# PATHOLOGICAL CONDITIONS AND ASSOCIATED FINANCIAL LOSSES FOR ORGAN CONDEMNATIONS IN CATTLE SLAUGHTERED IN SIAYA COUNTY, KENYA

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# DEDICATION

To my parents Ernest Musebe Achola and Rebecca M'mbone Achola who I regard as the pillar in my education, my wife Fridah and son Andrew, my brothers Emmanuel and Erick and sisters Maureen, Doreen and Annette.

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# LIST OF ABBREVIATIONS AND ACRONYMS

| | | |
|---|---|---|
| ANOVA | - | Analysis of variance |
| CAMP | - | Christie, Atkins, and Munch-Peterson test |
| CNS | - | Coagulase negative *Staphylococcus* species |
| DNA | - | Deoxyribonucleic acid |
| EDTA | - | Ethylenediaminetetraacetic acid |
| ELISA | - | Enzyme-linked immunosorbent assay |
| FAO | - | Food and Agriculture Organization of the United Nations |
| FEC | - | Fecal Egg Count |
| g | - | grams |
| GDP | - | Gross Domestic Product |
| GIS | | Geographic Information System |
| GPS | - | Global Positioning System |
| $H_2$ | - | Hydrogen gas |
| $H_2O_2$ | - | Hydrogen peroxide |
| H & E | - | Hematoxylin and eosin stain |
| HCL | - | Hydrochloric acid |
| IBM | - | International Business Machines corporation |
| ID | | Identification |
| kg | - | kilogram |
| KNBS | - | Kenya National Bureau of Statistics |
| KShs | - | Kenya shillings |

| | | |
|---|---|---|
| mL | - | millilitre |
| NaCl | - | Sodium chloride |
| PCR | - | Polymerase Chain Reaction |
| PTBA | - | Potassium Tellurite Blood Agar |
| SD | - | Standard deviation |
| TSI | - | Triple Sugar Iron |
| μm | | micrometre |
| UK | - | United Kingdom |
| UoN | - | University of Nairobi |
| US$ | - | United States dollar |

# ABSTRACT

Cattle production is an important economic activity in Kenya, but not fully maximized due to losses from disease conditions, some causing condemnation of organs at slaughter. To date, no studies have documented the conditions and quantified the associated financial losses in Siaya County, Kenya. This study aimed at documenting organ condemnations in cattle in slaughterhouses in the county, establish causes for the respective condemnations in selected slaughterhouses and to estimate the associated financial losses. The study was carried out in two phases involving a retrospective desk study (from 2013-2017), followed by a cross-sectional study (from 5th June 2018 - 4th July 2018). Data from slaughterhouse records were retrieved, causes for condemnation documented and monetary values of the condemned organs estimated in the retrospective study. The cross-sectional study involved post-mortem examination of organs in 3 selected slaughterhouses (Kaumara, Siaya, and Ugunja), collection of condemned organs and identification of the respective causes of the condemnations through pathological, parasitological and bacteriological laboratory techniques. Weights of condemned organs were measured and using their prevailing market prices, the respective financial loss was estimated. Descriptive statistics were used to summarize the data, while differences in means of organ condemnations over the years and for the selected slaughterhouses were analyzed using ANOVA and Bonferroni *post hoc* test to determine significance. Retrospective desk study results showed that out of 101,852 cattle slaughtered between 2013 to 2017, 27,888 (27.4%) organs were condemned mainly due to parasitic conditions 12,172 (12%), inflammatory conditions 8,084 (8%) and circulatory disturbances 3,060 (3%). The total direct financial losses incurred during the 5-year period was Kenya shillings (KShs.) 38,696,072 (US$ 383,129.40), with a mean annual loss of KShs. 7,739,214.40 (US$ 76,625.90). In the cross-sectional study, 75 out of 112 (67%) cattle slaughtered

had one or more organs condemned and 194 samples were collected for laboratory analyses. On characterization, parasitic infestations [hepatic fasciolosis 58 (51.8%), pimply guts/ Oesophagostomiasis 28 (25%) and hepatic hydatidosis 1 (0.9%)], were the major causes. Others were masseter muscle abscess by *Corynebacterium pseudotuberculosis* infection 1 (0.9%), splenomegaly [from congestion 1 (0.9%) and hemosiderosis 1 (0.9%)] and pulmonary blood aspiration from lack of stunning 2 (1.8%). *Fasciola gigantica* were identified morphologically as the cause of hepatic fasciolosis, while histopathological examination of 1 (0.9%) liver confirmed the presence of hydatid cysts. *Oesophagostomum* species larvae were recovered from 2 (25%) pimply gut nodules out of 8 suspected intestinal lesions digested in acid pepsin, while histopathological examination of 20 pimply gut nodules revealed the larval parts in 5 (25%) of them. As a result of these condemnations, a total of KShs. 94,469 (US$ 935) losses were incurred. There were no statistically significant differences (p=0.99) in condemnation of organs over the years and also for the three slaughterhouses. In conclusion, organs were condemned mainly due to parasitic and inflammatory conditions and laboratory methods if used, would be helpful in ascertaining the causes. Many organs were condemned at slaughter due to controllable parasitic, bacterial and conditions associated with poor slaughtering techniques; causing wastage of edible organs and heavy economic losses for the livestock industry. The occurrence of hepatic fasciolosis and hydatidosis suggested a possible transmission of the zoonotic agents to humans. The study recommends dissemination of the findings to all stakeholders and enhanced sensitization to farmers on measures of controlling the conditions at farm level and sensitization of slaughter workers on proper slaughter techniques to reduce losses in slaughterhouses. Further research is needed, employing more advanced methods like molecular techniques to determine a possible transmission of zoonotic diseases including hydatidosis and fasciolosis to humans.

# CHAPTER ONE: INTRODUCTION

Cattle have played a very special role in human history from the time they were tamed over 10,000 years ago. They are generally kept for meat, milk and milk products, hides, skins and for draught power. There are more than 1.43 billion cattle kept around the world (FAO, 2013). Cattle production is one of the most important economic activities in Kenya. In 2016, among the marketed agricultural products; cattle/calves and milk were valued at Kenya Shillings (KShs.) 84 and 23 billion respectively (KNBS, 2017). However, the potential of cattle production is not fully exploited due to ensuing losses from disease conditions (Tembo and Nonga, 2015). A vast majority of these conditions are frequently detected during meat inspection in slaughterhouses (GOK, 2016; Herenda *et al.,* 2000).

Disease conditions in cattle may cause partial or total condemnation of affected organs during meat inspection, therefore resulting in wastage of meat and losses of income for the livestock industry. Some of the conditions are not only zoonotic but may be sources of antimicrobial-resistant organisms making it difficult to treat the resultant disease and even other diseases in humans, hence making the community vulnerable (GOK, 2017). Some of the zoonotic diseases including anthrax and Rift Valley Fever may result in human epidemics (Cook *et al.,* 2017).

The Kenya Vision 2030 development plan aims at changing the country to an industrializing middle-income country with high-quality life for all its citizens before the year 2030. In the livestock sector, it aims at not only establishing 4 to 5 disease-free zones but also increasing the value and productivity. Improving animal health, including disease prevention and effective management (e.g. through vaccination, dipping or treatment) has a strong positive impact on the efficiency, productivity and value in livestock systems, thus, food security in general (FAO, 2017).

However, in order to know the particular livestock disease management route to undertake, proper diagnosis needs to be made (OIE, 2018). This can be done through observation of clinical manifestations on live animals and/ or through post mortem changes on dead animals (death having occurred either by natural causes or through the slaughter process) (OIE, 2018). This is followed by the collection of specimens and carrying-out of respective confirmatory laboratory tests (OIE, 2018). Clinical diagnosis by a veterinary practitioner may give a rough indication of the suspected disease, but it is usually not confirmatory (Stockham and Scott, 2013). Post mortem changes give added information which points to probable disease diagnosis; while laboratory techniques (bacteriology, parasitology, and histopathology) defines the disease condition (OIE, 2018).

When pathology/abnormality is detected in an organ, the general tendency is for the slaughter-house workers to simply discard the condemned organs or, at most, the meat-inspector (veterinarian) observing it, noting down the manifested lesions and recording a diagnosis (Herenda *et al.,* 2000). This is not sufficient to give a definitive diagnosis; one has to go further and carry out a laboratory confirmation. This will also give an indication of what is happening (the disease condition) at the farm level, that is: in the remaining animals in the respective farm(s). Even if the affected farm is not experiencing any deaths, the economic loss incurred by the farmer through morbidity losses (animals appearing healthy but not producing maximally) can be enormous (Tembo and Nonga, 2015).

Where animals are treated before they are slaughtered and the disease-causing agents are not viable, remnants of the respective genetic material can be detected. This is possible through the use of techniques such as polymerase chain reaction (P.C.R.) to confirm the diagnosis (Groundwater *et al.,* 2017). Additionally, special staining techniques and immunohistochemistry can also be used to detect respective antigens for confirmation of such conditions (Chauhan, 2010).

Since there are always animals being slaughtered; there is an ample number of condemned organs which, in addition to the economic loss to the farmer through not selling the organ(s), can be used as disease indicators for the respective farm/area (Jaja *et al.*, 2018). However, it is important to note that, in most cases, severely sick animals never reach slaughterhouses but are slaughtered at home and consumed by humans (Cook *et al.*, 2017). Studies on disease conditions and condemnation of organs in slaughterhouses, therefore, play a key role in animal disease surveillance (including zoonoses) and epidemiology (Tembo and Nonga, 2015). In Ethiopia for instance, a similar study by Belina and Melese (2017) reported that out of about 1,110 animals slaughtered, condemnation of organs at postmortem inspection was mainly due to hepatic fasciolosis 14%, hepatic hydatidosis 4%, cirrhosis 3%, pulmonary hydatidosis 6%, *Cysticercus bovis* infection (heart) 5% and Splenomegaly 3%. Consequently, high financial losses estimated at US$68,500.00 were incurred (Belina and Melese, 2017). In Kenya, a retrospective study in Kisumu municipality reported that liver condemnations were due to *Fasciola* 52% and 56% in 2007 and 2008 respectively, with financial losses incurred from the condemnation of liver alone estimated at US$ 12 thousand annually (Kanyari *et al.*, 2012). However, in Siaya County, no such previous published findings exist, hence necessitating this study.

This study, therefore, concentrated on documenting condemned organs in slaughterhouses in Siaya County, establishing the definitive cause (s) for the condemnation through laboratory diagnostic techniques and estimating the respective financial loss to the farmer/trader.

## 1.1 OBJECTIVES

### 1.1.1 Overall objective

To document pathological conditions, determine their causes and associated financial losses in cattle slaughtered in Siaya County, Kenya.

### 1.1.2 Specific Objectives:

i. To document organ condemnations in cattle in slaughterhouses in the study area

ii. To determine causes of the respective organ condemnations in selected slaughterhouses

iii. To estimate financial losses incurred as a result of the organ condemnations

## 1.2 Hypothesis

In Siaya County, many cattle organs are condemned at slaughter, due to various infectious and non-infectious causes; resulting in heavy economic losses to farmers and traders.

## 1.3 Justification of the study

Worldwide, conditions which lead to condemnation of organs in cattle in slaughterhouses cause significant economic and public health implications. The economic losses associated with them occur through: wastage of organs at slaughter, reduction in productivity of livestock, and diminished availability of consumable organs intended for human nutrition (Jaja *et al.,* 2018; Kanyari *et al.,* 2012).

Public health consequences occur since some of the conditions are zoonotic (Tembo and Nonga, 2015). At present, there are only a few reports and no published studies done confirming causes of the condemnation as well as quantifying the resultant direct financial losses in Siaya County. Identifying and documenting the conditions which lead to organ condemnation at slaughter, confirmation of their causes and estimation of the associated direct financial losses in cattle from selected slaughterhouses in Siaya County, Kenya, will provide added information, with respect to disease diagnosis and epidemiology in the study area.

Results of this study will, therefore, be useful for policy formulation to enable the application of appropriate and effective disease control programs and resources in Siaya County; an exercise

which will improve cattle husbandry and consequently alleviate poverty by increasing incomes in the community, improve food security and public/human health by increasing availability of beef and products, which are also safe.

# 2 CHAPTER TWO: LITERATURE REVIEW

## 2.1 Cattle production statistics

Globally, the population of cattle is reported to be 1.5 billion heads. Of all countries, Brazil has the highest cattle inventory - in excess of 211 million heads (14.4% of total), followed by India at 189 million (12.9%) and China 114 million (7.7%) among others. In Africa, Ethiopia is the largest producer of cattle at 54 million (3.7%), followed by Sudan 41.9 million (2.7%), Tanzania 24.5 million (1.7%) and Nigeria 20 million (1.4%), while Kenya has a population of 18 million contributing 1.2% of total world cattle population (FAO, 2013). Siaya County's population of cattle stands at 346,071, comprising 340,000 beef cattle and 6,071 dairy cattle (County Government of Siaya, 2018).

Kenya is among the largest producers of dairy and beef products in Sub-Saharan Africa, with an estimated population of 4 million improved dairy cattle and 14 million indigenous cattle (GOK, 2019). The beef industry is the fastest-growing, found mainly in the arid and semi-arid areas and powered by an increase in beef exports, rising human population, domestic incomes and urbanization (GOK, 2019). The dairy industry is most common among smallholders and is the largest component of the Agriculture sector contributing approximately 3.5% of the country's GDP and slightly more than 10% of the agricultural GDP (GOK, 2019). The industry supports about 2 million rural families as well as nearly 1 million jobs in the dairy value chain. In addition to meat, milk products, hides, and skins, cattle in Kenya are also kept for prestige, capital resources and cultural necessities such as paying the bridal price (GOK, 2019).

## 2.2 Challenges affecting cattle production in Kenya

Cattle production faces many challenges which include insufficient breeding services, poor cattle husbandry practices, insufficient extension and advisory services, insufficient water, feeds and

feeding especially during frequent droughts, cattle disease outbreaks (including foot and mouth disease, lumpy skin disease, East Coast Fever and trypanosomosis) and pests, high cost of inputs, low accessibility to markets, occasional flooding and insecurity (GOK, 2019; Onono *et al.*, 2013). Cattle diseases are responsible for high economic losses due to morbidities and mortalities, including rejection of organs during meat inspection in slaughterhouses (Tembo and Nonga, 2015). Apart from their effect on cattle production, cattle diseases are also a serious health hazard to humans especially when zoonoses including anthrax and brucellosis occur (Muendo *et al.*, 2012).

### 2.3 Cattle slaughterhouses in Kenya

Slaughterhouses in Kenya are regulated by the Meat Control Act (Local Slaughterhouse) regulations CAP 356 (Revised 2012) that generally ensure proper operations in slaughterhouses and safety of meat for human consumption. These regulations cover elements of the slaughter process including location, layout/ building structure, categorization, facilities (such as changing rooms, toilets hand wash facilities), waste disposal, staff personal hygiene, meat inspection, record-keeping, meat containers and transportation (GoK, 2016). There are three types of slaughterhouses in Kenya: category A is a large slaughterhouse which can process over 40 cattle per day and is authorized to supply meat to any part of the country; category B is medium in size and can process 6-39 cattle per day and is authorized to supply meat to towns or urban centers within its locality. The lowest category is C (slaughter slab), processing less than 5 cattle per day and supplying a town center within its locality; this latter category constitutes a vast majority of slaughter facilities in rural Kenya. The rest are uncategorized/ informal facilities which include "backyard" slaughtering done outside the regulations which may facilitate unlawful livestock trade or slaughtering of dead or diseased animals, thus endangering consumers and the public in general (Cook *et al.*, 2017).

## 2.4 Conditions causing organ condemnations in slaughter cattle

Conditions leading to organ condemnation in cattle in slaughterhouses have been documented globally, in various countries including USA, Tanzania, Ethiopia, and in Kenya (Rezac *et al.,* 2014; Tembo and Nonga, 2015; Lati *et al.,* 2015; Gathogo *et al.,* 2012). The causes of the conditions in slaughter stock have been found to be mainly parasitic and bacterial infections (Mandefro *et al.* 2015; Yibar *et al.* 2015). Bacteria may cause lesions by the production of pus, destruction of tissues through toxins or replication inside host cells causing granulomas or pyogranulomas (Cheville, 2006). On the other hand, invertebrate parasites such as helminths produce lesions through obstruction by masses of parasites or granulomas (Cheville, 2006). Condemnation involves a portion or whole organ which appears abnormal or diseased; or when it is suspected that the affected organ condition may present a health hazard or appear abhorrent to humans (Herenda *et al.,* 2000; GOK, 2016).

In the USA, out of about 1,460 cattle slaughtered, the following conditions were observed: liver abscess (30%), rumenitis scars (10%) and bovine respiratory disease complex lesions (10%), among others (Rezac *et al.,* 2014). At ante-mortem (AM) inspection of cattle, a study by Lati *et al.* (2015) in Ethiopia reported physical abnormalities such as localized swelling, cuts, branding among others. At post mortem (PM), the study reported that condemnations were due to hepatic fasciolosis (12 %), cystic hydatidosis: [liver (18%) and lung (12%)] and *Corynebacterium bovis*: [heart (2%) and whole carcass (0.4%)].

In Tanzania, using both prospective and retrospective studies, the following data were collected: (11%) lungs, (7%) intestines, (6%) livers, (4%) kidneys and (0.1%) carcasses were condemned (Tembo and Nonga, 2015). The condemnations were due to pulmonary emphysema (3 %), hepatic fasciolosis (5 %), pimply gut (6 %), renal congenital cysts (2 %), hepatic hydatidosis (3 %) and

tuberculosis (carcass) (2 %) (Tembo and Nonga, 2015). In an earlier study, also in Tanzania, Mellau *et al.* (2010) characterized pulmonary diseases in slaughtered cattle sheep and goats as pneumonia, hydatidosis, emphysema, abscesses, anthracosis, pleurisy, calcified cysts, melanosis, and bovine pulmonary tuberculosis.

In Kenya, previous studies have focused on specific diseases, parasites, or organs (Kanyari *et al.*, 2012; Gathogo *et al.*, 2012; Kithuka *et al.*, 2002). A retrospective study in Kisumu municipality reported that liver condemnations were due to *Fasciola* 52% and 56% in 2007 and 2008 respectively; other conditions were cystic echinococcosis and *Stilesia hepatica* infestations (Kanyari *et al.*, 2012). However, in Siaya County, no studies have been carried out nor published so far documenting the disease conditions and the associated financial losses in slaughterhouses.

## 2.5 Identifying causes for organ condemnations

### 2.5.1 Routine meat inspection in Kenya

Routine meat inspection is carried out according to the Meat Control Act Cap 356 Laws of Kenya, by any Veterinary Officer, or any other animal health officer appropriately authorized in writing by the Director of Veterinary Services (GoK, 2016). The inspecting officer ensures that only apparently healthy, normal animals are slaughtered. This ensures that the meat is free from disease, wholesome and of no risk to human health. This is achieved through ante-mortem and post-mortem inspection of the slaughter animals.

Ante-mortem inspection is usually carried out within 24 hours of slaughter. The inspector conducts screening of all animals destined for slaughter. Animals which are dirty, diseased as well as those treated with antimicrobials and other drugs including acaricides are separated and detained or condemned all together (GoK, 2016).

Routine post-mortem inspection is carried out immediately after the completion of dressing. All organs and carcass portions are separated and inspected using vision, incision, palpation and olfaction techniques, detecting any pathological lesions pertinent to the wholesomeness of meat. Detection of pathological conditions, parasites or other abnormalities warrant removal and condemnation of the affected portions or a whole organ. The inspecting officer is then required to keep a daily record book of slaughter and organ condemnations carried out in the facility (GoK, 2016).

The post-mortem (PM) examination of organs enables the description of gross lesions observed in the most unbiased and exhaustive way, providing features including color, consistency, distribution, shape, and size, and interpreting the findings (Pires *et al.,* 2012). The gross lesions observed during post-mortem examination of organs are often suggestive of a particular pathological process or disease. However, these lesions are frequently not specific and require additional laboratory diagnostic techniques to confirm or determine a definitive diagnosis (Pires *et al.,* 2012; Chauhan, 2010). Additionally, the quality of the PM examination often depends upon the background, training, and experience of the professional carrying out the procedure (Pires *et al.,* 2012).

### 2.5.2 Laboratory diagnostic techniques

Laboratory diagnostic techniques are fundamental in identifying causes for organ condemnation. The beginning point for the laboratory diagnosis of animal conditions is the collection of appropriate samples (OIE, 2018). This must be carefully done, labeled accurately, transported in appropriate containers (some need transport media), submitted in time, in good state and number to facilitate valid results (OIE, 2018). Unfortunately, a vast majority of slaughterhouses in Kenya and in Siaya county do not have laboratory facilities.

#### 2.5.2.1 Identification of microbial pathogens

Microbial infections in cattle and other species may be caused by bacteria, viruses, fungi, and parasites (Quinn *et al.*, 2016). The respective specimens for identification of microbial causes are normally selected based on the observed signs and symptoms and should be indicative of the disease condition or process, submitted on time and in sufficient quantities (Baron, 1996). Microbiological examination involves techniques such as direct examination of specimens, culture, microbiological identification, serodiagnosis and antimicrobial susceptibility (Groundwater *et al.*, 2017; Priyanka *et al.*, 2016).

Direct examination of specimens, shows gross pathology; while the use of microscopy may help identify microorganisms. Additionally, techniques such as immunofluorescence and immunoassays (like Enzyme-linked immunoassay – ELISA), may identify specific microbial agents while genetic probes identify genus or species-specific DNA or RNA material.

Culture involves the isolation of microbial agents and often utilizes specialized media (Quinn *et al.*, 2016; Zhao et al., 2014). Culture-based methods not only detect live pathogens but are also cost-effective, have high success rates and are used in most clinical microbiology laboratories (Priyanka *et al.*, 2016). However, it is important to note that culture is not suitable for some bacterial pathogens like *Mycobacterium tuberculosis* which are slow-growing; taking several weeks (Groundwater et al., 2017).

In microbiological identification, an examination of colonial and cellular morphology may enable preliminary identification. Additionally, growth characteristics in certain conditions, utilization of various carbohydrates and other substrates, immunoassays and genetic probes are also employed (Zhao *et al.*, 2014). Microorganisms, especially bacteria can also be tested *in vitro* to confirm

whether they are susceptible to certain antimicrobial agents (Quinn *et al.,* 2016), for their identification.

#### 2.5.2.2 Identification of parasitic causes

##### 2.5.2.2.1 Hemoparasites

Blood smears stained using Romanowsky dyes, including Giemsa and examined under an oil immersion objective (×100 objective lens) is frequently used in the detection of hemoparasites such as trypanosomes, babesial and theilerial piroplasms, and rickettsial diseases including anaplasmosis and ehrlichiosis in suspected live or dead animals. Similarly, needle biopsies of enlarged lymph nodes are commonly stained for diagnosis of trypanosomes or theilerial meronts (Taylor *et al.,* 2016). Organ smears are also commonly prepared from lungs, kidney, spleen, heart muscle and liver, taken from suspected animals at necropsy to detect blood parasites such as *Babesia* and *Anaplasma* species (OIE, 2013).

##### 2.5.2.2.2 Helminths

Adult parasites (such as *Dictyocaulus spp*. in bovine lungs), can be recovered by cutting down from trachea to small bronchi using a pair of scissors. Observed worms are then removed and put into sample bottles containing saline and counted immediately. Lungs can also be perfused with water and the adult worms recovered and counted/examined immediately (Taylor *et al.,* 2016; Mahmood *et al.,* 2014).

For other parasites such as Fasciola, infected livers are removed and sliced, any flukes seen grossly are removed intact and collected into ethanol thereafter processed for specialized examination and taxonomic identification (Taylor *et al.,* 2016; Sepulveda and Kinsella, 2013).

*Echinococcus granulosus* infection of livestock (CE) is most frequently diagnosed during necropsy and usually during meat inspection for cattle, small ruminants, and pigs (OIE, 2019). The

completely developed CE metacestodes in the intermediate host are generally recognizable as unilocular, fluid-filled cysts sited most frequently in the viscera (mostly in the liver and/or the lungs). However, the sensitivity of diagnosis of CE at meat inspection is quite low since the detection of early infections may be missed; other conditions such as congenital cysts exhibit similar lesions. The specificity of diagnosis can be improved by histopathological, immunohistochemical or PCR analysis of suspect lesions (Craig *et al.,* 2015).

Histopathology is also useful in the diagnosis of many other parasites in tissues. *Oesophagostomum spp.* can be seen in nodules under a microscope, similarly *Fasciola spp*. can be visualized in liver sections (Chamuah *et al.,* 2016; Salmo *et al.,* 2014), including the tissue reaction as a result of the presence of the parasites.

Small portions of intestinal tissues with nodules may be digested in pepsin/hydrochloric acid (pepsin-HCL) and the sediment examined using a microscope for the presence of *Oesophagostomum spp.* larvae (Kommu *et al.,* 2017). This method is popular in most countries since it is both inexpensive and less labour intensive (Taylor *et al.,* 2016).

Diagnosis of helminths depends on Faecal Egg Counts (FEC) and microscopy for eggs, larvae, and hemoparasites. The morphological identification of the parasites even to the genus level is a significant challenge. Additionally, these methods are labour-intensive and time-consuming, also requiring experienced personnel (Taylor *et al,* 2016).

Immunologically based diagnostic tests such as ELISA have been developed, but are only capable of measuring a single parasite species for each test, and detection of current infection is a challenge since antibodies can persist long after infection. These methods also involve invasive procedures like blood sample collection with veterinary supervision, making it expensive to the farmer (Taylor *et al.,* 2016).

DNA-based diagnostic methods such as PCR are not widely available or used in practice but have considerable potential in parasite diagnosis especially and including parasite detection, quantification, identification to species level and resistance detection and can be extremely sensitive and specific. However, DNA-based diagnostic methods have challenges which include: DNA template preparation, multi-species nature of helminth parasite infections in animals and genetic diversity inherent in parasites. Also, specialized equipment and materials are required, and are time consuming and labour-intensive (Taylor *et al.*, 2016).

#### 2.5.2.3 Histopathological identification of condemnation causes

Histopathological examination of tissue specimens is an important diagnostic tool for animal as well as human disease of different causes such as infectious, parasitic and non-infectious (e.g. neoplastic, deficiency diseases and intoxications) (Sołtysiak *et al.*, 2014).

This technique is particularly useful where culture is not practical, or culture samples were improperly collected or transported for analysis or the infectious pathogen is slow-growing or fastidious and offers discernment into interactions concerning the pathogens and effect on the host organism (e.g. tissue reactions) (Gupta *et al.*, 2009). When combined with appropriate clinical information, histopathological manifestations seen in tissue may offer sufficient information to properly pinpoint a specific cause for the disease condition. For instance, the existence of caseous granulomas suggests infection by *Mycobacterium tuberculosis* (Suvarna *et al.*, 2013).

In this technique, routine Hematoxylin and Eosin (H&E) can be used to visualize microorganisms; however, others can only be visualized using special histochemical stains. Such special stains include; Gram stain for bacteria, Ziehl-Neelsen (ZN) for mycobacteria, Mann's methyl blue-eosin stain for demonstration of Negri bodies in rabies virus infection, Giemsa stain for parasites and

Periodic Acid-Schiff (PAS) stain for fungi and tissue protozoal parasite (Suvarna *et al.,* 2013 ; Gupta *et al.,* 2009).

Specificity in certain instances is a limitation in histopathology which necessitates extra diagnostic techniques such as immunohistochemistry and molecular techniques [e.g. *in situ* hybridization or fluorescent in situ hybridization (FISH) and PCR] which are more rapid and specific, to further confirm the diagnosis (Gupta *et al.,* 2009).

### 2.6 Public health risks associated with the conditions

Zoonotic diseases including bovine tuberculosis, leptospirosis, fasciolosis, hydatidosis, and brucellosis have been reported in slaughtered cattle (Cook, 2017; Tembo and Nonga, 2015; Mellau *et al.,* 2010). These diseases are not only a health risk to slaughterhouse workers and butchers but also to meat consumers. Slaughterhouse studies in Kenya, Tanzania, and Ethiopia have reported the occurrence of such zoonotic conditions in workers and cattle (Cook, 2017; Tembo and Nonga, 2015; Mellau *et al.,* 2010). Usually, organs exhibiting these conditions are either trimmed or condemned whole at postmortem inspection in slaughterhouses to ensure safe and wholesome meat intended for human consumption (GoK, 2016). In Siaya County, however, causes of a majority of the conditions have not been characterized and documented.

### 2.7 Financial losses associated with condemnation of organs

Various researchers around the world have reported huge financial losses for the as a result of condemnation of organs in cattle in slaughterhouses. The estimation of these financial losses was frequently based on the computation of the following parameters: number of organ (s) condemned, percent of the respective organ (s) involved (condemned) or their weight and the prevailing market price per kilogram (Jaja *et al.,* 2018; Belina and Melese, 2017; Kanyari *et al.,* 2012).

In Nekemte slaughterhouse in Ethiopia, annual direct financial losses as a result of organ condemnation were estimated to be US$ 52 thousand (Lati *et al.,* 2015). In Dodoma slaughterhouse in Tanzania, a month's financial losses were estimated to be US$ 9 thousand (Tembo and Nonga, 2015). Elsewhere in a study by Jaja *et al.,* (2018) in South Africa, from the year 2010 to 2012 and from July to December 2013, losses of US$ 34,192 and US$ 2,570 respectively were incurred as a result of organ condemnation in cattle in slaughterhouses.

In Kenya, a retrospective study using slaughterhouse records in Kisumu municipality reported that average monetary losses due to parasitic conditions of the liver alone stood at US$ 12 thousand annually between 2007 and 2008 (Kanyari *et al.,* 2012). In Siaya County, no previous studies have been carried out to quantify these losses.

# 3 CHAPTER THREE: MATERIALS AND METHODS

## 3.1 Study area and sites

The study was carried out in Siaya County, Kenya (Figure 3.1.). The county has a surface area of approximately 3,540 km², located between Latitude 0º 26´ South and 0º 18' North and about Longitude 33º 58' East through 34º 33' East. It has a population of 842,304 persons (KNBS, 2015). It is bordered by 5 counties namely: Busia to the North and North-West, Kakamega to the North-East, Vihiga to the East, Kisumu to the South-East and Homa Bay to the South and South-West. To the west is Lake Victoria (KNBS, 2015). The county receives a bi-modal rainfall with long rains in the months of March and June and short rains in the last quarter of the year between September and December (GOK 2013).

Agriculture is the pillar of the county's economy; it is the main source of occupation and livelihoods for farmers and other actors in the value chain. The county is divided into two agro-ecological zones; the Upper Midland and the Lower Midland. On the Upper Midlands, the average annual rainfall ranges between 800mm – 2,000mm while lower midland areas receive rainfall averaging between 800 – 1,600mm (KNBS, 2015). Between the years 2013 and 2015, the county's mean annual rainfall was 1,406 mm (Abura *et al.,* 2017). Temperatures fluctuate with altitude increasing from 21° C in the North East to approximately 23° C on the shorelines of Lake Victoria whereas, in the South, it varies around mean minimum temperature of 16° C and mean maximum temperature of 29° C (GOK 2013). The climatic conditions in the county favour both crop and livestock production (KNBS, 2015). Most livestock species in the county are kept under extensive farming systems. A small number of farmers have also switched to intensive livestock farming, thus increasing the output of various livestock products (County Government of Siaya, 2018). The county's livestock population consist of: dairy cattle (7,024), beef cattle (340,000), Sheep

(157,608), Goats (286,680), pigs (14,509), rabbits (14,359) and Poultry (891,836) (County Government of Siaya, 2018).

There are 29 cattle slaughter facilities in the study area. Ten are located in Ugenya, 9 in Ugunja and 4 in Alego Usonga Sub-Counties; Bondo, Rarieda and Gem have 2 slaughter facilities each. Of these, 2 are category "B" (medium-sized slaughterhouses), while the majority are Category C (slaughter slabs). The remaining are uncategorized/ informal and do not operate on a regular or daily basis, according to records in Sub-County veterinary offices.

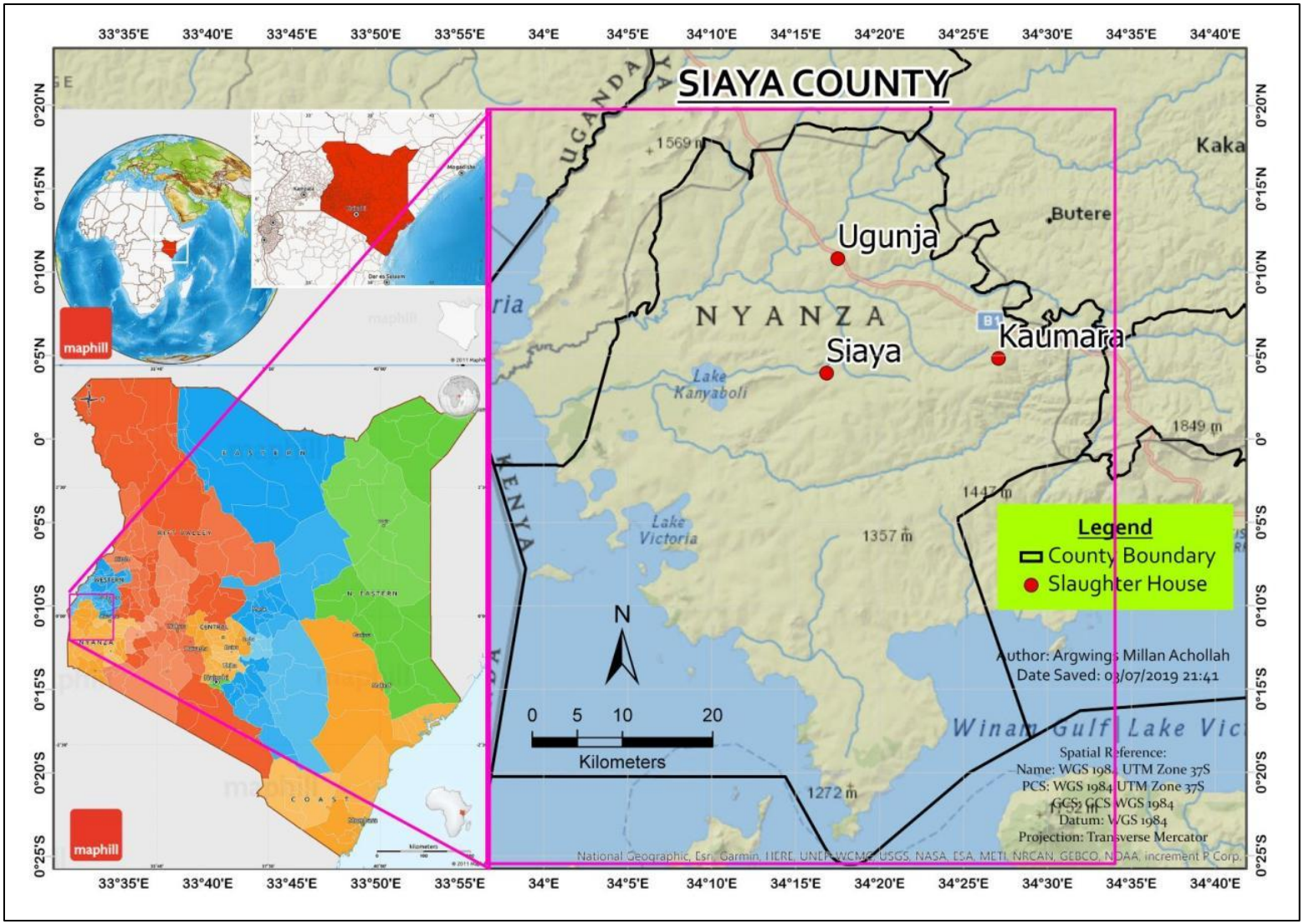


**Figure 3.1 Location of the study area and slaughter facilities visited. Generated from maphill.com, Google Earth Pro and ARC GIS version 10.5 software**

## 3.2 Study design

This study was carried out in two phases involving a retrospective desk study (secondary data), followed by a cross-sectional study (primary data). The retrospective desk study entailed analysis of data from the slaughterhouse records; documenting the condemned organs and the recorded cause (s) for the condemnation, which was mainly based on observed gross lesions. The monetary value of the condemned organs, at that time, was separately estimated and this gave the respective estimated financial loss to the farmer or trader. The cross-sectional study, on the other hand, involved post mortem inspection of the slaughtered carcasses and eviscerated organs, gross examination of condemned organs and collection of specimens for laboratory analyses at the University of Nairobi, Department of Veterinary Pathology Microbiology and Parasitology. The monetary value of the condemned organs was also separately estimated, using the current prices and organ weight; this also gave the respective estimated financial loss incurred by the farmer or trader.

## 3.3 Selection of slaughterhouses for the cross-sectional study

Three slaughterhouses namely; Siaya, Kaumara and Ugunja Slaughterhouses in Alego-Usonga, Gem and Ugunja sub-counties respectively, were selected through convenience sampling method (Appendix 8.7). They were selected based on their high throughput of slaughter cattle (compared to other slaughter facilities within Siaya County), proximity to the respective urban centres and shorter distance traveled between the facilities (therefore easily accessible) and available resources for the investigator; this allowed prompt post mortem (PM) inspection, data collection, recording, sample collection, transportation, laboratory, and statistical analysis.

### 3.4 Study animals and sampling method used for the cross-sectional study

Study animals were apparently healthy adult cattle of both sexes and mainly indigenous zebu breeds brought for slaughter at Kaumara, Siaya and Ugunja slaughterhouses. The animals originate from markets and farms mainly within Siaya County itself and the bordering counties according to GOK (2013). Convenience sampling method was used to select the study animals (Daniel and Cross, 2013). This was based on their availability at the respective slaughterhouses and were consecutively included in the sample in order of their arrival.

### 3.5 Sample size determination for the cross-sectional study

The following equation earlier on provided by Yamane (1973) was used to guide sample size determination for the cross-sectional study, assuming a 90% confidence level and p = 0.1.

$$\boldsymbol{n = \frac{N}{1 + N(e^2)}}$$

Where,

$n$ = the required sample size, $N$ = cattle population size in Siaya County = 346,071 (GoK, 2013) and $e$ = level of precision (0.1). Thus, $n$=346,071/ [1+346,071(0.1)$^2$] = 99.97, ≈ 100.

The sample size was divided at a ratio of 3:2:2 for Kaumara: Siaya: Ugunja respectively, to obtain the required size for the respective slaughterhouses. The study animals were consecutively selected in order of arrival for slaughter/inspection one slaughterhouse at a time until a convenient sample size totaling 112 animals was reached.

### 3.6 Study methodologies

#### 3.6.1 Retrospective desk study: Documenting organ condemnation in cattle slaughtered in Siaya County

Monthly meat hygiene records (from all slaughter facilities in the study area) located at the Siaya County Veterinary offices for a 5-year period (January 2013 to December 2017) were retrieved (in

the month of February 2018), reviewed, recorded and analyzed in order to document organ condemnations. The data comprised the number of cattle slaughtered in Siaya County during the period, the number of organs condemned, as well as all the respective reasons/causes for the condemnation.

### 3.6.2 Cross-sectional study: Establishing causes for organ condemnation

#### 3.6.2.1 Identification of study animals

Each animal (cattle) presented for slaughter at the respective slaughter facilities, were consecutively assigned a unique identity number (based on the respective slaughterhouse and sub-county), in order of their appearance. Demographic details namely: breed, sex, and origin were then recorded in printed forms (Appendix 8.11) prior to slaughter.

#### 3.6.2.2 Post-mortem (PM) examination of organs in selected slaughterhouses

Post mortem examination/inspection of organs from each cattle was carried out as described by the Laws of Kenya (GoK, 2016) at Kaumara, Siaya and Ugunja slaughterhouses. This entailed visual/observation, palpation, incision (when necessary) and olfaction methods for each organ. The observed lesions were described and interpreted to make a tentative diagnosis and recorded accordingly.

#### 3.6.2.3 Gross morphological characterization

Criteria used to describe and identify gross lesions observed included: location (in relation to the organ), distribution, color, size, shape/demarcation, consistency and texture on palpation or incision, number of the lesions and special elements such as odour, as described by Pires *et al.* (2012). The gross morphological lesions and other suspected abnormalities and parasites observed were recorded in printed forms for each animal (Appendix 8.11). Photographs of condemned

organs were captured using a digital camera SH100 (Samsung, Seoul, South Korea). Condemned organs which showed lesions or other abnormalities were then purposively sampled for further characterization of possible causes.

#### 3.6.2.4 Sample collection for laboratory investigation

Sterile cotton swabs were used to collect samples from lesions that required bacteriological isolation and identification according to the method provided by Tille (2017). The swabs were then separately placed in Amie's transport media (Delta lab, Rubí, Barcelona, Spain), labeled, placed in sterile plastic ziplock bags (Beaufy group, Qingdao, China). The specimens were then packed in a plastic insulated cool box with frozen ice packs and transported for laboratory analyses.

For hemoparasites, impression smears were prepared from suspected organs (enlarged spleens) using the method provided by (OIE, 2013). The smears were then air-dried and fixed in absolute methanol (Finar chemicals, Ahmedabad – Gujarat, India), labeled and transported to the laboratory for microscopic examination.

Portions of condemned livers suspected to be infested with trematodes were examined visually, incised and/or gently squeezed to expel any worms inside bile ducts. Visible adult trematodes were recovered from the exposed tissues, washed in warm water and fixed immediately in 70% ethanol (Scharlab – Sentmenat, Barcelona, Spain) (Pires *et al.*, 2012) for taxonomic identification as described by (Taylor *et al.*, 2016). Portions of the respective livers were also preserved in 10% neutral buffered formalin for routine histopathology as described by Suvarna *et al.* (2013).

Portions of intestines with greyish white/pink nodules (pimply gut lesions) suspected to have nematode worms were dissected out and each set (of nodules) per animal randomly assigned into two groups (one for histopathology and the other for digestion in pepsin-HCL) using IBM-SPSS statistics version 21 software (IBM, New York, USA), to demonstrate the nematode larvae. The

portion intended for pepsin-HCL digestion was washed in clean water, placed in Zip lock bags and packed in a 12-litre cooler box with frozen icepacks, for processing using the method described by Ministry of Agriculture (1986). The second portion was collected in 10% neutral buffered formalin for routine histopathology and staining with Hematoxylin and Eosin as described by Suvarna *et al,* (2013). Other samples from condemned organs intended for histopathology (liver, spleen, lungs) were also preserved in 10% neutral buffered formalin in plastic sample containers for histopathology.

#### 3.6.2.5 Bacterial isolation and identification

Isolation and identification of bacterial pathogens were performed by use of established procedures as provided by Tille (2017). Swabs were streaked separately onto Blood Agar and MacConkey Agar plates and incubated aerobically at 37° C overnight or up to 48 hours. Following incubation, each colony was observed to determine its morphology. Subsequent characterization tests done included: Gram staining and biochemical tests namely: oxidase, catalase, Indole production, Methyl red, Citrate utilization, Urease test, Triple Sugar Ion (TSI), Sucrose, Lactose, Christie-Atkinson-Munch-Peterson (CAMP), coagulase, and growth in Potassium Tellurite Blood Agar (PTBA) (Tille, 2017).

#### 3.6.2.6 Identification of parasites

##### 3.6.2.6.1 Hemoparasites

Impression smears prepared from suspected organs (enlarged spleens) were stained with Giemsa stain using the method described by OIE (2013). The slides were examined using a light microscope (Leica Microsystems, Heerbrugg, Switzerland) under oil immersion objective (×1000 magnification) for the presence of hemoparasites.

Demonstration of the suspected parasites in the slides by this method was considered positive for hemoparasites. An impression smear was considered negative for the hemoparasites if no parasite was detected after examining all the fields of the slides, under an oil immersion lens (×1,000 magnification) (Manamperi *et al.,* 2018).

#### 3.6.2.6.2 Hepatic trematodes (liver flukes)

The recovered trematode**s** were stained with Aceto carmine stain, according to the method provided by the International Institute of Parasitology (1994). The trematodes were examined using a stereomicroscope (Wild Heerbrugg, Heerbrugg, Switzerland) for their morphological features and for species identification as described by Taylor *et al.* (2016).

The leaf-shaped adult flukes which are broader anteriorly than posteriorly, grey-brown in colour, measuring about 2.5–3.5 cm long and 1 cm wide with a conical anterior end and distinct shoulders marked off from the body were considered to be *Fasciola hepatica*. Other adult flukes bigger than Fasciola hepatica, measuring up to 7.5 cm long and 1.5 cm wide, with a more transparent body, more leaf-like in shape, a very short conical anterior end and barely distinguishable shoulders were taken to be *Fasciola gigantica*. The morphometric parameters (Appendix 8.18) of hepatic trematodes were measured in millimeters (mm) as described by Halakou *et al*. (2017) using a calibrated light microscope (Leica Microsystems, Heerbrugg, Switzerland). Calibration was carried out as described in International Institute of Parasitology (1994); such that 1 ocular division = 25μ of the stage micrometer.

#### 3.6.2.6.3 Intestinal nematodes

Each set of intestinal specimens per animal prepared for routine histopathology were examined using a light microscope (Leica Microsystems, Heerbrugg, Switzerland) at 40×, 100×, 400×

magnification to demonstrate the presence of nematode larvae within the nodular lesions as well as tissue reaction.

For intestinal specimens digested in pepsin – HCL, the digested material in separate sample containers was washed, diluted as described by Ministry of Agriculture (1986) and drawn with a wide-mouthed plastic pipette and every drop examined in a petri dish using a light microscope (Leica Microsystems, Heerbrugg, Switzerland) for presence of nematode larvae. Nematode larvae recovered were examined for key features including head and tail characteristics as described by Taylor *et al.* (2016).

#### 3.6.2.7 Histopathological characterization of lesions

The formalin-fixed specimens were paraffin-embedded (FFPE), sectioned at a 5µm thickness, stained with Hematoxylin and Eosin stain and mounted on frosted microscope slides as described by Suvarna *et al.* (2013).

Tissues which had abnormal color were additionally subjected to special staining techniques to characterize the pigment. Techniques used were Prussian blue method for hemosiderin (in spleen sample) and Fontana Masson method for melanin (in a liver sample) as described by Dey (2018). The slides were examined using a light microscope (Leica Microsystems, Heerbrugg, Switzerland) to identify and characterize tissue alterations and or causes. Photomicrographs of the lesions were captured using a digital camera E-330 (Olympus Imaging Corporation, Tokyo, Japan) mounted on the light microscope.

### 3.6.3 Estimation of direct financial losses attributed to organ condemnation

Direct financial losses (DFL) due to organ condemnation for past years (retrospective study) and those encountered in the cross-sectional study were computed using Microsoft (MS) Excel 2016 application software (Microsoft Corporation, Washington, USA). These financial losses were

calculated based on the type and number of organs condemned, estimated weights and the prevailing monetary value of the organs at the said period, using the equation shown below, adapted from Mwabonimana *et al.* (2010).

$$\boldsymbol{DFL} = \sum(\boldsymbol{NC} \times \boldsymbol{WT} \times \boldsymbol{RP}),$$

Where;

***DFL*** = Direct financial losses, ***NC*** = Number of the condemned organ (livers / lungs / kidneys / intestines / hearts / muscle etc.), ***WT*** = Weight (kg) of the condemned organ, ***RP*** = Prevailing retail prices (KShs.) of the respective condemned organ during the particular period.

The prevailing market prices for meats and organs over the years were obtained through a key informant semi-structured interview with a total of 15 randomly selected local butcher men (Appendix 8.5). The weights of condemned organs for the retrospective study were considered to be: liver 4 kg, lungs 4 kg, kidney 1 kg, Stomach and intestines 4 kg and heart 1.1 kg (Raji *et al.,* 2010). For the cross-sectional study, weights of condemned organs were measured using a 10 kg hanging scale and 1.2 kg benchtop scale (Kern and Sohn, Balingen, Germany).

### 3.7 Mapping for the cross-sectional study

Slaughterhouses visited were georeferenced using Garmin GPSMAP64S device (Garmin Ltd, Kansas, USA). The locations of slaughterhouses were mapped and presented using Google Earth Pro (Google LLC, California, USA) and ARC GIS version 10.5 (Esri, California, USA) software and maphill.com (Maphill, Kaprova, Czech Republic).

### 3.8 Data analysis

Collected data were entered into MS Excel 2016 (Microsoft Corporation, Washington, USA) in HP notebook (Hewlett Packard Inc. California, USA) for processing, and exported to IBM-SPSS

statistics version 21(IBM, New York, USA) for statistical analysis. Average retail prices for the meats and organs over the years were computed in MS Excel 2016 (Microsoft Corporation, Washington, USA). Descriptive statistics namely: total counts, mean, maximum, minimum, range and proportions (%) were used to summarize the data, also in the MS Excel 2016 software. Proportions of organ condemnations were computed using the formula shown below, adapted from Jaja *et al.* (2018);

$$\textbf{Proportion of organ condemnations} = \frac{\textbf{number of organs condemned}}{\textbf{Total number of cattle slaughtered}}$$

For inferential statistics, differences in means of organ condemnations over the years (for the retrospective desk study) and also between the three slaughterhouses (for the cross-sectional study) were analyzed using ANOVA and Bonferroni *post hoc* test in IBM-SPSS (IBM, New York, USA) to determine whether there was significance. A significant difference was considered at $p \leq 0.05$ at 95% confidence level.

### 3.9 Research approval

Approval to undertake this research was obtained from the University of Nairobi and County Director of Veterinary Services, Siaya County, Kenya (Appendix 8.1 and 8.2).

# 4 CHAPTER FOUR: RESULTS

## 4.1 Retrospective study results

### 4.1.1 Cattle organs condemned and the respective conditions as recorded in slaughterhouses in Siaya County, Kenya

A total of 101,852 cattle (mean annual: 20,370 cattle) were slaughtered and inspected between the year 2013 and 2017 in Siaya as shown in Appendix 8.3. Out of these animals, 27,888 (27.4%) organs were condemned for various reasons (conditions). The most condemned organ was the liver 18,031 (17.7%), followed by lungs 3,402 (3.3%) and kidneys 2,758 (2.7%). Gastrointestinal tract 1,941 (1.9%) and heart; 1,756 (1.7%), were the least condemned organs (Figure 4.1). There was no statistically significant difference ($p$=0.99) in condemnation of organs over the 5-year period.

The conditions causing organ condemnation were categorized as Inflammatory processes, circulatory disturbances, disturbances of cell metabolism, parasitic conditions, concretions, disturbances in development, mineralization, pulmonary emphysema, and hydronephrosis.

Parasitic conditions 12,172 (12%), caused the highest number of condemnations followed by inflammatory conditions 8,084 (8%), circulatory disturbances 3,060 (3%) and disturbances in mineralization 2,865 (3%). The other conditions were: disturbances in development 380 (0.4%), emphysema in lungs 371 (0.4%), hydronephrosis 441 (0.4%), disturbance in cell metabolism 288 (0.3%) and concretions 227 (0.2%) (Appendix 8.4).

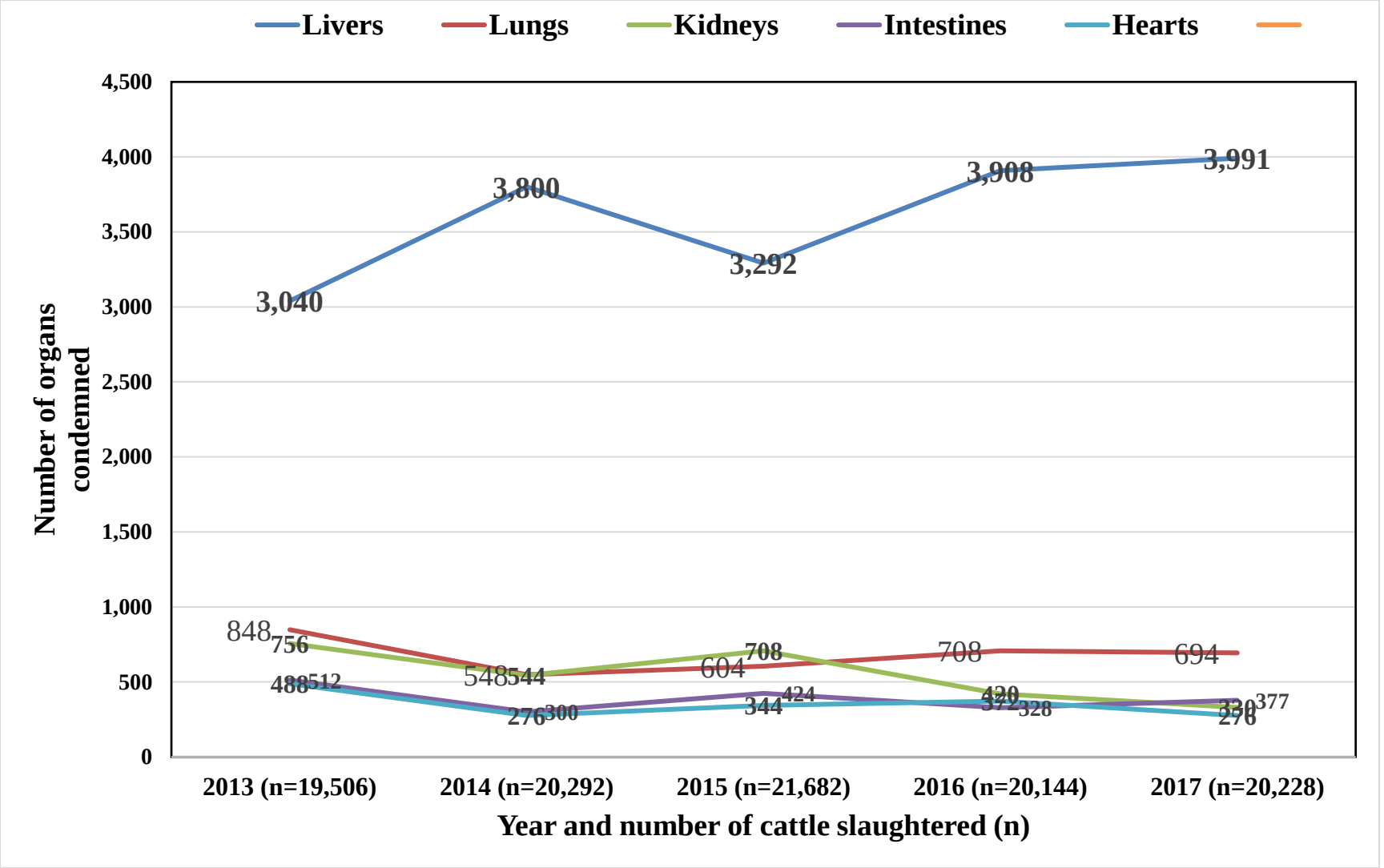


**Figure 4.1 The trend of organ condemnation in slaughterhouses in Siaya County, from the year 2013 to 2017**

Inflammatory conditions comprised liver cirrhosis, abscessation of the liver, lung and heart, pneumonia, pleurisy, hydropericardium, nephritis, enteritis, peritonitis and pericarditis. Disturbances of cell metabolism included: pigmentation due to melanosis of the lung and heart; while parasitic conditions included: hepatic fasciolosis, hydatidosis of the lung and liver, *Stilesia hepatica* infection in the liver, oesophagostomosis (pimply gut - intestines), paramphistomosis (stomachs) and cysticercosis of the heart.

Circulatory disturbances were namely: telangiectasis (liver), congestion (lung), hemorrhages of the gastrointestinal tract (GIT) and heart and infarcts of the kidneys. Disturbances in development included congenital cysts in kidneys. Calcified cysts (in the liver) were categorized as disturbances in mineralization. Other conditions were: concretions (stones) and hydronephrosis in kidneys and emphysema in the lungs.

Hepatic fasciolosis 9,082 (8.9%) was the most common cause of condemnation, followed by liver cirrhosis 3,484 (3.4%), liver calcification 2,865 (2.8%) and pneumonia 1,019 (1%); while cysticercosis of the heart 63 (0.1%) and melanosis also of the heart 129 (0.1%) accounted for the least number of organ condemnations (Figure 4.2).

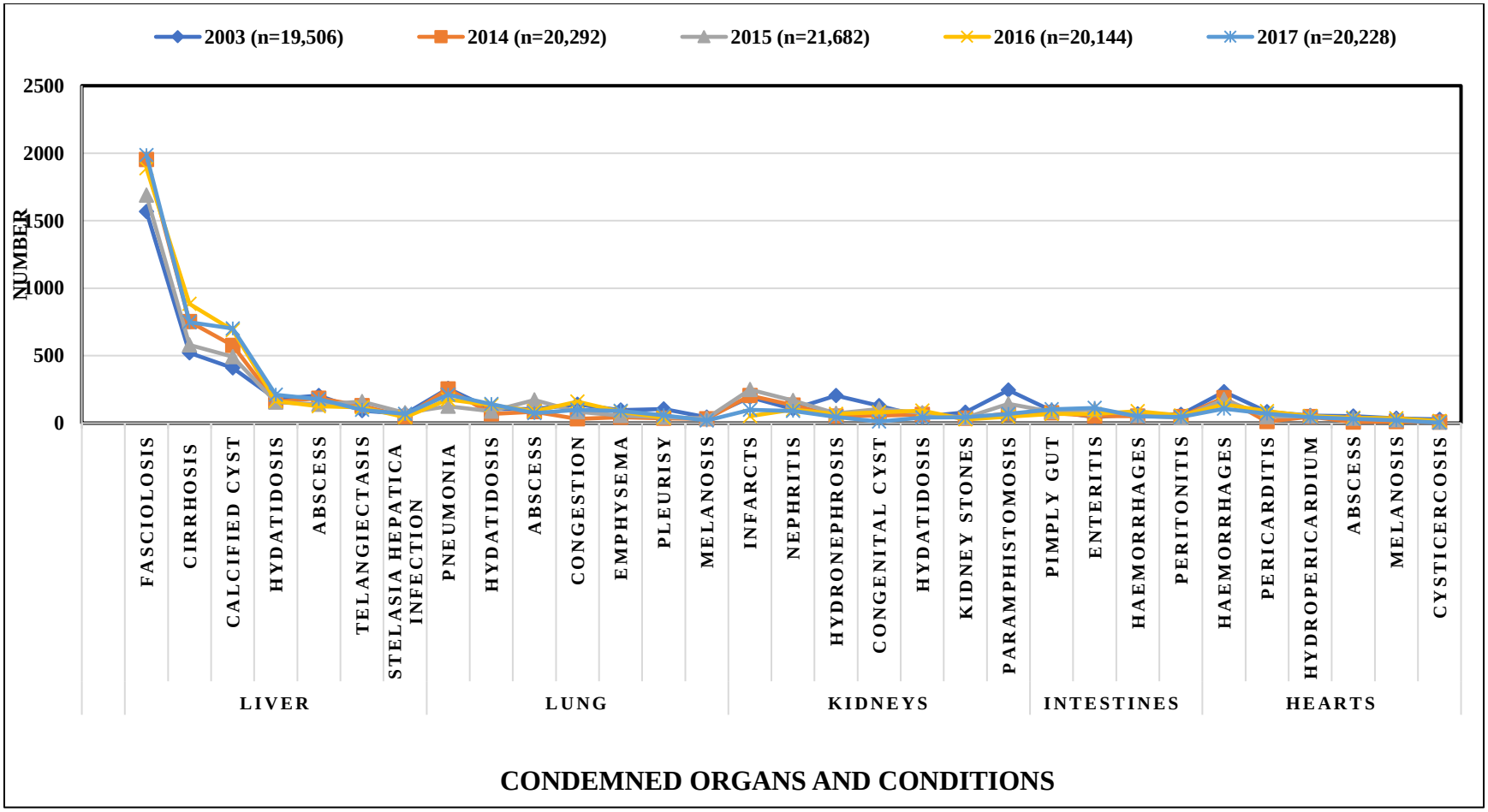


**Figure 4.2 The trend of pathological conditions and respective organs condemned in Siaya County from 2013 – 2017**

### 4.1.2 Direct financial losses associated with organ condemnation during the study period 2013 – 2017

Average retail prices of organs obtained from butcher-men, used for calculation of direct financial losses from organ condemnation during the period 2013 -2017 are presented in Table 4.3. During the period, there was an increase in prices for all organs in the year 2015.

**Table 4. 1. Average retail prices per kg of organs from slaughtered cattle (during the period 2013-2017) in Siaya County, Kenya.**

| Organ | Year and respective price per kg of organs | | | | |
|---|---|---|---|---|---|
| | **2013** | **2014** | **2015** | **2016** | **2017** |
| Muscle | 300 | 300 | 360 | 360 | 360 |
| Liver | 400 | 400 | 460 | 460 | 460 |
| Lung | 200 | 200 | 240 | 240 | 240 |
| Intestines | 200 | 200 | 240 | 240 | 240 |
| Hearts | 400 | 400 | 460 | 460 | 460 |
| Spleen | 400 | 400 | 460 | 460 | 460 |
| Kidney | 400 | 400 | 460 | 460 | 460 |

The total direct financial losses due to condemnation of organs during the 5-year period stood at Kenya shillings (KShs.) 38,696,072 (US$383,129.40) with a mean annual loss of KShs. 7,739,214.40 (US$ 76,625.90) (Table 4.4). During the period, highest losses were incurred in 2017 (US$ 87,056), followed by 2016 (US$ 85,379.70) while the lowest losses were incurred in 2013 (US$ 64,050.70) (Table 4.4).

**Table 4. 2. Calculated direct financial losses associated with organ condemnation during the period 2013-2017**

| Monetary value | Year, respective financial losses and mean annual | | | | | | |
|---|---|---|---|---|---|---|---|
| | **2013** | **2014** | **2015** | **2016** | **2017** | **Total** | **Mean Annual** |
| **KShs.** | 6,469,120 | 7,267,040 | 7,543,904 | 8,623,352 | 8,792,656 | 38,696,072 | 7,739,214.4 |
| **US$ (KShs. 101)** | 64,050.7 | 71,950.9 | 74,692.1 | 85,379.7 | 87,056 | 383,129.4 | 76,625.9 |

## 4.2 Cross-sectional study results

### 4.2.1 Phenotypic characteristics and county of origin of slaughtered cattle

During the cross-sectional study carried out from 5th June 2018 - 4th July 2018, a total of 112 cattle were slaughtered and inspected in the three slaughterhouses. Forty-six (41%) were slaughtered at Kaumara, 34 (30.4%) at Siaya and 32 (28.6%) at Ugunja slaughterhouse. Of these animals, 70 (62.5%) were males while 42 (37.5%) were females. All the animals slaughtered were adults, 108 (96.4%) were Zebu breed, 3 (2.7%) Friesian/Zebu crosses and 1(0.9%) was a Friesian. Sixty-seven of all the slaughter animals (60%) originated from Siaya County. The rest were sourced from other counties namely: 27 (24%) from Busia, 12 (10.7%) Homabay, 4 (3.6%) Migori and 2 (1.8%) Kakamega. At Kaumara, 38 (82.6%) slaughtered cattle originated from Siaya, 7(15.2%) Homabay and 1(2.2%) Migori County. For Siaya slaughterhouse, cattle originated from following counties: 11 (32.4%) Siaya, 15 (44.1%) Busia, 5(14.7%) Homabay and 3 (8.8%) Migori; while at Ugunja slaughterhouse, 18 (56.3%) cattle originated from Siaya, 12 (37.5%) from Busia and 2(6.3%) from Kakamega (Appendix 8.8).

### 4.2.2 Number and type of organ condemnations during the cross-sectional study

Of the 112 cattle which were slaughtered, 75 (67%) of them had at least one condition, while 37(33%) had no organs condemned. At Kaumara slaughterhouse, of the 46 cattle slaughtered, 30 (65.2%) of them had at least 1 pathological condition [25 (83.3%) animals had 1 type of condition while 5 (16.7%) had more than 1], while 16 (34.8 %) had no organs condemned. For Siaya slaughterhouse, out of 34 cattle slaughtered, 25 (73.5%) had at least 1 pathological condition [17 (68%) had 1 type of condition and 8 (32%) had more than 1]; nine (26.5%) of the slaughtered animals had no organs condemned. For Ugunja slaughterhouse, out of the 32 slaughtered cattle, 20 (62.5%) were found with at least 1 pathological condition [15 (75%) of them had 1 condition

while 5 (25%) had more than 1 condition]; twelve (37.5%) animals had no organs condemned (Appendix 8.9).

Among the 112 sets of organs, 59 livers (52.7%) were the most condemned. Of these, 31 (27.7%) were condemned as whole organs, while 28 (25%) were partially condemned. For Kaumara slaughterhouse, 17 (70.8%) livers were condemned as whole organs while 7 (29.2%) were partially condemned. Nine (40.9%) livers were condemned as whole organs at Siaya slaughterhouse while 13 (59.1%) were partially condemned. At Ugunja slaughterhouse, 5 (38.5%) livers were condemned as whole organs while 8 (61.5) were partially condemned. All 28 intestines [Kaumara 9 (32.1%), Siaya 8 (28.6) and Ugunja 11 (39.3)] and 1 (0.9%) masseter muscle at Siaya slaughterhouse were partially condemned, while 2 (1.8 %) spleens [Kaumara 1 (0.9 %) and Siaya slaughterhouse 1 (0.9%)] and 2 (1.8 %) lungs [Kaumara 1 (0.9 %) and Siaya slaughterhouse 1 (0.9%)] were condemned as whole organs (Appendix 8.10).

### 4.2.3 Number of samples collected and the respective laboratory tests performed

In total, 194 samples were collected for laboratory analysis (Appendix 8.12); 97 (50%) of them parasitology, 91 (46.9%) for histopathology and 6 (3.1%) for bacterial isolation and identification. Sixty-five (33.5%) of all the samples were collected at Kaumara, 95 (49%) Siaya and 34(17.5%) at Ugunja slaughterhouse; most of the samples were collected from the liver, 155 (79.9%) followed by GIT, 28 (14.4%). Other samples were collected from the spleen 6(3.1%), lungs 4(2.1%) and masseter muscle 1(0.5%) (Appendix 8.12).

### 4.2.4 Conditions and causes for organ condemnation in selected slaughterhouses

For the cross-sectional study, the following conditions manifested in respective condemned organs.

#### 4.2.4.1 Hepatic fasciolosis

Hepatic fasciolosis was diagnosed in 58 (51.8%) of all cattle inspected and 98.3% of all livers condemned during post mortem inspection. With respect to Kaumara, Siaya and Ugunja slaughterhouses, 24 (52.2%), 21 (61.8%) and 13 (40.6%) livers, were infested with liver flukes, respectively.

##### 4.2.4.1.1 Gross morphological appearance of the respective condemned organs

Grossly, all the 58 infected livers exhibited extensive areas of cream-yellow, firm, hard, distended and engorged bile ducts demonstrating the typical pipe-stem liver disease (Figure 4.1); while in addition, 33 (56.9%) of them exhibited hepatomegaly.

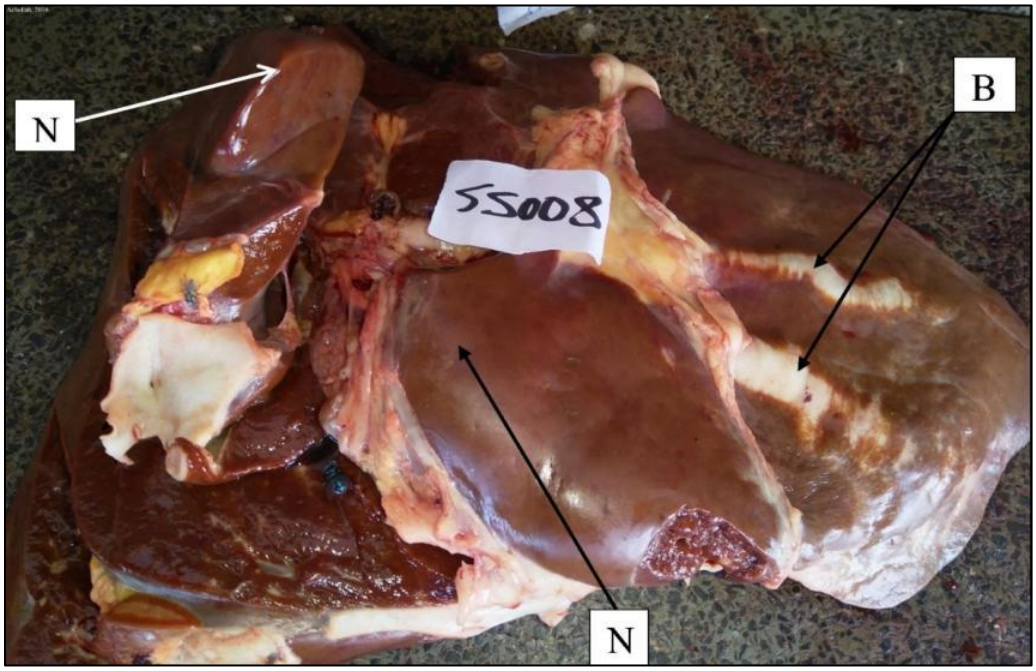


**Figure 4.3 Visceral surface of liver from slaughtered animal (ID: SS008) illustrating distended/dilated bile ducts (B) characteristic of "pipe-stem" liver disease. Normal parts (N) are indicated**

On cut surface, grey-brown to black hard gritty materials were observed in dilated and thickened bile ducts in 57 (98.3%) condemned liver samples. Several twisted red-brown/pale leaf-shaped adult liver flukes and black/brown gritty material regarded as excrement were found in bile ducts in different areas in all the Fasciola-infested livers (Figure 4.2). Black soft and somewhat circular sharply demarcated focal areas of pigmentation were also observed in 1 (1.7%) of them. One (1.7%) of the Fasciola-infested livers had multiple abscesses. Additionally, 1 (0.9%) infested liver (from Cattle ID: SS012), exhibited a pale-grey to pink, locally extensive indurated area (of the left hepatic lobe) with thickened capsule and uneven surface covered by an off-white (pale pink) soft but tough material.

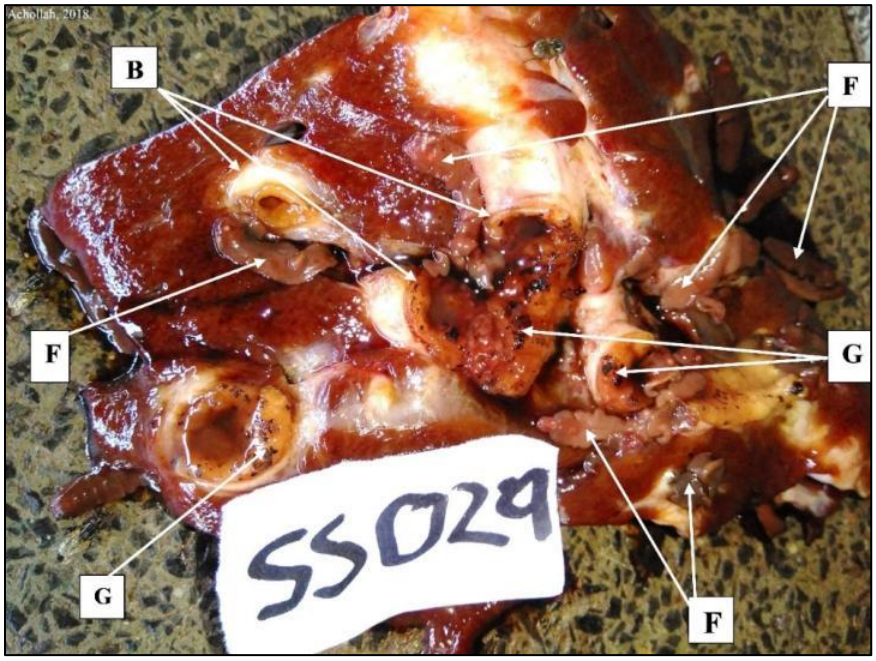


**Figure 4.4 Cross-section of Fasciola-infested liver obtained from the slaughtered animal (ID: SS029) demonstrating dilated/ thick-walled bile ducts (B); twisted, reddish-brown leaf-shaped liver flukes (F) and black/brown gritty material within bile ducts (G)**

#### 4.2.4.1.2 Histopathological manifestations of the respective condemned organs

On carrying out a histopathological examination of the 58 fluke-infected liver specimens, 55 (94.8%) showed loss of normal hepatic lobular architecture (Figure 4.3), inflammatory reactions in which there was a marked alteration of the portal area with mononuclear inflammatory cell infiltrates accompanied by proliferation of fibrous connective tissue. In addition, hepatic arterial vasculitis, and proliferation of bile ducts, periductal fibrosis, and congestion of portal veins with erythrocytes was also evident in the portal areas of 55 (94.8%) of them (Figure 4.4).

In the parenchyma of 54 (93.1%) of these liver samples, vacuolar degeneration/cell swelling was observed in which hepatocytes were enlarged, with a rather pale cytoplasm and centrally located nuclei. Additionally, accumulation of yellowish-brown pigment (bile) in bile canaliculi were observed in 47 (81%), accompanied by infrequent pyknosis of hepatocytes nucleus in hepatic parenchyma. Forty-five infested liver samples (77.6%) showed congested central veins in hepatic parenchyma (Figure 4.5). Microscopic examination of the black pigmented areas of 1 (1.7%) of the infected livers revealed an occasional accumulation of fine yellowish-brown or black granules intermixed with hepatocytes with pale cytoplasm and infiltrates of lymphocytes, plasma cells eosinophils and fibroblasts (Figure 4.6).

The locally extensive indurated area exhibited a marked alteration the normal hepatic lobular architecture. Scattered foci of regenerative hepatocytes, separated by bands of fibrous connective tissue were also noted in the parenchyma. In portal triads, distortion and occurrence of hypertrophied and hyperplastic bile ducts accompanied by mononuclear cell infiltrates, numerous small thin-walled blood vessels and fibrous connective tissue proliferation were observed (Figure 4.9). Elsewhere in the parenchyma, accumulation of brownish pigment among hepatocytes, hepatic tissue regeneration of varying sizes between many haphazardly arranged bands of fibrous

connective tissue were observed. Also, in other areas of the parenchyma, marked hepatocyte necrosis, mononuclear inflammatory infiltrates and replacement of hepatic mass with heavy fibrous connective tissue was observed characteristic of hepatic fibrosis (Figure 4.9).

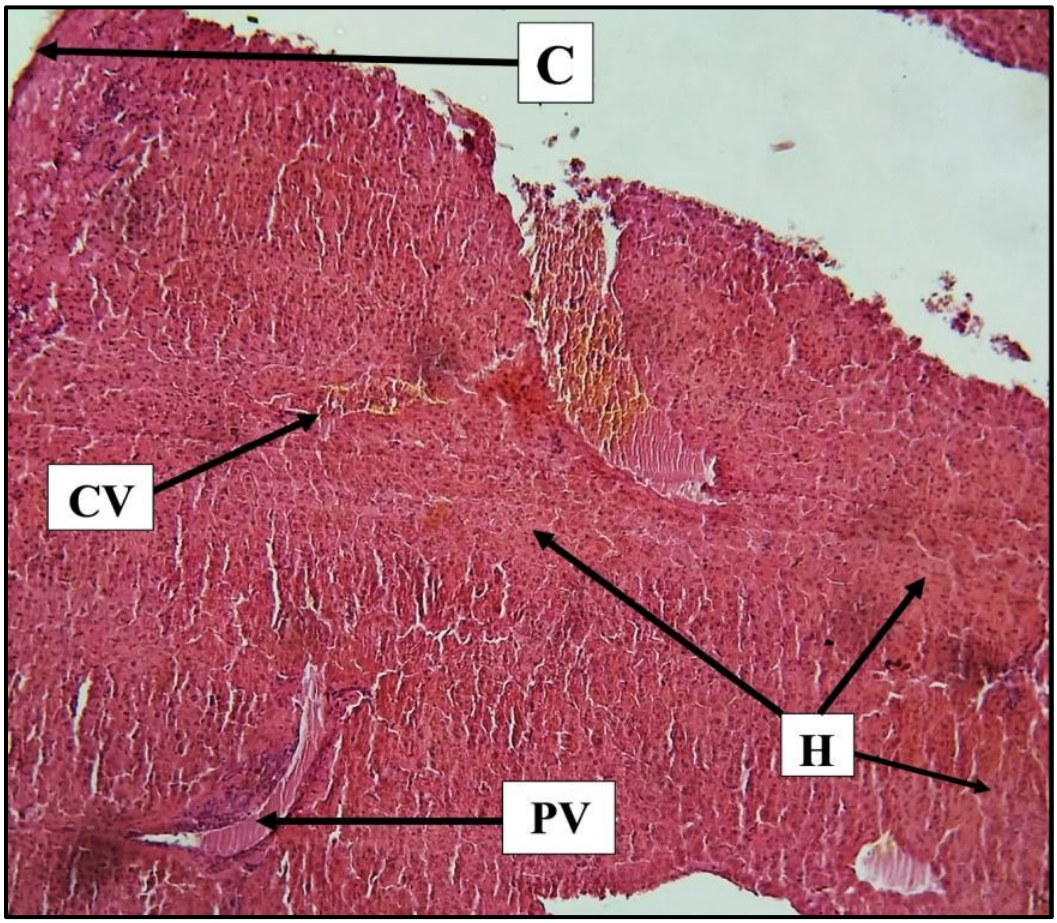


**Figure 4.5 Photomicrographs of liver tissue from slaughtered animal (ID: SS033), showing loss of normal hepatic lobular architecture, congested central vein (CV) and portal vein (PV). Hepatocytes and liver capsule are indicated by (H) and (C) respectively (H&E X40)**

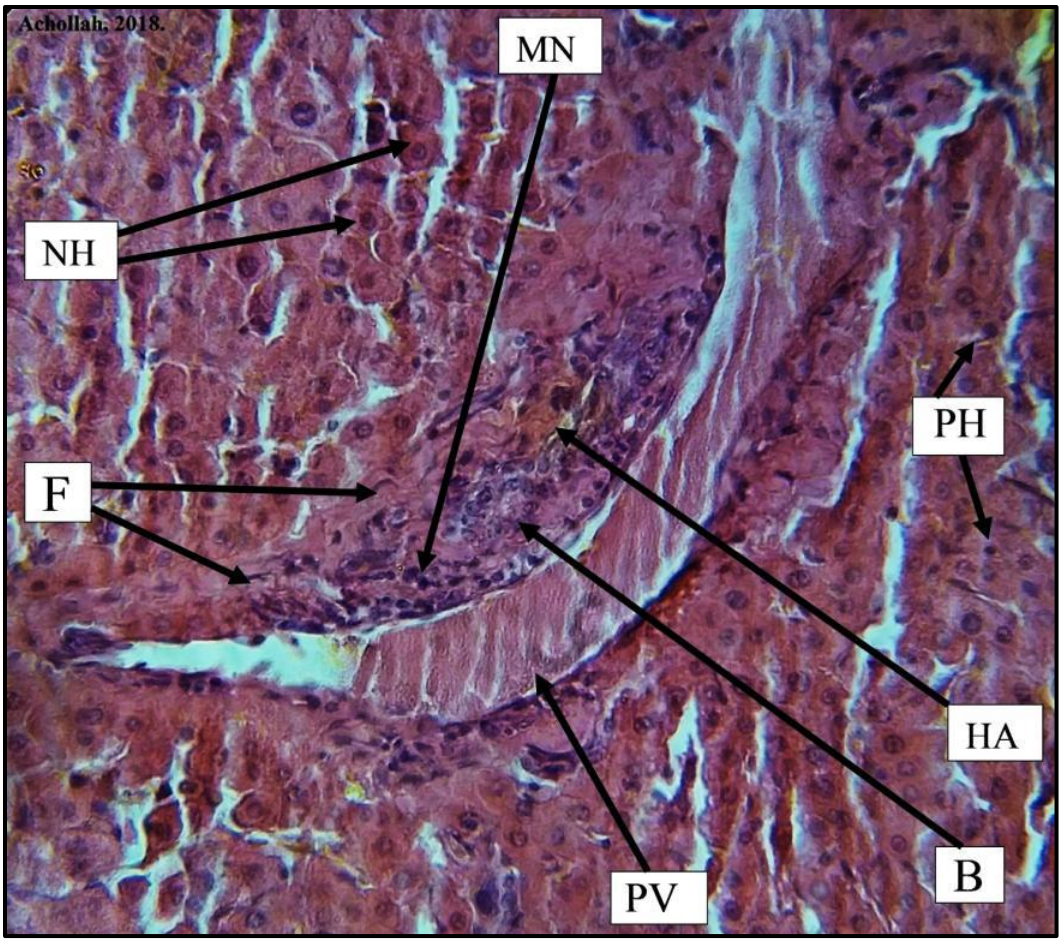


**Figure 4.6 Photomicrographs of liver tissue from slaughtered animal (ID: SS033), (Portal area), showing normal hepatocytes (NH), disruption and proliferation of bile duct epithelium (B), disruption of a branch of hepatic artery (HA) and portal vein (PV), mononuclear cells infiltration (MN), proliferation of fibrous connective tissue (F) and pyknosis of hepatocytes (PH) (H&E X100)**

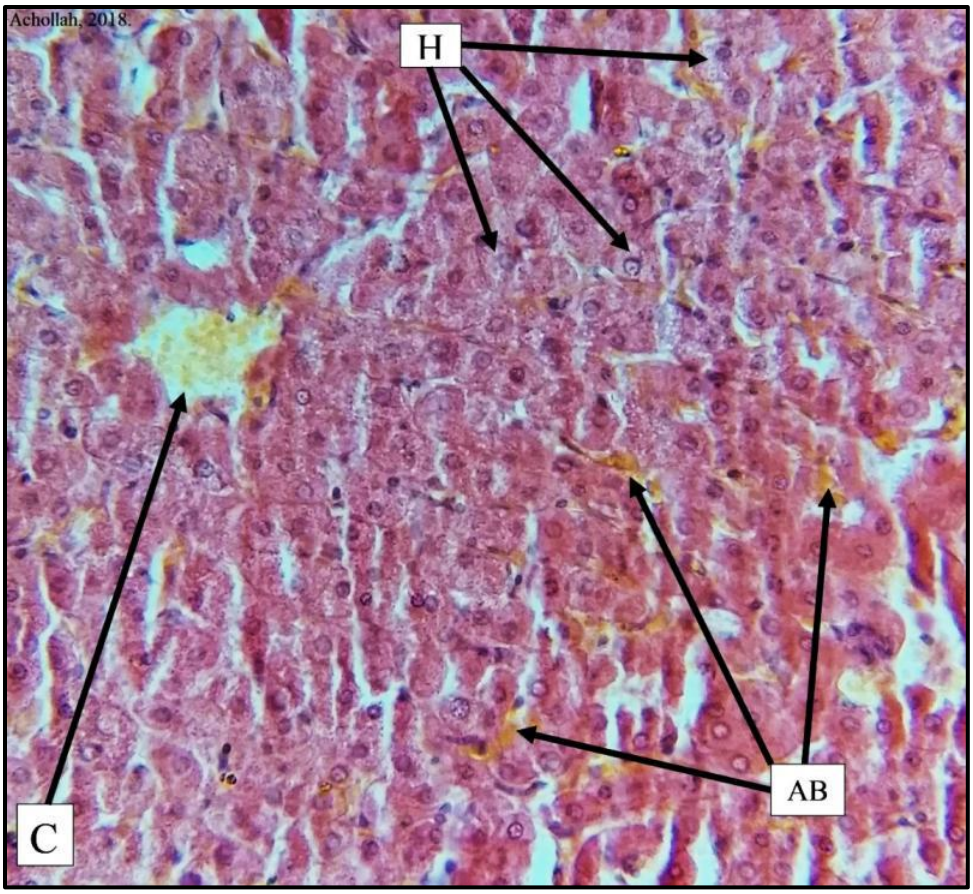


**Figure 4.7 Photomicrographs of liver tissue from slaughtered animal (ID: SS033), showing congested central vein (C), accumulation of yellowish-brown pigment (bile) in bile canaliculi (AB), vacuolar degeneration of hepatocytes (H) (H&E X100)**

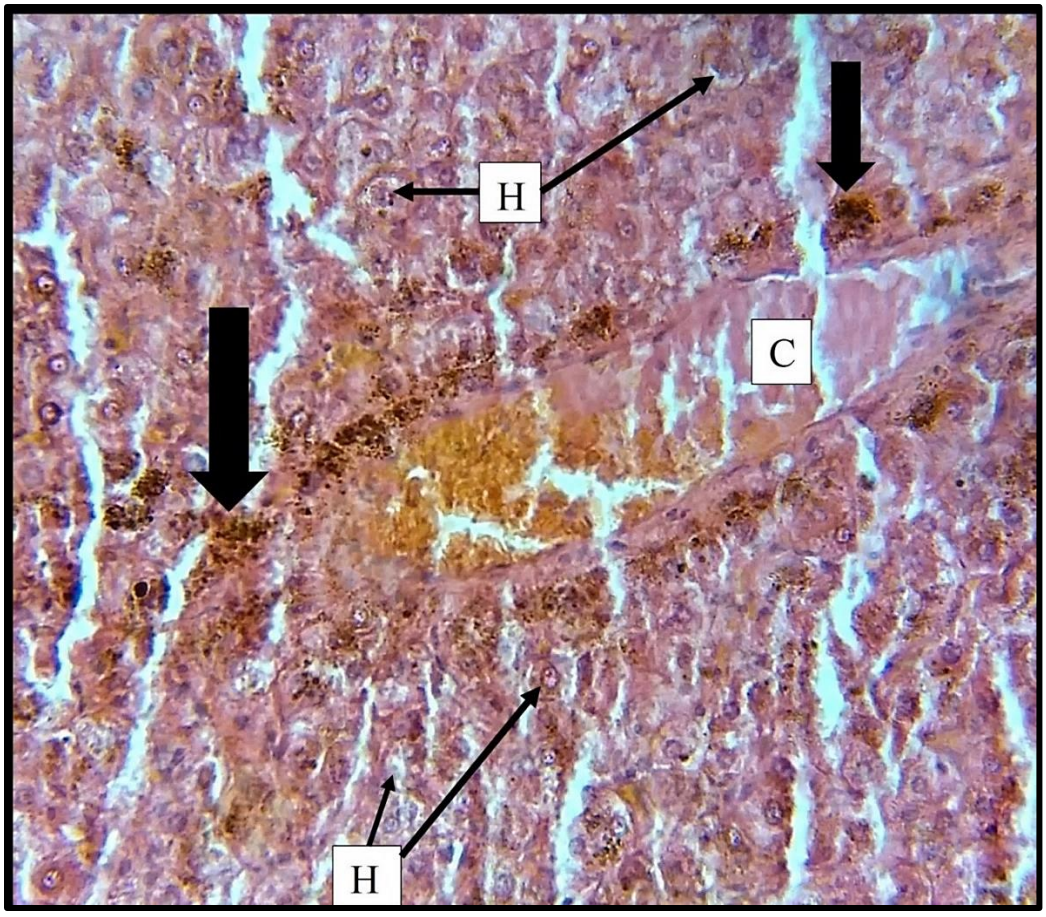


**Figure 4.8 Photomicrographs of liver tissue from slaughtered animal (ID: SS033) showing accumulation of fine yellowish-brown to black granules (thick arrows) (fluke puke) admixed with hepatocytes with pale cytoplasm (H) and mononuclear cell infiltrates and fibroblasts, central vein (C) (H&E X400)**

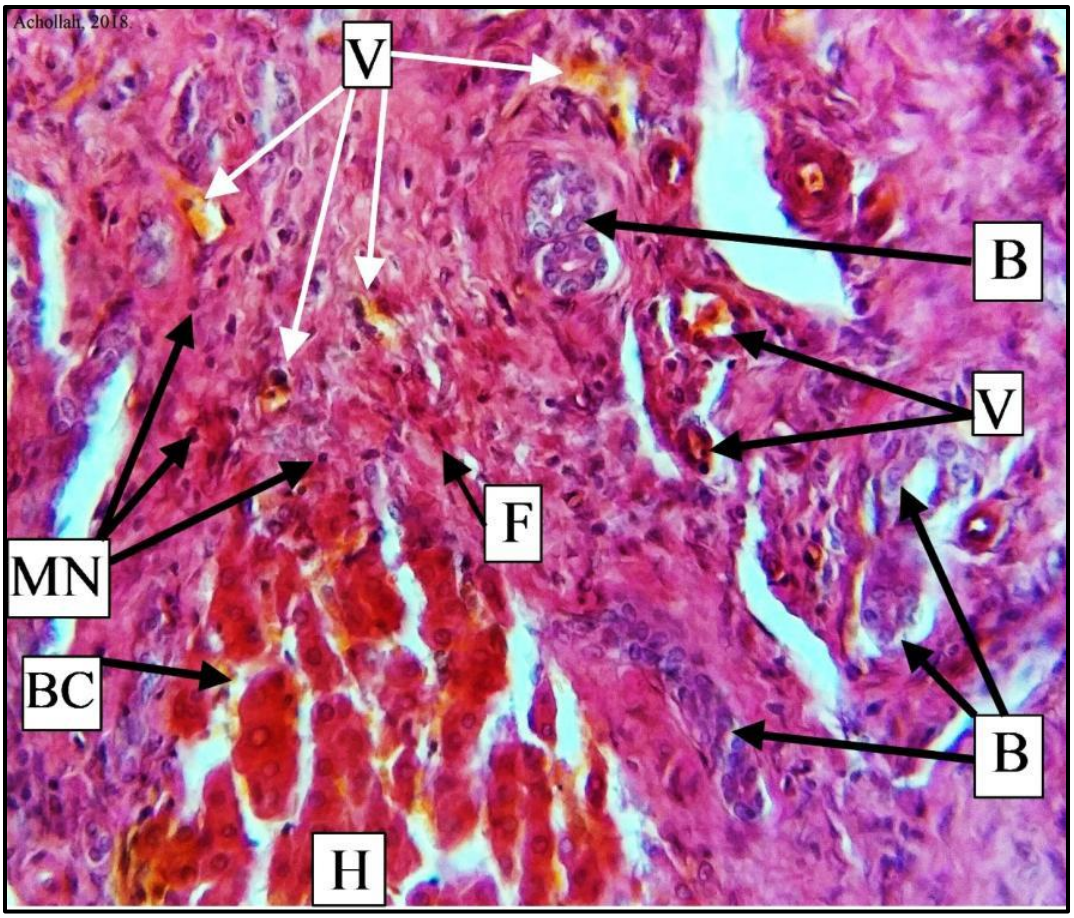


**Figure 4.9 Photomicrograph of liver sections from slaughtered animal (ID: SS012), showing distortion of portal triad, with haphazardly arranged fibrous connective tissue (F), mononuclear inflammatory infiltrates (MN), numerous small thin-walled blood vessels (V), proliferation of bile ducts (B) and accumulation of brownish material (BC) in the adjacent regenerative hepatocyte nodule (H) (H&E X400)**

#### 4.2.4.1.3 Identification of the liver flukes recovered from slaughtered cattle

From the 58 infected livers, a total of 87 liver flukes were collected for morphological analysis in the laboratory. All were pale grey or brown, 18-43mm long (mean: 30.41mm), transparent, leaf-shaped with a short conical anterior end and had barely distinguishable shoulders (Figure 4.10). The morphological appearance (Figure 4.10) and morphometric parameters measured (Appendix 8.18 were consistent with *Fasciola gigantica*.

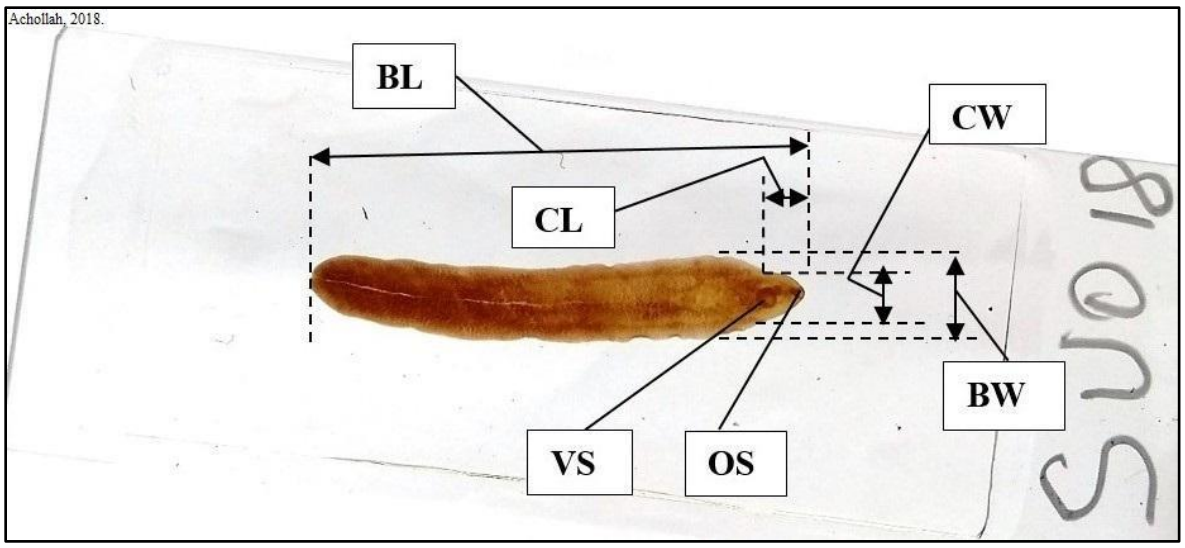


**Figure 4.10 Morphological appearance of liver fluke specimen recovered from slaughtered animal (ID: SU018), showing the long transparent and leaf-shaped body with barely distinguishable shoulders. The ventral sucker (VS) and oral sucker (OS) are indicated on the anterior part. Some of the morphometric parameters measured are indicated: body length (BL), body width (BW), cone length (CL) and cone width (CW).**

#### 4.2.4.2 Hepatic hydatidosis

Hepatic hydatidosis was diagnosed in a condemned liver of one slaughtered animal (0.9%) during post mortem inspection (Cattle ID: SU001); the condition was observed in Ugunja slaughterhouse.

##### 4.2.4.2.1 Gross morphological appearance of the condemned organ

The respective liver had the following gross lesions: chocolate-colored, enlarged with visible greyish-white, soft to firm multifocal areas of fluid (clear)-filled cysts ranging in size from 1.5 cm to 3 cm in diameter and protruding above the surface (Figure 4.11). Cutting through the liver, cavities lined by a white membrane and other fluid-filled cysts at different depths in the liver parenchyma were observed, characteristic of hepatic hydatidosis.

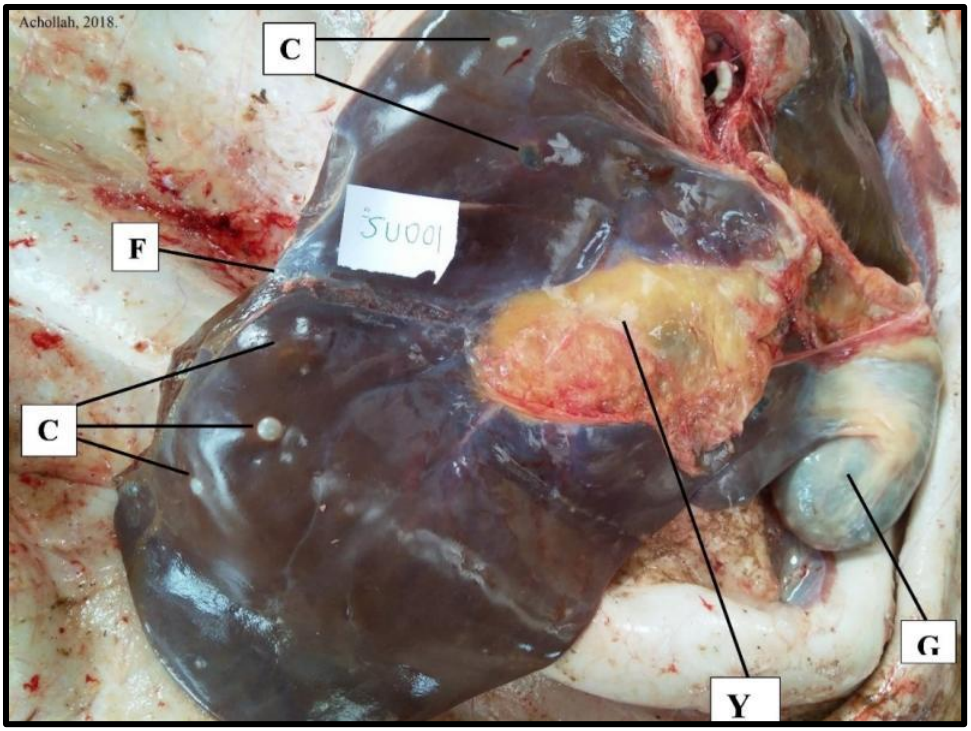


**Figure 4.11 Diaphragmatic surface of liver from the animal (ID: SU001) diagnosed with hepatic hydatidosis showing greyish-white soft to firm multifocal fluid-filled swellings (C) within the parenchyma. Gall bladder (G), a section of the falciform ligament (F) and fat tissue covering part of the coronary ligament (Y) are indicated**

#### 4.2.4.2.2 Histopathological manifestations of the condemned organ

On carrying out histopathological examination, the liver revealed multifocal, fibrous laminated cyst-walls, compressing structures in the hepatic parenchyma (Figure 4.12). The cyst-wall consisted of an inner layer with nuclei-like structures, an acellular laminated middle layer and a fibrous outer layer with inflammatory cells (Figure 4.13). Variable degeneration of hepatocytes, inflammatory cell infiltrations, congestion of hepatic sinusoids as well as central veins was observed in areas of the parenchyma adjacent to the cyst walls (Figure 4.14). Mild/scattered peri-cystic inflammatory infiltrates of eosinophils, lymphocytes, macrophages and occasionally neutrophils were also seen in other areas of the hepatic parenchyma adjacent to the cyst-wall (Figure 4.15). In other areas, hepatic portal veins were congested; these were also located in

adjacent portal triads accompanied by fibrous connective tissue proliferation and increased number of bile ducts. Nuclei-like structures were seen lining the innermost layer (germinal layer) of the cyst walls (Figure 4.16), while mononuclear inflammatory cells infiltrated the outer layer.

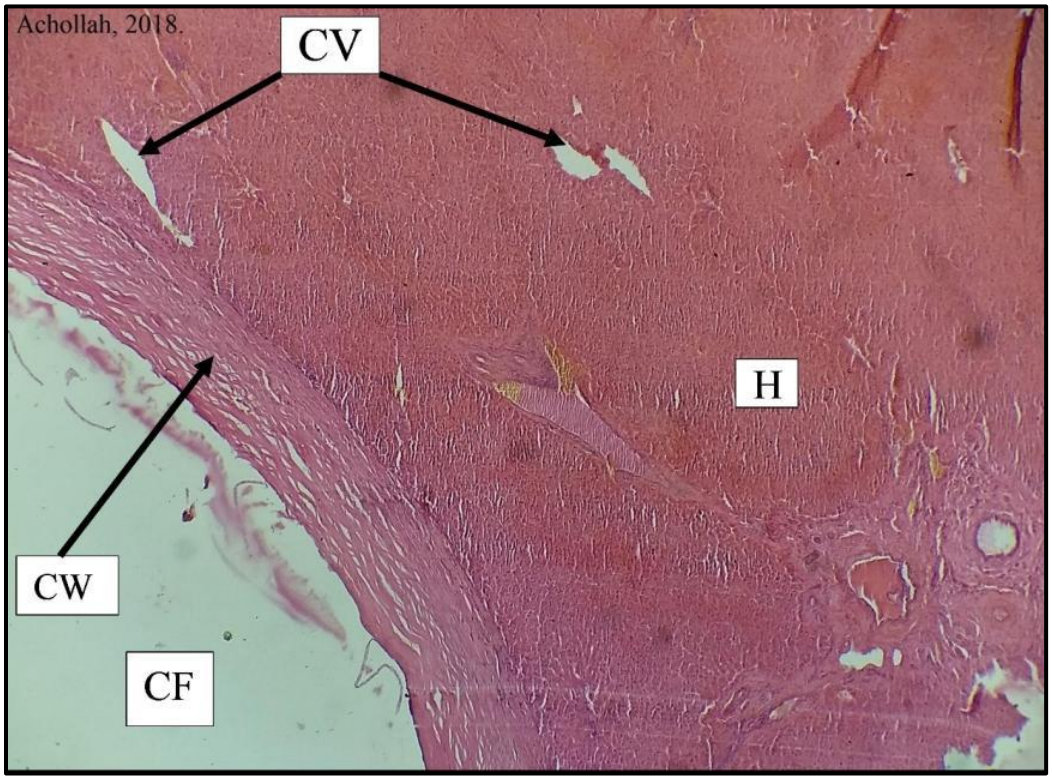


**Figure 4.12 Photomicrograph of liver tissue from the slaughtered animal (ID: SU001) diagnosed with hepatic hydatidosis, showing a section of the cyst wall (CW) within hepatic parenchyma (H), with compressed central veins (CV). Cyst fluid (CF) is indicated (H&E X40)**

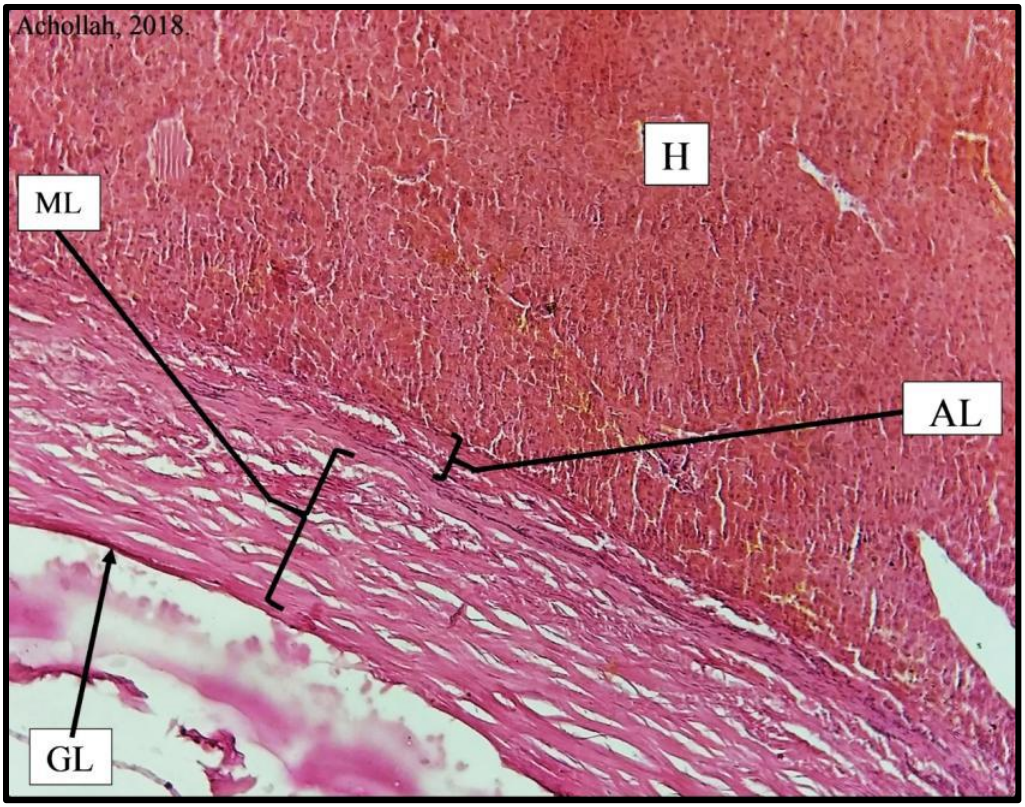


**Figure 4.13 Photomicrograph of liver tissue from the slaughtered animal (ID: SU001) diagnosed with hepatic hydatidosis, showing, layers of the cyst wall: germinal (innermost) layer (GL), eosinophilic laminated middle layer (ML) and adventitial (outer) layer (AL). Hepatocytes (H) are indicated (H&E X100)**

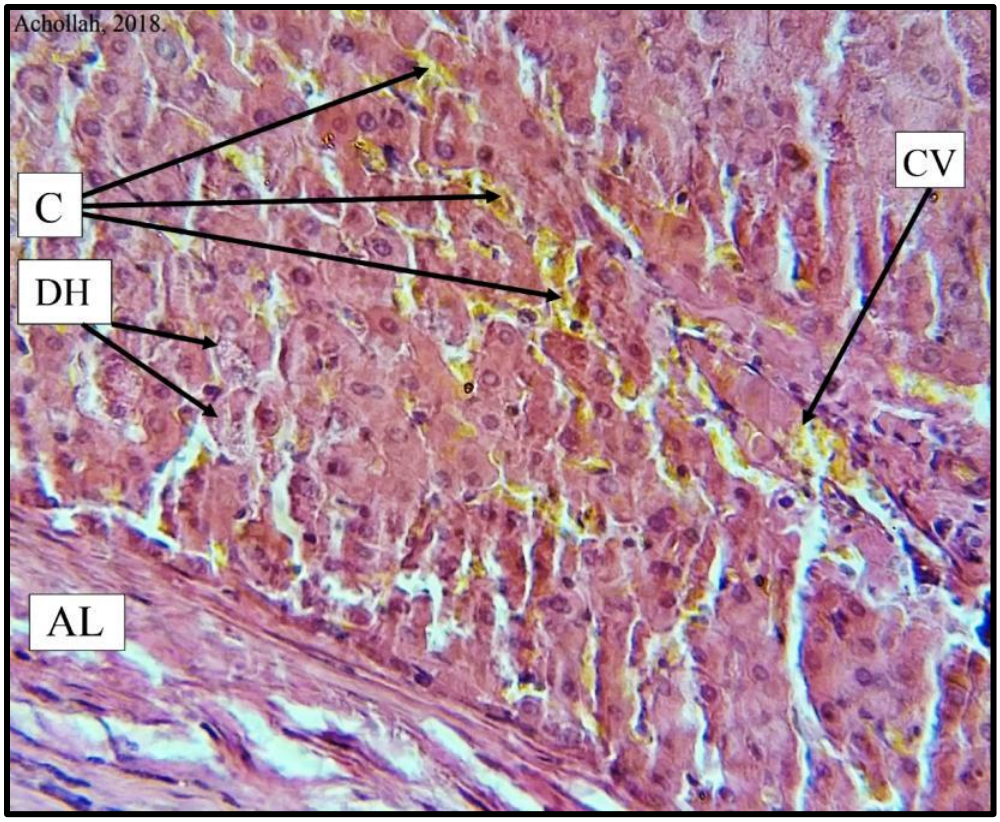


**Figure 4.14 Photomicrograph of liver tissue from slaughtered animal (ID: SU001) diagnosed with hepatic hydatidosis, showing congestion of hepatic sinusoids (C) and central veins (CV) and degenerating hepatocytes of varying degrees (DH). Adventitial layer (AL) is indicated (H&E X400)**

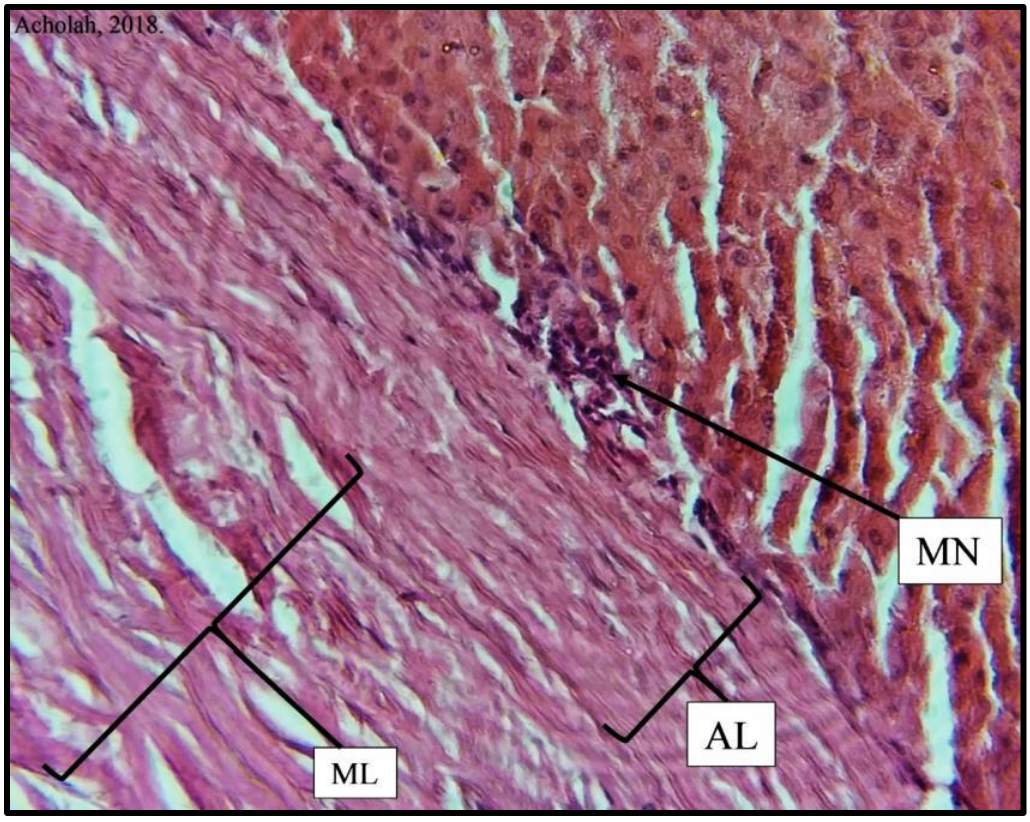


**Figure 4.15 Photomicrograph of liver tissue from slaughtered animal (ID: SU001) diagnosed with hepatic hydatidosis showing mild peri-cystic mononuclear inflammatory infiltrates, a few neutrophils and fibrous connective tissue (MN). The middle layer (ML) and adventitial layer (AL) are shown (H&E X400)**

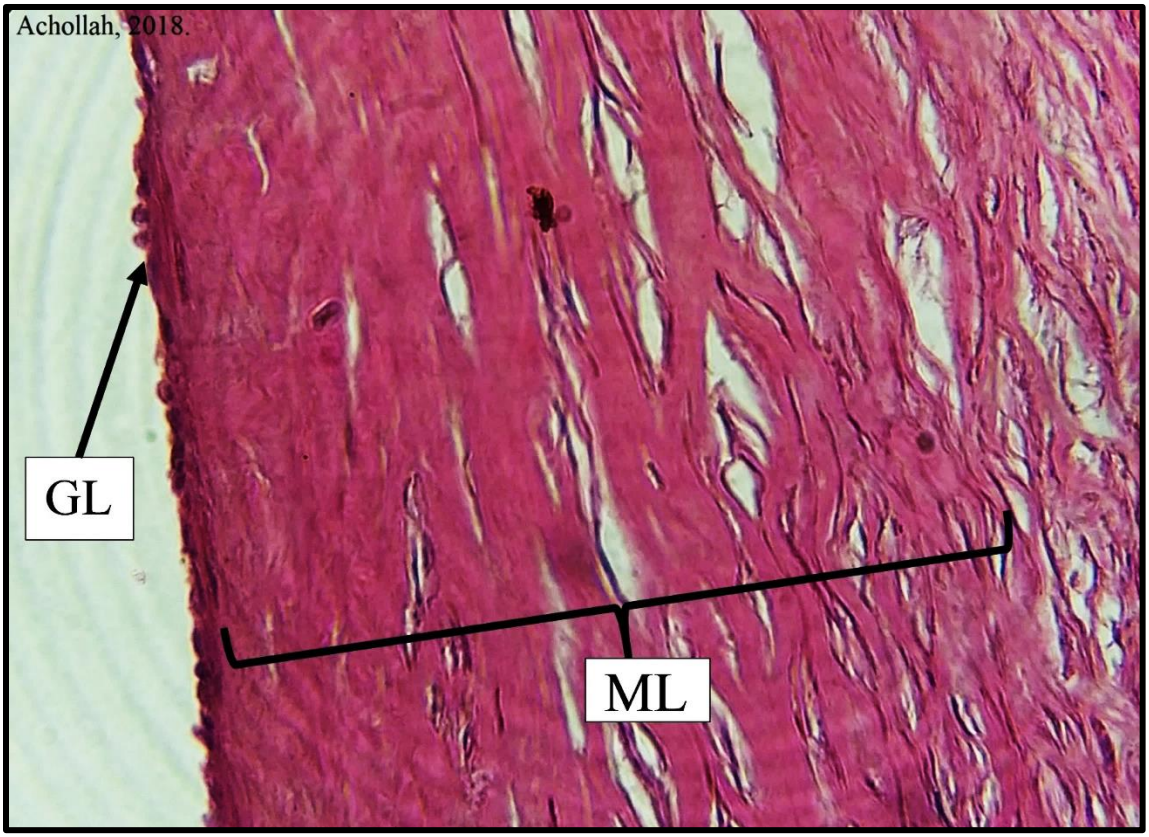


**Figure 4.16 Photomicrograph of liver tissue from slaughtered animal (ID: SU001) diagnosed with hepatic hydatidosis, showing the germinal layer (GL) of the cyst wall with nuclei like structures, laminated middle layer (ML) (H&E X400)**

#### 4.2.4.3 Intestinal nematodiasis (pimply gut)

Intestinal nematodiasis (pimply gut) was diagnosed in 28 (25%) out of the slaughtered cattle during post mortem inspection. With respect to Kaumara, Siaya and Ugunja slaughterhouses, 5 (17.9%), 19 (67.9%) and 4 (14.3%) of the 28, had intestinal nematodiasis.

##### 4.2.4.3.1 Gross morphological appearance of the respective condemned organ

Grossly, nodules measuring 3 – 5 mm in diameter were observed projecting from the serosal surfaces of the intestines; 20 (71.4%) in small intestines and 8 (28.6%) in large intestines. All 28

nodular swellings were hard; 10 (35.7%) of which were greyish/ pale green, while the rest were off white/pink 18 (64.3%) (Figure 4.17).

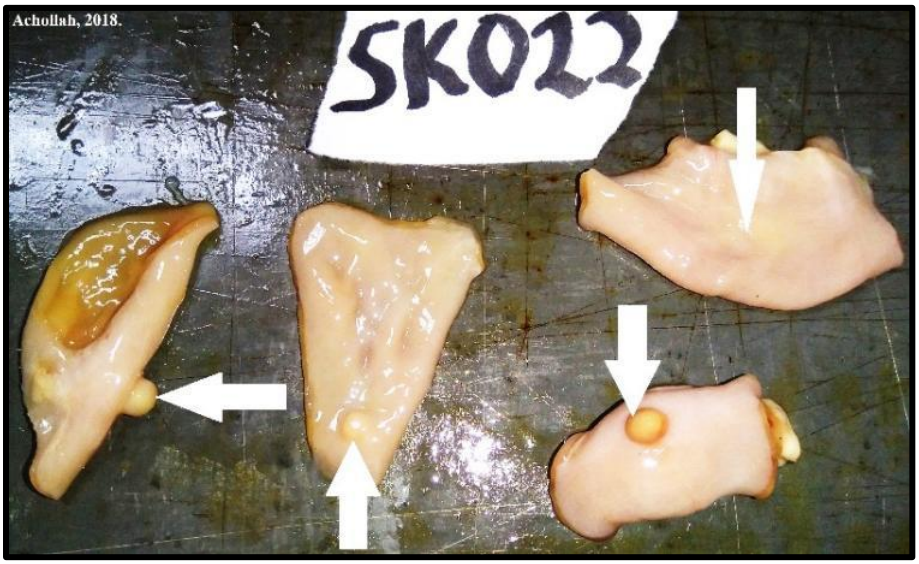


**Figure 4.17 Portions of the small intestine from animal (ID: SK022) showing multifocal nodular swellings (arrows) on the serosal surfaces of small intestines**

#### 4.2.4.3.2 Histopathological manifestations of the respective condemned organ

On carrying out histopathological examination of the 20 (71.4%) intestinal specimens, focal to multifocal irregular /round-shaped structures were seen within the wall of intestines (Figure 4.18); with 10 (50%) of them located in the tunica serosa, 5 (25%) in sub-mucosa and the other 5 (25%) displacing the tunica muscularis.

In all specimens, the tunica mucosa adjacent to the nodules showed sloughing, disorganized architecture, increased population of goblet cells and mixed inflammatory infiltrates comprising lymphocytes, plasma cells and a few neutrophils (Figure 4.19).

The lamina muscularis adjacent to the nodule was disrupted and also revealed mixed inflammatory infiltrates (Figure 4.20). Congestion of veins was observed in the submucosa of all the nodules examined, these were also accompanied by heavy mixed inflammatory infiltrates comprising lymphocytes, plasma cells, macrophages and many eosinophils (Figure 4.20).

On examination of the tunica muscularis in all nodules, disruption and presence of mixed inflammatory infiltrates – macrophages, lymphocytes, and eosinophils, and fibrous connective tissue proliferation was observed (Figure 4.21). A thin fibrous capsule surrounded the nodules within the intestinal walls in all specimens examined (Figure 4.22); the centre of the nodules was composed of eosinophilic necrotic cellular debris, giant cells, lymphocytes, eosinophils, and a few neutrophils, also centred on the nematode larvae and its remnants (Figures 4.23, 4.24 and 4.25). Deep purple staining zones (regarded as calcification) were observed in 5 (25%) of the nodules. Examination of the tunica serosa also revealed mixed inflammatory infiltrates comprising macrophages, eosinophils a few lymphocytes and giant cells. Larval parts were observed in 5 (25%) of the nodules.

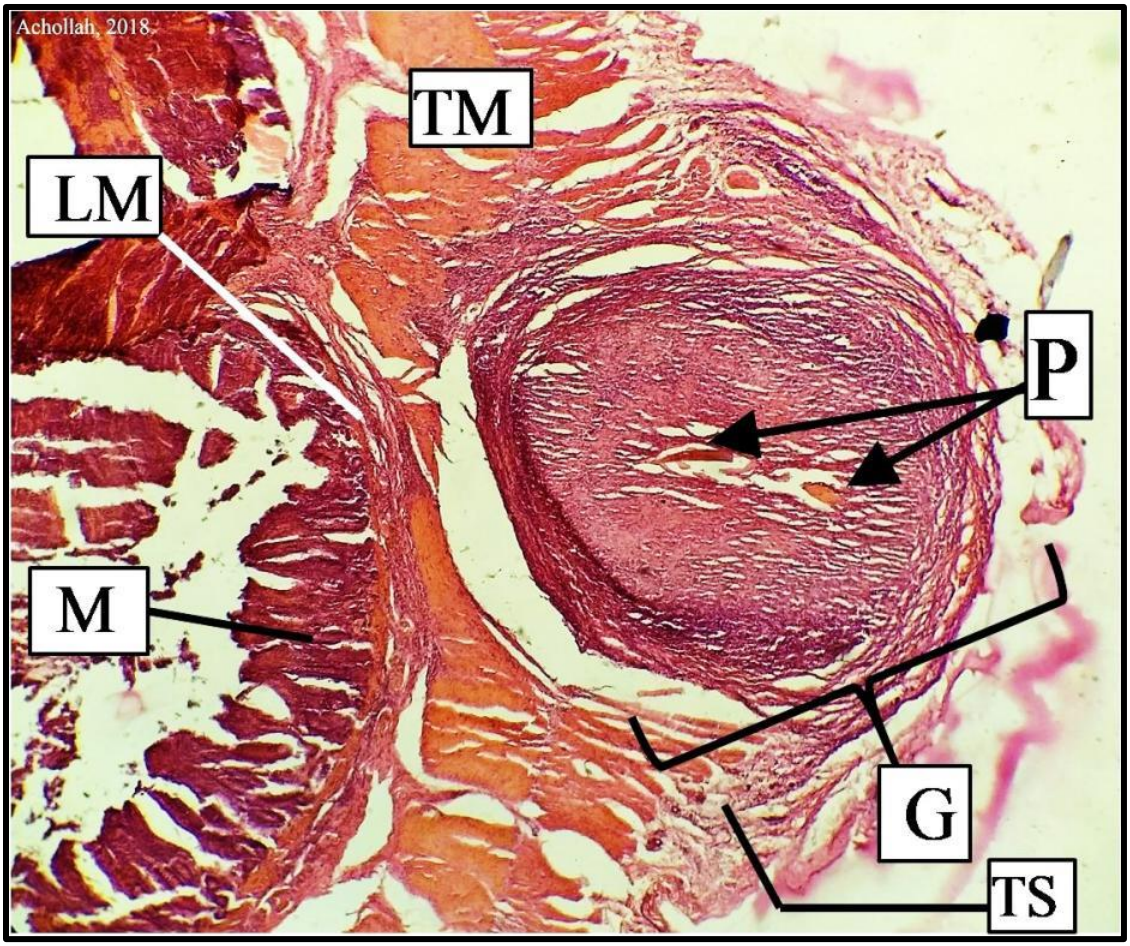


**Figure 4.18 Photomicrograph of the nodular lesion in the intestinal wall from slaughtered animal (ID: SK001), showing circular/uneven shaped nodule (G) displacing the tunica muscularis (TM) and serosa (TS), parasite parts (P) located at the centre. Lamina muscularis (LM) and mucosa (M) are indicated (H & E X40).**

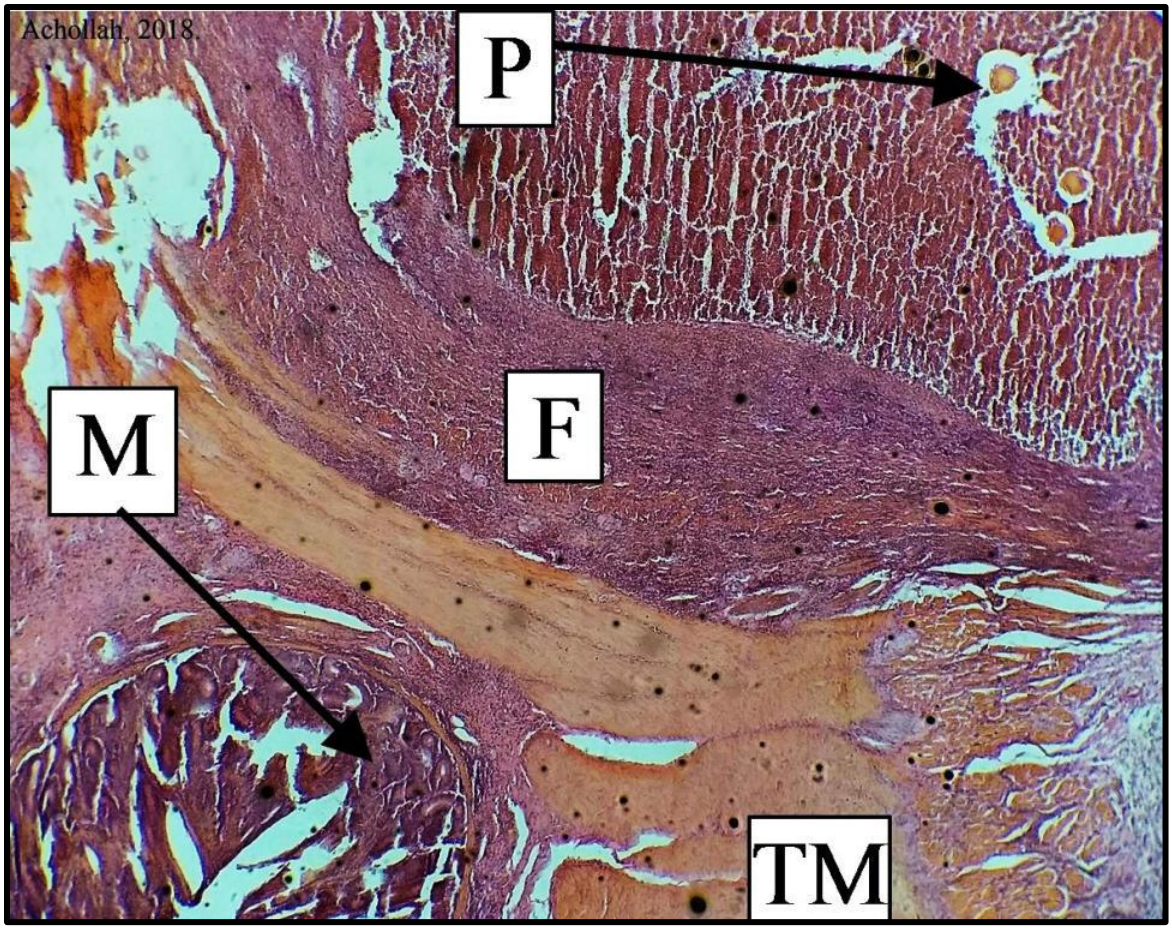


**Figure 4.19 Photomicrograph of the nodular lesion in the intestinal wall from slaughtered animal (ID: SK026), showing nodule with parasite parts (P) surrounded by a thin fibrous capsule (F) disrupting and displacing the tunica muscularis (TM). Mucosal layer (M) is indicated (H & E X40).**

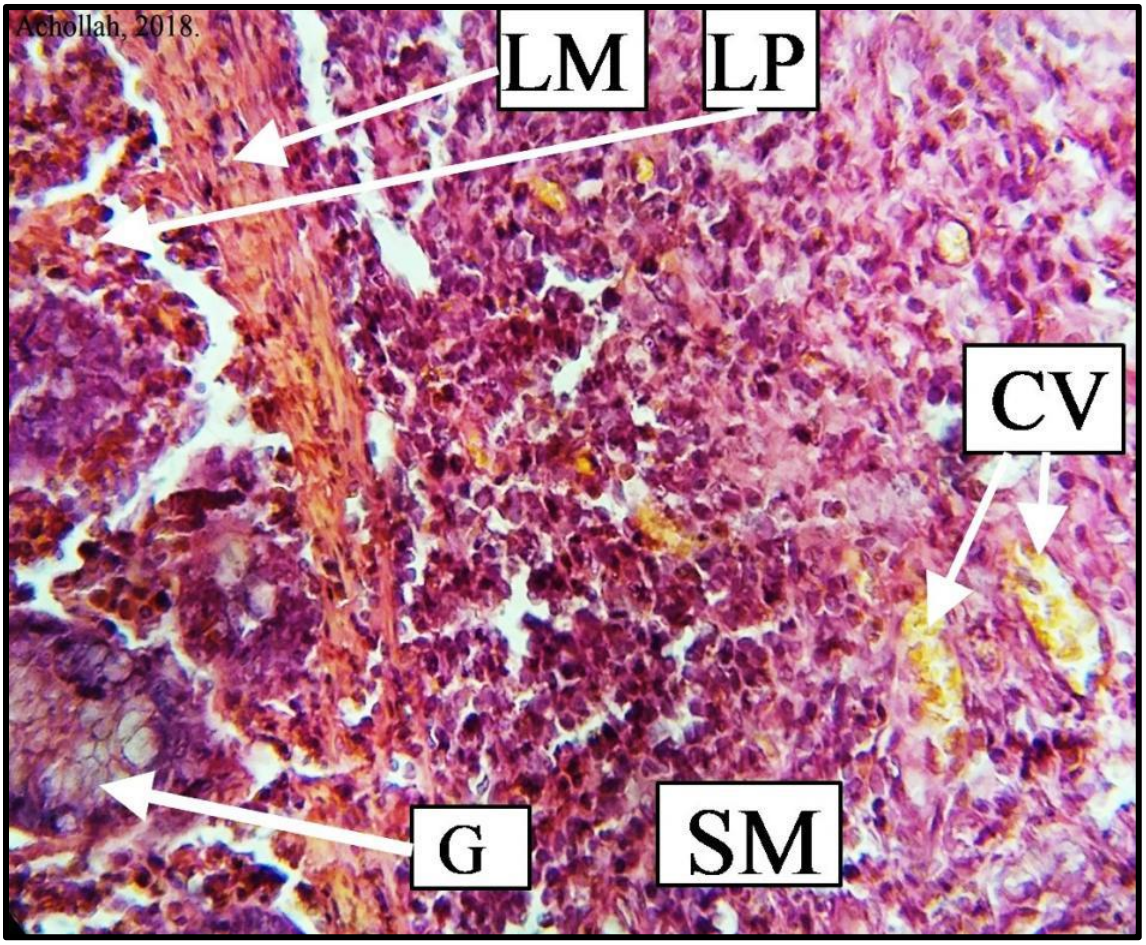


**Figure 4.20 Photomicrograph of the nodular lesion in intestinal wall from slaughtered animal (ID: SK026), showing a disorganized tunica mucosa with hyperplasia of goblet cells (G); disrupted lamina muscularis (LM) and lamina propria (LP) with mixed inflammatory infiltrates; submucosa (SM) showing heavy mixed inflammatory infiltrates and congested veins (CV) (H & E X100)**

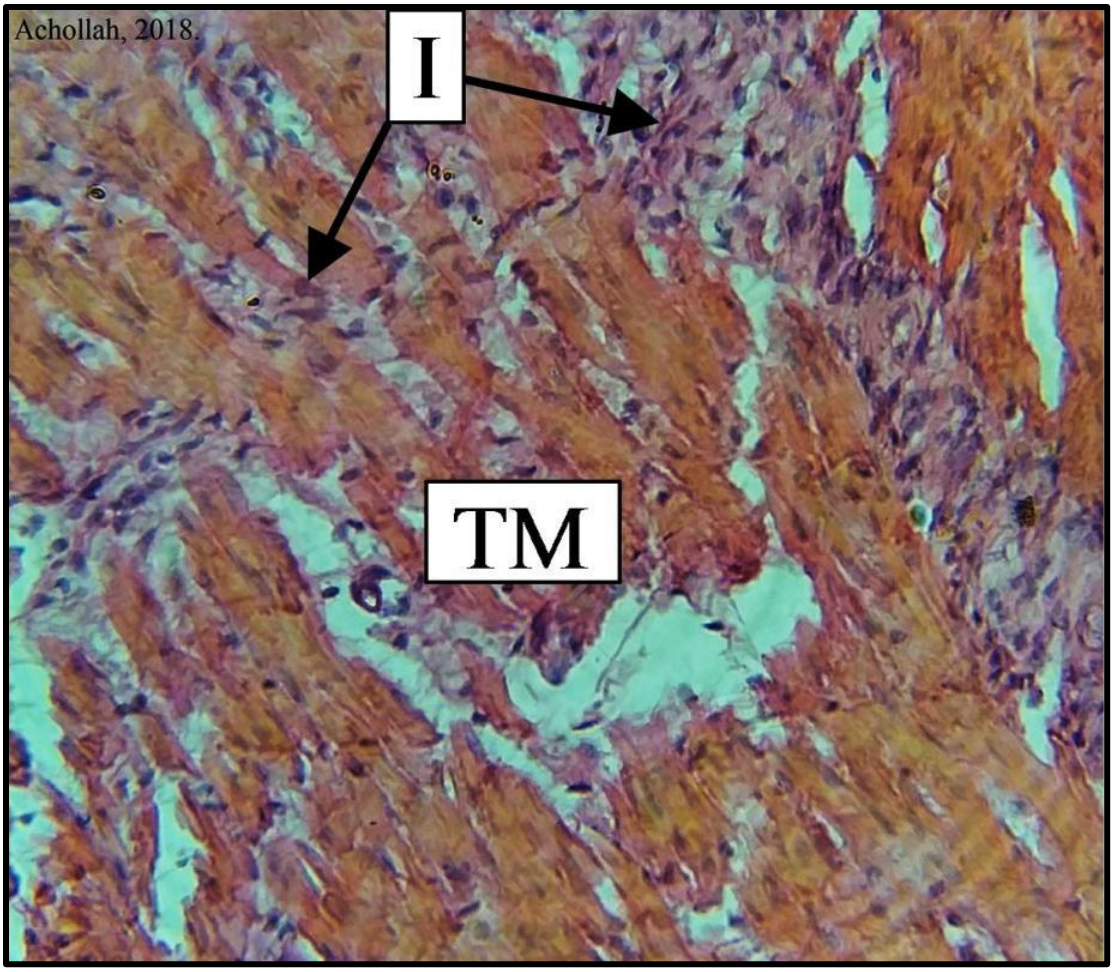


**Figure 4.21 Photomicrograph of the nodular lesion in the intestinal wall from slaughtered animal (ID: SK026), disruption of the tunica muscularis (TM) with mixed inflammatory infiltrates and fibrous connective tissue (I) (H & E X100)**

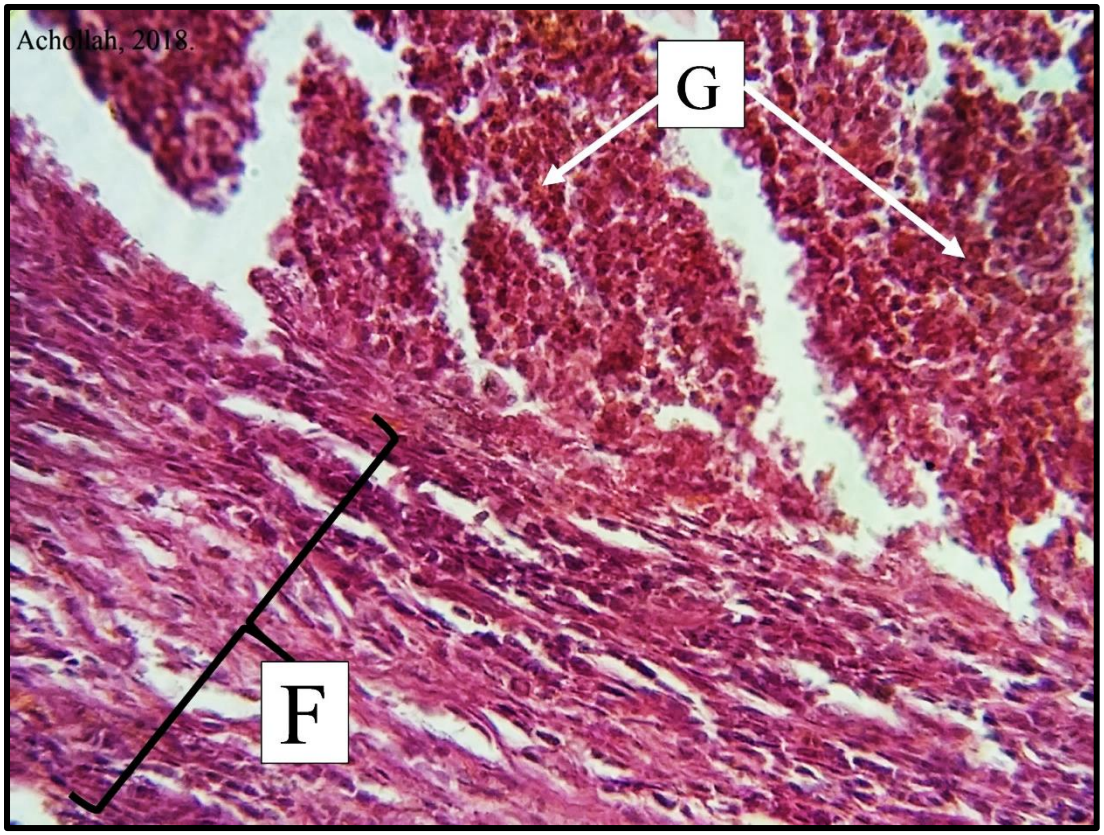


**Figure 4.22 Photomicrograph of a section of the nodular lesion in the intestinal wall from slaughtered animal (ID: SK026), showing fibrous capsule (F) surrounding the inner core of the nodule (G) of mixed necrotic inflammatory cells (H & E X100)**

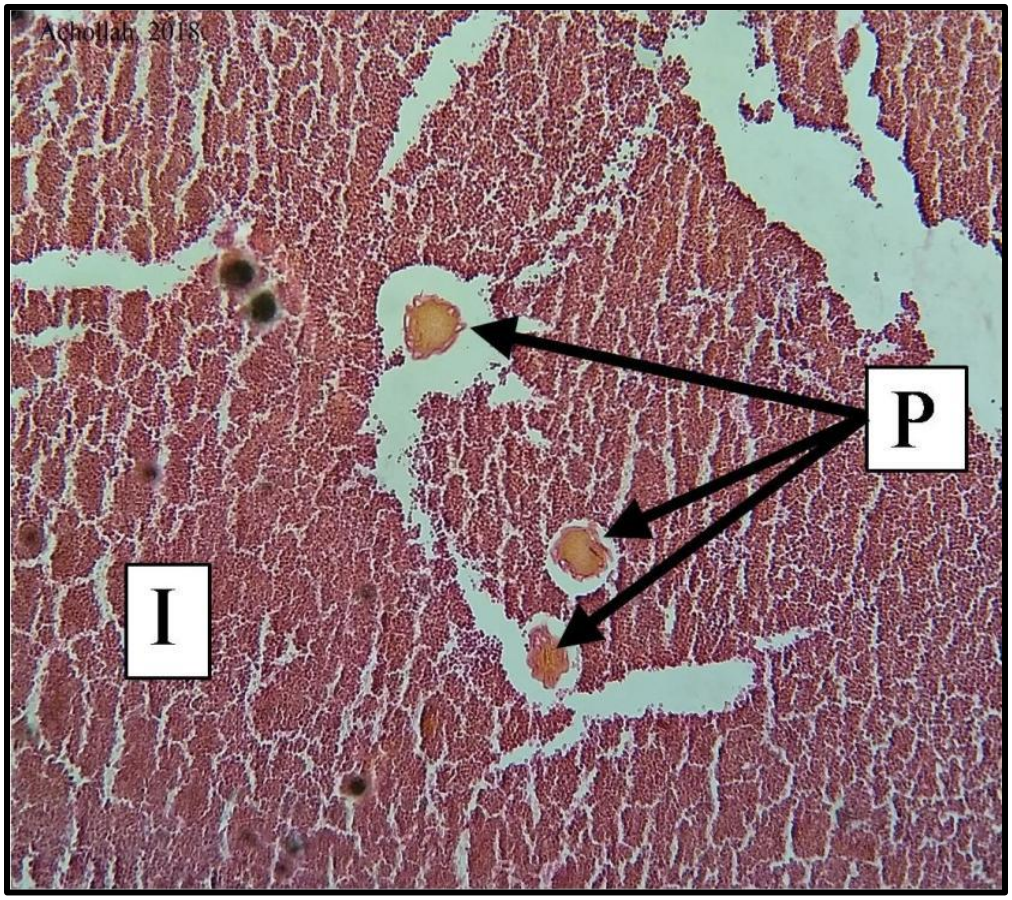


**Figure 4.23 Photomicrograph of the nodular lesion in the intestinal wall from slaughtered animal (ID: SK026), showing the central core of mixed cellular infiltrates (I) centred on the parasite parts (P) (H&E X100)**

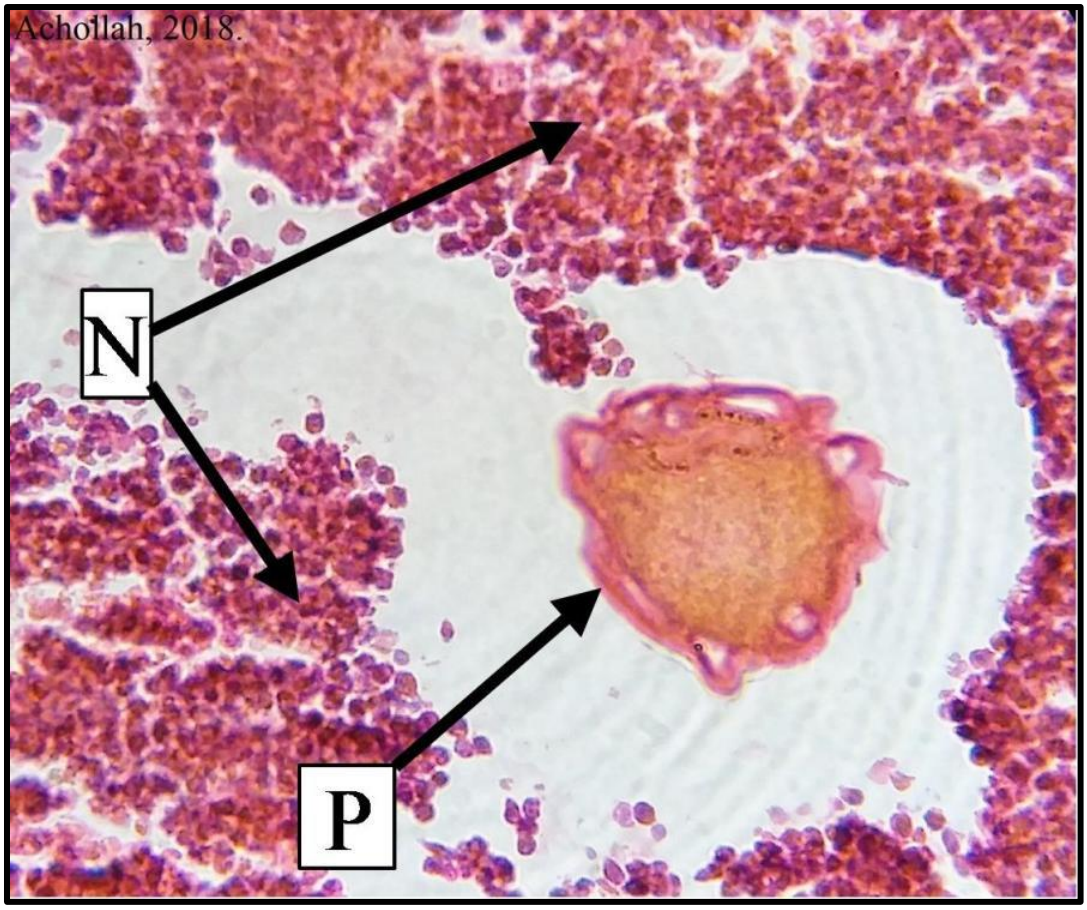


**Figure 4.24 Photomicrographs of the nodular lesion in the intestinal wall from slaughtered animal (ID: SK026), showing necrotic inflammatory cellular debris (N) centred on the parasite part (P) (H & E X400)**

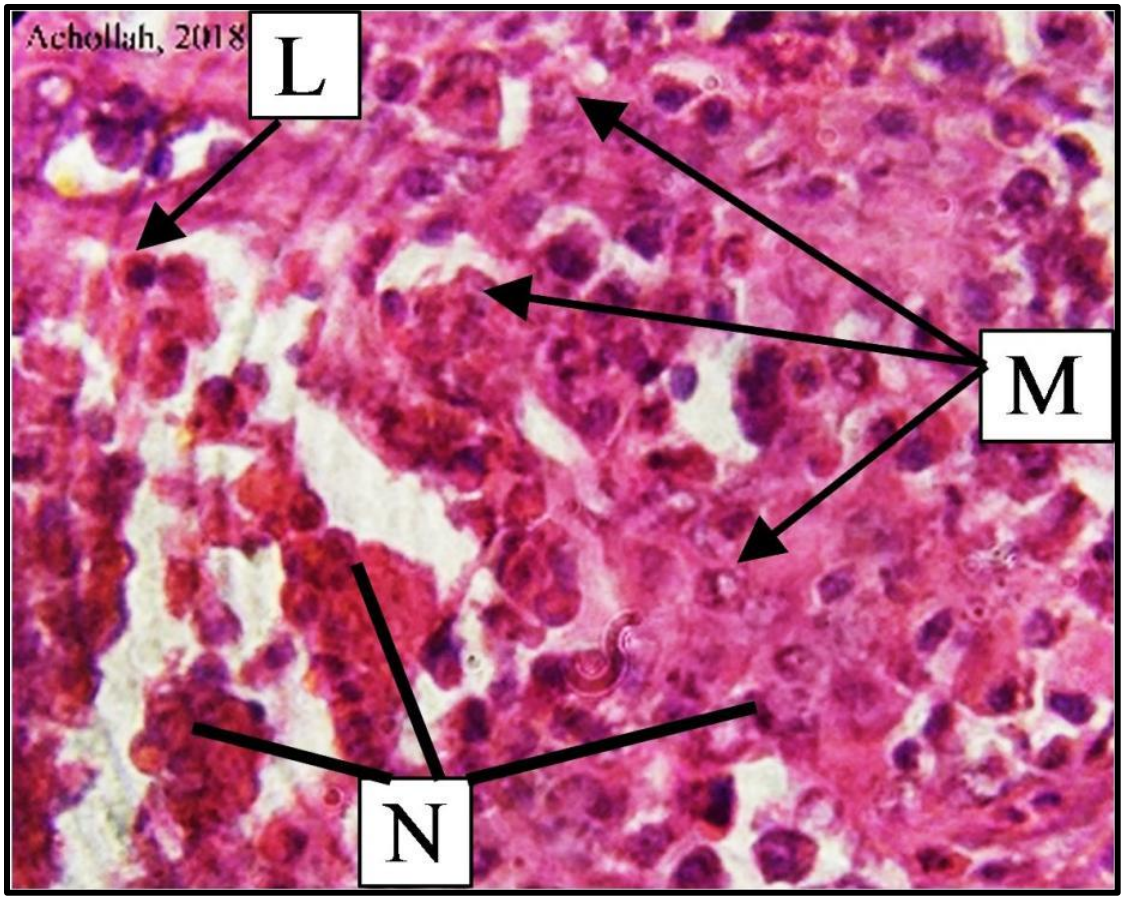


**Figure 4.25 Photomicrographs of the nodular lesion in the intestinal wall from slaughtered animal (ID: SK026), showing a section of the central core with necrotic cell debris (N): macrophages (M) and lymphocytes (L) (H&E X630)**

#### 4.2.4.3.3 Parasitological analysis of the recovered nematode larvae

Of the 8 (28.6%) randomly selected intestinal nodules digested in pepsin – HCL, the nematode larvae were recovered from 2 (25%) of them. Microscopic examination of the recovered larvae showed broad, rounded heads, long cephalic space and filamentous tail sheath consistent with *Oesophagostomum* species (Figure 4.26).

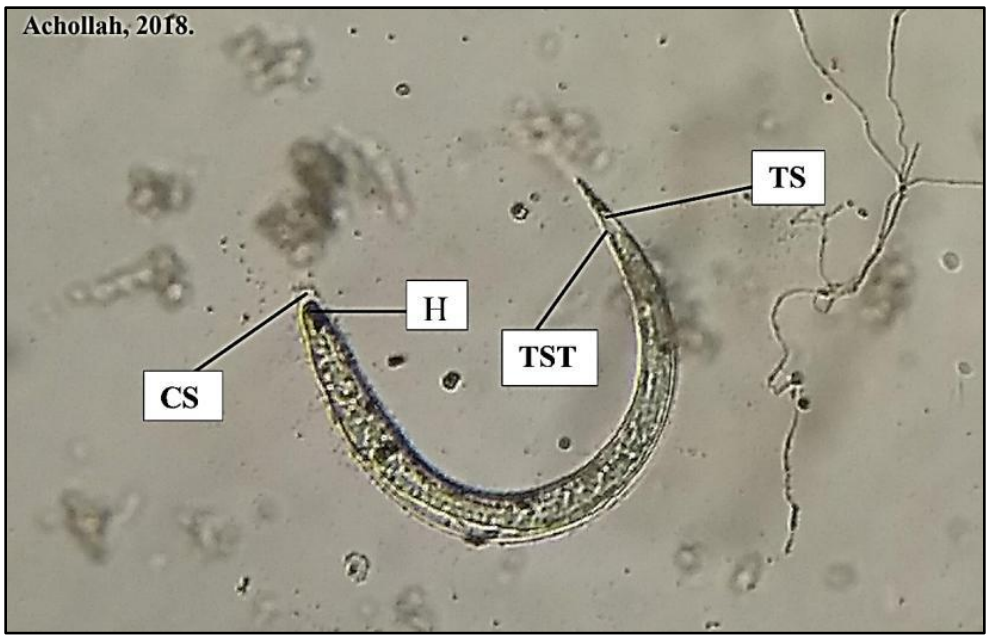


**Figure 4.26 Photomicrograph of nematode larvae recovered from nodules in the small intestinal wall of a slaughtered animal (ID: SU023), through pepsin-HCL digestion. Broad rounded head (H), cephalic space (CS), filamentous tail sheath (TST) and long tail space (TS), are indicated (X40)**

#### 4.2.4.4 Pulmonary blood aspiration

Pulmonary blood aspiration was diagnosed in 2 (1.8%) of the slaughtered cattle, ID: SK040 and SS004, from Kaumara and Siaya slaughterhouses respectively (Figures 4.27 A and B).

##### 4.2.4.4.1 Gross morphological appearance of the respective condemned organs

Grossly, both lungs had dark-red multiple foci with feathery boundaries under the visceral pleura with a soft and spongy consistency (Figure 4.27 A and B). Cutting through the lung and trachea, for animal number SK040, un-clotted blood mainly and a few small scattered pieces of ingesta were observed in the trachea, bronchi, and bronchioles; while cattle number SS004 revealed only blood in the trachea, bronchi and bronchioles.

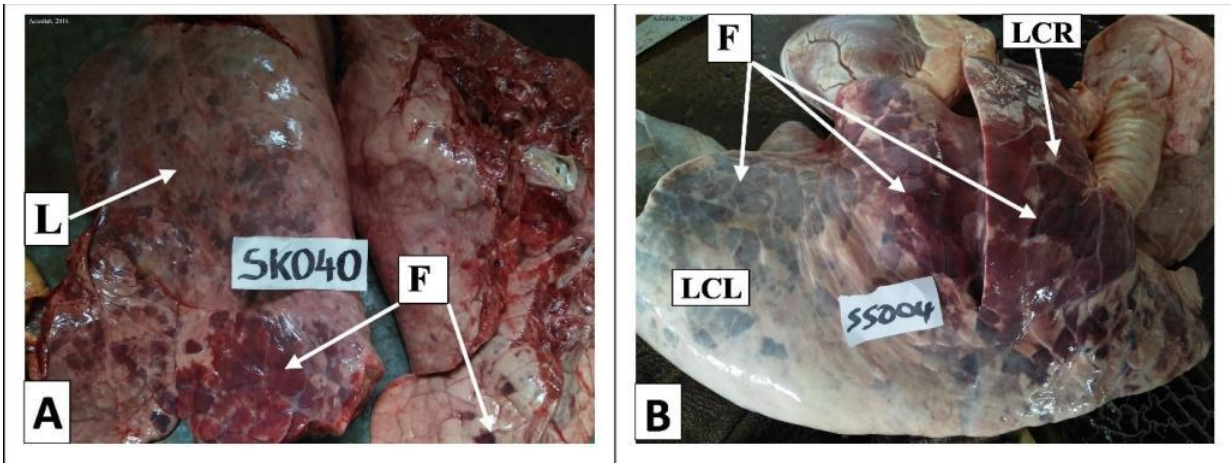


**Figure 4.27 Gross morphological appearance of lungs from slaughtered animal [ID: SK040 (A) and SS004 (B)] diagnosed with pulmonary blood aspiration. A, showing dark-red multiple foci (F) of varying sizes under the visceral pleura. Left caudal lobe (L) most affected. B, shows dark red multiple foci (F) under the visceral pleura; affected left caudal lobe (LCL) and left cranial lobe (LCR) are indicated**

#### 4.2.4.4.2 Histopathological manifestations of the respective condemned organs

On carrying-out histopathological examination, lungs from cattle (ID: SK040) revealed an abundant presence of erythrocytes within alveolar sacs and bronchioles with lack of inflammatory cells. Grey/black irregular shaped structures (pieces of ingesta) were also observed intermixed with erythrocytes and no inflammatory infiltrates (Figure 4.28). Lungs from cattle (ID: SS004), also revealed an abundant presence of erythrocytes within alveoli, alveolar sacs, bronchi and bronchioles with intact walls and lack of inflammatory cellular infiltrates, characteristic of pulmonary blood aspiration (Figure 4.31 – 4.32).

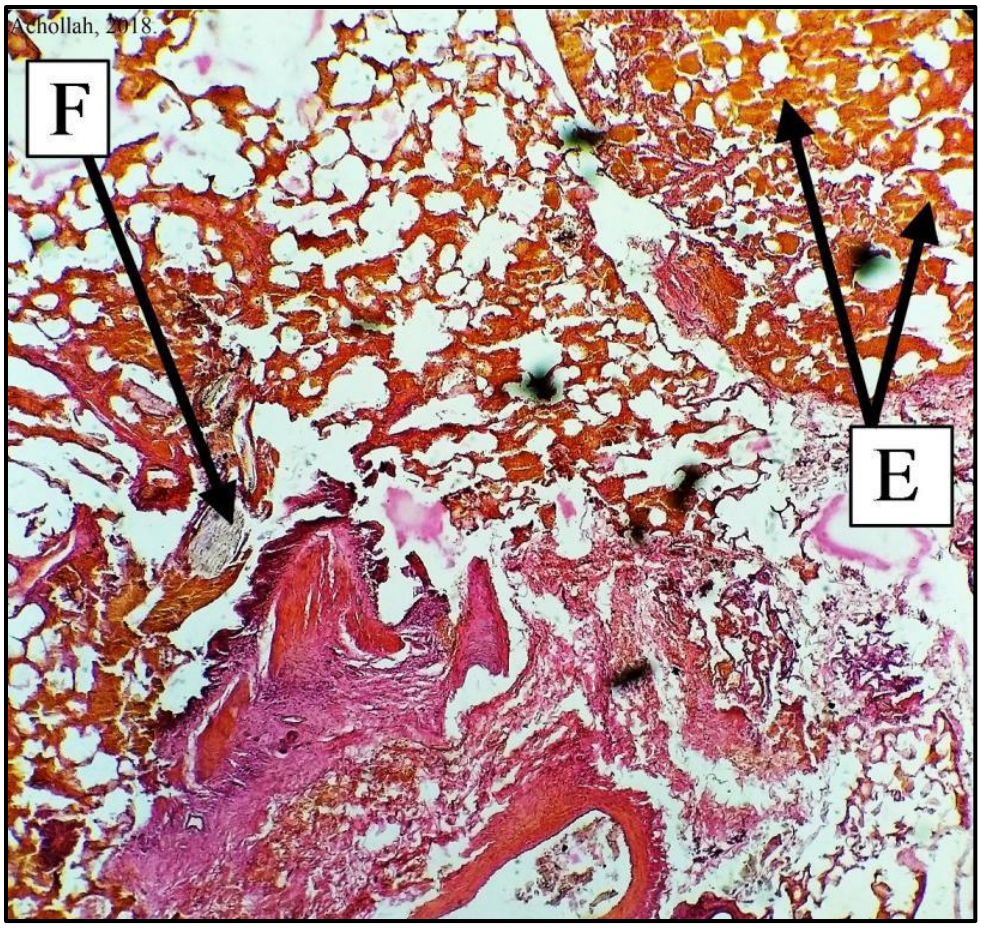


**Figure 4.28 Photomicrographs of lungs from slaughtered animal (ID: SK040) diagnosed with blood aspiration showing the presence of abundant erythrocytes (E) in alveoli, alveolar sacs, foreign material (pieces of ingesta) (F) among erythrocytes (H&E X40)**

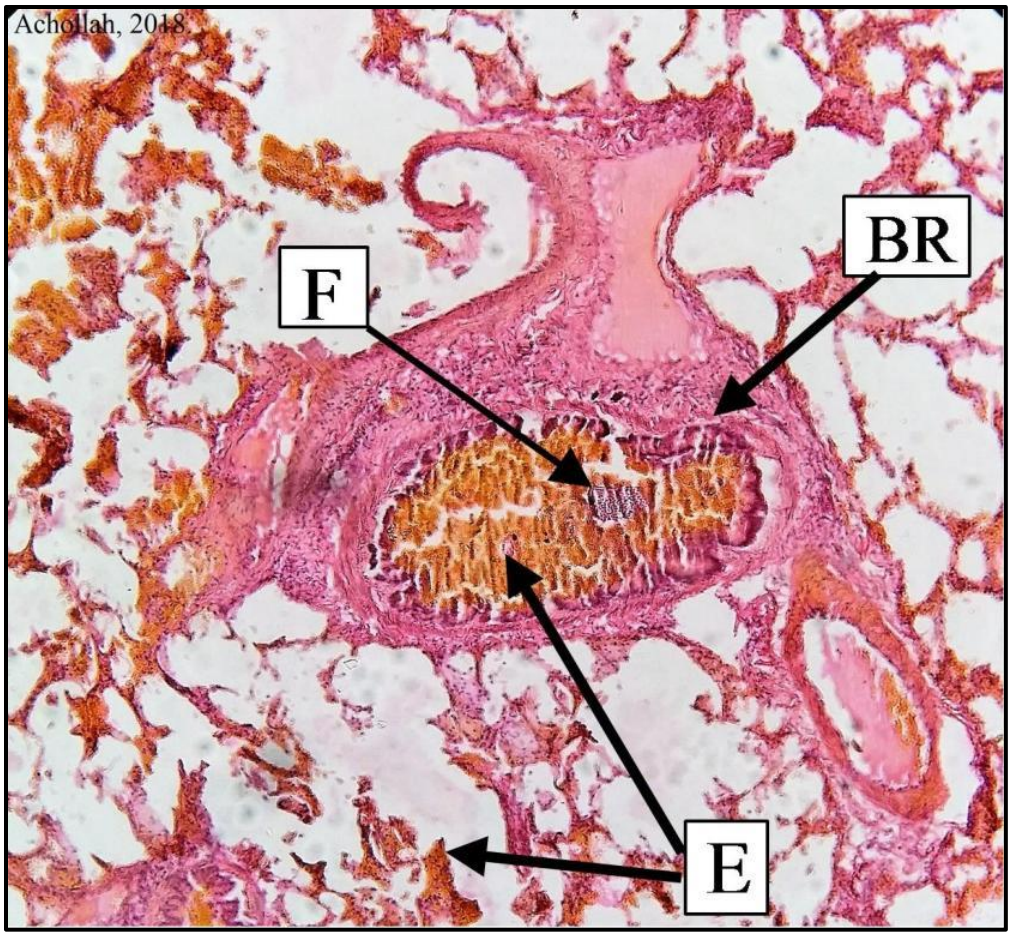


**Figure 4.29 Photomicrographs of lungs from slaughtered animal (ID: SK040) diagnosed with blood aspiration, showing bronchiole (BR) filled with erythrocytes (E), foreign material (F), (H&E X100)**

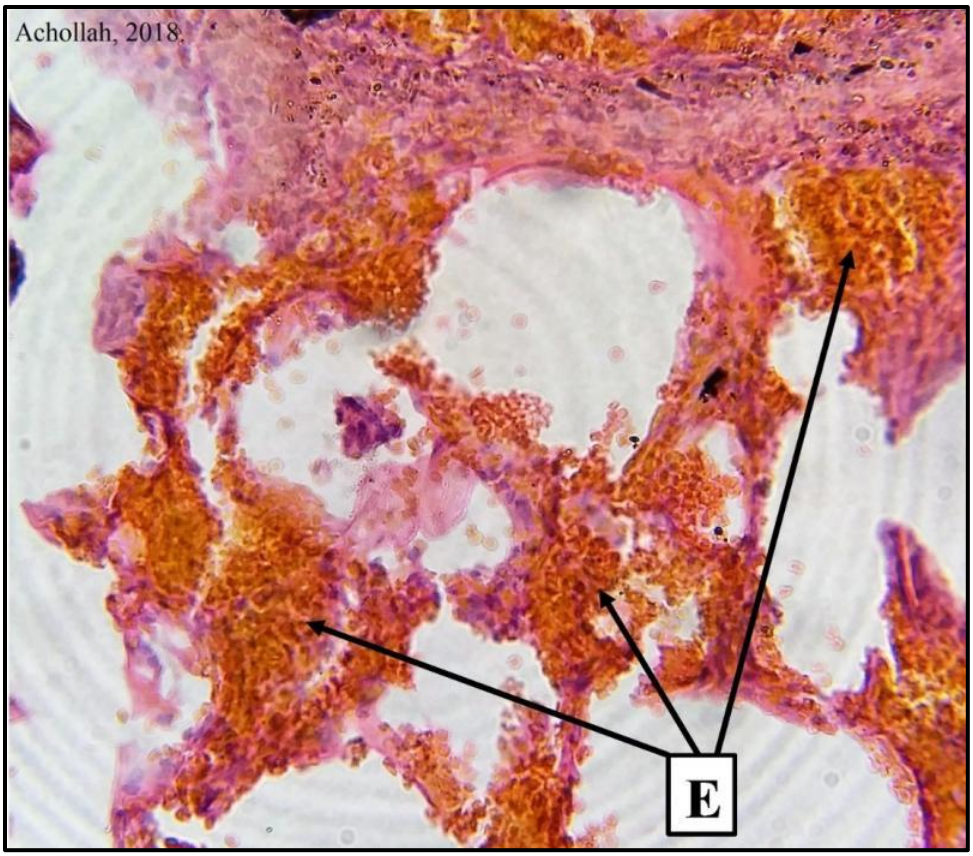


**Figure 4.30 Photomicrographs of lungs from slaughtered animal (ID: SK040) diagnosed with blood aspiration, showing erythrocytes (E) and a homogenous pink staining material within alveoli (H&E X400)**

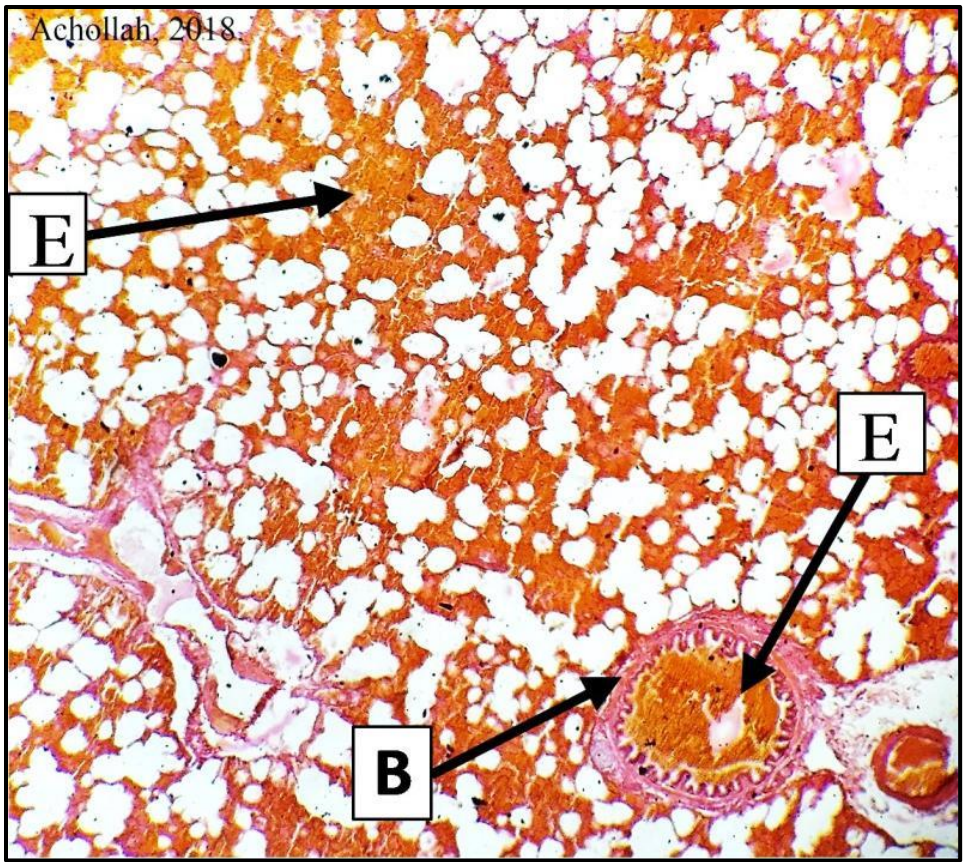


**Figure 4.31 Photomicrograph of lungs from slaughtered animal (ID: SS004) diagnosed with blood aspiration, showing abundant erythrocytes (E) in alveoli, alveolar sacs and bronchus (B) (H&E X40)**

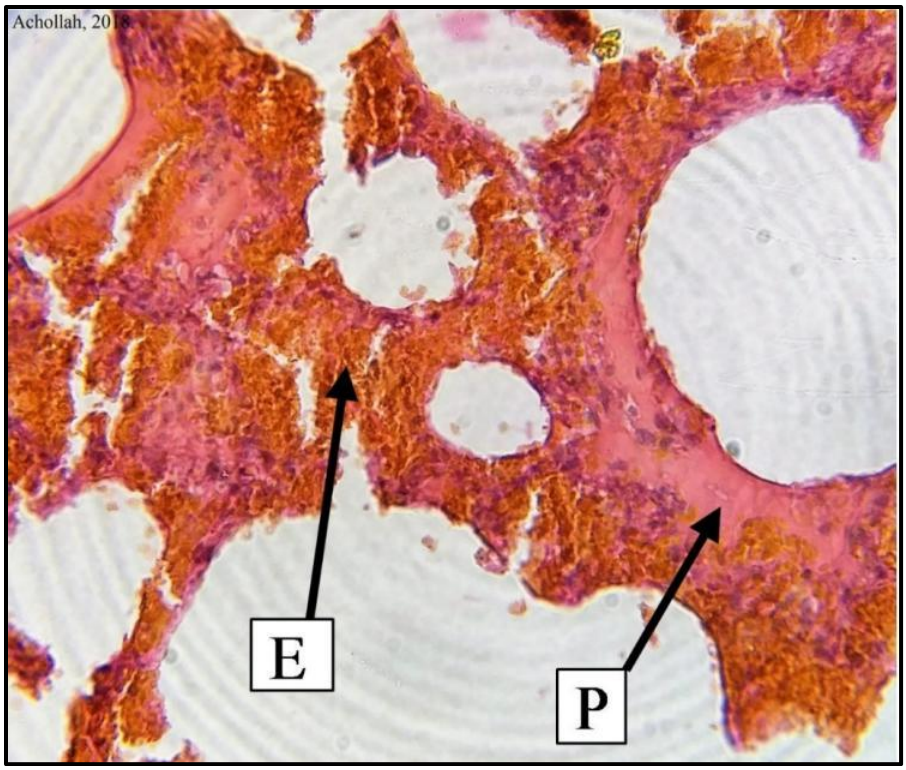


**Figure 4.32 Photomicrograph of lungs from slaughtered animal (ID: SS004) diagnosed with blood aspiration, showing erythrocytes (E) and a homogenous ink staining substance (P) within alveoli with intact walls and absence of inflammatory cells (H&E X 400)**

#### 4.2.4.4.3 Bacteriological isolation from respective condemned organs

Lung samples from cattle (ID: SK040) inoculated in blood agar yielded two types of isolates; the first isolate exhibited the following colonial morphology: large, round light grey/off-white, glistening moist colonies with slightly raised centers, fixed margin and non- hemolytic type. Inoculation in MacConkey agar revealed large, round and moist, red/pink colonies which are lactose fermenters. Gram reaction revealed pink (gram-negative) rod-shaped bacteria, while biochemical tests revealed the following: oxidase (-ve), catalase test (-ve), indole test (+ve), methyl red (+ve), citrate (-ve), urease test (-ve) and Tripple Sugar Iron (TSI): Acid slant (yellow), acid butt (yellow) with gas production. These results were consistent with *Escherichia coli* isolates.

The second isolate exhibited the following colonial morphology; off-white, pinpoint, α - haemolytic colonies in blood agar, with no growth realized in MacConkey agar. Gram reaction for the isolate revealed gram-positive cocci in chains while biochemical reactions revealed the following: oxidase (-ve), catalase test (-ve), CAMP test (-ve). These results were consistent with α - haemolytic *Streptococcus spp*. isolates.

Lung samples from cattle (ID: SS004) inoculated in blood agar yielded two types of isolates; the first isolate exhibited the following colonial morphology: small cream-yellow, non-hemolytic colonies with no growth exhibited in MacConkey agar. Gram reaction revealed gram-positive pleomorphic coccobacilli, while biochemical tests had the following: oxidase (-ve), catalase test (+ve), indole test (-ve), methyl red (+ve), citrate (-ve), urease test (-ve) and Tripple Sugar Iron (TSI): Alkaline butt alkaline slant; no gas and no $H_2S$ production, glucose (-ve), sucrose (-ve), and growth in potassium tellurite blood (PTBA) agar revealed grey-black colonies. These results were consistent with *Corynebacterium spp*. isolates.

The second isolate from the lung sample (ID: SS004) exhibited the following colonial morphology; small white colonies, raised center, smooth edges, non- hemolytic, with no growth realized in MacConkey agar. Gram reaction for the isolate revealed gram-positive cocci in clusters while biochemical reactions revealed the following: oxidase (-ve), catalase test (+ve), indole test (-ve), methyl red (+ve), citrate (-ve), urease test (-ve) and Tripple Sugar Iron (TSI): Alkaline butt alkaline slant; no gas and no $H_2S$ production, glucose (+ve, no gas produced), sucrose (-ve), CAMP test (-ve) and coagulase test (-ve). These results were consistent with coagulase-negative *Staphylococcus spp*. isolates.

#### 4.2.4.5 Masseter muscle abscess

Masseter muscle abscess was diagnosed in one slaughtered animal (0.9%) (ID: SS017) at Siaya slaughterhouse.

##### 4.2.4.5.1 Gross morphological appearance of the condemned organ

Gross examination of the affected part revealed a greyish, fluctuant, non-mobile swelling, measuring 15 cm in diameter on the left masseter muscle. Cut section revealed an abundant yellowish brown/red fluid measuring nearly 1,000 ml admixed with necrotic tissue debris.

##### 4.2.4.5.2 Bacteriological isolation from the abscess

Swabs of the abscess from cattle (ID: SS017) inoculated in blood agar yielded two types of isolates; the first isolate exhibited the following colonial morphology: small, milky, β - haemolytic colonies with no growth realized in MacConkey agar. Gram reaction revealed gram-positive, small rod-shaped/ slightly curved bacteria and forming chains, while biochemical tests had the following: oxidase (-ve), catalase test (-ve), indole test (-ve), methyl red (+ve), citrate (-ve), urease test (-ve) and Tripple Sugar Iron (TSI): Alkaline butt alkaline slant; no gas and no $H_2S$ production, glucose (+ve, gas produced), sucrose (-ve), and growth in potassium tellurite blood (PTBA) agar revealed grey-black colonies. These results were consistent with *Corynebacterium pseudotuberculosis* isolates.

The second isolate exhibited the following colonial morphology; greyish, medium-sized, β-haemolytic, with no growth realized in MacConkey agar. Gram reaction for the isolate revealed gram-positive cocci in chains while biochemical reactions revealed the following: oxidase (-ve), catalase test (-ve), indole test (-ve), methyl red (-ve), citrate (-ve), urease test (-ve) and Tripple Sugar Iron (TSI): Alkaline butt, alkaline slant; no gas and no $H_2S$ production, glucose (+ve and

gas produced), sucrose (-ve) and CAMP test (+ve); these results were consistent with *Streptococcus agalactiae* isolates.

#### 4.2.4.6 Splenomegaly

Splenomegaly was diagnosed in 2 slaughtered cattle (1.8%), (Cattle ID: SK009 and SS001) at Kaumara and Siaya slaughterhouse respectively.

##### 4.2.4.6.1 Gross morphological appearance of the respective condemned organs

On gross examination, the respective spleen from animal ID: SK009 was dark red, soft, diffusely enlarged and bulging when cut. For animal ID: SS001, the respective spleen was dark red and diffusely enlarged as well, rather firm, bulging and oozing little blood when cut.

##### 4.2.4.6.2 Histopathological manifestations of the respective condemned organs

On carrying-out histopathological examination on spleen sample ID: SK009, the respective splenic section stained with Hematoxylin and Eosin showed a rather disorganized architecture with marked deposition of brownish granular pigment, regarded as hemosiderin, mainly in the red pulp. Generally, the red pulp showed an increase in intact and fragmented erythrocytes and macrophages with brownish granular pigment (Figure 4.33 and 4.34).

Using the Prussian blue staining method, the pigment deposits stained intensely blue, thus confirming splenic hemosiderosis (Figures 4.35, 4.36 and 4.37).

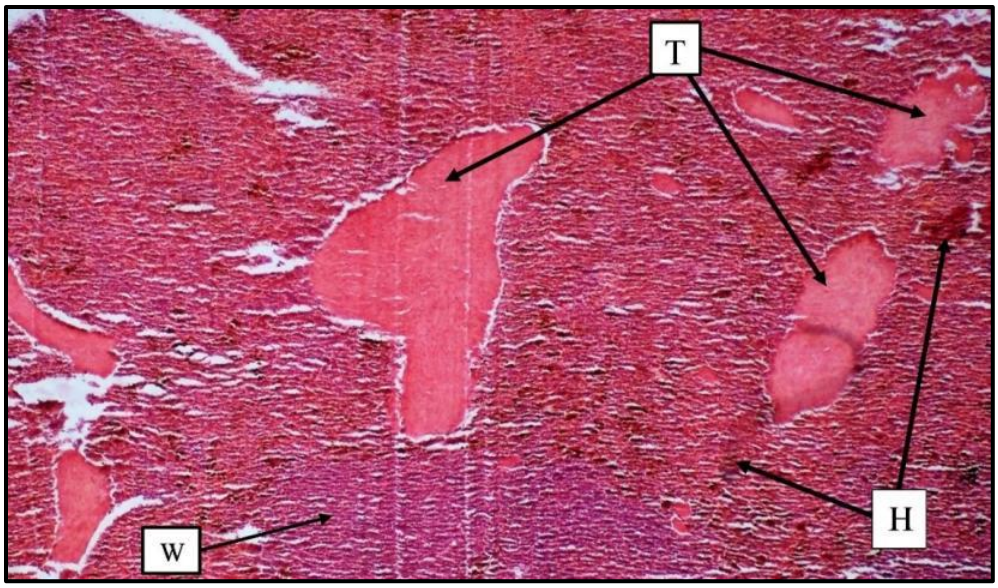


**Figure 4.33 Photomicrograph of spleen section from a slaughtered animal (ID: SK009), showing a disorganized architecture, brownish pigment deposits (H) within the red pulp. White pulp (W) and splenic trabecula (T) are indicated (H&E X40)**

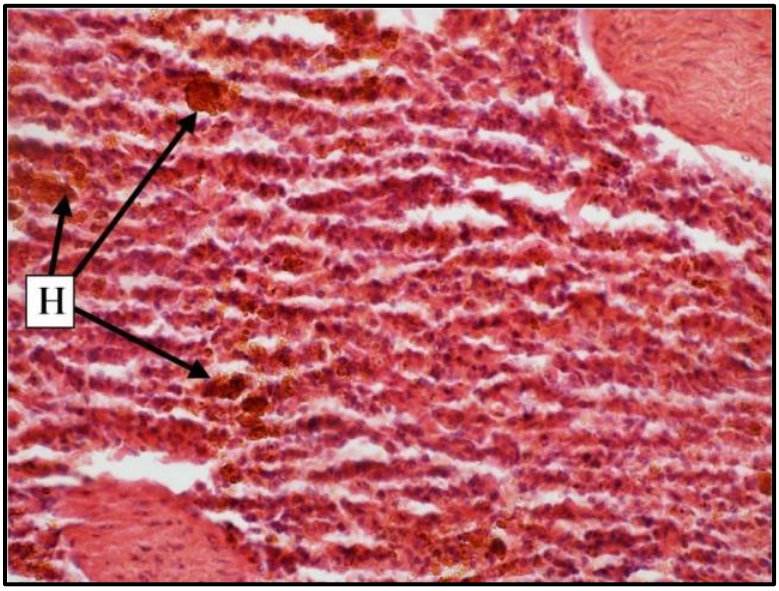


**Figure 4.34 Photomicrograph of spleen section from a slaughtered animal (ID: SK009), showing the large coarse brownish/golden brown pigment granules (hemosiderin) within cells (H) (H&E X400)**

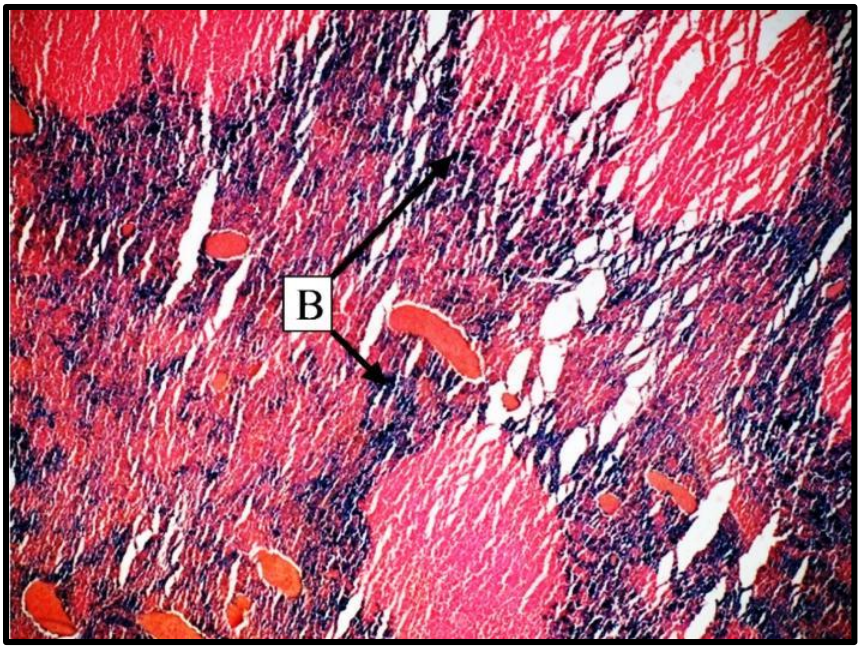


**Figure 4.35 Photomicrograph of spleen section from slaughtered animal (ID: SK009), after staining with Prussian Blue, showing intense blue staining pigment (B) within splenic red pulp (X40)**

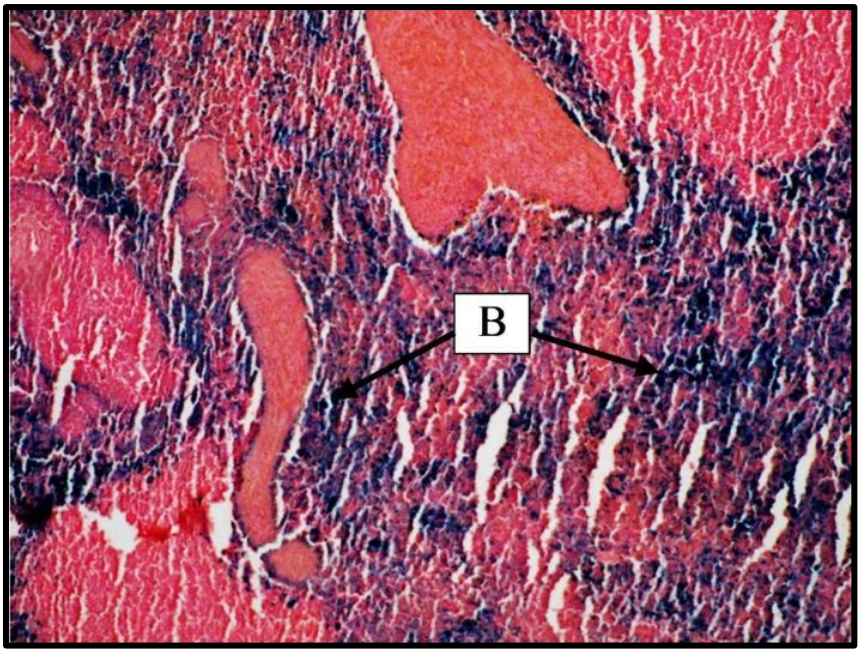


**Figure 4.36 Photomicrograph of spleen section from slaughtered animal (ID: SK009), after staining with Prussian Blue, showing intense blue staining pigment (B) in the red pulp (X100)**

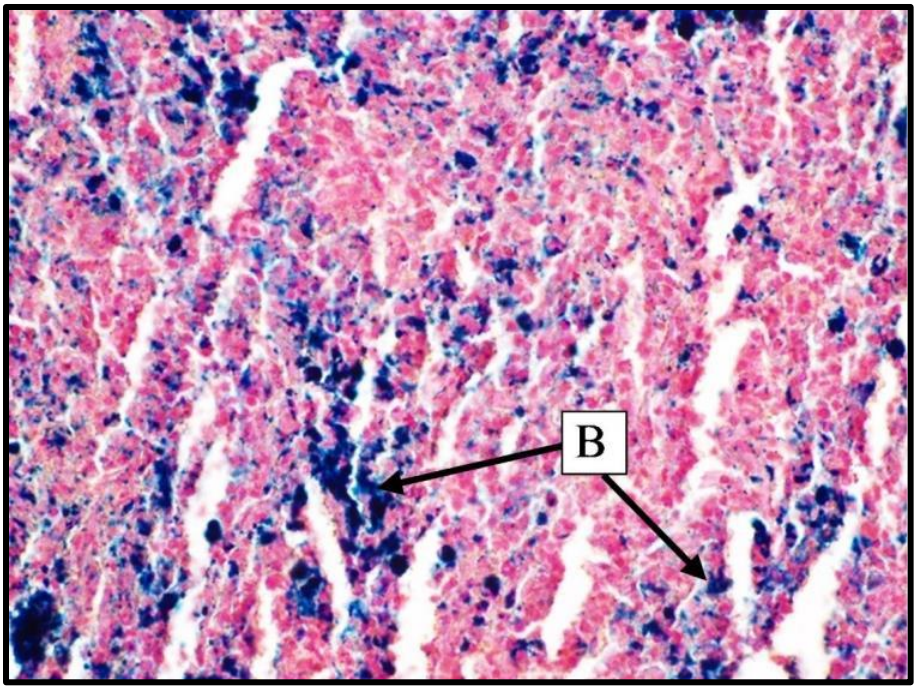


**Figure 4.37 Photomicrograph of spleen section from slaughtered animal (ID: SK009), after staining with Prussian Blue, showing the intracellular location of the blue staining pigment (B), nuclei (red) (X400)**

On histopathological examination of spleen sample ID: SS001, the red pulp was expanded and its vascular spaces distended with erythrocytes, characteristic of splenic congestion. The lymphoid tissues of the white pulp were reduced, broadly separated, generally lacking the normal noticeable lymphoid follicles (Figures 4.38). Additionally, an increased presence of neutrophils was observed in the red and white pulp.

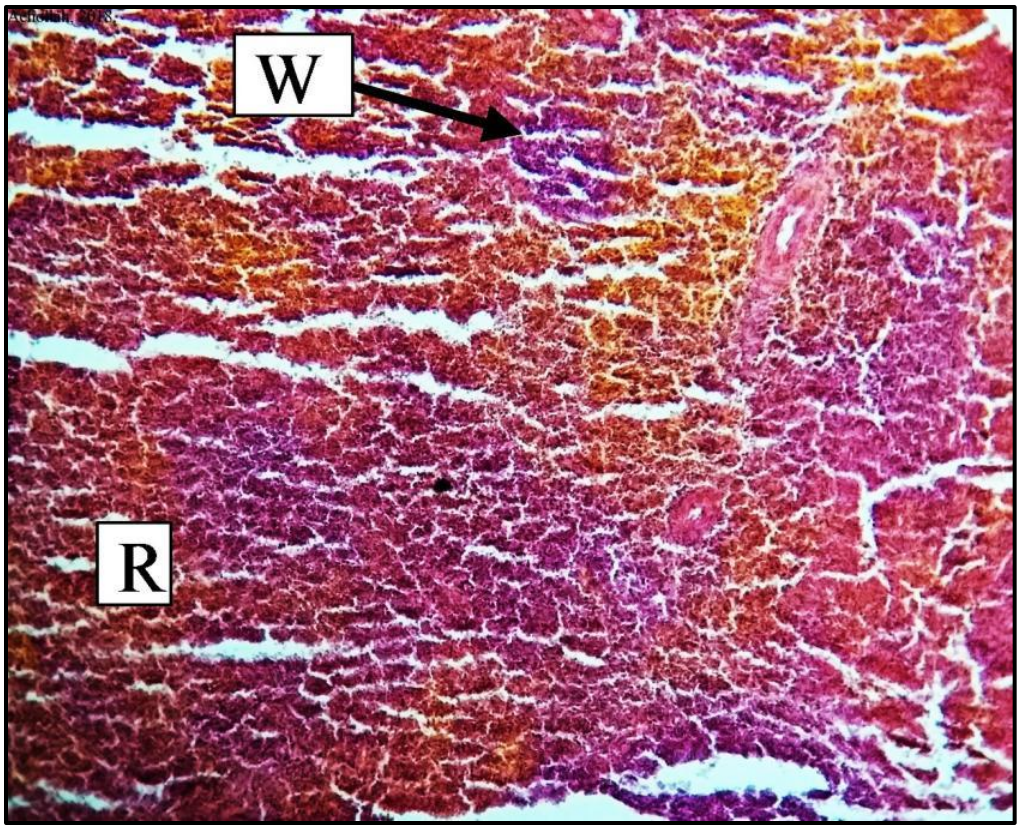


**Figure 4.38 Photomicrograph of splenic section from a slaughtered animal (ID: SS001) after staining with Hematoxylin and Eosin, showing the prominent red pulp (R), barely noticeable white pulp (W) (X100)**

#### 4.2.4.6.3 Microscopic findings from impression smears from the respective condemned organs

On microscopic examination of impression smear from spleen sample ID: SK009, multifocal areas with grey/brown granular material of varying sizes, irregular-shaped/ fragmented erythrocytes and a few scattered polymorphonuclear cells were observed (Figure 4.39 A).

For spleen sample ID: SS001, an increased presence of polymorphonuclear cells (neutrophils), macrophages and erythrocytes were observed in all the fields (Figure 4.39 B).

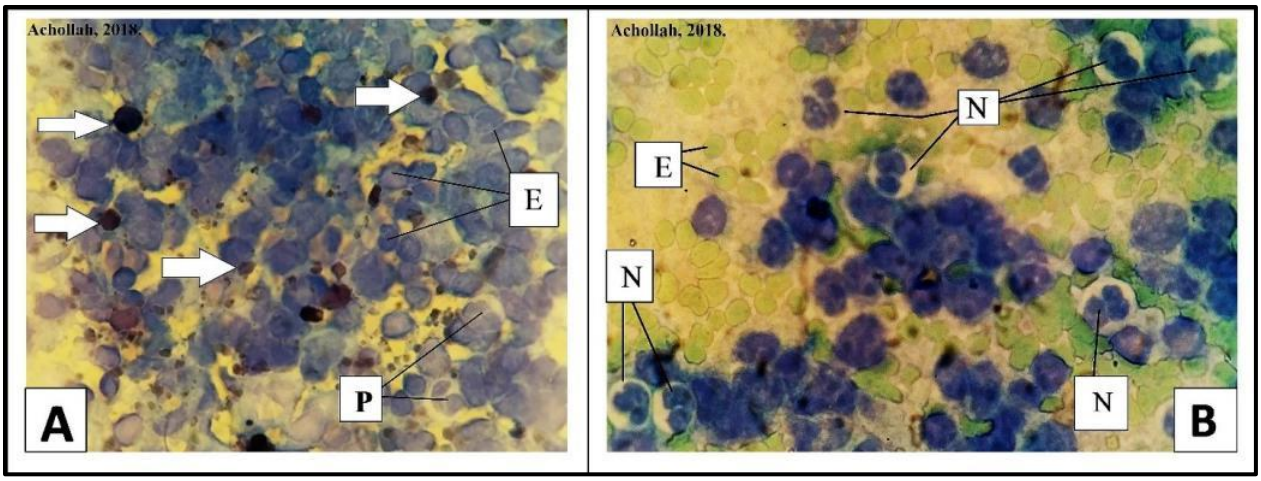


**Figure 4.39 Impression smear of spleen stained with Giemsa from slaughtered animals ID: SK009(A) and SS001(B). A, showing grey/brown granular material of varying sizes (arrows) interspersed with irregular shaped/ fragmented erythrocytes (E) and polymorphonuclear cells (P). B, showing polymorphonuclear cells (neutrophils) (N), macrophages and erythrocytes (E)**

#### 4.2.4.6.4 Bacteriological isolation from the respective condemned organs

Samples of the respective spleen (from animal ID: SK009) inoculated in blood agar exhibited the following colonial morphology: Small, white to grey β – haemolytic colonies; with no growth realized in MacConkey agar. Gram reaction revealed gram-positive cocci forming chains, while biochemical tests had oxidase (-ve), catalase test (-ve) and CAMP test (-ve). These results are consistent with β-hemolytic *Streptococcus spp.* isolates.

The respective spleen sample from animal ID: SS001 inoculated in blood agar yielded two types of isolates. The following colonial morphology was observed in the first isolate: Small size, yellow colonies, irregular edges, non-hemolytic with no growth exhibited in MacConkey agar. Gram reaction revealed gram-positive, short rod-shaped/ coccobacilli. biochemical tests had the following: oxidase (-ve), catalase test (+ve), indole test (-ve), methyl red (+ve), citrate (-ve), urease test (-ve) and Tripple Sugar Iron (TSI): Alkaline butt alkaline slant; no gas and no $H_2S$ production, glucose (+ve; no gas produced) and sucrose (-ve). These results are consistent with *Corynebacterium pilosum* isolates (Appendix 8.17).

The second isolate exhibited the following colonial morphology; yellow, small-sized, non – haemolytic colonies, with no growth realized in MacConkey agar. Gram reaction for the isolate revealed gram-positive, short rods while biochemical reactions revealed oxidase (-ve), catalase test (+ve), indole test (-ve), methyl red (-ve), citrate (-ve), urease test (+ve) and Tripple Sugar Iron (TSI): Alkaline butt, alkaline slant; no gas and no $H_2S$ production, glucose (-ve and no gas produced), sucrose (-ve) and growth in PTBA (-ve); these results are consistent with *Corynebacterium spp.* isolates (Appendix 8.17).

**Table 4. 3. Summary of conditions causing organ condemnation, numbers condemned and their percentage representation out of the 112 slaughtered animals during the cross-sectional study (5th June, 2018-4th July 2018)**

| Organs | Conditions | Category | Number Condemned | Percentage |
|---|---|---|---|---|
| Livers | Fasciolosis | Parasitic | 58 | 51.8 |
| | Hydatidosis | Parasitic | 1 | 0.9 |
| Intestines | Intestinal nematodiasis (pimply gut) | Parasitic | 28 | 25 |
| Lung | Blood aspiration | Poor slaughtering technique | 2 | 1.8 |
| Spleen | Splenomegaly | Disturbance in cell metabolism (Hemosiderosis) | 1 | 0.9 |
| | Congestion | Circulatory disturbance | 1 | 0.9 |
| Muscle | Abscess | Inflammatory processes | 1 | 0.9 |

## 4.3 Financial losses associated with organ condemnation during the period 5th June, 2018-4th July 2018 (Cross-sectional study)

During the cross-sectional study, KShs. 94,469.30 (US$. 935.30) loss was incurred as a result of organ condemnation at Kaumara, Siaya and Ugunja Slaughterhouses in Siaya County (Table 4.6). The amount of financial losses recorded in each slaughterhouse corresponded with the number of animals slaughtered. The highest losses were incurred at Kaumara slaughterhouse KShs. 41,918.80

(US$ 415) followed by Siaya at KShs. 32,114.50 (US$ 318). Ugunja slaughterhouse registered the lowest financial loss of KShs. 20,436 (US$ 202).

**Table 4. 4. Organs condemned, respective weights, prevailing prices and the computed financial losses (KShs and US$) in slaughterhouses during the period 5th June, 2018-4th July 2018 (Cross-sectional study)**

| Organ | Slaughterhouse, weights of condemned organs and the respective prevailing prices | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Kaumara | | | Siaya | | | Ugunja | | | |
| | WT | KShs/ kg | Total | WT | KSh/ kg | Total | WT | KShs/ kg | Total | TOTAL |
| Liver | 79 | 500 | 39,600 | 59.5 | 500 | 29,750 | 36 | 500 | 18,000 | 87,350 |
| GIT | 0.9 | 260 | 228.8 | 0.825 | 260 | 214.5 | 9.4 | 260 | 2,436 | 2,879.3 |
| Spleen | 2.1 | 500 | 1,050 | 1.5 | 500 | 750 | 0 | 500 | 0 | 1,800 |
| Lungs | 4 | 260 | 1,040 | 4 | 260 | 1,040 | 0 | 260 | 0 | 2,080 |
| Muscle | 0 | 360 | 0 | 1 | 360 | 360 | 0 | 360 | 0 | 360 |
| Number of cattle slaughtered | | | 46 | | | 34 | | | 32 | 112 |
| Total (KShs.) | | | 41,919 | | | 32,115 | | | 20,436 | 94,469 |
| Mean | | | | | | | | | | 31,490 |
| Total $ (KShs 101) | | | 415 | | | 318 | | | 202 | 935 |

**WT** – the weight of the respective condemned organs in kilograms.

# 5 CHAPTER FIVE: DISCUSSION

This study aimed at documenting organ condemnations in cattle from slaughterhouses in Siaya County, establishing the definitive cause (s) for the condemnation through laboratory diagnostic techniques and estimating the respective financial loss incurred by the cattle traders or farmers.

Condemnation of organs during the retrospective study was mainly attributed to parasites (such as fasciolosis and hydatidosis), inflammatory processes (liver cirrhosis) and disturbance in calcification (hepatic calcification). The condemnation level was much lower than those reported recently in Kombolcha Elfora (69%) in Ethiopia (Sheferaw and Abdu, 2017), but comparable to the one obtained in Dodoma (29%), Tanzania (Tembo and Nonga, 2015).

Considering specific causes of condemnation, big differences emerged as compared to other studies. For instance, Sheferaw and Abdu (2017) in Ethiopia reported condemnations to be due to: fasciolosis (liver) 26%, pneumonia 16%, cirrhosis (liver) 0%, hydatid cyst (liver) 4%, hydatid cyst (lung) 10% and nephritis 15% Moje *et al.* (2014), also in Ethiopia, reported condemnations to be due to: fasciolosis (liver) (18%), liver cirrhosis (25%), calcification (liver) 10%, pneumonia (8.5%), hydatid cyst (lung: 54%, liver: 41%); and Raji *et al.* (2010), in Nigeria, who reported condemnations due to the following conditions: hepatic fasciolosis 23%, haemonchosis, 12%, pericarditis, 17%, pneumonia 9%, liver cirrhosis 10%, emphysema (lungs) 5%, and abscesses in liver, lungs and the kidneys 5%. In Migori County in Kenya, a study by Kere *et al.* (2019) reported that about 3% lungs and 2% livers of cattle slaughtered between the year 2007 to 2016, were condemned as a result of cystic echinococcosis (hydatidosis).

Variations in proportions of conditions in different localities could also be related to geographical locations which impact local climatic conditions, management systems, the population of livestock, prevalence and distribution of animal diseases and vectors (Tembo and Nonga, 2015).

In Siaya county, the population of cattle could have been lower, translating to a smaller proportion of conditions observed at slaughter compared to Sheferaw and Abdu (2017), Moje *et al*. (2014) and Raji *et al.* (2010) in Ethiopia and Nigeria respectively.

High number of parasitic conditions in both retrospective and cross-sectional study could be attributed to the ubiquitous nature of helminth parasites of cattle and other animals and the near-perfect environmental conditions in the tropics and subtropics, favoring their survival and spread. The retrospective study found no statistically significant difference (p=0.99) in condemnation of organs in Siaya County over the years. This could be attributed to the origin of slaughtered animals which was mainly within Siaya County (60%) (GoK, 2013); implying a vast majority of the animals could have been exposed to the same environmental/disease conditions.

A large number of organ condemnations corresponded to the high amounts of finances lost (mean annual of KShs. 7,739,210 (US$ 76,626). This was considered very high compared to a mean of US$5,759.00 reported in East Wollega in Ethiopia in a study by Moje *et al.* (2014). The variations in the prevalence of diseases, the number of animals slaughtered and retail market prices for these localities could be responsible for the differences in calculated financial losses (Mohammed *et al.*2018). In the retrospective study, the annual financial losses also increased from the year 2013 to 2017 despite the relatively similar organ condemnation rates. This can be attributed to the retail price changes of organs which increased from the year 2015 to 2017, implying that farmers, livestock traders, butchers and other players in the cattle production industry were deprived of their incomes. Consumers of beef and their products were also deprived of important nutrients such as proteins, vitamins, and minerals as a result.

The loss of 198.2 kg of edible meat at slaughter during the 1-month cross-sectional study at Kaumara, Siaya and Ugunja slaughterhouses, also indicated a heavy loss of the key source of

protein for consumers as well as income for farmers and livestock traders. The scale of financial losses is in this study is comparable to those encountered in other countries such as Ethiopia and Tanzania (Belina and Melese, 2017; Tembo and Nonga, 2015). For the respective slaughterhouses, the highest losses were registered from Kaumara, followed by Siaya, while Ugunja slaughterhouse had the lowest financial losses; the magnitude of financial losses was found to vary according to the number of animals slaughtered and therefore the number of organs condemned.

Parasitic causes of condemnation comprised hepatic fasciolosis, hydatidosis, and pimply gut. In hepatic fasciolosis, the major lesions observed were attributed to obstruction of bile ducts. These lesions are similar to those of hepatic fasciolosis encountered at Sulaimani abattoir, Qeregol region in Iraq (Salmo *et al.* 2014) and in Bangladesh (Affroze *et al.,* 2013). The lesions are associated with repeated fluke infestations in which progressive inflammation and fibrosis in portal units occur as a result of the direct irritation by flukes, biliary stasis and concomitant infections (Cullen and Stalker, 2016).

One fluke-infested liver grossly had circumscribed areas with black pigment. Histopathological examination of the pigment revealed a yellowish-brown/black fine granule in hepatic parenchyma intermixed with hepatocytes and inflammatory infiltrates; consistent with "fluke puke" (iron-porphyrin pigment) associated with hepatic fasciolosis (Cullen and Stalker 2016). In addition, the histochemical staining of the pigment was negative for both iron and melanin. The hepatic fibrosis diagnosed in this study was also attributed to fasciolosis, where migration of immature liver flukes plus bile duct obstruction frequently lead to chronic inflammation and fibrosis, consistent with the findings of Rashid *et al.* (2018).

All the parasites recovered from affected livers were identified morphologically as *Fasciola gigantica*. The study in Dagoreti slaughterhouse complex in Nairobi, Kenya by (Kithuka *et al.,*

2002) also reported that all Fasciola species recovered from infested livers were *Fasciola gigantica*. The morphometric characteristics measured in the present study were consistent with the standardized ones (Valero *et al.,* 2006). The measurements are comparable to those of Halakou *et al.* (2017) in Iran, in which body length of 26.8 – 32.1 mm with a mean of 29.45 and body width of 5.9 – 8.1 mm with a mean of 7.0 were recorded for *Fasciola gigantica*. These results were also comparable to those of Hussein and Khalifa (2010) in Qena Governorate, Egypt in which the measured *Fasciola gigantica* ranged from 20mm – 39 mm with a mean of 30.4 for body length and 11 – 13 mm with an average of 11.9mm for body width.

Fasciola parasite frequently occurs in tropical areas of Africa and Asia (Taylor *et al.,* 2016); causing a decline in wool quality, decreased meat and milk production and poor weight gain in young animals (Salmo *et al.,* 2014). The high proportion of *Fasciola gigantica* in this study differed from those of Asfaw (2018) in Ethiopia, who morphologically identified 69 (59.9%) as *F. hepatica*, 13 (11%) *F. gigantica* and 33 (29%) as mixed species and immature ones. This high proportion of hepatic fasciolosis indicates a possible zoonotic risk; drinking contaminated water or consuming metacercaria in vegetables predisposes to human infection (Nyindo and Lukambagire, 2015).

Visible greyish-white fluid-filled cysts of varying sizes were observed bulging from the surfaces of a condemned liver. At histopathological examination, these were confirmed to be hydatid cysts (Cullen and Stalker 2016). The gross and microscopic findings recorded agreed with the findings of Singh *et al.* (2016) in Punjab (India). Congestion of hepatic sinusoids, central veins and hepatocyte degeneration observed in the present study was caused by pressure from the developing fluid-filled hydatid cysts; consistent with the study of Verma and Swamy (2009) in Rajasthan (India). The occurrence of cystic echinococcosis in people is closely related to that of domestic

herbivores (Mandal and Mandal, 2012), therefore its occurrence in this study indicates a possible human exposure. People may act as aberrant intermediate hosts in case they consume food contaminated with the fecal matter of an infected dog, or directly from it.

Most of the pimply gut nodules observed in this study did not have the nematode larvae. This agrees with the study of Chamuah *et al.* (2016) in Jharnapani, India who also reported that most of the nodules he found (66.7%) lacked the immature nematode larvae. This occurs either from the death and fragmentation of the parasites as a result of immunological as well as other host reactions or their exit out of the nodules back into the gut lumen in the progression of their development (Nwosu *et al.,* 2012). The fourth-stage larvae (L4) usually attach to or penetrate the intestinal wall, later emerge to the mucosal surface, wander through to the colon before maturing into an adult stage (Taylor *et al.,* 2016). The nodules causing condemnation were therefore caused by *Oesophagostomum spp. Oesophagostomum radiatum* mainly affect cattle and water buffalo and may cause fatal infection or severe fetid diarrhea, anorexia and weight loss in young stock, while formation of nodules in intestines of adult animals may cause rejection/condemnation of the affected intestines or carcass (if emaciation and edema is present) at slaughter (Taylor *et al.,* 2016; Herenda *et al.,* 2000) .

Pulmonary blood aspiration is frequently encountered at slaughter as observed by Cullen and Stalker (2016). The absence of inflammatory signs in the affected lungs observed in the present study agreed with the findings of El-Siddige (2003) in Ethiopia. *Escherichia coli* and *Streptococcus spp.* were isolated from lung sample SK040, while *Corynebacterium spp.* and coagulase-negative *Staphylococci* were isolated from lung sample SS004. The absence of inflammatory changes in the lung samples seem not to be characteristic of the bacterial organisms isolated; the occurrence of these organisms in the lung tissue could have accompanied the entrance

of the aspirated materials. Previous studies have also isolated similar bacterial organisms from normal lung tissues of camels at slaughter in Athi River, Kenya (Mutua, *et al.,* 2017). Pulmonary blood aspiration in lungs, observed in this study can be attributed to lack of stunning during the slaughter process; in which the loss of consciousness is prolonged, leading to aspiration of blood as described by Gregory *et al*. (2009) in the UK.

*Corynebacterium pseudotuberculosis* nitrate-reducing biotype, causes ulcerative lymphangitis and abscesses in cattle and horses, while Streptococci group of bacteria are not only frequent commensals on mucous membranes with many infections considered opportunistic, but also cause pyogenic infections, suppurative lesions or septicemia in a wide range of animal species. However, *Streptococcus agalactiae* causes chronic mastitis in the same species (Quinn *et al*., 2016). For the present study, therefore, the isolation of *Streptococcus agalactiae* from the masseter muscle abscess was considered a contaminant during the slaughter process, while *Corynebacterium pseudotuberculosis* was taken as the main cause of the abscess.

Enlargement and discoloration led to the condemnation of two spleens (ID: SK009 and SS001). Special histochemical staining performed on the first spleen (ID: SK009) confirmed the brownish pigment granules as hemosiderin. Impression smears also confirmed large grey-brown granules of various sizes, while bacterial isolation and identification results were consistent with β-haemolytic *Streptococcus* isolates. However, no hemoparasites were observed in all fields of the impression smears. This splenic enlargement was caused by massive accumulation of hemosiderin by other causes not established by this study, different from the β-haemolytic *Streptococcus* isolated. These organisms are usually found or isolated in septicemias, abscesses and many other suppurative conditions (Quinn *et al.,* 2016). For the second spleen sample (ID: SS001), microscopic lesions were mainly: congested vascular spaces in the red pulp and scanty white pulp and presence of

many neutrophils, while impression smears showed an increased presence of neutrophils, macrophages and erythrocytes and no hemoparasites detected. Bacterial isolation and identification results from this sample were consistent with *Corynebacterium pilosum* and other *Corynebacterium* species isolates. Many *Corynebacterium species* exist in mucous membranes as commensals, are considered opportunistic pathogens and can also cause contamination during the slaughter process. In cattle, *Corynebacterium* species causes a wide range of diseases including subclinical mastitis (*C. bovis*), mastitis (*C. ulcerans*), Cystitis and pyelonephritis (*C. renale* type I and *C. pilosum*), abscesses and ulcerative lymphangitis (*C. pseudotuberculosis* nitrate-reducing biotype) (Quinn *et al.*, 2016). The lesions in this spleen sample suggested splenic congestion and could have been caused by other agents, different from the isolated bacteria.

# 6 CHAPTER SIX: CONCLUSIONS AND RECOMMENDATIONS

## 6.1 Conclusions

- Parasitic conditions, inflammatory conditions, circulatory disturbances, disturbances of cell metabolism, concretions, disturbances in development and mineralization accounted for condemnation of a large number (27.4%) of edible organs of cattle from slaughterhouses in Siaya County, Kenya. It is necessary to use laboratory methods such as bacteriological, parasitic and histopathological techniques as this will ascertain the causes of such conditions/ condemnations in slaughterhouses.
- A large number of organs from 67% of all cattle slaughtered were condemned due to parasitic and bacterial conditions which are controllable at the farm level, and conditions caused by poor slaughtering techniques. The occurrence of hepatic fasciolosis and hydatidosis in this study suggested a possible transmission of the zoonotic agents to people
- Financial losses incurred as a result of condemnation of organs were high [mean annual loss of KShs. 7,739,214.40 (US$ 76,625.90) between 2013 and 2017, and mean of KShs. 31,490 (US$. 312) for the cross-sectional study], indicating heavy economic loses for the livestock industry

## 6.2 Recommendations

1. There is a need to disseminate the present research findings to all stakeholders including the County Director of Veterinary Services and Livestock, animal health practitioners, farmers, stock traders through pieces of training, workshops, conferences and journals
2. There is a need for use of laboratory methods in addition to gross morphological lesions observed in slaughterhouses to enhance accurate diagnosis of the disease conditions and to enable the application of effective control measures
3. Enhanced sensitization and upscaling of extension services to farmers by the Veterinary Services Department on measures to effectively control the disease conditions at farm level and sensitization of slaughterhouse workers on recommended slaughter techniques to reduce losses of edible organs and the associated financial losses in slaughter facilities are also needed
4. Further research needs to be undertaken, employing more advanced laboratory methods such as molecular techniques to determine a possible transmission of the zoonotic diseases (hydatidosis and fasciolosis) to people

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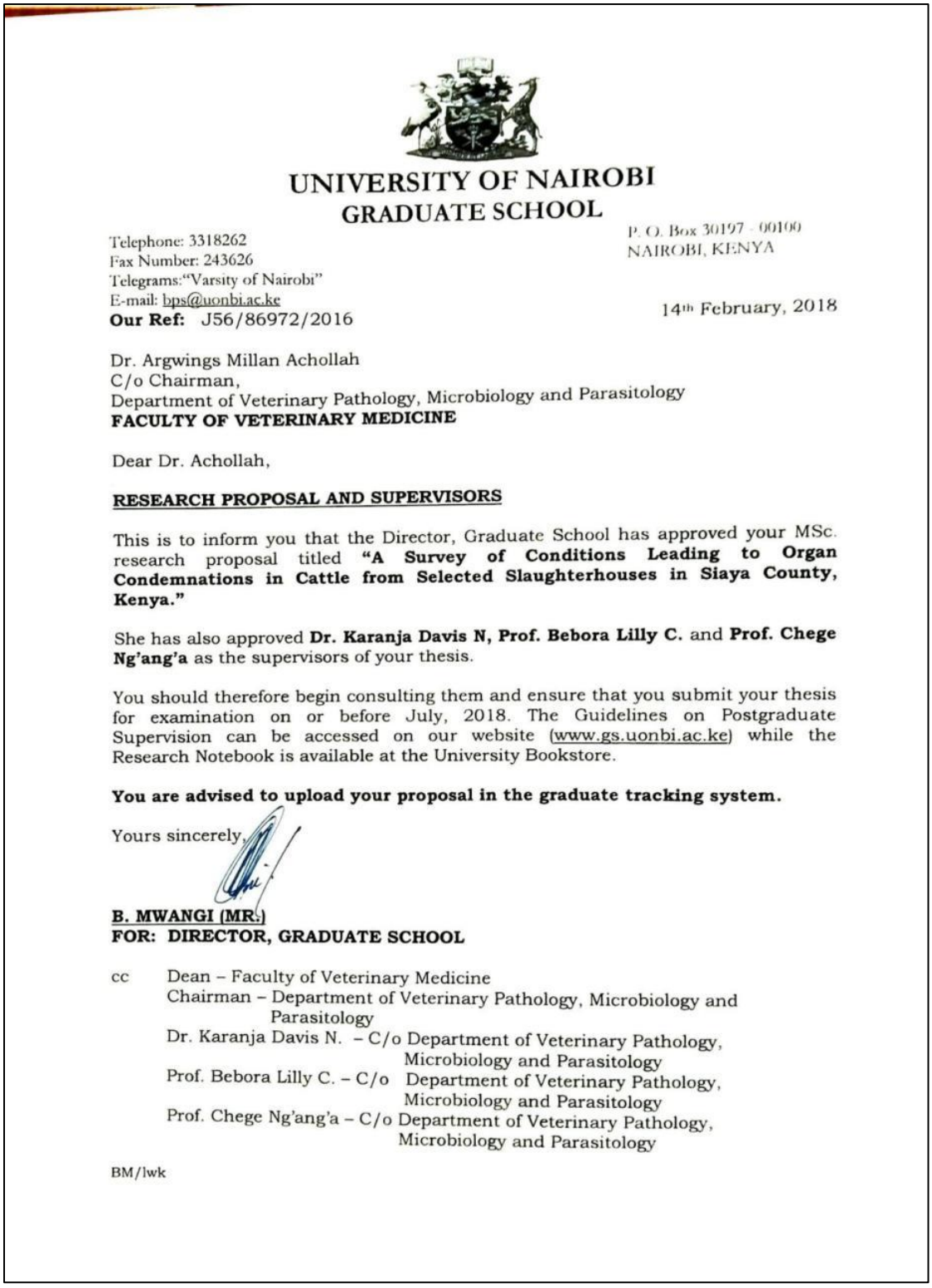
# 8 APPENDICES

## Appendix 8. 1. Research approval from Graduate school, University of Nairobi



UNIVERSITY OF NAIROBI
GRADUATE SCHOOL

Telephone: 3318262
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**Our Ref:** J56/86972/2016

P. O. Box 30197 - 00100
NAIROBI, KENYA

14th February, 2018

Dr. Argwings Millan Achollah
C/o Chairman,
Department of Veterinary Pathology, Microbiology and Parasitology
**FACULTY OF VETERINARY MEDICINE**

Dear Dr. Achollah,

**RESEARCH PROPOSAL AND SUPERVISORS**

This is to inform you that the Director, Graduate School has approved your MSc. research proposal titled **"A Survey of Conditions Leading to Organ Condemnations in Cattle from Selected Slaughterhouses in Siaya County, Kenya."**

She has also approved **Dr. Karanja Davis N, Prof. Bebora Lilly C.** and **Prof. Chege Ng'ang'a** as the supervisors of your thesis.

You should therefore begin consulting them and ensure that you submit your thesis for examination on or before July, 2018. The Guidelines on Postgraduate Supervision can be accessed on our website (www.gs.uonbi.ac.ke) while the Research Notebook is available at the University Bookstore.

**You are advised to upload your proposal in the graduate tracking system.**

Yours sincerely,

**B. MWANGI (MR.)**
**FOR: DIRECTOR, GRADUATE SCHOOL**

cc Dean – Faculty of Veterinary Medicine
Chairman – Department of Veterinary Pathology, Microbiology and Parasitology
Dr. Karanja Davis N. – C/o Department of Veterinary Pathology, Microbiology and Parasitology
Prof. Bebora Lilly C. – C/o Department of Veterinary Pathology, Microbiology and Parasitology
Prof. Chege Ng'ang'a – C/o Department of Veterinary Pathology, Microbiology and Parasitology

BM/lwk

**Appendix 8. 2. Approval from the County Director of Veterinary Services – Siaya County**

REPUBLIC OF KENYA

COUNTY GOVERNMENT OF SIAYA
DEPARTMENT OF AGRICULTURE, FOOD, LIVESTOCK AND FISHERIES

VETERINARY SERVICES

County Director of Veterinary Services
Siaya County
P.O. Box 529
SIAYA
21st May, 2018.

REF: SYACTY/VET/TRAINING/S/CVOL.1/.....7.....

Dr. Argwings Millan Achollah
P.O Box 78 – 40612
Sawagongo

**RE: AUTHORIZATION OF RESEARCH PROJECT (MASTER OF SCIENCE IN VETERINARY PATHOLOGY AND DIAGNOSTICS)**

Your research on **Survey of conditions leading to condemnation of organs in cattle from selected slaughterhouses in Siaya County, Kenya,** from 4th June, 2018 – 13th July, 2018 at Kaumara, Siaya town and Ugunja slaughterhouses refers.

This office has no objection in conducting the research in Siaya County slaughterhouses. The officers in charge of the mentioned slaughterhouses are hereby requested to provide the necessary support.

COUNTY DIRECTOR VETERINARY SERVICES SIAYA COUNTY P.O. Box 529 - 40600 SIAYA

Dr. Gladys A. Oyoko
County Director of Veterinary Services
Siaya County.

Copy to: : Chief Officer, Agriculture, Livestock and Fisheries
: Director County Human Resource Management
: County Secretary
: Secretary County Public Service Board

**Appendix 8. 3. The annual number of cattle slaughtered and organs condemned (%) during the period 2013 – 2017, in Siaya County**

| Condemned organs | Year, number of cattle slaughtered (n), organs condemned annually, the percentage in brackets and mean annual | | | | | | |
|---|---|---|---|---|---|---|---|
| | **2013** | **2014** | **2015** | **2016** | **2017** | **Total** | **Mean Annual** |
| | n=19,506 | n=20,292 | n=21,682 | n=20,144 | n=20,228 | n=101,852 | 20,370 |
| Livers | 3,040 (15.6) | 3,800 (18.7) | 3,292 (15.2) | 3,908 (19.4) | 3,991 (19.7) | 18,031 (17.7) | 3,606 |
| Lungs | 848 (4.3) | 548 (2.7) | 604 (2.8) | 708 (3.5) | 694 (3.4) | 3,402 (3.3) | 680 |
| Kidneys | 756 (3.9) | 544 (2.7) | 708 (3.3) | 420 (2.1) | 330 (1.6) | 2,758 (2.7) | 552 |
| Intestines | 512 (2.6) | 300 (1.5) | 424 (2.0) | 328 (1.6) | 377 (1.9) | 1,941 (1.9) | 388 |
| Hearts | 488 (2.5) | 276 (1.4) | 344 (1.6) | 372 (1.8) | 276 (1.4) | 1,756 (1.7) | 351 |
| Total number of condemned organs | 5,644 (29) | 5,468 (27) | 5,372 25) | 5,736 (28) | 5,668 (28) | 27,888 (27.4) | 5,578 |

**Appendix 8. 4 Number of cattle slaughtered per year (n), organs condemned (percentage), respective pathological conditions and mean annual figures in Siaya County, Kenya during the period 2014 – 2017**

| Organ | Conditions diagnosed | 2013 | 2014 | 2015 | 2016 | 2017 | Total (%) | Mean annual |
|---|---|---|---|---|---|---|---|---|
| | | n=19,506 | n=20,292 | n=21,682 | n=20,144 | n=20,228 | n=101,852 | n= 20,370 |
| Liver | Fasciolosis | 1568 (8.0) | 1952 (9.6) | 1688 (7.8) | 1888 (9.4) | 1986 (9.8) | 9,082 (8.9) | 1816 |
| | Cirrhosis | 520 (2.7) | 752 (3.7) | 580 (2.7) | 884 (4.4) | 748 (3.7) | 3,484 (3.4) | 697 |
| | Calcified cyst | 408 (2.1) | 576 (2.8) | 492 (2.3) | 688 (3.4) | 701 (3.5) | 2,865 (2.8) | 573 |
| | Hydatidosis | 180 (0.9) | 160 (0.8) | 156 (0.7) | 164 (0.8) | 211 (1.0) | 871 (0.9) | 174 |
| | Abscess | 204 (1.0) | 184 (0.9) | 140 (0.6) | 124 (0.6) | 178 (0.9) | 830 (0.8) | 166 |
| | Telangiectasis | 92 (0.5) | 128 (0.6) | 160 (0.7) | 116 (0.6) | 99 (0.5) | 595 (0.6) | 119 |
| | *Stelasia hepatica* infection | 68 (0.3) | 48 (0.2) | 76 (0.4) | 44 (0.2) | 68 (0.3) | 304 (0.3) | 61 |
| Lung | Pneumonia | 256 (1.3) | 252 (1.2) | 124 (0.6) | 176 (0.9) | 211 (1.0) | 1,019 (1) | 204 |
| | Hydatidosis | 104 (0.5) | 68 (0.3) | 88 (0.4) | 132 (0.7) | 141 (0.7) | 533 (0.5) | 107 |
| | Abscess | 104 (0.5) | 84 (0.4) | 172 (0.8) | 88 (0.4) | 75 (0.4) | 523 (0.5) | 105 |
| | Congestion | 140 (0.7) | 32 (0.2) | 84 (0.4) | 160 (0.8) | 99 (0.5) | 515 (0.5) | 103 |
| | Emphysema | 96 (0.5) | 44 (0.2) | 56 (0.3) | 84 (0.4) | 91 (0.4) | 371 (0.4) | 74 |
| | Pleurisy | 104 (0.5) | 36 (0.2) | 44 (0.2) | 40 (0.2) | 58 (0.3) | 282 (0.3) | 56 |
| | Melanosis | 44 (0.2) | 32 (0.2) | 36 (0.2) | 28 (0.1) | 19 (0.1) | 159 (0.2) | 32 |
| Kidneys | Infarcts | 192 (1.0) | 204 (1.0) | 248 (1.1) | 52 (0.3) | 98 (0.5) | 794 (0.8) | 159 |
| | Nephritis | 104 (0.5) | 132 (0.7) | 168 (0.8) | 100 (0.5) | 91 (0.4) | 595 (0.6) | 119 |
| | Hydronephrosis | 204 (1.0) | 52 (0.3) | 72 (0.3) | 68 (0.3) | 45 (0.2) | 441 (0.4) | 88 |
| | Congenital cyst | 128 (0.7) | 56 (0.3) | 104 (0.5) | 80 (0.4) | 12 (0.1) | 380 (0.4) | 76 |
| | Hydatidosis | 48 (0.2) | 64 (0.3) | 76 (0.4) | 92 (0.5) | 41 (0.2) | 321 (0.3) | 64 |
| | Kidney stones | 80 (0.4) | 36 (0.2) | 40 (0.2) | 28 (0.1) | 43 (0.2) | 227 (0.2) | 45 |
| Intestines | Paramphistomosis | 244 (1.3) | 72 (0.4) | 144 (0.7) | 48 (0.2) | 68 (0.3) | 576 (0.6) | 115 |
| | Pimply gut | 92 (0.5) | 76 (0.4) | 84 (0.4) | 68 (0.3) | 102 (0.5) | 422 (0.4) | 84 |
| | Enteritis | 44 (0.2) | 52 (0.3) | 100 (0.5) | 68 (0.3) | 112 (0.6) | 376 (0.4) | 75 |
| | Haemorrhages | 68 (0.3) | 52 (0.3) | 56 (0.3) | 88 (0.4) | 50 (0.2) | 314 (0.3) | 63 |
| | Peritonitis | 64 (0.3) | 48 (0.2) | 40 (0.2) | 56 (0.3) | 45 (0.2) | 253 (0.2) | 51 |
| Hearts | Haemorrhages | 232 (1.2) | 188 (0.9) | 176 (0.8) | 140 (0.7) | 106 (0.6) | 740 (0.8) | 168 |
| | Pericarditis | 84 (0.4) | 12 (0.1) | 48 (0.2) | 88 (0.4) | 71 (0.4) | 303 (0.3) | 61 |
| | Hydropericardium | 56 (0.3) | 48 (0.2) | 52(0.2) | 52 (0.3) | 43 (0.2) | 251 (0.6) | 50 |
| | Abscess | 52 (0.3) | 8 (0.04) | 36 (0.2) | 40 (0.2) | 32 (0.2) | 168 (0.2) | 34 |
| | Melanosis | 36 (0.2) | 12 (0.1) | 24 (0.1) | 36 (0.2) | 21 (0.1) | 129 (0.1) | 26 |
| | Cysticercosis | 28 (0.1) | 8 (0.04) | 8(0.04) | 16 (0.1) | 3 (0.03) | 63 (0.1) | 13 |

**Appendix 8. 5 Key informant Semi-Structured interview for selected butcher men on retail prices of meat and offals in Siaya County, Kenya**

**UNIVERSITY OF NAIROBI**
**COLLEGE OF AGRICULTURE AND VETERINARY SCIENCES**

**Serial No. …….** **Date: ……**

**KEY INFORMANT SEMI-STRUCTURED INTERVIEW FOR BUTCHER MEN ON PRICES OF MEAT AND OFFALS IN SIAYA COUNTY, KENYA**

| **A. INTRODUCTION:** |
|---|
| **Name**: Dr. Argwings Achollah Millan, MSc Student, University of Nairobi<br>**Name of Project**: Establishing causes and associated financial losses for organ condemnations in cattle slaughtered in Siaya County, Kenya. |
| **PURPOSE OF INTERVIEW:** To obtain market prices of meat and offals during the period 2013-2018, to help complete the above project. |
| **CONFIDENTIALITY:** In the reports that I will submit, your responses during this interview will not be quoted with your name and that of your butchery. |
| **VERBAL CONSENT:** Would you like to participate in this interview? ☐Yes / ☐No |
| **B. MAIN QUESTIONS:** |
| What is the name of your butchery?..... For how many years have you operated it?..... |
| In the last 5 years, what were your retail prices per kilogram (kg) of meats (meat on bone/muscle) and offals such as liver, lungs, stomachs and intestines, hearts, spleen and kidneys in your butchery per year? |
| What are your current retail prices per kilogram (kg) of meats (meat on bone/muscle) and offals such as Liver, lungs, stomachs and intestines, hearts, spleen and kidneys in your butchery? |
| **C. CONCLUSION** |
| Is there any other information that you would like to add that you feel is important for this interview on price/kg of meats and offals? |

**D. RETAIL PRICES OF MEATS AND OFFALS**

| **ORGAN** | **YEAR** | | | | | |
|---|---|---|---|---|---|---|
| | **2013** | **2014** | **2015** | **2016** | **2017** | **2018** |
| Liver | | | | | | |
| Lungs | | | | | | |
| Kidneys | | | | | | |
| Stomachs, intestines/GIT | | | | | | |
| Muscle (meat) | | | | | | |
| Spleen | | | | | | |
| Heart | | | | | | |

……Thank you!.....

**Appendix 8. 6. Average retail prices of meat and offals obtained from selected butcher men in Siaya County**

| ORGAN | YEAR | | | | | |
|---|---|---|---|---|---|---|
| | 2013 | 2014 | 2015 | 2016 | 2017 | 2018 |
| Muscle | 300 | 300 | 360 | 360 | 360 | 360 |
| Liver | 400 | 400 | 460 | 460 | 460 | 500 |
| Lung | 200 | 200 | 240 | 240 | 240 | 260 |
| Stomachs and Intestines/GIT | 200 | 200 | 240 | 240 | 240 | 260 |
| Hearts | 400 | 400 | 460 | 460 | 460 | 500 |
| Spleen | 400 | 400 | 460 | 460 | 460 | 500 |
| Kidney | 400 | 400 | 460 | 460 | 460 | 500 |

**Appendix 8. 7. Characteristics of the three selected slaughterhouses and their geospatial location in Siaya County**

| Characteristic | Slaughterhouse | | |
|---|---|---|---|
| Slaughterhouse name (code) | Siaya (SS) | Kaumara (SK) | Ugunja (SU) |
| Location (Sub-County) | Alego-Usonga | Gem | Ugunja |
| GPS coordinates | 0°03'57.0"N 34°16'51.2"E | 0°04'49.9"N 34°27'05.7"E | 0°10'48.9"N 34°17'31.3"E |
| Ownership | Public | Private | Private |
| Category of Slaughterhouse (A-large, B-medium, C-Slaughter slab) | B | B | C |
| Approved slaughterhouse throughput (cattle units per day) | 6-39 | 6-39 | ≤5 |
| Average slaughter per day (cattle units) | 6 | 8 | 6 |
| Export/local slaughterhouse | local | local | local |
| Stunning | No | No | No |
| Source of water | Piped water and borehole | Piped water and borehole | Borehole |
| Fence | Yes | Yes | No |
| Lockable gate | No | Yes | No gate |
| Artificial light | Yes | Yes | No |

**Appendix 8. 8. Phenotypic characteristics and county of origin of cattle slaughtered and examined in the cross-sectional study and % representation during period 5th June 2018 to 4th July 2018**

| Sex | Slaughterhouse | | | |
|---|---|---|---|---|
| | **Kaumara (%)** | **Siaya (%)** | **Ugunja (%)** | **Total (%)** |
| Male | 21(45.6) | 25 (73.5) | 24 (75) | 70(62.5) |
| Female | 25 (54.3) | 9 (26.5) | 8 (25) | 42 (37.5) |
| Total | 46 (41.1) | 34 (30.4) | 32(28.6) | 112 |
| **Breed** | | | | |
| Zebu (%) | 44 (95.7%) | 32 (94.1) | 32 (100) | 108 (96.4) |
| Exotic (%) | 1(2.2) | 0 | 0 | 1(0.9) |
| Zebu/Exotic cross (%) | 1(2.2) | 2 (5.9) | 0 | 3(2.7) |
| **County of origin (%)** | | | | |
| Siaya | 38 (82.6) | 11(32.4) | 18 (56.3) | 67 (60) |
| Busia | 0 | 15 (44.1) | 12 (37.5) | 27 (24) |
| Homabay | 7(15.2) | 5 (14.7) | 0 | 12 (10.7) |
| Migori | 1(2.2) | 3 (8.8) | 0 | 4 (3.6) |
| Kakamega | 0 | 0 | 2 (6.3) | 2(1.8) |
| TOTAL (n) | 46 (41.1) | 34 (30.4) | 32(28.6) | 112 |

**Appendix 8. 9. Number and % of pathological conditions in cattle slaughtered during the period 5th June 2018 to 4th July 2018**

| **Criterion** | **Slaughterhouse (%)** | | | **Total (%)** |
|---|---|---|---|---|
| | **Kaumara** | **Siaya** | **Ugunja** | |
| **1.** Number of animals with ≥ 1 pathological conditions (%) | 30 (65.2) | 25 (73.5) | 20 (62.5) | 75 (67) |
| • Number with 1 condition (%) | 25 (83.3) | 17 (68) | 15 (75) | 57 (76) |
| • Number with > 1 condition | 5 (16.7) | 8 (32) | 5 (25) | 18 (24) |
| **2.** Number of animals with no condemnation | 16 (34.8) | 9 (26.5) | 12 (37.5) | 37 (33) |
| Total animals inspected | 46 | 34 | 32 | 112 |

**Appendix 8. 10. Number of partial or whole organ condemnation and % representation out of 112 animals slaughtered and inspected during the cross-sectional study**

| Organ | **Slaughterhouse** | | | | | | **Total** | | **Total number of organs condemned** |
|---|---|---|---|---|---|---|---|---|---|
| | **Kaumara** | | **Siaya** | | **Ugunja** | | | | |
| | **WC[1]** | **PC[2]** | **WC[1]** | **PC[2]** | **WC[1]** | **PC[2]** | **WC[1] (%)** | **PC[2] (%)** | |
| Liver | 17 (70.8) | 7 (29.2) | 9 (40.9) | 13 (59.1) | 5 (38.5) | 8 (61.5) | 31 (27.7) | 28 (25) | **59 (52.7)** |
| GIT[3] | 0 | 9 | 0 | 8 | 0 | 11 | 0 | 28 | 28 (25) |
| Lungs | 1 | 0 | 1 | 0 | 0 | 0 | 2 (2) | 0 | 2 (1.8) |
| Spleen | 1 | 0 | 1 | 0 | 0 | 0 | 2 (2) | 0 | 2 (1.8) |
| Muscle | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 1 (0.9) |

*[1] Whole organ condemnation; [2] Partial organ condemnations;[3]Gastrointestinal tract*

**Appendix 8. 11. Slaughter cattle data capture form used during the cross-sectional study**

**SLAUGHTER CATTLE DATA CAPTURE SHEET**

**Name of the research project: ……Investigator: ……Date: ……**

**Name of Slaughterhouse: …… County: ……. Sub-County: …… GPS Location: ……...**

**Cattle ID: ……. Sex: ……. Breed: ……. Origin/Source: ……**

| Organ | Lung | Heart | liver | Kidney | Spleen | Muscle | Tongue | Gut |
|---|---|---|---|---|---|---|---|---|
| Description of gross lesion | | | | | | | | |
| Tentative diagnosis | | | | | | | | |
| Partial/total condemnation | | | | | | | | |
| Weight of organ condemned | | | | | | | | |
| Laboratory test required | | | | | | | | |
| Sample Collected | | | | | | | | |
| Laboratory results | | | | | | | | |
| Laboratory diagnosis | | | | | | | | |

**Appendix 8. 12. Number of samples collected from slaughterhouses and respective laboratory tests performed during the study period 5th June 2018 to 4th July 2018**

| Organs | Slaughterhouse | | | NO. of samples collected | Tests performed | | |
|---|---|---|---|---|---|---|---|
| | [1]SK | [2]SS | [3]SU | | Parasitological | Histopathology | Bacteriological |
| **Liver** | **55** | **70** | **30** | **155** | **87** | **67** | **1** |
| **GIT** | **5 (17.9)** | **19 (67.9)** | **4 (14.3)** | **28** | **8** | **20** | **0** |
| **Spleen** | **3** | **3** | **0** | **6** | **2** | **2** | **2** |
| **Lungs** | **2** | **2** | **0** | **4** | **0** | **2** | **2** |
| **Muscles** | **0** | **1** | **0** | **1** | **0** | **0** | **1** |
| **TOTAL** | **65 (33.5)** | **95 (49)** | **34 (17.5)** | **194** | **97(50%)** | **91(46.9%)** | **6(3.1%)** |

*[1]Kaumara, [2]Siaya and [3]Ugunja slaughterhouse*

**Appendix 8. 13. Bacterial isolation and identification criteria and data capture form used during the cross-sectional study (5th June 2018 to 4th July 2018)**

| Cattle /sample ID | Growth in blood Agar | Growth on MacConkey agar | Gram stain | Oxidase test | Catalase test | Indole | Methyl Red | Citrate | Urea | TSI | Coagulase test | CAMP test | Growth in PTBA agar | Remarks |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | | | | | | | | | | | | | | |
| 2 | | | | | | | | | | | | | | |
| 3 | | | | | | | | | | | | | | |
| 4 | | | | | | | | | | | | | | |
| 5 | | | | | | | | | | | | | | |
| 6 | | | | | | | | | | | | | | |

**Appendix 8. 14. Bacterial isolation and identification records for animal ID: SK040**

| **Cattle ID** | **SK040** | **SK040** |
|---|---|---|
| **Organ** | lung | lung |
| **Growth in blood agar** | Large, round light grey/off white glistening, moist colonies, slightly | Off-white, pinpoint colonies, α - haemolytic |
| **Growth on MacConkey** | Large, round, moist, red/pink colonies – lactose fermenters | No growth |
| **Gram stain (color, shape)** | Pink (Gram-negative) rod-shaped | Gram-positive cocci in chains |
| **Oxidase test** | -Ve, No color change | -Ve; no colour change |
| **Catalase test** | -Ve, No bubbles | -Ve, no bubbles |
| **Indole test** | +Ve, Wine-red ring | N/A |
| **Methyl red test** | +Ve, Red colour | N/A |
| **Citrate test** | -Ve, Green colour | N/A |
| **Urea test** | -Ve, no colour change | N/A |
| **TSI test** | Acid slant (yellow), acid butt (yellow) and gas production | N/A |
| **Glucose** | N/A | N/A |
| **Sucrose** | N/A | N/A |
| **PTBA test** | N/A | N/A |
| **CAMP test** | N/A | -Ve, no enhanced zone of hemolysis |
| **Coagulase test** | N/A | N/A |
| **Genus/ species** | *Escherichia coli* | α - haemolytic *Streptococcus species* |

**Appendix 8. 15. Bacterial isolation and identification records for animal ID: SSO04 and SK009**

| **Cattle ID** | **SK009** | **SS004** | |
|---|---|---|---|
| **Organ** | spleen | lung | |
| **Growth in Blood agar** | Small white –grey colonies; β -haemolytic; | Small cream/yellow colonies; non-haemolytic | Small white colonies, raised center, smooth |
| **Growth on MacConkey** | No growth | No growth | No growth |
| **Gram stain** | Gram +Ve cocci forming chains | Gram +Ve, pleomorphic/cocco bacili | Gram +Ve cocci in clusters |
| **Oxidase test** | -Ve; no colour change | -Ve, no colour change | -Ve, No colour change |
| **Catalase test** | Ve; no bubbles | +Ve, copious bubbles | +Ve, copious bubbles |
| **Indole test** | N/A | -Ve | -Ve |
| **Methyl red test** | N/A | +Ve | +Ve, Red color |
| **Citrate test** | N/A | -Ve | -Ve |
| **Urease test** | N/A | -Ve | -Ve |
| **TSI test** | N/A | Alkaline butt, alkaline slant, no | Alkaline butt, Alkaline slant, no gas, no $H_2S$ |
| **Glucose** | N/A | -Ve | +Ve, no gas |
| **Sucrose** | N/A | -Ve | -Ve |
| **PTBA agar** | N/A | Grey-Black colonies | N/A |
| **CAMP test** | -Ve, no enhancement of hemolysis | N/A | -Ve |
| **Coagulase test** | N/A | N/A | -Ve, tube, and slide coagulase test; no clots |
| **Genus/ species** | β-hemolytic *Streptococcus* species | *Corynebacterium spp.* | Coagulase-negative *staphylococcus spp.* |

**Appendix 8. 16. Bacterial isolation and identification record for animal ID: SS017**

| **Cattle ID** | **SS017** | |
|---|---|---|
| **Organ** | muscle | |
| **Growth in Blood agar** | small, milky, β - haemolytic | Greyish, medium-sized, β-haemolytic |
| **Growth on MacConkey** | No growth | No growth |
| **Gram stain** | Gram-positive, small rod-shaped, slightly curved, form | Gram-positive cocci in chains |
| **Oxidase test** | -Ve no color change | -Ve; no color change |
| **Catalase test** | -Ve | -Ve; |
| **Indole test** | -Ve | -Ve |
| **Methyl red test** | +Ve | -Ve |
| **Citrate test** | -Ve | -Ve |
| **Urease test** | -Ve | -Ve |
| **TSI test** | Alkaline butt, Alkaline slant, no gas, no $H_2S$ | Alkaline butt, alkaline slant, no gas, no $H_2S$ |
| **Glucose** | +Ve; gas produced | +Ve, gas produced |
| **Sucrose** | -Ve | -Ve |
| **PTBA agar** | Grey-black colonies | N/A |
| **CAMP test** | -Ve, no enhancement of hemolysis | +Ve; enhanced haemolysis on blood agar |
| **Coagulase test** | N/A | N/A |
| **Genus/ species** | *Corynebacterium pseudotuberculosis* | *Streptococcus agalactiae* |

**Appendix 8. 17. Bacterial isolation and identification records for animal ID: SS001**

| **Cattle ID** | **SS001** | |
|---|---|---|
| **Organ** | spleen | |
| **Growth in Blood agar** | Small size, yellow colonies, irregular edges, non-hemolytic | Small-sized yellow colonies, non -haemolytic |
| **Growth on MacConkey** | No growth | No growth |
| **Gram stain** | Gram-positive, short rod-shaped/ coccobacilli | Gram-positive; short rods |
| **Oxidase test** | -Ve; no color change | -Ve; no colour change |
| Catalase test | +Ve | +Ve; copious bubbles |
| **Indole test** | -Ve | -Ve |
| **Methyl red test** | +Ve | -Ve |
| **Citrate test** | -Ve | -Ve |
| **Urease test** | -Ve | +Ve |
| **TSI test** | alkaline butt, alkaline slant, no gas, no $H_2S$ | Alkaline butt, alkaline slant, no gas, $H_2S$ produced, |
| **Glucose** | +Ve; no gas produced | -Ve |
| **Sucrose** | -Ve | -Ve |
| **PTBA agar** | N/A | -Ve |
| **CAMP test** | N/A | N/A |
| **Coagulase test** | N/A | N/A |
| **Genus/ species** | *Corynebacterium pilosum* | *Corynebacterium spp.* |

**Appendix 8. 18. Morphometric data (maximum and minimum values, mean and standard deviation) of adult liver flukes recovered from slaughtered cattle at Kaumara, Siaya and Ugunja Slaughterhouses**

| Morphometric parameter | Maximum | Minimum | Mean | Standard deviation |
|---|---|---|---|---|
| Body length (mm) | 43 | 18 | 30.41 | 5.79 |
| Body width (mm) | 11 | 2.75 | 5.87 | 1.48 |
| Cone length (mm) | 2.75 | 0.75 | 2.13 | 0.46 |
| Cone width (mm) | 3.55 | 1.375 | 2.67 | 0.59 |
| Oral sucker maximum diameter (mm) | 0.875 | 0.375 | 0.64 | 0.10 |
| Oral sucker minimum diameter (mm) | 0.7 | 0.25 | 0.43 | 0.09 |
| Ventral sucker maximum diameter (mm) | 1.5 | 0.875 | 1.16 | 0.13 |
| Ventral sucker minimum diameter (mm) | 1 | 0.375 | 0.64 | 0.15 |
| Distance between anterior end and ventral sucker (mm) | 2.8 | 1.2 | 2.12 | 0.42 |
| Distance between ventral sucker and oral sucker (mm) | 2.5 | 1.075 | 1.95 | 0.39 |
| Pharynx length (mm) | 0.825 | 0.25 | 0.53 | 0.13 |
| Pharynx width (mm) | 0.55 | 0.225 | 0.36 | 0.09 |
| Body area ($mm^2$) | 328.13 | 38.86 | 143.63 | 54.54 |
| Pharynx area ($mm^2$) | 0.29 | 0.05 | 0.15 | 0.07 |
| Body length/ body width ratio | 9.07 | 3.45 | 5.35 | 1.14 |
| Pharynx length/ pharynx width ratio | 2.54 | 1 | 1.49 | 0.34 |