactions between different communities through livestock trade, movement of livestock from one place to another for cultural obligations including use of livestock to pay dowry and other cultural functions, improper disposal of infested organs and dog faeces and failure to deworm dogs and livestock potentially lead to spread of hydatidosis from endemic areas to areas where the disease has never been reported. According to the current studies, it is feared that the parasite is disseminated along livestock marketing chains (Odero, 2015). This may pose risk to human and

livestock population in Busia, being that it offers market to livestock from endemic areas.

2.7 World Health Organization response on Cystic Hydatidosis

World Health Organization assists countries to develop and implement pilot projects leading to the validation of effective CH control strategies by 2020. Working with the veterinary and food safety authorities as well as with other sectors is essential to attain the long-term outcomes of reducing the burden of disease and safeguarding the food value chain (WHO report, 2011). World Health Organization supports capacity building through training courses targeting medical and paramedical personnel, focused on the clinical management of cystic echinococcosis in rural areas of affected countries, (WHO report, 2011). Morocco is piloting a project aimed at decentralizing diagnostic and therapeutic techniques and promoting the puncture, aspiration, injection, re-aspiration (PAIR) strategy in rural and hyper-endemic areas. Mongolia has recognized the importance of echinococcosis as a public-health problem and, at the request of the Ministry of Health, WHO in 2010 conducted an initial situation analysis. The analysis focused on implementing early diagnosis and building a basic surveillance system covering humans and animals to understand the actual burden of the disease (WHO report, 2011). No significant investment for echinococcosis has been made and programmed progress has therefore stalled. China is integrating echinococcosis prevention, control and treatment in their economic and development plans to raise attention to the vast problem in the country, and especially in the Central Asian Republics. Early detection of *E. granulosus* and *E. multilocularis* infestations, especially in low-resource settings, is still needed in addition to the evaluation of clinical treatment options. Further assessment and potential commercialization of a vaccine for *E. granulosus* recombinant oncosphere antigen (EG95) is on trial in sheep

to impede *E. granulosus* infestation of lambs. This could supplement control measures such as the treatment of dogs and culling of older sheep. One DALY can be thought of as one lost year of "healthy" life. The sum of these DALYs across the population, or the burden of disease, can be thought of as a measurement of the gap between current health status and an ideal health situation where the entire population lives to an advanced age free of disease and disability, (WHO report, 2011).

2.8 Treatment of Cystic Hydatidosis

For simple cases of cystic hydatidosis, the most common form of treatment in humans is open surgical removal of the cysts combined with chemotherapy using albendazole and or mebendazole before and after surgery, (Luo *et al*, 2013; Erzurumlu *et al*., 2000). However, if there are cysts in multiple organs or tissues, or the cysts are in risky locations such as the brain, surgery becomes impractical. For inoperable cases, chemotherapy and or puncture-aspirate-injection-reaspiration (PAIR) become alternative options of treatment, (Patkowski *et al*., 2017; Da Silva, 2010). In the case of alternative treatment using just chemotherapy, albendazole is preferred twice a day for 1–5 months, (Keshmiri *et al*., 2001).

Medical treatment implemented at doses between 800 and 1,200 mg/day (10–20 mg/kg day) for 3–4 months achieves cure rates of hepatic cysts that vary from 28.5% to 43%, with a rate of relapse between 3% and 22%, whereas the cure rates of pulmonary hydatid cysts reach 73%, (Alvela-Suárez *et al*., 2014). In addition, medical treatment with albendazole before surgery reduces relapses for CH, (Gollackner *et al*., 2000). An alternative to albendazole is mebendazole for at least 3 to 6 months. PAIR is a minimally invasive procedure that involves three steps: puncture and needle aspiration of the cyst, injection of a scolicidal solution for 20–30 minutes, and cyst-re-aspiration

and final irrigation. Patients who undergo PAIR typically take albendazole or mebendazole from 7 days before the procedure until 28 days after the procedure (Alvela-Suárez *et al*., 2014; Luo *et al*., 2013). While open surgery still remains the standard for cystic hydatidosis treatment, there are studies which suggest that PAIR with chemotherapy is more effective than surgery in reducing disease recurrence, morbidity and mortality. In addition to the three above mentioned treatments, there are currently research and studies looking at new treatment involving percutaneous thermal ablation (PTA) of the germinal layer in the cyst by means of a radiofrequency ablation device. This form of treatment is still relatively new and requires much more testing before being widely used, (Rigter *et al*., 2004).

An alternative to open surgery is laparoscopic surgery, which is an example of excellent cure rates with minimal morbidity and mortality, (Schipper *et al*., 2002). Radiofrequency thermal ablation has also proved to be a safe method to destroy the germinal layer. This method can be done by percutaneous approach using a kind of needle-electrode employed in the ablation of liver tumors. However, further investigations need to be carried out before it can be recommended as an effective percutaneous treatment (Da Silva, 2010).

2.9 Prevention and Control of Cystic Hydatidosis

Hydatidosis is preventable through various ways which target interruption of the life cycle. Since the route of infection is hand to mouth, hygiene and frequent hand washing for at least 20minutes using potable water constitutes an important preventive measure. *E. granulosus* can be prevented by preventing dogs from accessing livestock carcasses or slaughter wastes from farms, households, abattoirs or butchers; deworming dogs with anti-helmintics (praziquantel) to kill the adult tapeworm; detecting cysts at meat

inspection, thus targeting infected farms or communities and vaccinating sheep (or other livestock) to protect against the development of the larval stage of *E. granulosus.* The cycle of *E. granulosus* in wildlife is not easy to control, but by discouraging scavenging, and implementing hygiene, the infestation to domestic animals and spread to humans can be reduced.

Controlling *E. multilocularis* in wildlife is difficult because of the cycle between foxes and rodents, but reduction in transmission has been achieved through use of praziquantel baits to foxes and dosing of owned dogs where spill-over into the dog population occurs. There are different strategies that are currently being used to prevent and control cystic hydatidosis. Most of these methods try to prevent and control cystic hydatidosis by targeting the major risk factors for the disease and the way it is transmitted. For instance, improved water sanitation attempt to target poor education and poor drinking water sources and health education programs focused on CH and its agents which are both risk factors for contracting hydatidosis (Craig *et al*., 2016).

Furthermore, since humans often come into contact with *Echinococcus* eggs via touching contaminated soil, animal faeces and animal hair, another prevention strategy is improved hygiene (Craig *et al*., 2016). In addition to targeting risk factors and transmission, control and prevention strategies of cystic hydatidosis also aim at intervening at certain points of the parasite's life cycle, in particular, the infestation of hosts like dogs that reside with or near humans. Vaccination of sheep with an *E. granulosus* recombinant antigen (EG95) offers encouraging prospects for prevention and control. Small-scale EG95 vaccine trials in sheep indicate high efficacy and safety with vaccinated lambs not becoming infested with *E. granulosus*. A program combining vaccination of lambs, deworming of dogs and culling of older sheep could lead to

elimination of cystic echinococcosis disease in humans in less than 10 years, (Craig *et al*., 2016). Boiling livers and lungs that contain hydatid cysts for at least 30 minutes has been proposed as a simple, efficient and energy- and time-saving way to kill the infectious larvae. Prevention programs involve deworming of dogs, improved food inspection and slaughterhouse hygiene, and public education campaigns, (Craig *et al*., 2016).

The control program consists of three main components namely, at the human intermediate host level, mass screening and treatment through surgery, PAIR and chemotherapy can be applied. At the definitive host level, controlling dog population through humane killing of stray dogs, sterilization of female dogs before their first breeding age and regular deworming of all owned dogs are important control measures. Community public health education to create awareness on the transmission mode and dangers of the infestation is a key control and prevention measure. The disease is more prevalent in arid pastoral areas and the respective governments give little priority to such areas because they contribute low GDP hence low priority focus of these governments to these areas (Magambo *et al*., 2006).

Long-term CH control measures include public health education with primary health care and veterinary public health activities, such as the improvement of slaughter hygiene and dog registration (Irabedra *et al*., 2016).

2.10 Factors Hindering Control of Cystic Hydatidosis

The control of CH is directly linked to social, political and economic situations, and sometimes to religious practices of the people in the affected areas. Social and political instability and poverty, favor the spread of the disease owing to uncontrolled animal slaughtering and viscera disposal, uncontrolled animal movement through trade,

transboundary movement of animals through the international porous borders, nomadic nature of pastoral communities, lack of long term funding, uncontrolled movement of dogs in pastoral areas, inadequate veterinary services and inadequate knowledge on health education and information of the people are barriers facing effective control programs and interventions for CH (Battelli, 2009; Magambo et al., 2006).

2.11 Conceptual Framework for Cystic Hydatidosis

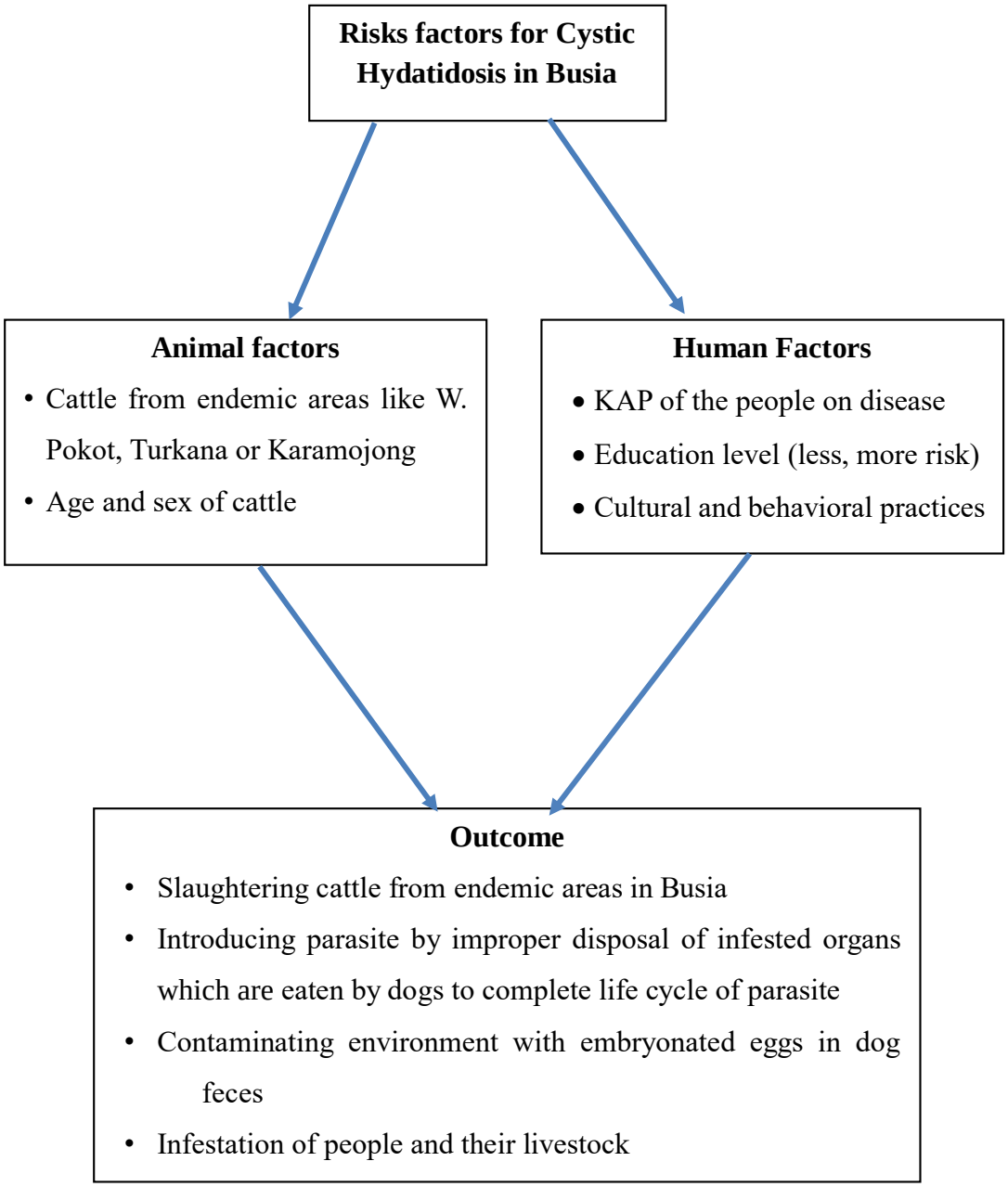

Figure 2.2: Conceptual framework of risk factors associated with Cystic Hydatidosis in slaughtered cattle at Busia town abattoirs, Kenya – 2018

CHAPTER THREE

METHODS

3.1 Study Design

This was a cross-sectional study conducted on slaughtered cattle at Amerikwai and Busia municipal abattoirs.

3.2 Study Site

The study was conducted at the two abattoirs in Busia town. Busia lies between latitude 0º and 0º 45 north and longitude 34º 25 east. Its altitude is undulating and rises from about 1,130m above sea level at the shores of Lake Victoria to a maximum of about 1,500m. It has a total population of 111,345 (KNBS, 2009). The main economic activity is trade with neighboring Uganda, in Busia town (being a border town) the county headquarters and largest town in Busia County. Away from town, the area economy is heavily reliant on fishing and agriculture on subsistence farming like growing cassava, white and yellow maize, sweet potatoes, ground nuts, soya beans sorghum, finger millet, yams, bananas, and beans on small pieces of land, usually an acre or less. Other economic activities include raising livestock on small scale, mainly cattle which acts as drought animals, some goats, pigs, fish farming, sheep and usually some free range chickens. Cash crops such as sugarcane are also grown in Busibwabo and Nasewa area. A few people engage in commercial poultry production especially broilers to meet the high demand of chicken meat by the fast growing Busia Town population.

Busia Municipal abattoir slaughters an average of 13 cattle per day while Amerikwai abattoir slaughters an average of four cattle per day.

3.3 Livestock Population in Busia

Table 3.1: Cattle, sheep, and goats population in Busia (KNBS, 2009)

County	Cattle	Sheep	Goats	**Total**
Busia	132,804	24,561	58,506	**215,871**

Source: KNBS, 2009 population and housing census report

3.4 Sampling

3.4.1 Identification of the origin of cattle for slaughter at the abattoirs

The study focused on the value chain of beef consumed in Busia town and its environs. There was a general observation by the people of Busia that many animals slaughtered in Busia town abattoirs come from West Pokot, Maasailand (Narok), Turkana and the eastern part of Uganda. These regions are endemic for CH and present a risk of spreading hydatid disease to livestock and human population of Busia community. The meat value chain assessment began from the butcheries which were selling beef after slaughter and worked both up and down stream to identify the origin of the animals being slaughtered at the abattoirs for local consumption. The study began by visiting all the butcheries to establish the abattoirs where they slaughtered their animals or get their meat for sale from. All the butcheries which were visited in Busia Town got or slaughtered meat from Busia Municipal and Amerikwai abattoirs. These two abattoirs were visited to establish the origins or sources of animals for slaughter at the abattoirs.

3.4.2 Study population

The study covered all age categories of male and female cattle destined for slaughter at the Busia Municipal and Amerikwai abattoirs. Owners of these cattle of adult age, who consented by signing an informed consent form were enrolled for KAP assessment.

3.4.3 Sample size estimation

The sample size of cattle to be inspected at the two abattoirs was calculated using Cochrane formula, 1977;

$$\boldsymbol{n = \frac{(z)^2\, pq}{d^2}}$$

The following assumptions were made;

- n=minimum sample size
- p= (25.8%, which is the highest prevalence for cattle recorded in Turkana and Maasailand (Suswa area), Kenya (Addy, 2012).
- q= 1-p (1-0.258) =0.742
- z= 1.96 for a 95% confidence interval
- d= desired precision or error margin (0.05)
- $\boldsymbol{n = \frac{(1.96)^2\, x\, 0.258\, x\, 0.742}{0.05^2} = 294}$

The sample size of 294 was subject to adjustment depending on the degree of sampling. This sample size was proportionately divided between the two abattoirs based on average monthly slaughter figures per abattoir. Average monthly slaughter figures for Busia abattoir was 375 carcasses and for Amerikwai was 128 carcasses (375 + 128 = 503). These average figures were calculated from monthly meat inspection reports for the two abattoirs for a period of three years. The sample size calculated for each abattoir is as shown below;

Busia slaughterhouse = 375/503 * 294 = 220

Amerikwai slaughterhouse = 128/503 * 294 = 74.

3.5 Eligibility

The sample size was achieved by sampling all cattle whose owners consented through informed consent at the two abattoirs during the study period.

3.5.1 Inclusion criteria

All carcasses of cattle slaughtered at the two abattoirs, whose owners consented, were eligible for inspection for cystic hydatidosis.

Consenting adult respondents (18 years and above) were interviewed for the knowledge, attitude and practice assessment.

3.5.2 Exclusion criteria

Cattle whose owners could not be reached or traced for KAP assessment were not eligible.

Carcasses and organs condemned for other infections like liver flukes, tuberculosis, abscesses, and emphysema were not eligible for consideration into the study.

Sheep and goats were not included in the study.

3.6 Piloting the Study

Piloting of the study was done at Bumala abattoir to test the questionnaire and to establish the average amount of time required in the administration of each questionnaire. During piloting, a total of 11 carcasses were inspected and 13 respondents were assessed on KAP. Out of the eleven carcasses, none of them was positive for cystic hydatidosis.

3.7 Data Collection

3.7.1 Data collection tools

3.7.1.1 Questionnaire for cattle traders and abattoir workers on KAP

The questionnaire was administered to the owners of the animals as well as the abattoir workers including flayers, abattoir cleaners and meat inspectors to assess their level of knowledge, attitude and practice on the disease. The interviews were conducted in a private setup within the abattoirs to maintain confidentiality. We used unique codes to identify participants on the questionnaires to prove voluntary consent, but not on the collected specimen labels. The KAP assessment helped to identify the knowledge gap of the study participants on the disease. The questionnaire was translated into the language in which the participants were most comfortable and recorded in English. The interviewers were well trained in administering the questionnaire. In cases where children or proxies came to the abattoir with animals for slaughter, their parents or guardians were traced to their homes for KAP assessment. The variables in the questionnaire included socio-demographic information of the participants and demographic information of cattle and also highlighted KAP.

We assigned scores to knowledge questions in the questionnaire. A correct response earned a score of one (1) while an incorrect or *"I don't know"* response got a zero (0) score. Adequate knowledge was considered as being a total score above or equal to half (≥5) of the overall score (10). Those who considered keeping dogs and livestock in same homestead to increase the risk of hydatidosis were considered to have a good attitude. Regular deworming of livestock and dogs, good meat hygiene and proper disposal of infested and infected organs and dog faeces were considered as good practices.

3.8 Routine Meat Inspection of Cattle

3.8.1 Ante-mortem inspection

During data collection, ante mortem inspections were done. Ante-mortem inspection was conducted on animals awaiting slaughter at the lairages. In the ante mortem inspection, pre-slaughter examinations of animals were conducted and information concerning age, breed, sex and origin of each study animal was properly recorded. The ages of the cattle were estimated based on dentition and conventionally grouped into age groups (Khan *et al*., 2003; Schwartz and Dioli, 1992). Animals were subsequently marked for easier traceability of their carcasses during meat inspection. In some cases, estimation of age was done at the time as post mortem inspection. Knowledge, attitude and practice assessment was conducted at the same time as ante-mortem and post-mortem inspections were done.

3.8.2 Post-mortem inspection

Post-mortem examination was done during the routine meat inspection in which standard meat inspection procedures including visual observation, palpation and incision were used to determine the infestation status of each carcass *(*Meat Control Act, CAP 356). Organs including the livers, lungs, hearts, kidneys, spleens and whole carcasses were inspected for the presence of *Echinococcus* cysts. The organ(s) from which the cysts were recovered were also recorded.

3.9 Laboratory Data

3.9.1 PCR analysis for Cystic Hydatidosis

A total of 14 whole cysts were collected from bovine carcasses, placed in sterile bags for PCR. One sterile bag was used for hydatid cyst(s) obtained from each infested animal organ and was labelled according to the date, abattoir and origin of the animal.

The labelled samples were transported in cool boxes with ice packs at 2 - 4ºC to The International Livestock Research Institute (ILRI) laboratory in Busia where PCR was done to confirm if the cysts were due to hydatid disease. These samples were collected from the positive carcasses which were got from the overall sample size of 294.

3.10 Data Management

3.10.1 Data entry

The collected data were entered in an excel sheet data base for cleaning and verification using Ms. Excel.

3.10.2 Data analysis

Data analysis was done using Epi Info 3.4.3. Descriptive statistics was done to summarize the data and measures of central tendency and dispersion for continuous variables were calculated.

3.10.2.1 Prevalence

The prevalence of CH was estimated by dividing the total number of positive cases by the total number of carcasses inspected at the two abattoirs during the study period. Frequencies, proportions and 95% Confidence Interval (CI) for categorical variables were also calculated.

3.10.2.2 Risk factors

Bivariate analysis and logistic regression were used to determine the risk factors associated with adequate knowledge, good attitude and practice among the study participants. From the bivariate analysis, variables that had p-values of ≤ 0.1 were entered into a multivariate regression model. The final model was arrived at using backward stepwise elimination method where variables with p-values ≤ 0.05 were

considered to have statistical associations with adequate knowledge, good attitude and practice as dependent coefficients.

3.11 Ethical Considerations

Ethical approval for this study protocol was obtained from the Institutional Review and Ethics Committee of Moi University (IREC) No. 0001850 (Appendix 8.6). The permission to access the abattoirs was sought from Busia County Director of Veterinary Services (DVS), (Appendix 8.7). Entry into the abattoir compounds was achieved through verbal consent from the abattoir management although a written permission would have been necessary.

3.12 Data Integrity

The questionnaires which were used to collect data were safely kept in a cabinet under lock. The key was kept by me to minimize chances of accessing the data by unauthorized persons. The soft copy of the data was stored in a computer with an access password known to me only.

3.13 Disposal of Specimen and Data

The questionnaires which were used for interviews on KAP were shredded and properly disposed of at appropriate time to avoid being accessed by unauthorized persons for any secondary use. The biological samples and tissues which were not used were also disposed of appropriately to avoid environmental contamination or being accessed by any unintended user.

3.14 Dissemination of Data

Results of the study have been submitted to a journal for dissemination through publication. The results have also been disseminated to Busia community through

public barazas, media, seminars and stakeholder meetings. The findings have been disseminated through a presentation in scientific conference organized by the Kenya Veterinary Association, Nyanza Branch held at Kisii Agricultural Training Institute in November 2018.

CHAPTER FOUR

RESULTS

4.1 Demographic Results

4.1.1 Inspected carcasses

The study was conducted from 14th May to 27th June 2018. In the study, a total of 302 cattle carcasses were inspected during the study period. Two hundred and twenty two (73.51%) were male and 80 (26.49%) were female. Out of the total number of carcasses inspected, the proportion inspected at Busia Municipal abattoir were 231 (76.49%, CI: 70.86-81.63). Cattle originating from Busia County were 188 (62.25%) of the total number of carcasses inspected Other sources of cattle included Bungoma County, Kakamega County, Siaya County, West Pokot County and Uganda (Figure 4.1)..

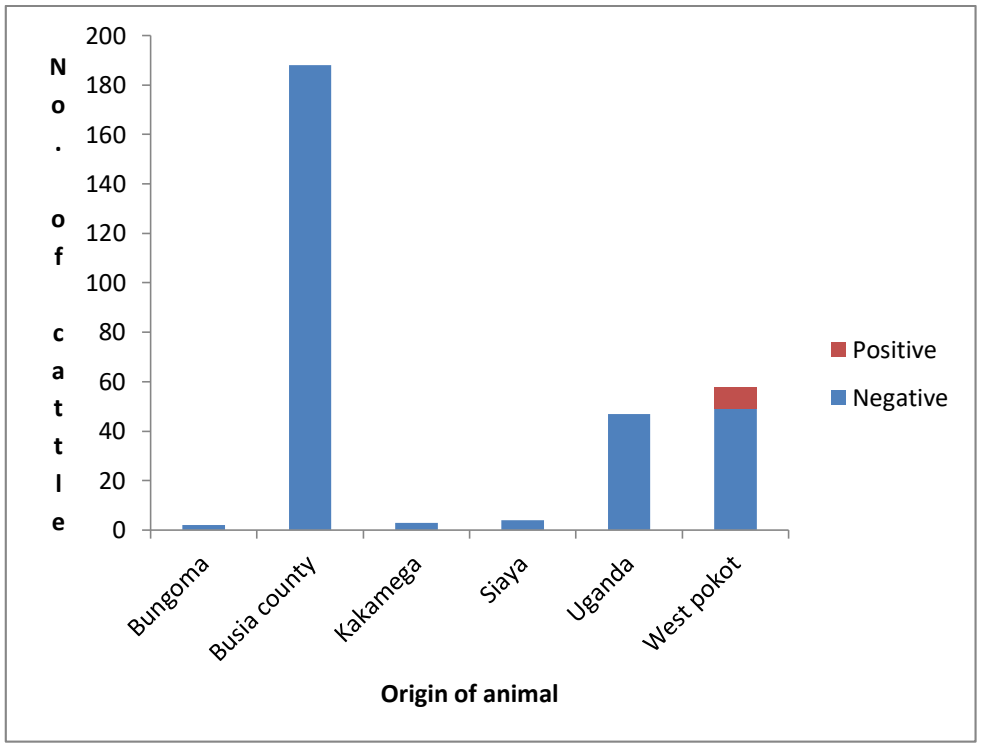

Figure 4.1: Distribution of source of cattle by county and their infestation status, Busia town abattoirs 2018 (n=302)

Local breeds of cattle comprised 295 (97.68%, CI: 95.08-98.98) of all the slaughtered cattle during the study period. The inspected carcasses consisted of 222 (73.51%) male animals. Among the carcasses inspected, 144 (47.68%) were aged between 4-6 years and 18 (5.96%, CI: 3.67-9.42) were aged above nine years. The proportion of carcasses which were positive for hydatid cysts on morphological meat inspection on different organs were nine which gave an estimated prevalence of 2.98%. Among the nine positive cases, eight were female; six positive females had their ages ranging from 7-9 years while two had ages ranging from 4-6 years. The one positive male cattle was more than nine years old (Table 4.1).

Table 4.1: Proportions of cattle by age, gender and infestation status at Busia town abattoirs 2018 (n=302)

Approximate age group (years)	**Male**		**Total male**	**Female**		**Total female**	**Total carcasses**
	Positive	Negative		Positive	Negative		
1-3	0	13	13	0	5	5	**18**
4-6	0	101	101	2	41	43	**144**
7-9	0	93	93	6	23	29	**122**
>9	1	14	15	0	3	3	**18**

Among the nine positive carcasses, five (55.56%) had multiple organ infestations. Main infested organs were liver (n=7, 77.78%) (Plate 4.1) and lung (n=4, 44.44%) (Plate 4.2). The total samples collected from the nine positive carcasses for PCR confirmation were 14. All the positive carcasses were from West Pokot County thus giving a reported prevalence of 15.52% (9/58, CI: 10.38-23.44) from West Pokot County.

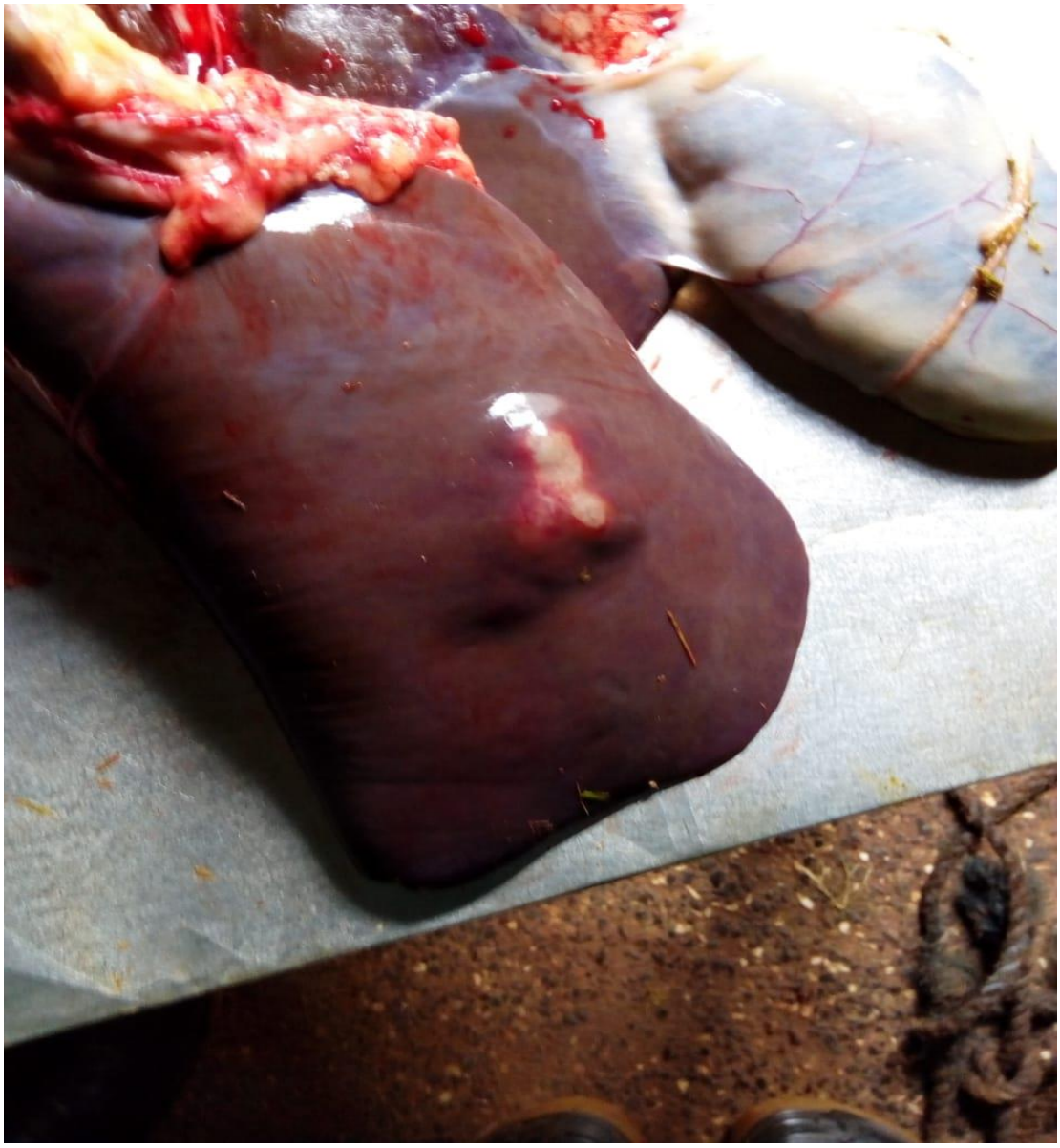

Plate 4.1: Photograph of a cystic liver of a bull slaughtered at Busia municipal abattoir, Busia town 2018

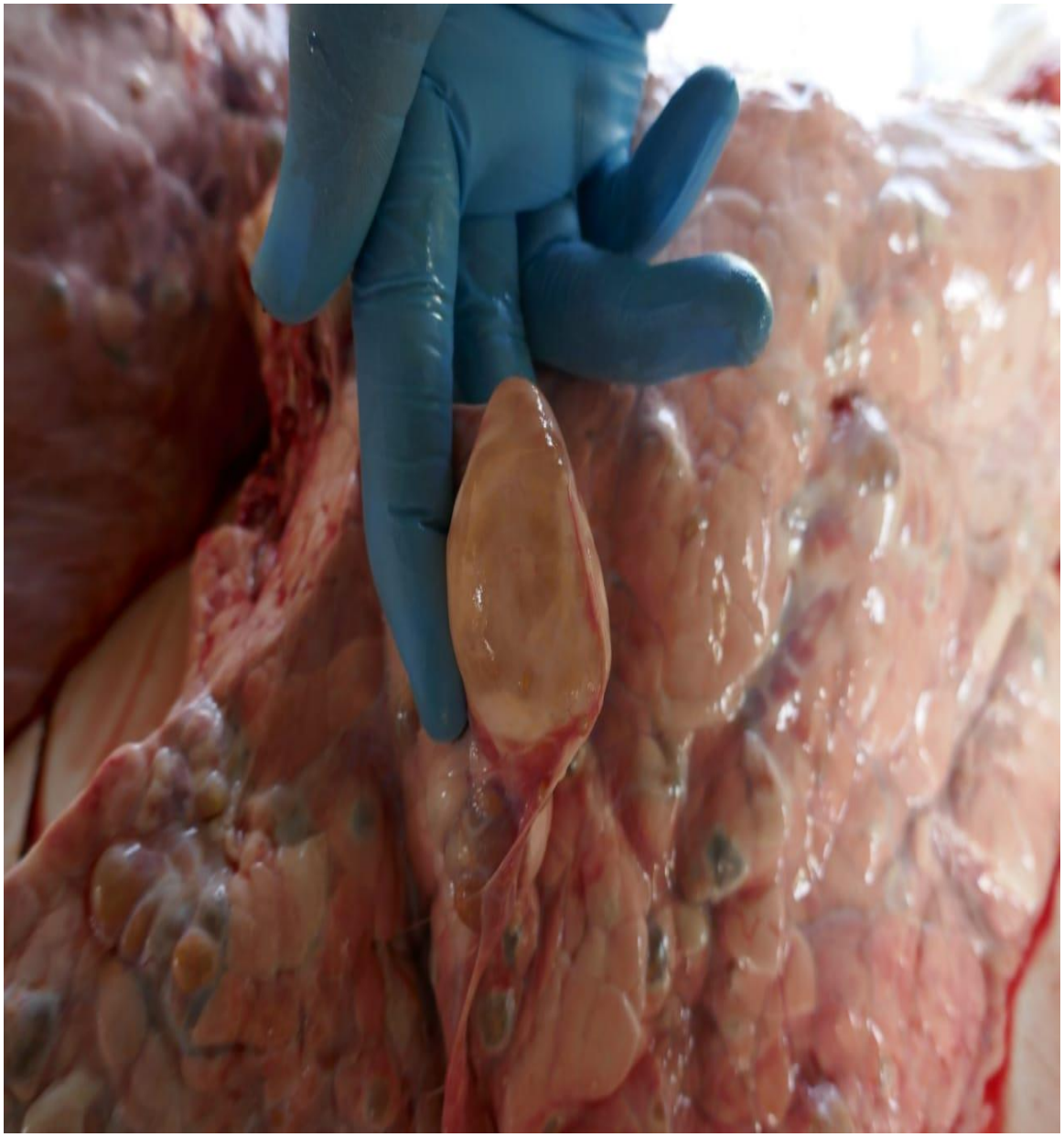

Plate 4.2: Photograph of cystic lung of a female bovine slaughtered at Busia municipal abattoir, Busia town 2018

4.1.2 Questionnaire results on knowledge, attitude and practice survey findings

A total of 310 study participants were interviewed on KAP to assess their level of awareness and understanding of the disease.

4.1.2.1 Gender

Male participants were 260 (83.87%, 95% CI: 79.19 – 87.69) of the study participants.

4.1.2.2 Age

The median age of the study participants was 41 years and the range was 21-69 years.

4.1.2.3 Religion

Christians were 267 (86.13%, 95% CI: 81.66 – 89.68), Muslims were 40 (12.90%, 95% CI: 9.48 – 17.27) and the rest were not practicing any religion.

4.1.2.4 Occupation

The participants who were in self-employment were 177 (57.10%), those who were unemployed were 98 (31.61%) and those in formal employment were 35 (11.29%). Livestock farmers were 158 (50.97%, 95% CI: 45.27 – 56.65) of the study participants (Table 4.2). The study participants who had been in the trade or farming for more than 10 years were 156 (51.66%), those who had been in the trade/farming for 5-10 years were 96 (31.79%), those who had been in the trade/farming for 2-4 years were 46 (15.23%) and 12 (3.97%) had been in the trade/farming for less than two years.

4.1.2.5 Monthly income

Their approximate monthly income ranged differently, with 158 (52.32%) getting 10000-25000 each month from the trade/farming, 73 (24.17%) getting 26000-40000, 53 (17.54%) were getting less than 10000 and 16 (5.30%) were getting more than 40000 from the trade each month.

4.1.2.6 Level of education

When we asked about their level of education, 116 (37.42%, 95% CI: 32.06 – 43.06) had completed primary education, 92 (29.67%, 95% CI: 24.71 – 35.15) had completed secondary education and 58 (18.71%, 95% CI: 14.62 – 23.60) did not have formal education (Table 4.2).

Table 4.2:Level of education of study participants and their role in the beef value chain, Busia town abattoirs, 2018

Factor	Frequency	Percentage	Confidence interval (CI)
Level of education (n=310)			
None	58	18.70	14.62 – 23.60
Primary completed	116	37.42	32.06 – 43.06
Secondary completed	92	29.68	24.71 – 35.15
Tertiary completed	44	14.20	10.01 – 20.67
Role in value chain (n=310)			
Livestock farmer	158	50.97	45.27 – 56.65
Butchery owner	65	20.97	16.66 – 26.01
Livestock trader	39	12.58	9.20 – 16.92
Butchery attendant	21	6.77	4.34 – 10.32
Butcher man/flayer	17	5.48	3.33 – 8.80
Meat inspector	5	1.61	0.60 – 3.94
Intern/student	3	0.97	0.25 – 3.04
Abattoir cleaner	2	0.65	0.11 – 2.57

4.2 Bivariate and multivariate analysis of knowledge on Cystic Hydatidosis

On assessment of knowledge on hydatid cyst, 197 (63.55%, 95% CI: 57.89 – 68.86) of the study participants admitted to have heard about the disease and 53 (26.90%) of the 197 knew the cause of the disease. The participants who did not know the transmission mode were 257 (82.90%, 95% CI: 76.42 – 89.02). Among the participants who knew the transmission mode, 28 (53.83%) knew that pastures were contaminated by dog feces

while the rest knew that pastures were contaminated by human or other livestock feces. The role of dogs in the transmission of the disease was not known to 257 (82.90%, 95% CI: 84.16-91.63). The effects of hydatidosis on livestock were known to 175 (56.45%) of the respondents, out of which 161 (92.00%) were butchery owners. On average, the participants who had adequate knowledge on hydatidosis were 40 (12.90%). The general findings on the study results on bivariate analysis was that, gender (P <0.0354), religion (P<0.04467), occupation (p<0.0024), age above 35 years (p<0.0018) and literacy level (p<0.0001) had statistical association with knowledge (Table 4.3); however, religion (p>0.2234) and gender (P>1.5805) did not have a statistical association with the disease at multivariate analysis (Table 4.3).

Table 4.3: Bivariate analysis with knowledge as a coefficient of other variables

Coefficient	**95% confidence intervals**	**Standard error**	**Z value**	**P value**
Age (> 35 years)	88.44 (51.79 – 945.74)	1.0478	3.417	0.0046
Education (primary or less)	56.13 (40.33 – 194.67)	0.27990	- 4.361	0.0012
Gender	83.87 (0.87 – 112.44)	1.3300	4.328	0.0354
Marital status (single)	8.71 (2.45 – 44.12)	1.5539	- 5.069	6.5437
Occupation (butchery owner)	51.94 (33.56 – 98.89)	0.4361	- 2.800	0.0085
Religion (none)	0.97 (0.56 – 2.44)	0.4361	- 2.800	0.0447

Table 4.4: Multivariate logistic regression with knowledge as random effect variable to age, level of education and occupation

Coefficient	Odds ratio (95% CI)	Standard error	Z value	P value
Age (> 35 years)	73.43 (21.71 – 1342.72)	1.0216	3.417	0.0018
Education (primary or less)	56.13 (40.33 – 194.67)	0.27990	- 4.361	0.0001
Gender	83.87 (0.87 – 112.44)	1.0293	4.328	1.5805
Occupation (butchery owner)	0.47 (0.12 – 0.86)	0.4361	- 2.800	0.0024
Religion (none)	0.97 (0.56 – 2.44)	0.4361	- 2.800	0.2234

4.3 Attitude by Study Participants on Cystic Hydatidosis

4.3.1 Risk of hydatid disease

When participants were assessed on their attitude on the disease, 162 (54.00%) disagreed that there is a risk of hydatid disease transmission to livestock or humans by having a dog on the same compound with livestock while 91 (30.33%) agreed, 32 (10.67%) strongly agreed and 15 (5.00%) strongly disagreed to the question.

4.3.2 Importance of deworming dogs

Among the participants who answered the question regarding the importance of deworming dogs to control the disease, 130 (43.33%) disagreed that deworming dogs is important in controlling the disease while 119 (39.67%) agreed, 43 (14.33%) strongly agreed and eight (2.67%) strongly disagreed.

4.3.3 Disposing of condemned organs as a waste of food

The participants who disagreed that disposing or condemning infected organs was a waste of food were 159 (51.96%) while 95 (31.05%) strongly disagreed, 42 (13.73%) agreed and 10 (3.27%) strongly agreed.

4.3.4 Deworming livestock and keeping them clean as a reflection of status

Those who agreed that keeping their livestock dewormed and clean is a reflection of their status were 177 (57.65%) while 88 (28.66%) strongly agreed, 42 (13.68%) disagreed and none of them strongly disagreed. Factors associated with good attitude included education level ($p<0.024$) of the participant.

4.4 Practice

4.4.1 Deworming livestock

Assessment of study participants on their practice on the disease revealed that, 256 (85.62%, 95% CI: 81.12 – 89.39) dewormed their livestock and 124 (48.44%, 95% CI: 42.17 – 54.74) of the 256 (85.62%) dewormed their livestock after every 3 months (Table 4.5).

4.4.2 Keeping dogs at home and deworming them regularly

The participants who kept dogs at home were 221 (71.99%, 95% CI: 66.55 – 76.87). None of the dog keepers was a Muslim. Among the dog keepers who dewormed their dogs were 93 (42.08%, 95% CI: 35.49 – 48.89) and 37 (39.78%, 95% CI: 29.78 – 50.46) of those who dewormed their dogs did it at the recommended deworming interval of three months (Table 4.5).

Table 4.4: Deworming interval for cattle and dogs by study participants in Busia, 2018

Deworming interval	**Livestock n=256**		**CI**	**Dogs n=93**		**CI**
Coefficient	Freq	Percentage		Freq.	Percentage	
3 months	124	48.44	42.17 – 54.74	37	39.78	29.78–50.46
Occasionally	72	28.12	22.70 – 34.06	41	44.09	33.80 – 54.76
6 months	33	12.89	9.04 – 17.62	7	7.53	3.08 – 14.90
1 year	26	10.16	6.74 – 14.53	7	7.53	3.08 – 14.90
> 1 year	1	0.39	0.01 – 2.16	1	1.07	0.03 – 5.85

4.4.3 Disposing dog faeces at home

Among the dog keepers, 95 (42.99%, 95% CI: 36.37 – 49.80) disposed of faeces by burying, 64 (28.96%, 95% CI: 23.07 – 35.42) did nothing to dog feces and 19 (8.60%, 95% CI: 5.26 – 13.10) threw the feces somewhere in the fence or nearby bushes (Table 4.6).

4.4.4 Feeding dogs on raw meat

The dog keepers who admitted that they fed their dogs on raw meat including raw infected organs were 120 (54.30%, 95% CI: 47.48 – 61.00).

4.4.5 Slaughtering animals at home

The study participants who admitted that they sometimes slaughtered animals at home were 196 (63.25%, 95% CI: 58.78 – 69.80). However 115 (58.67%, 95% CI: 51.44 – 65.64) of the meat slaughtered at home was not inspected by qualified meat inspectors and 85 (43.37%, 95% CI: 36.32 – 50.62) of infected organs of animals slaughtered at home were fed to dogs (Table 4.6).

Table 4.5: Disposal of infected organs of animals slaughtered at home and dogs feces by study participants, Busia town 2018

Factor	Freq	Percentage	CI
Disposing infected organs at home (n=196)			
Feed to dogs	85	43.37	36.32 – 50.62
Take to condemnation pit	66	33.67	27.10 – 40.75
Bury	17	8.67	5.13 – 13.52
Cook and eat	14	7.14	3.96 – 11.69
Throw away	11	5.61	2.83 – 9.82
Burn	3	1.54	0.32 – 4.41
Disposing dog feces (n=221)			
Bury	95	42.99	36.37 – 49.80
Do nothing	64	28.96	23.07 – 35.42
Dump in pit latrine	43	19.46	14.45 – 25.30
Throw away	19	8.59	5.26 – 13.10

4.5 Laboratory Results

Out of the 14 hydatid cyst samples collected, 13 (92.86%) were positive for *Echinococcus* on conventional polymerase chain reaction test (sensitivity=92% and specificity=95%) (Figure 4.4). The samples whose bands were visualized in ultra violet (UV) trans-illuminator were interpreted as positive on PCR test.

The molecular test did not go to the sequencing level to identify the genotypes of *E. granulosus* which were circulating in the cattle that were slaughtered at the two abattoirs during the study period.

CHAPTER FIVE

DISCUSSION

The reported prevalence of 2.98% in this study shows that Busia is at risk of infestation with cystic hydatidosis. Cattle from endemic areas including West Pokot, eastern Uganda, Maasailand and Turkana, some of which are infested with hydatid cysts, end up at Busia town abattoirs and therefore are risks of spreading the disease to the local animal and human populations at large through improper disposal of their infested organs. The reported prevalence shows the extent at which the infestation can easily spread over time from its known endemic areas. This trend of spread is very important to the public health stakeholders in Busia and other non-endemic regions that the disease is no longer a problem to pastoral communities only, (Odero, 2015; Addy, 2012; Njoroge *et al*., 2002).

The study further found that animals slaughtered at Busia municipal abattoir were almost three times as many as those slaughtered at Amerikwai abattoir, owing to the fact that Busia municipal abattoir is the biggest abattoir in Busia County. The study also found that most of the animals slaughtered at the Busia town abattoirs during the study period did not come from outside Busia County. However the source of animals for slaughter at the two abattoirs depends on the season of the year as we were told by the meat inspectors and some of the study participants. In dry seasons, the pastoral communities from West Pokot, Turkana, Maasailand and Eastern Uganda especially Karamoja District, destock or cull their cattle due to inadequate pastures and water caused by the drought. Some of the destocked or culled animals end up in Busia livestock markets like Bumala market where they are sold to butchery owners in Busia town or local livestock traders and therefore end up at the Busia and or Amerikwai abattoirs. Being that the study was conducted towards the end of the long rains, the

pastoralists had enough pastures and water for their livestock hence did not destock or cull their animals for sale. This gives the reason why most of the cattle slaughtered at the two abattoirs during the study period came from within Busia County. Origin of cattle was confirmed from the Animal Movement Permits and No Objection certificates filed in the Busia veterinary offices and also from discussions and interviews held with livestock traders and farmers who brought their animals for slaughter.

Local breeds of cattle formed the majority of the slaughter figures most likely because, they form the biggest proportion of cattle population in Busia County compared to cross breeds and exotic breeds hence readily available for slaughter (KNBS, 2009). Male cattle formed higher percentage of the cattle slaughter figures than females. This could be because they grow faster, are huger, heavier in weight and attain their mature weight earlier than their female counter parts and therefore give higher returns in profit after sale of their carcasses as butchery owners buy cattle for slaughter based on weight and body size. During the study period, cattle between the ages of 4-6 years formed the major proportion of slaughter figures. The local breeds of cattle (zebu), being the majority of cattle in Busia County, have slow growth rates and late maturity. Majority of the zebu bulls get their optimum weight at an average age of four years (48 months) (Nogueira, 2004).

The findings of the KAP survey showed that the beef value chain in the study area was dominated by men as men have culturally been cattle traders and men tend to dominate livelihood activities that generate a lot of income. Women were either butchery owners or farmers, but not livestock traders, butchers or flayers which are masculine jobs. This finding is similar to studies done in Uganda (Omadang *et al*., 2017) and Pakistan (Khan *et al*., 2018).

The risk factors that may contribute to the introduction of the parasite to Busia communities include low knowledge, poor attitude and bad practices by study participants, porous border migration of cattle from Eastern Uganda (Karamojong communities), uncontrolled movement of cattle from endemic areas which leads to entry of infested animals into Busia Town, sex and age of cattle (Nungari *et al*., 2019; Othieno *et al*., 2018). Most of the study participants, especially farmers, did not have much knowledge about hydatidosis. They did not know the causes, transmission modes or the role played by dogs in transmission of the disease. They did not know the control measures of the disease. Among the people who knew the effects of cystic hydatidosis, butchery owners were the majority as they were more familiar with the direct losses on income due to condemnation of infested organs and carcasses which is similar to findings in a study done in Pakistan (Khan *et al*., 2018). The losses due to condemnations of infested organs and carcasses were impacting heavily on the profits of butchery owners hence having great effect on their income. Results from this study indicate that CH is not familiar to none pastoral communities like Busia. This is consistent with studies in Tanzania (Ernest *et al*., 2009) and Ethiopia (Gebremichael *et al.*, 2013). Participants above the age of 35 years appeared to be more knowledgeable about the disease than younger people. "The statistical association between age and knowledge on hydatid disease could be due to the cumulative experience and insights about the disease that accrues with age (Dawit *et al*, 2013)".

A large number of the participants disagreed that, there is a risk of transmission of the disease by keeping a dog on the same compound with the livestock and this could be contributed by low level of education by the study participants as more than half of them had primary education and below. Majority of them also did not find it important to deworm dogs as a control measure for hydatidosis, though more than half of the

participants agreed that deworming livestock and keeping them clean is a true reflection of someone's status. Condemnation of infested organs, according to more than half of the study participants is not a waste of food as it is a way of controlling disease transmission from animals to humans. However some of the study participants who responded to that question said that condemning infested organs or carcasses was a waste of food. There is low knowledge and poor attitude on the disease by participants which may be contributed to by low literacy level. "Inadequate knowledge and information are barriers facing the effectiveness of interventions" (Battelli, 2009).

The number of study participants who dewormed their livestock was more than three quarters; however more than a quarter of them did not have a regular deworming interval for their livestock. Less than half of the dog keepers dewormed their dogs at the recommended interval of three months. The findings on number of dog keepers who dewormed their dogs is consistent with a study done in Uganda (Omadang *et al*., 2017), but contrasted by studies done in Ethiopia where dog keepers were 71.40% of the study participants and none of them dewormed their dogs (Gebremichael *et al.*, 2013) and in Pakistan where dog keepers were 63.67% and 68.40% of them dewormed their dogs (Khan *et al*., 2018). None of the dog keepers was a Muslim and this could be explained by the fact that the Muslim religion considers the dog as 'unclean' and discourages the Muslim followers from keeping dogs (Macpherson, 1983).

Deworming cattle and dogs regularly, at least after every three months, kills the adult worms and the larval stage of the parasite. This helps in breaking the life cycle of the parasite hence can effectively lead to elimination of the parasite in livestock, dogs and the environment and is also an important strategy of eliminating the parasite in humans (Craig *et al*., 2016).

The dog owners did not know the risk contained in improper disposal or handling of dog faeces in terms of transmission of CH. This makes controlling the disease difficult being that the dog faeces, with infective larvae of the parasite, contaminates the environment hence exposing the livestock and human population to risk of infestation. Controlling cystic hydatidosis is less effective without the support of dog-owners, and this support can only be obtained if the people have a clear understanding of the life cycle of the parasite and of risk factors for human and livestock infestations (Mastin *et al*., 2015).

Slaughtering animals at home, especially in funerals or social ceremonies is rampant, but qualified meat inspectors are rarely contacted to inspect such carcasses. Failure to call qualified meat inspectors to inspect meat slaughtered at home leads to improper disposal of infested organs and carcasses of backyard slaughters, but also risks transmission of other zoonotic diseases to humans. Rampant home slaughters and failing to call meat inspectors was also observed in a study conducted among pastoral communities in Greater Kapoeta of South Sudan (Wumbiya, 2017). Infested organs and carcasses of cattle slaughtered at home are eaten by the people, fed to dogs or disposed of in places where dogs can readily access them. This is done due to lack of knowledge on the dangers posed by improper handling of infected organs in terms of zoonotic disease transmission to humans. Feeding dogs on possibly infested raw meat or organs as done by majority of dog keepers also promote perpetuation of the parasite in dogs hence in the environment through dog feces (Mastin *et al*., 2015). Introduction of meat inspection services in backyard slaughters can help to combat hydatid disease by ensuring that infested organs and carcasses are properly disposed of.

Our findings revealed that inadequate deworming of dogs and livestock, poor disposal of dog faeces and infested organs of animals slaughtered at home are risk practices by Busia communities. This is consistent with studies conducted in Tanzania, Uganda and Ethiopia (Dawit et al., 2013; Othieno *et al*., 2017; Ernest *et al*., 2009).

Further results of this study reveal that female cattle are more likely to be infested with the parasite than male cattle ($p<0.05$), similar to a study done in Tabriz area, Northwest of Iran (Mirzaei *et al*., 2015), and a study done in Central Ethiopia (Assefa *et al*., 2015). This is because; females are kept for longer periods as they are used for breeding purposes. However, both male and female cattle are at risk of contracting the infestation (Odero, 2015). The study findings confirmed that the infestation rates of cattle increase significantly with age. Cystic hydatidosis is a chronic disease which is normally diagnosed at post mortem in cattle. Therefore, the older the animal, the easier it if for the cyst to grow to a size where it can easily be noticed by visual observation during meat inspection, (Mirzaei *et al*., 2015; Assefa *et al*., 2015). Livers are the main infested tissue, which is similar to a study done in slaughter houses in Maasailand (Addy, 2012), but contrary to findings of other current studies which have found that lungs are the main infested tissue (Mirzaei *et al*., 2015; Assefa *et al*., 2015; Tembo and Nonga, 2015). The parasite can infest any organ in the body, but livers and lungs account for almost 90% of all organ cases in the body (Mirzaei *et al*., 2015).

Long-term CH control measures include public health education with primary health care and veterinary public health activities, such as the improvement of slaughter hygiene and meat inspection and dog registration (Parodi and Masala, 2005). The public health education campaign may however be hampered by the low literacy level among the people involved in beef value chain and dog keepers. Observation of proper hygiene

in veterinary public health activities like meat slaughtering, regular deworming of dogs and livestock are important strategies in controlling or fully eliminating *Echinococcus* parasite from our environment. Regular deworming of dogs can be realized through registration and licensing of all dogs and practicing responsible dog ownership by dog keepers (Patkowski *et al*., 2017).

The fact that Busia town borders Uganda may complicate the control efforts. The Karamoja District of Uganda which also sells its livestock to Busia markets is a known endemic focus of hydatid disease. Cattle rustlings in this tribe are common occurrences and international efforts may be necessary to accomplish any meaningful control of the disease (Macpherson, 1983).

5.1 Study Limitations

The prevalence which has been estimated by this study might be lower or higher than expected because of failure to include cattle whose owners could not be traced and therefore not having a chance to establish their infestation status and so the estimated risk may be lower or higher than reported. Furthermore, this study could not establish the source of infestation for the sampled carcasses.

Identification of appropriate primers for the isolated *Echinococcus* DNA took long, making the study not to be completed in time. Inappropriate primers were bought two (2) times by international livestock research institute (ILRI) for PCR in the laboratory in Busia and each procurement process took not less than two (2) weeks before delivery of the primers. In each case we isolated *E. granulosus* DNA two times, but both turned negative when we ran them on PCR at the Busia laboratory. Failing to get positive results on PCR in the two occasions might be explained by the following statement; “Characterization of *Echinococcus* strains seems to be essential for the establishment

of a preventive and control strategy in every endemic area, but using DNA based methods for strain/genotype characterizations of *E. granulosus* has some difficulties, especially access to an efficient and pure concentration of DNA and proper primers" (Rahimi *et al*., 2007; Harandi *et al*., 2002). However, we finally got the appropriate primers and did the PCR test to confirm that the collected samples were due to hydatid cysts; though one of the samples calcified hence it was difficult to extract DNA from it for the PCR test in the laboratory.

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CHAPTER SIX

CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

The reported prevalence of 2.98% shows that Busia has a non-negligible risk of infestation with cystic hydatidosis which may worsen with time given that the study site is connected to areas perceived to be endemic for the disease (West Pokot, Turkana, Maasailand and Karamojong community in Eastern Uganda) via livestock trade. Livestock from West Pokot, some of which have cystic hydatidosis infestations and end up being slaughtered in Busia town, pose a risk of spreading the disease to Busia town communities.

Among the risk factors which may contribute to the spread of this disease to Busia include introduction of infested livestock from endemic areas like West Pokot due to uncontrolled movement of such livestock, improper disposal of infested carcasses or organs from such animals, if accessed by dogs, completes the life cycle of *E. granulosus*, failure to deworm dogs, poor meat hygiene especially for carcasses which are slaughtered at home and low literacy level of the people involved in beef value chain in Busia Town.

Furthermore, communities in Busia town are unfamiliar with CH and engage in behaviors that may increase their risk to infestation by the disease due to low knowledge, poor attitude and bad practice on the disease. Such behaviors include feeding raw infested offal to dogs and poor disposal of dog faeces. Failure to do meat inspection by qualified meat inspectors exposes the life of the human population in the study area to other zoonotic diseases.

Cystic hydatidosis is an important disease in both livestock and human in Kenya. The trend of spread over time should be a warning to Busia public health authorities that the disease can easily spread to the area and that it is no longer a problem to pastoral communities only.

6.2 Recommendation

Cystic hydatidosis is an important but neglected zoonotic disease which should be put under constant surveillance and regularly reported about by Public Health authorities in Busia and Kenya at large and taking local action when breakdowns are identified.

In order to control the spread of *E. granulosus* to non-endemic areas like Busia, there should be controlled livestock movement from endemic areas like West Pokot into the study site and screening them for CH at the livestock markets before they are allowed for slaughtering in Busia, improved meat hygiene, deworming dogs with anti-helmintics (praziquantel) to kill the adult tapeworm, detecting cysts at meat inspection, thus targeting infested farms in endemic areas and vaccinating livestock to protect them against the development of the larval stage. It is also imperative that dog owners in the study area be made aware of the dangers of feeding raw infested offal to dogs since home slaughter is a common feature in the study site and most of the infested organs are not properly disposed of at home.

Due to poor KAP on CH by Busia community there is need to implement information, education and communication campaigns to improve KAP on CH in the area. The authors recommend commencement of Busia community public health education (PHE) to improve knowledge, attitude and practices on the disease and improve veterinary public health activities. It will also create awareness on the transmission

mode and dangers of infestation by dogs or improper disposal of infested organs as a key control and prevention measure.

Future studies can focus on the source of infestation for these animals which are slaughtered at the study site. Further studies of the sociological aspects that may enhance transmission of CH to Busia and focus on prevalence in humans and dogs in Busia can be undertaken to help in stopping the spread of this disease to the study site.

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APPENDICES

Appendix I: Informed consent form in English

Title of study: Risk assessment of cystic hydatidosis in cattle slaughtered at Busia Town abattoirs -Kenya, 2018.

Dear Respondent…

I am Henry Joash Otieno Ogutu, a student at Moi University, School of Public Health. I am currently working on my research project on cystic hydatidosis and I have selected Busia County as my study area. Cystic hydatidosis is a zoonotic disease of public health importance. It is transmitted from animals to humans when people eat food contaminated with feces containing eggs from dog tapeworm (*Echinococcus granulosus*). As a member of this community your participation in this study will be highly appreciated. The interview will take about 25 minutes. Your participation in this study will help us assess the level of hydatidosis awareness. You will not receive any direct incentive for participating in this study. Some of the questions may be sensitive; you do not have to answer any question that you are not comfortable with. Your response will be handled confidentially. There are minimal risks for participating in this study and you can choose to withdraw at any time. Feel free to ask any questions that you might have regarding this study.

Informed Consent:

I have been fully informed about the study, the risks and benefits of it. I had the opportunity to ask questions which were satisfactorily answered. I therefore consent to voluntarily participate in the study.

Name of participant……..…

Signature/ thumb print of participant……Date…….

Name of researcher/research assistant……...…...

Signature…….. Date ……...

Contact person for questions/clarifications; Joash Ogutu 0721 516 798

Appendix II: Study Questionnaire in English

Topic: Risk assessment of cystic hydatidosis in cattle slaughtered at Busia Town abattoirs -Kenya, 2018.

Questionnaire Code ……..……

Unique ID……

Date…….....

Slaughter house……..……..

Sub-county……

Ward……...……..

Village……..

Interviewers Name/ID……..…

GPS COORDINATES…….…..…..

PART A: DEMOGRAPHIC DATA OF THE INTERVIEWEE

D1. Gender (1) Male (2) Female

D2. Age group (1) 18-30 years (2) 31-44 years (3) 45-59 years (4) Above 59 years

D3. Religion (1) Christian (2) Muslim (3) Hindu (4) None 5) Others (specify) ……

D4. Occupation; (1) Formal employment (2) Self-employed (3) Unemployed

D5. Level of education; (1) None (2) Primary (3) Secondary (4) Tertiary (5) University

D6. Marital status; (1) Single (2) Married (3) Separated (4) Divorced (5) Widowed

D7. Role in the value chain; (1) Livestock farmer (2) Livestock trader (3) Butchery owner (4) Butchery attendant (5) Butcher man/slaughter man/flayer (6) Meat inspector (7) Abattoir cleaner (8) Student/intern

D8. Duration of time in trade/farming; (1) < 2 years (2) 2-4 years (3) 5-10 years (4) > 10 years

D9. Which livestock do you keep/trade in? (1) Cattle (2) Sheep (3) Goats (4) Pigs (5) All

D10. Approximate income per month in Ksh; (1) <10,000 (2) 10,000-25,000 (3) 26,000-40,000 (4) > 40,000

PART B: LIVESTOCK DEMOGRAPHIC DATA

L1. Origin of the animal (1) Within Busia county (2) Pokot (3) Turkana (4) Uganda (5) Trans Nzoia (6) Bungoma (7) Siaya (8) Maasailand (9) Kakamega

L2. Location/village of origin if possible…….

L3. Breed of the animal (1) Local breed (2) Cross breed (3) Exotic breed

L4. Sex; (1) Male (2) Female

L5. Approximate age of the animal in years; (1) <1 (2) 1-3 (3) 4-6 (4) 7-9 (5) >9

L6. Hydatidosis infection status at meat inspection; (1) Positive (2) Negative

L7. Organ(s) infected (list all) (1) Liver (2) Lung (3) Heart (4) Intestines (mesenteric organs) (5) Carcass (6) others (specify)…….

PART B: KNOWLEDGE, ATTITUDE AND PRACTICE AMONG INTERVIEWEES

Knowledge

K1. Have you heard about hydatid disease? (1) Yes (2) No

K2. If yes above, do you know the causes of hydatidosis? (1) Yes (2) No

K3. Have you ever been educated on hydatidosis? (1) Yes (2) No

K4. Which of the following ways is it transmitted to animals? (1) By grazing on contaminated pastures or water (2) Through biting flies (3) Don't know

K5. How does pasture get contaminated? (1) Dog feces (2) Human feces (3) Other livestock feces (4) Don't know

K6. Do you know that dogs play a role in transmission of hydatidosis? (1) Yes (2) No

K7. How is hydatidosis treated in livestock? (1) Using medicine (2) Using medicine and surgery (3) No treatment (4) Don't know

K8. Do you know how hydatidosis can be controlled? (1) Yes (2) No

K9. If yes, name the control methods that you know

1)

2)

K10. What are the effects of hydatid infection to livestock?

1)

2)

Attitude

A1. There is a risk of hydatidosis transmission by having a dog in the same homestead with livestock. (1) Strongly agree (2) Agree (3) Disagree (4) Strongly disagree

A2. It is necessary to deworm dogs as a control measure for hydatidosis. (1) Strongly agree (2) Agree (3) Disagree (4) Strongly disagree

A3. Disposing meat/offal with cysts is a waste of food. (1) Strongly agree (2) Agree (3) Disagree (4) Strongly disagree

A4. I should keep my livestock dewormed and clean all the time as this reflects my status.

(1) Strongly agree (2) Agree (3) Disagree (4) Strongly disagree

Practice

P1. Do you deworm your livestock? (1) Yes (2) No

P2. If yes, after how long do you deworm your livestock? (1) Every 3 months (2) Every 6 months (3) Every 1 year (4) > 1 year (5) Occasionally

P3. Which dewormer do you normally use to deworm your livestock? (1) Albendazole (2) Levamisole (3) Mebendazole (4) Don't know (5) Other (specify)……

P4. Do you keep dogs at home? (1) Yes (2) No

P5. Do you deworm your dogs? (1) Yes (2) No

P6. If yes, after how long do you deworm your dogs? (1) Every 3 months (2) Every 6 months (3) Every 1 year (4) > 1 year (5) Occasionally

P7. Do you sometimes slaughter animals at home? (1) Yes (2) No

P8. Do you call meat inspector to inspect meat slaughtered at home? (1) Yes (2) No

P9. What do you do with infected organs of animals slaughtered at home? (1) Cook and eat (2) Bury (3) Burn (4) Feed dogs (5) Throw away (6) Give other people (7) Take to condemnation pit

P10. Do you feed your dogs on raw meat? (1) Yes (2) No

P11. How do you dispose of dog feces at home? (1) Throw away (2) Burry (3) Burn (4) Do nothing (5) Dump in pit latrine

P12. How is condemned meat disposed at the abattoir? (1) Sold to people (2) Fed to dogs (3) Thrown in condemnation pit (4) Taken away by owner of slaughtered animal

P13. Does the meat inspector always give reasons for condemning the meat? (1) Yes (2) No

Appendix III: Consent form in Kiswahili

Mada ya utafiti: Mchango wa minyororo ya uuzaji wa mifugo na jukumu la maarifa ya wadau, mtazamo na mazoezi ya kueneza cystic hydatidosis mjini Busia, Kenya, 2018.

Mhojiwa mpendwa …

Mimi ni Henry Joash Otieno Ogutu, mwanafunzi katika chuo kikuu cha Moi, shule ya afya ya umma. Kwa wakati huu ninafanya utafiti juu ya cystic hydatidosis na nimechagua kaunti ya Busia kuwa eneo langu la kuufanya utafiti huu. Ugonjwa huu unasambazwa kutoka kwa wanyama hadi kwa binadamu wakati watu wanapokula chakula kilichochafuliwa na kinyezi kilichoko na mayai ya minyoo ya mbwa. Kama mwenyeji wa kijiji hiki, kushiriki kwako kwenye utafiti huu utathaminiwa sana. Mahojiano yatachukuwa dakika ishirini na tano (25). Kushiriki kwako kwa utafiti huu utatusaidia kutathmini kiwango cha ufahamu wa hydatidosis. Hautapokea motisha yoyote kwa kushiriki katitka utafiti huu. Sio lazima ujibu maswali yanayokufanya usiwe huru. Majibu yako yatawekwa siri. Kuna hatari ndogo kwa kushiriki katika utafiti huu lakini uko huru kutoka wakati wowote. Kuwa huru kuuliza maswali yoyote unayohusu utafiti huu.

Idhini ya habari:

Nimeelezwa habari kamili kuhusu huu utafiti, hatari na mazuri yake. Nilipata nafasi ya kuuliza maswali na niliridhika na majibu niliyopewa. Ninakubali kushiriki kwa utafiti huu kwa hiari.

Jina la mhojiwa ……

Sahihi/ kidole cha mhojiwa…… Tarehe …….

Jina la mtafiti/mhoji…….....

Sahihi…….. Tarehe …….

Utakayewasiliana naye kwa maswali/ufafanuzi; Joash Ogutu 0721 516 798

Appendix IV: Study Questionnaire in Kiswahili

Mada: Mchango wa minyororo ya uuzaji wa mifugo na jukumu la maarifa ya wadau, tabia na mazoezi ya kueneza cystic hydatidosis mjini Busia, Kenya, 2018.

Kodi ya dodoso ……..……

Kitambulisho……

Tarehe…….....

Kichinjio…….……..

Kaunti ndogo……

Wadi……...……..

Kijiji……..

Jina la mhoji/Kitambulisho……..…

GPS ……..…..…..

SEHEMU YA A: MASWALA KUHUSU MHOJIWA

D1. Jinsia (1) Mwanaume (2) Mwanamke

D2. Kikundi cha miaka (1) Miaka 18 – 30 (2) Miaka 31 – 44 (3) Miaka 45 – 59 (4) Juu ya miaka 59

D3. Dini (1) Mkristo (2) Muislamu (3) Mhindi (4) Hakuna

(5) Mengine (fafanua) ……

D4. Kazi (1) Ajira rasmi (2) Kujiajiri (3) Kukosa kazi

D5. Kiwango cha masomo (1) Hakuna (2) Msingi (3) Sekondari (4) Ya juu

D6. Hali ya ndoa (1) Hajaolewa (2) Ameolewa (3) Wametengana (4) Ametalakiwa (5) Amefiwa

D7. Jukumu katika ‘value chain’ (1) Mkulima wa mifugo (2) Mfanyibiashara wa mifugo (3) Mmiliki wa chumba cha kuuza nyama (4) Mhudumu wa chumba cha

kuuza nyama (5) Mchinjaji (6) Mhakiki/Inspekta wa nyama (7) Msafishaji wa kichinjio (8) Mwanafunzi

D8. Muda katika ukulima (1) <miaka 2 (2) Miaka 2 – 4 (3) Miaka 5 – 10 (4) > miaka 10

D9. Ni mifugo ipi umefuga/unauza (1) Ng'ombe (2) Kondoo (3) Mbuzi (4) Nguruwe (5) Yote

D10. Takriban ya mapato kila mwezi kwa Kshs (1) <10,000 (2) 10,000-25,000 (3) 26,000-40,000 (4) > 40,000

SEHEMU YA B : DATA YA MIFUGO

L1. Asili ya mfugo (1) Ndani ya Busia (2) West Pokot (3) Turkana (4) Uganda (5) Trans Nzoia (6) Bungoma (7) Siaya (8) Maasaini (9) Kakamega

L2. Lokesheni/Kijiji ya asili ikiwezekana ……

L3. Aina ya mfugo (1) Wa kienyeji (2) Mchanganyiko (3) Ya kigeni

L4. Jinsia (1) Mwanaume (2) Mwanamke

L5. Takriban umri wa mfugo kwa miaka (1) <1 (2) 1-3 (3) 4-6 (4) 7-9 (5) >9

L6. Hali ya uambukizi wa hydatidosis wakati wa ukaguzi wa nyama (1) Chanya (2) Hasi

L7. Kiungo/Viongo vilivyoathiriwa (orodhesha yote) (1) Ini (2) Mapafu (3) Moyo (4) Matumbo (5)Mzoga (6) Mengine (fafanua)…….

SEHEMU C: MAARIFA, MTAZAMO NA MAZOEZI MIONGONI MWA WAHOJIWA

Maarifa

K1. Umesikia kuhusu ugonjwa wa hydatid (1) Ndio (2) La

K2. Kama ndio, je wajua kinachosababisha hydatidosis? (1) Ndio (2) La

K3. Ushawahifunzwa kuhusu hydatidosis? (1) Ndio (2) La

K4. Inasambazwa kwa mifugo kupitia njia gani kati ya zifuatazo? (1) Kwa kulisha mifugo kwa malisho yaliyochafuliwa (2) Kupitia nzi wanaouma (3) Sijui

K5. Malisho yanachafuliwa kwa njia gani? (1) Kinyezi cha mbwa (2) Kinyezi cha binadamu (3) Kinyezi cha mifugo wengine (4) Sijui

K6. Je, wajua kuwa mbwa wana jukumu katika maambukizi ya hydatidosis? (1) Ndio (2) La

K7. Hydatidosis inatibiwa aje katika mifugo? (1) Kwa kutumia madawa (2) Kwa kutumia madawa na upasuaji (3) Hakuna matibabu (4) Sijui

K8. Je wajua jinsi hydatidosis yaweza dhibitiwa? (1) Ndio (2) La

K9. Kama ndio, taja njia za kudhibiti unazojua

1)

2)

3)

K10. Athari za maambukizi ya hydatid ni zipi kwa mifugo?

1)

2)

3)

Mtazamo

A1. Kuna hatari ya maambukizi ya hydatidosis ukiwa na mbwa pamoja na mifugo nyumbani (1) Nakubali sana (2) Nakubali (3) Sijakubaliana (4) Sijakubaliana sana

A2. Ni muhimu kuwapa mbwa dawa za minyoo kama njia moja ya kudhibiti hydatidosis (1) Nakubali sana (2) Nakubali (3) Sijakubaliana (4) Sijakubaliana sana

A3. Kutupa nyama / viungo vya ndani vilivyo na cyst ni kuharibu chakula (1) Nakubali sana (2) Nakubali (3) Sijakubaliana (4) Sijakubaliana sana

A4. Inafaa niwape mifugo dawa ya minyoo na kuwaweka wasafi kila wakati kuonyesha hali yangu (1) Nakubali sana (2) Nakubali (3) Sijakubaliana (4) Sijakubaliana sana

Mazoea

P1. Je, huwa unawapa mifugo wako dawa ya minyoo (1) Ndio (2) La

P2. Kama ndio, unawapa dawa ya monyoo baada ya muda gani? (1) Kila miezi 3 (2) Kila miezi 6 (3) Kila mwaka 1 (4) >mwaka 1 (5) Mara kwa mara

P3. Huwa unatumia dawa gani ya minyoo kwa mifugo wako? (1) Albendazole (2) Levamisole (3) Mebendazole (4) Sijui (5) Mengine (fafanua)……

P4. Je, Unafuga mbwa nyumbani? (1) Ndio (2) La

P5. Unawapa mbw dawa ya minjoo? (1) Ndio (2) La

P6. Kama ndio, unawapa mbwa dawa ya minyoo baada ya muda gani? (1) Kila miezi 3 (2) Kila miezi 6 (3) Kila mwaka 1 (4) >mwaka 1 (5) Mara kwa mara

P7. Je, Kuna wakati mwingine unachinja mifugo nyumbani? (1) Ndio (2) La

P8. Huwa unaita wakaguzi wa nyama kukagua nyama iliyochinjwa nyumbani? (1) Ndio (2) La

P9. Je, huwa unafanyia nini viungo vilivyoambukizwa ya mifugo waliochinjwa nyumbani? (1) Napika na kula (2) Nazika (3) Nachoma (4) Nawalisha mbwa (5) Natupa (6) Nawapa watu wengine (7) Natupa kwenye shimo la sahau

P10. Je, unawalisha mbwa wako nyama mbichi? (1) Ndio (2) La

P11. Je, unatupaje kinyesi cha mbwa nyumbani? (1) Natupa (2) Nazika (3) Nachoma (4) Sifanyi chochote (5) Natupa kwa choo

P12. Je, nyama iliyokataliwa inatupwa aje kwenye kichinjio? (1) Inauziwa watu (2) Inalishwa mbwa (3) Inatupwa kwenye shimo la sahau (4) Inachukuliwa na mwenye mfugo aliyechinjwa

P13. Je, mkaguzi wa nyama hupeana sababu ya kukataa nyama? (1) Ndio (2) La

Appendix V: Procedure for Polymerase Chain Reaction

Deoxyribonucleic Acid (DNA) Extraction

The collected samples were immediately frozen at -20^{0}C for around a month then processed and stored in 70% ethanol. For DNA extraction, up to 20mg of tissue samples was excised and placed in a nuclease-free microfuge tube. A digestion buffer A of 300 µL was added to the tissue. Twelve microliters of proteinase K was then added to the suspension and left to incubate at 55^{0}C for 1.5 hours. Three hundred microliters of buffer SK was added to the lysate and mixed by vortexing and then 300 µL of 100% ethanol was added. A micro spin column with collection tube was assembled and 600 µL of the mixture was applied to the spin column assembly. This was capped and centrifuged for three minutes at 8,000 rotations per minute (RPM). The flow-through was discarded and spin column reassembled with its collection tube. Centrifugation was repeated until all the lysate had passed through the column. To wash the bound DNA, 500 µL of wash solution A was applied to the column and centrifuged the unit for one minute at 14000RPM. After centrifugation, the flow-through was discarded and spin column reassembled with its collection tube; 500 µL of wash solution A was applied to the column and centrifuged the unit for two minutes at 14000RPM. The spin column was carefully detached from the collection tube and discarded the collection tube and flow-through. The spin column with DNA bound to the resin was assembled with a provided 1.7mL elution tube. Two hundred microliters of Elution Buffer B was added to the center of the resin bed then allowed to stand for 10 minutes. It was then centrifuged for one minute at 6000RPM. A portion of Elution Buffer B passed through the column which allowed for the hydration of the DNA to occur. Centrifugation at 14000RPM for an additional two minutes was done to collect the total elution volume. The purified genomic DNA was stored at -20^{0}C for one day to await PCR process.

Identification of *nad5* gene

Conventional PCR was carried out on all the 14 hydatid cysts DNA isolated. The PCR primers Mit-F/Mit-R were used to amplify a 562 bp fragment of the mitochondrion the NADH-Ubiquinone oxidoreductase (complex I), chain 5 N-terminus (*nad5* gene) of *E. granulosus* (Gen Bank accession No. ARO49807) (Table 3.2). The PCR amplification reactions containing 3 µL mtDNA, 0.5 µL each of the forward and reverse primers (this study), and 12.5 µL of *Taq* PCR Master Mix (Qiagen) in a final reaction volume of 28 µL. After denaturation at 95°C for 10 min, amplification cycles were performed for four-stage, 25 cycles of 95°C for 30 s, 58°C for 30 s, and 72°C for 30 s for seven cycles in stage one, 95°C for 30 s, 56°C for 30 s, and 72°C for 30 s for seven cycles in stage two, 95°C for 30 s, 55°C for 30 s, and 72°C for 30 s for seven cycles in stage three, 95°C for 30 s, 54°C for 30 s, and 72°C for 30 s for four cycles in stage four, followed by 72°C for 10 min and cooling to 10°C. PCR products were loaded on 1.2% (w/v) Hi-Standard Agarose gel (AGTC Bio-products Limited, Hessle, UK) in 1X Tris-Boric-EDTA and stained with 0.5 µg/ml Safe White Nucleic Acid Stain (NBS Biologicals, Cambridge-shire, UK). Electrophoresis was carried out for 40 min at 190 V. The bands were visualized in UV trans-illuminator and digitally photographed. (Norgen Biotek Corp; Genomic DNA Isolation Kit, Product No. 24700, 24750 or www.norgenbiotek.com)

Table 4.6 Primers used to amplify nad5 gene in this study

Primer Name	Primer	bases	Annealing	Size (bp)	Accession	Gene
Mit-F	GTTATGTTGCGGTAGCTATGTCAG	24	59.79	562	ARO49807	*nad5*
Mit-R	CAAACCGAGACGACACCAAC	20	59.42			

Appendix VI: Study Approval Letter by IREC, Moi University and MTRH

INSTITUTIONAL RESEARCH AND ETHICS COMMITTEE (IREC)

MOITEACHING AND REFERRAL HOSPITAL
P.O. BOX 3
ELDORET
Tel: 33471//2/3

MOIUNIVERSITY
SCHOOL OF MEDICINE
P.O. BOX 4606
ELDORET
Tel: 33471/2/3

Reference IREC/2016/FELP/G
Approval Number: 0001850

10th May, 2018

Henry Joash Otieno Ogutu,
Moi University,
School of Public Health,
P.O. Box 4606-30100,
ELDORET-KENYA.

INSTITUTIONAL RESEARCH & ETHICS COMMITTEE
10 MAY 2018
APPROVED
P. O. Box 4606 - 30100 ELDORET

Dear Mr. Ogutu,

RE: APPROVAL OF AMENDMENT

The Institutional Research and Ethics Committee has reviewed the amendment made to your proposal titled:-

"Risk Mapping of Cystic Hydatidosis in Cattle Slaughtered at Busia Town Abattoirs – Kenya, 2018

We note that you are seeking to make amendments as follows:-

1. To change the title to above from "An Assessment of the Epidemiology, Knowledge, Attitude and Practice on Cystic Hydatidosis in Ruminants in Busia County Abattoirs – Kenya, 2016".
2. To change the objective to "Identify and map hot spots for risk of transmission of hydatidosis to Busia town by cattle that come from outside the town or endemic areas through beef value chain.
3. Study site will also shift from four abattoirs to only one abattoir (Busia Municipal Abattoir).

The amendments have been approved on 10th May, 2018 according to SOP's of IREC. You are therefore permitted to continue with your research.

You are required to submit progress(s) regularly as dictated by your proposal. Furthermore, you must notify the Committee of any proposal change(s) or amendment(s), serious or unexpected outcomes related to the conduct of the study, or study termination for any reason. The Committee expects to receive a final report at the end of the study.

Sincerely,

DR. S. NYABERA
DEPUTY-CHAIRMAN
INSTITUTIONAL RESEARCH AND ETHICS COMMITTEE

cc: CEO - MTRH Dean - SPH Dean - SOM
Principal - CHS Dean - SOD Dean - SON

Appendix VII: Study Approval Letter by Busia County Director of Veterinary Services

	REPUBLIC OF KENYA **COUNTY GOVERNMENT OF BUSIA** DEPARTMENT OF AGRICULTURE AND ANIMAL RESOURCES	
Email : cdvsbusia2018@gmail.com	**DIRECTOR OF VETERINARY SERVICES** **BUSIA COUNTY** **P.O. BOX 261-50400** **BUSIA (K)**	
When replying please quote **CDVS/BSA/RESEARCH/VOL I/61**	Date:14TH MAY 2018	

Joash Henry Otieno Ogutu,
Moi University,
School Of Public Health
Box 3900-30100
Eldoret

Dear Joash,

RE: APPROVAL FOR RESEARCH IN BUSIA COUNTY: RISK ASSESSMENT OF CYSTIC HYDATIDOSIS IN CATTLE SLAUGHTERED AT BUSIA TOWN ABBATOIRS, KENYA 2018

Your letter dated 11th May 2018 on the above subject matter refers.

Your request to conduct research on Risk Assessment Of Cystic Hydatidosis In Cattle Slaughtered At Busia Town Abbatoirs, Kenya 2018 is hereby approved by The Director of Veterinary Services in consultation with the Chief Officer Agriculture and Animal Resources.

The County Government of Busia acknowledges the important role played by scientific research towards development of better livelihoods and innovations.

In this regard, you can carry out the research as per your approved protocol and you are hereby allowed access to the Busia County Abbattoir in the County as per your protocol.

We recommend that you work closely with the Veterinary authorities in Matayos Sub County, the meat inspectors and stakeholders therein and keep this office adequately updated and acknowledged.

Best wishes in your research.

14 2018

Regards,

Dr. Allan Fredrick Ogendo

County Director Veterinary Services Busia County

Cc: Chief Officer Agriculture And animal Resources, Busia County

Appendix VIII: Other figures

Map of Busia County

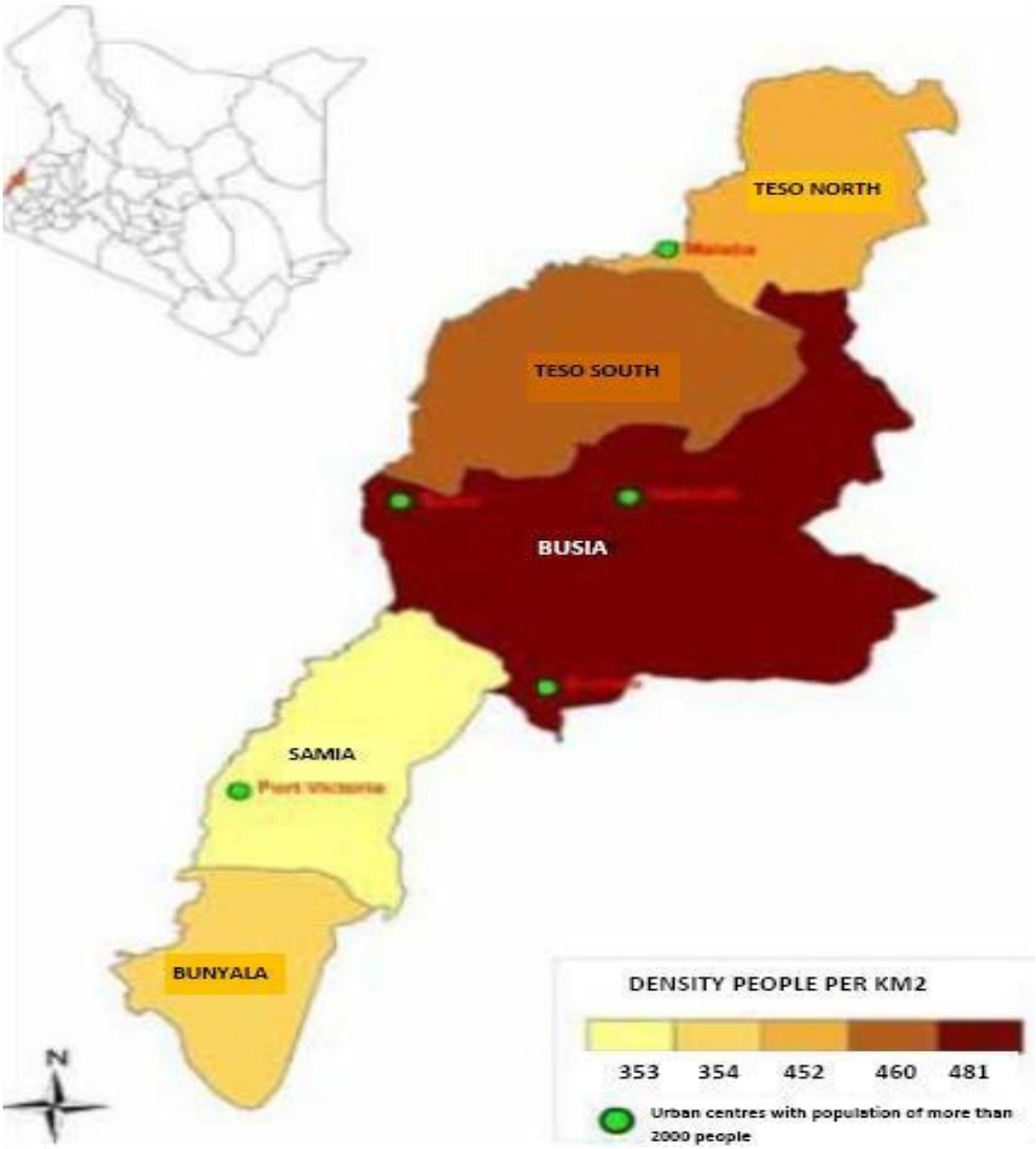

Figure 8.1: Map of Busia County
Source www.kenyampya.com

Location of Busia and Amerikwai abattoirs

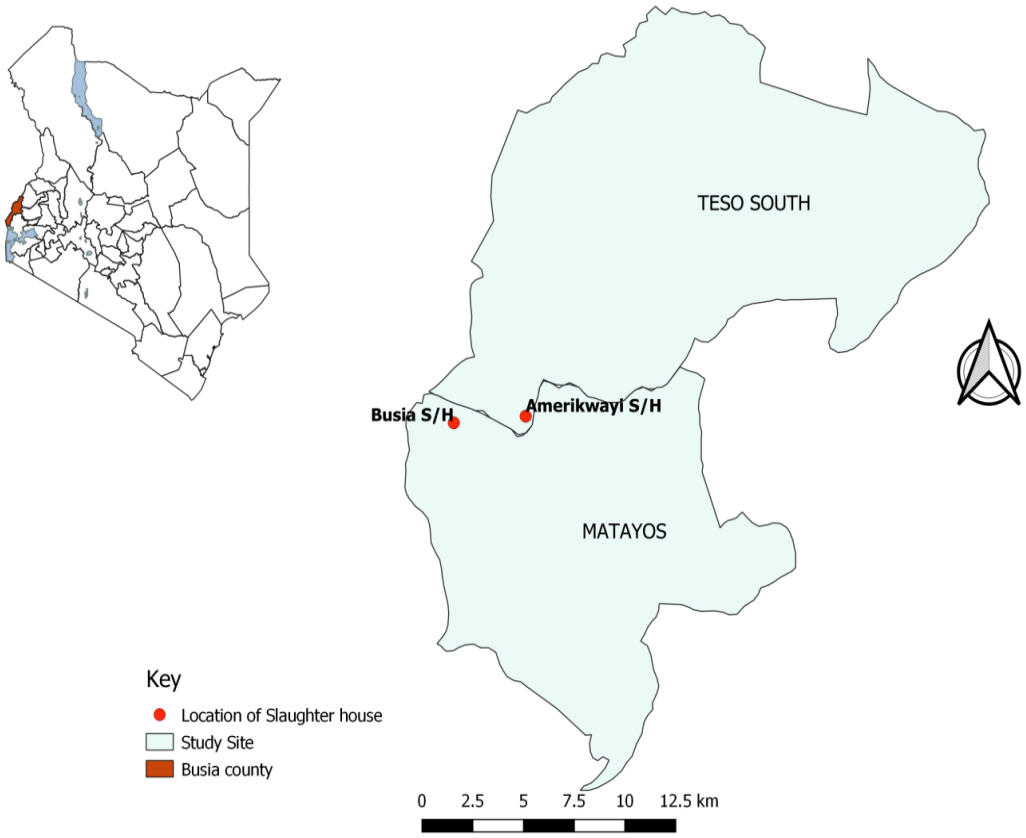

Figure 8.2: Location of Busia and Amerikwai abattoirs
Source www.kenyampya.com

Laboratory results

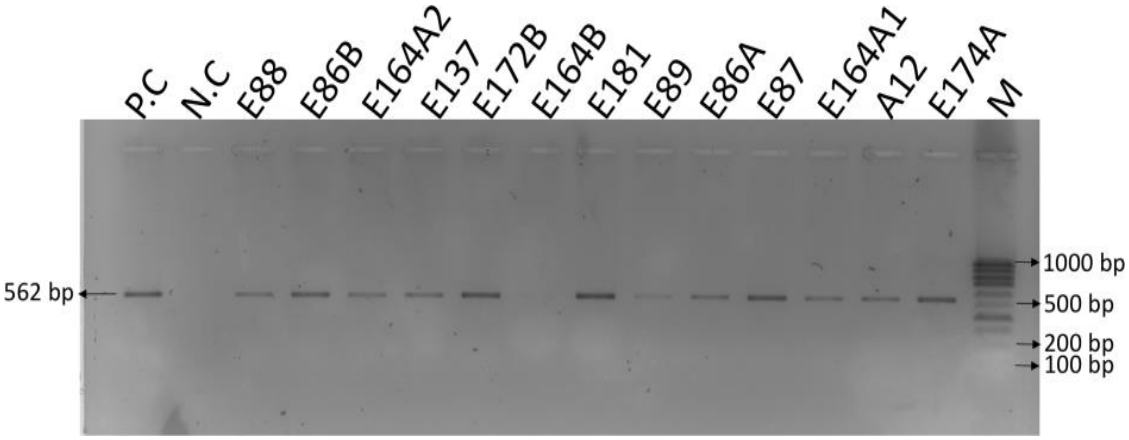

Figure 8.3: Results of PCR on samples collected from positives carcasses Busia abattoirs, 2018