Thesis Ref. No._____

PATHOLOGICAL CHARACTERIZATION OF PULMONARY LESION AND IDENTIFICATION OF ASSOCIATED BACTERIA AND PARASITE IN SHEEP AND GOAT AT DESSIE MUNICIPAL ABATTOIR, NORTHEAST ETHIOPIA

MSc Thesis

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JUNE, 2023

BISHOFTU, ETHIOPIA

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A thesis submitted to Addis Ababa University College of Veterinary Medicine and Agriculture in partial fulfillment of the requirements for the degree of Master of Science in Veterinary Pathology

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As member of the examining board of the final degree of Master of Science open defense, we certify that we have read and evaluated the thesis prepared by: Nuredin Teshale entitled "***Pathological Characterization of Pulmonary Lesion And Identification of Associated Bacteria and Parasite in Sheep and Goat at Dessie Municipal Abattoir, Northeast Ethiopia***" and recommended that it be accepted as fulfilling the thesis requirements for the degree of Masters of Science in Veterinary Pathology.

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DECLARATION

First, I declare that this thesis is my original work and that all sources of materials used for this thesis have been duly acknowledged. This has been submitted in partial fulfillment of the requirements for an advanced (MSc) degree at Addis Ababa University, College of Veterinary Medicine and Agriculture and is deposited at the University or College library to be made available to borrowers under rules of the Library. I solemnly declare that this thesis is not submitted to any other institution anywhere for award of any academic degree, diploma, or certificate.

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TABLE OF CONTENT **PAGES**

ACKNOWLEDGEMENTS

Above all thanks goes to Allah, the most gracious, the most merciful, lord of the universe for his innumerable favors, giving health and vigor to me during the study time.

I would like to express my sincere gratitude, admiration, and honors to my advisors, Dr. Jirata Shiferaw, for their extensive knowledge sharing, kind guidance, and proofreading this research paper (manuscript) from beginning to end. Their supervision has strengthened me to produce genuine work and helped me overcome challenges in my duties.

I am delighted to express my gratitude to Wollo University School of Veterinary Medicine for providing the necessary chemicals, reagents, and laboratory equipment for bacteriological and parasitological testing. I want to extend my gratitude to every staff member of the Wollo University School of Veterinary Medicine and special thanks to Dr. Abadi Amare for his valuable advice and devotion of his time in correcting the manuscript of my thesis. I also want to thank the Dessie municipal abattoirs as institution and Dr. Girma meat inspector at this office for facilitating sample collection and providing an opportunity to inspect the lung and the Animal Health Institute in Sebeta for their permission and allowance to perform histopathology.

I am thankful to my beloved all family, my wife and friends for their advice and moral support.

LIST OF TABLES **PAGES**

LIST OF FIGURES **PAGES**

LIST OF ANNEXES **PAGES**

LIST OF ABBREVIATIONS

AHI	Animal Health Institute
BALT	Bronchial Associated Lymphoid Tissue
BCS	Body Condition Score
CAPDC	Caprine Arthritis Encephalitis Pneumonia Disease Complex
CSA	Central Statistical Agency
DFEDB	Dessie Finance and Economic Development Bureau
DPX	Distrene Plasticizer/ Dibutyl Phthalate Xylene
ELISAs	Enzyme-linked Immunosorbent Assays
EMB	Eosin Methylene Blue
ESGPIP	Ethiopia sheep and goat productivity improvement program
FAO	Food and Agriculture Organization of the United Nations
H and E	Haematoxylin and Eosin
IMViC	Indole, Methylene red, Vogues Proscus and Commons Citrate test
RBC	Red Blood Cell
TSI	Triple Sugar Iron

ABSTRACT

Lung is susceptible and vulnerable to many infectious and non-infectious agents. A cross-sectional study design was conducted from December 2022 and May 2023 on major pulmonary lesion of sheep and goat slaughtered at Dessie municipal abattoir with the objective of characterizing the pulmonary pathological lesions, and isolating and identifying potential aerobic bacteria and parasite from pneumonic lung. A total of 420 (302 sheep and 118 goats) were examined for pulmonary lesions using standard techniques. Additionally, detailed histopathological investigation was also conducted on purposively selected lung lesions. Besides, pneumonic lesions were subjected to bacteriological and parasitological analysis. The overall abattoir prevalence of pulmonary abnormalities in sheep and goat was 341 (81.2%). The common gross lesions encountered were Pneumonia (55.5%), emphysema (7.1%), atelectasis (3.3%), Hydatid cyst (4.3%), congestion (2.4%), haemorrhage (3.1%), adhesion (0.7%), Melanosis (0.9%), and Bronchoectasis (0.7%). Pneumonia, hydatidosis, and pulmonary emphysema were significantly associated ($p<0.05$) with animal age groups. In the current studies, pneumonia was the most common disorder, accounting for 233 (55.5%). *Dictyocaulus filaria* (47.7%), *Mullerius capillaries* (23.2%) and *Protostrongylus rufescens* (17.16%) were isolated lung worm parasites. Whereas *E. coli* (20.6%), *Klebsiella Pneumoniae* (12.7%), *Mannheimia haemolytica* (10.8%), *Streptococcus species* (4.9%), *Staphylococcus aurous* (7.8%) and *Pasteurella multocida* (2.9%), *Pseudomonas* (6.9%), *Proteus* (1.9%), *Cornybacterium* (1%) were the isolated bacteria. Additionally, 18 (4.3%) Hydatid cysts were identified. The pulmonary lesion of sheep and goats in the study are relatively high prevalent and therefore, have great health and economic impacts. To formulate feasible and affordable control measures, more research on the causes of problems of the respiratory organs, as well as the isolation and identification of both aerobic and anaerobic bacteria, viruses, and fungi, is necessary.

Keywords: *Bacteria, Histopathology, Parasite, Pneumonia, Pulmonary Lesion, Sheep and Goats*

1. INTRODUCTION

Livestock has an important role in the subsistence and economic growth in Africa (Mohammed *et al*., 2022). Among the predominant livestock species, sheep play an important role in the socio-economic development of the majority of African countries. Ethiopia‘s estimated largest livestock population in Africa; currently supporting and sustaining the livelihoods of an estimated 80 percent of the rural community (Engdawork, 2019). According to CSA, (2020/21) estimates, Ethiopia has 70 million cattle, 42.9 million sheep, 52.5 million goats, 2.15 million horses, 10.80 million donkeys, 10.38 million mules, and about 8.1 million camels and 57 million poultry. In Ethiopia small ruminants play a significant role in the national economy. They provide more than 30% of the country's meat needs (domestic meat from these animals is also in high demand, especially during religious holidays) and earn money from exporting meat, primarily in the form of live animals and skins. They also produce wool, milk, manures for the land, and serve as an investment for farmers (Adem, 2016; Abatemam, 2018).

Because of different constraint such as malnutrition, inadequate animal production systems, deteriorating hygienic conditions, poor genetic potential of the local stock, marketing, social factors, structural limitations, and prevalent diseases, the productivity and economic benefits to farmers remain marginal and the productivity does not correspond to the size of the animals (Sissay *et al*., 2007). Diseases are one of the primary constraints of the productivity of food animals (Kidane *et al*., 2018).

Diseases affecting the respiratory system are generally the leading causes of morbidity and mortality in sheep and goat. In addition to mortality, respiratory infections limit growth rate, compromise animal wellbeing, and induce carcass condemnation (Mekibib *et al*., 2019; Mohammed *et al*., 2022). Lung is susceptible and vulnerable to many infectious and non-infectious agents causing several pathological changes indicative for disease conditions. This is because of their anatomical and histological particularities through the inhalation from the environment and or from the blood (Belkhiri, 2009; Mekibib *et al*., 2019).

At meat inspection a large number of specific diseases and nonspecific conditions may be diagnosed in the lung. Animals showing no clinical signs of diseases may be detected at slaughter and the true picture of these diseases and conditions could be documented and made available to the public (Jibat *et al*., 2008). According to the report of Benavides *et al*. (2015), the pathological alterations in the lung were observed in different gross and histological types that ranged in severity from mild to severe, acute to chronic and exudative to proliferative interstitial. Depending on the causative agents, different circumstances might lead to varieties of lung lesions. Pneumonia, hydatidosis, pulmonary calcification, abscess, pulmonary emphysema, tuberculosis, adhesions (pleurisy), pulmonary atelectasis, fibrosis, pulmonary haemorrhage and congestion are the main pulmonary pathology of the sheep (Radositis *et al*., 2007; Tsegaye and Tessema, 2016; Thannon, 2018).

According to the report of Adem (2016) pneumonia is account for around half of all sheep mortality and morbidity in Ethiopia's highlands. Pneumonia, among the inflammatory conditions of the lung tissues found in sheep all over the world. It is regarded one of the leading causes of death in the small ruminant (Mohammed *et al.,* 2022). Hydatidosis is one of the most important parasitic diseases of sheep and goat that cause huge economic losses due to reduction in carcass weight gain and condemnation of Organs (Abegaz and Mohammed, 2018). The emphysema lesions on lung can be examined grossly by appreciating pale, enlarged grayish-yellow, pearl like shiny, puffy, and crepitate feel upon observation and palpation of the lung. Atelectasis is result of collapse of the alveoli due to failure of the alveoli to inflate or because of compression of the alveoli (Zeryehun and Alemu, 2017).

Several investigations were undertaken to determine the prevalence, gross pathological lesions and economic significance of pulmonary abnormality of sheep and goat in Ethiopia and the result indicated varied prevalence. Among these, Mandefro *et al.* (2015); Assefa *et al*. (2017); Mishra *et al*. (2018); Denebo and Tafere (2022) reported 37%, 29.18%, 15.9% and 62.5% pulmonary gross pathological lesion respectively and Mekibib *et al*. (2019) revealed 17.13% prevalence, particularly pneumonia.

According to the report of Zeryehun and Alemu (2017); Jibat *et al*. (2008) and Mohammed *et al.* (2022) lung lesions rank among the major causes sizable economic loss each year as a result of mortality, inadequate weight gain, and the rejection of edible organs and corpses at slaughter and chemotherapeutic and vaccination programs. As with tuberculosis, hydatidosis, and several of these lung conditions are zoonotic and have serious public health implications (Jibat *et al*., 2008).

Since respiratory disease is a patter or dynamic feature; there is still a need for identifying the causes with histopathological characterization of respiratory diseases of sheep and goat slaughtered at abattoir in the study area. The gross and histological characterization of the lung disease specifically in sheep and goat at Dessie municipal Abattoir has not yet been studied. This study carried out on gross and histopathological lesions of sheep and goat lung infection together with identification of bacterial and parasitic agent.

Objective

- To determine the prevalence and frequency of different pathological lesion on the lung
- To Characterize gross and histopathological lesion of the lung
- To identify the bacterial and parasitic agents causing pneumonia of sheep and goat at Dessie municipal abattoir of the Northern Ethiopia.

2. **LITERATURE REVIEW**

2.**1. Anatomy and Histology of Lung**

The lungs are the major organs of the respiratory system and located in the thoracic cavity, protected by the thoracic cage and covered by pleura. The weights of lung in sheep are more than that of lung in goats. The color of lungs in sheep and goat is rose gray (Al_Sadi, 2005).

The lung consists of paired right and left, and are separated into a number of coordinated lobes by distinct, deep fissures which are apical, middle, caudal and accessory at the right side and the left consisted of apical (cranial and caudal), caudal, in goat the right was similar of the sheep but the apical was undivided only two lobes, cranial and caudal (Al_Sadi, 2005; Yousif and Dawood, 2019). Each pulmonary lobe is further subdivided by connective tissue into pulmonary lobules. Sheep and goats have well-lobated but poorly lobulated lungs (MacGavin and Zachery 2006). The pulmonary veins in sheep pass above the bronchial tree but in the goats their passage are between the bronchial trees (Kini, 2002).

The major bronchi divide into segmental and sub segmental bronchi. The bronchial tree in all lobes of the lung in sheep distributed from secondary bronchioles. The bronchi continue to divide into bronchioles, which are lack cartilage. The bronchioles further divide, ending in terminal bronchioles and extending into the respiratory bronchioles, which have alveoli budding from their walls (Kini, 2002). The diameters of bronchi, bronchiole and alveoli in sheep are more than in goats (Al_Sadi, 2005).

Histologically, the lungs are covered by visceral pleura. The pleura are lined by mesothelium; which is a serious membrane lined by a single layer of flattened squamoid cells called the mesothelium. The visceral pleura include some smooth muscle and connective tissue. The bronchi and bronchioles are lined by ciliated columnar epithelium. The bronchi are lined by a ciliated, pseudo-stratified columnar epithelium with goblet cells. The goblet cell is nonciliated columnar with cytoplasm distended by pale pinkish mucin and a basally located nucleus similar to that of the ciliated colurnnar cell. Mucosa, lamina propria, muscular mucosal tunic, and

adventitia make up the extra-lobular bronchi (MacGavin and Zachery, 2006). The bronchioles differ in structure from bronchi in several respects: The lamina propria is replaced by a smooth muscle layer, Lining epithelium is no stratified ciliate, have fewer goblet cells that subsequently disappear, lack cartilage in their walls. Clara cells are nonciliated cuboidal cell that line the terminal bronchioles (Kini, 2002; MacGavin and Zachery, 2006).

Alveolar ducts branch from respiratory bronchioles. The alveoli are lined by a single layer of two types of epithelial cells: type I and type II pneumocytes. The type I pneumocyte is a large, flat, and thin cell, have fewer organelles and are not metabolically active; their main function is gas exchange (MacGavin and Zachery, 2006; Kini, 2020). The second order alveolar cells appear interspersed among the first-order alveolar cells and appear clustered in interavleolar septa. Alveoli are separated from one another by a thin, highly vascularized layer of fine collagenous and elastic fibers. This layer, together with the squamous cells lining the adjacent alveoli, forms an alveolar septum. The alveolar septa are very delicate structures, consisting of two layers of nonnucleated epithelial cells and a capillary network (MacGavin and Zachery, 2006; Scurrell *et al*., 2007).

2.2. **Etiology of Pulmonary Abnormality**

The lung is a critical organ in the body, but vulnerable to a variety of disease-causing substances that enter the body by inhalation or through blood. These agents can be infectious or non-infectious. The etiology is complex and multifactor and includes both infectious and non-infectious factors. Non infectious factor, such as irritants that harm the lining of the respiratory tract, as well as environmental factors like the hot, humid climate of the tropics, which creates the ideal environment for these infections to occur. Toxicants, and mechanical induction of respiratory distress may also be the cause of these abnormal conditions. Stressors that contribute to the development of the disease and anything that causes the animal to have dropped in immunity (Plummer *et al*., 2012; Tijjani, 2012; Kumar *et al.*, 2014; Jesse *et al*., 2019).

The infectious agents associated with respiratory diseases of sheep include bacterial agents (*Mannheimia haemolytica, Pasteurella multocida*), viral agents (PI3 virus, Respiratory syncytial

virus and Ovine adenovirus), parasites (*Dictyocaulus filaria, Muellerius capillaries, Cystocaulus ocreatus, Protostrongylus rufescens* and hydatid cyst) and mycoplasma (*Mycoplasma ovipneumoniae* and *Mycoplasma arginini).* The viral and mycoplasma agents usually cause low grade respiratory disease with mild signs after an extended period of infection (Hashemnia *et al*., 2019). Mycoplasma interaction with the host´s cilia prevents normal ciliary activity, facilitating the invasion of the lower respiratory tract by other organisms, including *Mannheimia haemolytica* and *Pasteurella multocida. M. haemolytica* and *P. multocida*, the main causes of pneumonic pasteurellosis in sheep (Alarawi and Saeed, 2021).

Bacterial pneumonias are most common and they may act as a primary or secondary cause to it (Mishra *et al.,* 2018). In many cases, high humidity, dust, damp bedding, excessive heat, tight buildings with inadequate ventilation, and irritating gases such as ammonia compromise disease resistance and natural defense mechanisms in the sheep or goat, allowing pneumonia to develop. Weakness from a difficult birth, inadequate intake of colostrums, and other stresses contribute to the development of pneumonia in nursing animals. Often, a mild viral infection will occur, compromising the animal and allowing secondary bacterial infections to take place (Bell, 2008).

2.3. Pathogenesis of the Pulmonary Diseases

The pathogenesis of the respiratory diseases is multifactorial, and the combination of several infectious pathogens, host defense, environmental variables, and stress are the main contributors to the complex etiology of respiratory disorders tract. Depending upon the environmental, physiological, and etiological factors, respiratory conditions might be acute, chronic, and/or progressive in nature. The development of various types of pneumonia is caused by these predisposing conditions, which reduce the local resistance of the respiratory mucosa and permit the growth of infectious organisms in the respiratory tract (Yesuf *et al*., 2012). When viral and bacterial illnesses coexist, especially in unfavorable environmental circumstances, the situation worsens (Lacasta, 2008). The majority of sheep are still in good health because of their superior pulmonary defenses, which work to effectively clear these pathogens, despite the constant exposure of the ovine respiratory system to potentially hazardous species. The ovine respiratory system is frequently exposed to potentially harmful organisms, although the majority of sheep

are still in good condition thanks to their excellent pulmonary defenses which labor the effective clearance of these organisms. Inflammatory or disease-causing organisms can enter the lower respiratory tract when the mucociliary mechanism of the lung is disrupted, the pulmonary defenses' function is compromised, or the pulmonary tissues are damaged. There are numerous infectious agents that appear as a result of interactions between host defense mechanisms, viruses, bacteria, parasites, fungi, and environmental factors (Obaid and Khudiar, 2016).

2.4. Major Pulmonary Lesion

2.4.1. Pneumonia

Pneumonia is regarded a respiratory disease originate from an inflammatory reactions of the bronchioles and alveoli in the lung to infective agents and resulting in the consolidation of lung tissue. The word pneumonia is used for any inflammatory lesion in the lungs, regardless of whether it is exudative or proliferative, alveolar or interstitial. However, the word pneumonitis has been used by some as a synonym for pneumonia; however, others have restricted this term to chronic proliferative inflammation generally involving the alveolar interstitial and with little or no evidence of exudates (Zachary, 2017). Its histological forms are mild to severe, acute to chronic, and exudative to proliferative interstitial (Obiad and Khudiar, 2016). Lung enlargement, hyperemia, and sometimes oedema are characteristics of pneumonia (Denebo and Tafere, 2022). The histological character of pneumonia probably changes according to its etiological character, host immunity, environmental conditions, therapeutic attempts, and disease prognosis (Ahmad *et al*., 2016). The host (physiological and immunological), environmental stress, numerous agents (viral, bacterial, parasite, and mycoplasmal), environmental conditions, and poor management all interact to cause pneumonia (Jubb *et al*., 2015). Pneumonias can best be classified according to involvement of different pulmonary regions and anatomical sites and the gross and histological appearance of the affected lung tissue (Van Dijk *et al*., 2007; Mishra *et al*., 2018; Mekbib *et al*., 2019)**.**

Bronchopneumonia: In bronchopneumonia, the typical gross appearance of bronchopneumonia is of irregular consolidation in cranioventral regions. The cranial and middle lobes are most often

affected in those species having well-defined lobation. Consolidated lungs vary from dark red, through gray-pink, to more gray, depending on the age and nature of the process. Consolidation of the tissue is the most important gross criterion of pneumonia. The cut surface of infected lungs reflects the variability of involvement seen on the pleural surface. The severe inflammatory response around the bronchi and bronchiole, especially bronchio-alveolar junctions has been regarded as hallmark of bronchopneumonia. The inflammatory exudates collect in the bronchioles and bronchi, and alveolar lumens, hemorrhage and sometimes hyperplasia of associated bronchial lymphoid tissues (Yesuf *et al*., 2012; Chowdhury, 2018).

Bronchopneumonia is subdivided into three types depending up on the predominant exudates. Purulent bronchopneumonia: abundant neutrophils and few macrophages within the lumen of bronchi, bronchioles and alveoli. Fibrinous bronchopneumonia: predominant exudates are fibrin with few neutrophils and macrophages. Fibrinoprulent bronchopneumonia: neutrophils and fibrins were seen almost in equal amount in the exudates (Yesuf *et al*., 2012).

Suppurative bronchopneumonia: Grossly, irregular dark red to grey-pink consolidation particularly in the cranial, middle and accessory lobes, solid and pale with suppurative exudates in the bronchi and bronchioles and abssation of foci scattered throughout the affected part of lung parenchyma in the chronic phase as well as spherical onion-like appearance of abscess in the mediastinal lymph node (Singh *et al*., 2017; Mohammed *et al*., 2022). Microscopically; the inflammatory process in suppurative bronchopneumonia is generally confined to individual lobules and there were numerous polymorphonuclear infiltrating the alveolar spaces, bronchi, and bronchioles, as well as macrophages, sloughed epithelial cells, necrotic debris, as well as various quantity of mucus and fibrin (Chowdhury, 2018; Mohammed *et al*., 2022). Vascular congestion and inflammatory exudates were seen in the alveoli of most of the affected lungs (Rashid *et al*., 2014; Singh *et al*., 2017).

Fibrinous Pneumonia: the inflammation affects many adjacent lobules, and the exudates spreads swiftly through the lung tissue, eventually affecting the entire lobe (Zachery, 2017). The lesions are primarily in the cranial, cardiac, and accessory lobes, and they are characterized grossly by patches of consolidation with irregular shapes and dark red color. The injured lungs

were very challenging to cut due to pulmonary oedema, congestion, and fibrin accumulation. Typically, the pleural surface of the affected area was covered in a thin fibrin coating. Pleural thickening and cut surfaces showed focal to diffuse patches of concentrated, hard tissue in the lungs (Singh *et al*., 2017; Mohammed *et al*., 2022).

Histopathologically, Lungs with fibrinous bronchopneumonia were characterized by inflated pleura and alveoli due to fibrinous exudation and neutrophilic exudation, profuse fibrinous exudates visible fibrin strand in the interlobular septa, and an inflammatory zone around the respiratory bronchiole (Chowdhury, 2018). Numerous capillaries are congested, multifocal areas of necrosis, various levels of fibrinous exudates, and the bronchial epithelium is desquamated. The lumens of the alveoli and bronchioles contain inflammatory exudates that are primarily composed of mononuclear cells and contain few neutrophils. Consisting of parenchyma consolidation, lymphoid tissue hyperplasia, and desquamation of the bronchial epithelium, the endothelium damage linked to fibrinous pneumonia (Singh *et al*., 2017; Al Kharawi, *et al*., 2019; Mohammed *et al*., 2022).

Interstitial Pneumonia: Grossly, most parts of the affected lungs were enlarged and frequently seen in dorsocaudal regions. Moreover, the affected lungs are pale, rubbery in texture, meaty in appearance (Figure 1), gray-white foci on the cut surface and resisted the pressure (Mekibib *et al.*, 2019; Mohammed *et al*., 2022). Failure of the lungs to collapse and the presence of rib imprints on the pleural surface. Consistently increased tracheobronchial lymph nodes are present (Sukanta, 2018). Interstitial pneumonia may develop due to the initial injury and the inflammation process in endothelium, alveolar epithelium and bronchiolar epithelium, which later leads to the infiltration of leukocytes and thickening of interalveolar septa (Mishra *et al*., 2018).

Microscopically, Due to lymphocyte infiltration, the growth of smooth muscle and fibroblasts, and the obvious hyperplasia of type II pneumonocytes, the alveolar wall have thickened. There is hemorrhage in the alveoli, a rounded shape to the bronchi, thickening of the interalveolar septa caused by the growth of fibrous connective tissue and accumulation of macrophages, reactive cells, and inflammatory cells in the bronchial lumen. Some instances had substantial, clearly

defined lymphoid nodules developing in the area of nearby bronchi as a result of lymphoid hyperplasia (Yesuf *et al*., 2012; Chowdhury, 2018; Al-Karawi *et al*., 2019; Mohammed *et al*., 2022). Moreover, lymphofollicular proliferation with follicle-like aggregations, peribronchial and perivascular cuffing (Mekibib *et al*., 2019).

Granulomatous pneumonia: Granulomatous pneumonia is characterized caseous or noncaseous granulomas, hard multifocal nodules may be present in varying numbers and different size dispersed randomly throughout the lungs. These foci characterized by white color, well-circumscribed, variably sized nodules, which are surrounded by a clear discrete, red and hemorrhagic area (Mahdi *et al*., 2015). Microscopically, Granulomatous pneumonia is a lesion surrounded by mononuclear cells and multinucleated giant cells (Yesuf *et al*., 2012). The nodules had a central necrotic area that looked like a cheesy substance, and they were encircled by a zone of layers made up of various inflammatory cells and a zone of fibrous connective tissue (Mahdi *et al*., 2015).

Verminous pneumonia: Verminous pneumonia, which affects sheep, is a chronic and prolonged infection, clinically characterized by respiratory discomfort and pathologically by bronchitis and bronchopneumonia. It is an infection of the lower respiratory tract that can result in bronchitis, pneumonia, or both (Alemneh and Tewodros, 2016). The parasites *Dictyocaulus filaria, Protostrongylus rufescens,* and *Mulerius capillaries,* which are part of the two separate groups Trichostrongyloidea and Metastrongyloidea, are what cause verminous pneumonia, often known as ovine lung worm (Chakraborty *et al.,* 2014; Hailu, 2019). *Dictyocaulus filaria* occurred in the trachea, bronchi and bronchioles of sheep. This lungworm has a knob on its head, is white in color, and is thread-like in appearance. A *Dictyocaulus filaria* adult male lung worm's posterior end contains a short bursa with a short, robust, dark-brown, "boot-shaped" spicule (Dar *et al*., 2012). *Muellerius capillaries* are also occurred in bronchi, bronchioles and alveoli. This lungworm is thin (thus the common name hairworms), medium-sized (not longer than 3 cm), and has a bent tail. *Protostrongylus rufescens* adults were found in the bronchioles as *Dictyocaulus filaria,* despite the fact that it produces pulmonary nodules that resemble those of *Muellerius capilaris*. This is grayish reddish in color, has wavy tail and nodules similar to those of *Muellerius capilaris* (Adem, 2016; Wodaje *et al*., 2021)

Small ruminant lungworms have a direct and indirect life cycle (Adem, 2016). The life cycle of Dictyocaulus filaria is linear. It takes less time to reach an infectious stage. Larvae undergo two moults after hatching as part of their basic life cycle, and they contract the infection by consuming free L3. In the indirect life cycles of *Mullerius capillaries* and *Protostrongylus refescene*, the first two moults frequently take place in intermediate hosts (snails or slugs), and infection of the final host is brought on by ingestion of the intermediate host (Hailu, 2019). *P. refescens* has indirect life cycle that requires longer time and wet or rainy warmer season to complete their complex life cycle in the presence of suitable intermediate hosts that create favorable condition for sporadic distribution, dry or short rainy season does not favor the development of the snail intermediate hosts (Kebede *et al*., 2014).

According to the type and intensity of host-parasite interaction, the lungs are a primary hub for parasitic migration, and a range of parasites that pass through them have varying effects on the body. Each lungworm's predilection place determines how pathogenic it is on a relative basis. *D. filaria* lives in the trachea and bronchi so aspirated eggs, larvae and debris affect a large volume of lung tissue. It is therefore the most pathogenic specie (Alemneh and Tewodros, 2016). If the migrating parasites are numerous, huge, or especially when the host reacts hypersensitivity to them, severe and potentially fatal lung lesions may form. They cross the gut's wall and migrate to the lungs through the lymphatic system and the blood stream. The larvae enter the lungs, pass through the alveolar mucosa to the alveolar lumen, and then stay in the bronchi and bronchioles where they mature into adults and begin to lay eggs. The host's lungs respond by developing nodules that are encased in connective tissue and contain necrotic material, egg masses and worms (Adem, 2016).

Gross pathology; the lungs showed raised emphysematous patches, depressed consolidated areas, and dirty white to irregular or nodular lesions spread across the various lobes, particularly in the diaphragmatic lobes (Jubb *et al*., 2016). On cut surface, edematous foam and mucus mixed with massive numbers of nematodes fill the terminal bronchioles of the diaphragmatic lobes (figure 2). Sometimes formation of granulomas, grossly, are gray, non caseated nodules and may be confused with that resemble at the earlystages of tuberculosis (Dar *et al*., 2012; Zachary, 2017).

The macroscopic lung lesions in sheep and goats differ in a few ways. Goats showed more superficial lesions than sheep, whose lesions frequently advanced into deeper lung tissue (Panayotova-Pencheva and Alexandrov, 2010; Ayana and Chane, 2013). In contrast to sheep, where *M. capillaris* lesions are more frequently sub pleural, they are more frequently interstitial in goats. Large-scale tissue congestion along the biggest bronchi, which was dark-red to grey, was the main feature of the gross abnormalities in the lungs of ruminants infected with bigger species of Protostrongylus. Alveoli, terminal bronchi, and bronchioles were more severely injured in the lungs of goats infected with *protostrongylids* than the interstitium (Smith, 2015).

Microscopically, Infiltration of inflammatory cells present in the vascular wall and around bronchiole (Dar *et al*., 2012). There are also inflammatory infiltrates in the bronchial mucosa; alveolar edema; hyperplasia of BALT caused by persistent immunologic stimuli; hypertrophy and hyperplasia of bronchiolar smooth muscle because of increased contraction and decreased muscle relaxation; and a few eosinophilic granulomas around the eggs and dead larvae (Jubb *et al*., 2016; Zachary, 2017). Cross sections of the adult worms were seen in the bronchi and bronchiole. Interstitial pneumonic areas were seen which revealed thickening of interalveolar wall and mononuclear cell infiltration. Hemorrhages were observed in the bronchiolar and alveolar areas of the lung (Dar *et al*., 2012).

Aspiration pneumonia: Aspiration pneumonia is a type of lower respiratory tract infection that categorized under non-infectious conditions and is caused by the inhalation of foreign material, often in liquid form, reaching the lungs through the airways, which triggers a strong inflammatory response. The degree and prognosis are strongly depending on the nature of the material, the bacteria that are carried with it and the distribution of the material in the lungs (Plummer *et al*., 2012; Caswell and William, 2019). A single or a small group of animals are frequently affected by inhalational pneumonia. This distinguishes it from most bronchopneumonia that is caused by inhalation of tiny droplets or particles. The condition most frequently affects lambs raised in bottles, after improper dousing or dipping, or in laterally recumbent animals after inhaling rumen contents or a foreign body. The physical and chemical characteristics of the aspirated material typically influence both the gross and microscopic lesions of aspiration pneumonia. Apart from the inherent inflammatory and necrotic nature of

the aspirated fluid, most bacteria from the nasopharynx was flushed down the respiratory tree and reach the lungs by gravitational drainage and cause lesions ranging from bronchopneumonia to granulomatous in type (Fentahun, 2017). Grossly, dark brown, congested and somewhat meaty in consistency area was seen in the affected lungs. Moreover, gray to green-brown foul-smelling exudates were expressed from small airways upon compression (Mekibib *et al*., 2019). Microscopically, aspiration pneumonia characterized by the presence of aspirated foreign material in tissue (Mohammed *et al*., 2022)

Embolic pneumonia: Embolic pneumonia can occur in animal at any age. Multifocal hemorrhagic and/or purulent foci are seen or felt scattered in all or most lobes. These are usually round and not rectangular. Excessively septic, brown/black, embolic foci have a most likely source in open wounds of ligaments, tendons, and joints. Many septic lung lesions of various sizes and age, with connective buildup, suggests a prolonged shower. In most species, embolic material from the right heart does not cause infarctions unless extensively distributed, as the lung has a duel blood supply with the bronchial artery supplying the oxygen to help prevent the infarction. Initially, while the acute liver rupture may kill quickly, the lung may not have a septic odor, but if the necropsy is delayed the lung may have a definite septic odor as embolic organisms may be rapidly multiplying (King *et al*., 2014).

2.4.2. Hydatid cyst (Hydatidosis)

Hydatidosis is one of the main parasitic causes of lung lesion during post- mortem inspection. The liver and lungs are frequently where parasites are detected in intermediate hosts, though they can develop in a number of organs (Thompson and McManus, 2001). About 70% of hydatid cysts in sheep are located in the lungs, where they partially embed in the lung tissue while typically causing very little local reactivity and no related clinical symptoms. Parasites can develop in a variety of organs in the intermediate hosts but are often found in the liver and lung (Bell, 2008). The presence of cysts in the lungs is likely to be associated with tissue damage and functional disorders, and this has been indicated by the histological changes observed in infected organs (Kumari *et al.*, 2017).

The life cycle of hydatidosis involves two mammalian hosts. The adult cestode inhibits the small intestine of carnivores (definitive host) and produces eggs containing infective oncosphers. the adult cestode generates eggs with infectious oncosphers and inhibit the small intestine of carnivores (the final host). Cestodes segments and proglotids with free eggs were released into the environment from the last host's intestinal system. The larval stage (metacestod), which develops in the visceral organs typically after egg ingestion by food animals (intermediate hosts) like, sheep and goats typically produces numerous protoscolices, each of which has the potential to develop into an adult cestode after being consumed by the carnivore definitive host (Thompson and Mc Manus, 2001).

Grossly, the pulmonary parenchyma included visible oval, hard, fluid-filled cysts (Hashemnia *et al.*, 2019). Cysts are filled with clear to slightly turbid fluid and feel soft and doughy to the touch. Upon fluid aspiration, the cyst deflated, and its fluid within was discovered to be clear to slightly turbid. the cyst membrane, which appeared creamy white, could be easily removed from the organ. Some cysts, on the other hand, seemed rigid, had inspissated contents, and were calcified, gritty, and difficult to cut. Hydatid cyst contains semisolid material on which there may be deposition of calcium salts to form calcified cyst (Bell, 2008). Calcified cysts have a gritty sound upon incision with knife and when observed grossly the cyst is white or grey and irregularly rounded and frequently honey combed (Zeryehun and Alemu, 2017). The collected cysts were carefully incised and examined for protoscolices, which looks like white dots on the germinal epithelium, in hydatid fluid so as to classify cysts as fertile or infertile. In the lungs, a germinal layer was observed separating from the laminated layer in places (Kebede *et al*., 2019).

Histopathologically, the cysts were surrounded by an infiltrate of mixed inflammatory cells, including giant cells and eosinophils and also a layer of granulation tissue in some cases. the cyst wall contained lamellar hyaline layer, thin syncytial germinal layer, and fibrous connective tissue. Degenerated laminated membranes had significant inflammatory cell infiltration on the fibrous capsule's inner side (Hashemnia *et al*., 2019). The granulation tissue in which there was mild lymphoplasmacytic and palisade macrophage, the multinucleated giant cell was found on a wide zone of concentrically arranged fibro-vascular granulation tissue, and the adjoining alveolar tissue had atelectasis or emphysema (Kumari *et al*., 2017).

2.4.3. Neoplasm's

Pulmonary adenomatosis: Pulmonary adenomatosis is a chronic, progressive pneumonia that affects sheep. Histologically, it is characterised by adenomatous ingrowths of the alveolar walls, and clinically, it is characterised by the production of copious amounts of watery mucus from the lungs that are expelled by the nose. The histology is distinctive and indicative of the disease (John and Sons, 2008). The lungs are grossly expanded, thick, and congested, and the bronchi are filled with foam. The diseased lungs have a thick, hard firmness, with many, variably sized nodules that are grayish-white in colour and contain greyish fluid. The neoplastic pulmonary alveoli are bordered by cuboidal to columnar cells that are proliferating and generating papillary projections that extend into the alveolar lumenae, with lymphocytes making up the majority of the leukocytic cell infiltration in the parabronchial regions. Inflammatory cells, namely macrophages, lymphocytes, plasma cells, and giant cells with few neutrophils in their lumenae, were seen in the bronchioles along with hyperplasia of the lining epithelial cells that generated papillary projections (El-Mashad *et al*., 2020).

Fibrosarcoma: Fibrosarcoma is created microscopically when malignant fibroblasts mingle with collagen fiber to form whorls. These malignant cells displayed severe anaplasia and pleomorphism, taking on avariety of nucleus shapes and sizes (El- Mashad *et al*., 2020).

2.4.4. Abnormalities of inflation

Atelectasis: Atelectasis is a lung lesion that occurs when the alveoli collapse because they are unable to inflate (Denebo and Tafere, 2022). The lungs seemed grossly distinct from the surrounding pulmonary tissue, well-separated, and slightly depressed beneath the normal inflated lung surface. A significant amount of lung fibrosis or large abscesses may be discovered next to or between atelectasis. The atelectic patches are dark red in colour and have a flabby or firm texture. According to El-Mashad *et al*. (2020) the predominant pattern of lesions was lobular. The alveolar walls in the atelectasis area were parallel and close together, and collapsed alveoli were visible under a microscope (Kumar *et al*., 2014; Hashemnia *et al*., 2019). Atelectasis may be seen beside large sized abscess or in between areas of lung fibrosis (El-Mashad *et al*., 2020).

Emphysema: Emphysema develops as a result of the breakdown of lung connective tissue, including the elastic and supportive pulmonary parenchyma. When there is an abnormal permanent accumulation of air in the lungs brought on by an obstruction to the airflow or by prolonged gasping breaths during slaughter processes, it also happens in areas of lung fibrosis, bronchopneumonia, and granulomatous in type lung disorder circumstances (Radostits, 2007; Mellau *et al*., 2010; Zeryehun and Alemu, 2017).

Grossly, Air bubbles of various sizes were present in the interlobular septa and lung parenchyma. The emphysematous areas appeared pale in colour, blocked above the surrounding lung surface, and palpably swollen and crepitate due to the buildup of air in the pulmonary parenchyma. (Hashemnia *et al*., 2019; El-Mashad *et al*., 2020). Microscopically, emphysema was primarily seen in patients who also had bronchitis and other pneumonias. The emphysematous regions showed enlarged air gaps distal to the terminal bronchiole and swollen alveoli. The interalveolar septa were usually ruptured leading to formation of giant alveoli. Other associated findings were bronchial and bronchiolar epithelial hyperplasia (Hashemnia *et al*., 2019).

2.4.5. Circulatory disorder

Pulmonary Congestion: grossly, clearly visible as a mottled, reddish, heavier-than-normal lung. Pneumonia does not exist if the lung is not hard. When the lung is cut open, a lot of blood might leak into the airways. This condition, which has no known etiology, is nearly always brought on by blood pooling in the lungs after being compelled to leave the liver and vena cava and the muscles due to rigour mortis and gastrointestinal gas production, respectively. This is yet another non-lesion that is best ignored. Even histologically, any animals that haven't been bled out ought to have blood on them (King *et al*., 2014).

Pulmonary hemorrhage: grossly, patchy due to congestion and sporadic haemorrhage. The peribronchial, interlobular septal, and inter-alveolar areas all had engorged blood vessels under a microscope. Red blood cells partially or completely engulfed the alveoli and inter-alveolar septa. (Singh *et al*., 2017).

2.4.6. Lung Abscesses

Localized accumulation of pus (dead neutrophils) that is separated from the surrounding tissue by a fibrous capsule created as a result of an infection is known as an abscess. It looks like a huge palpable lesion filled with fluid that is changing. *Arcanobacterium pyogenes* and Staphylococcus aureus are the most frequent pyogenic bacteria that result in animal abscesses when there has been prior lung damage, such as after an acute pneumonic event or secondary to ovine pulmonary adenocarcinoma (jaagsiekte). They might also develop after embolic dissemination from another infection focus. *Fusobacterium necrophorum* can cause abscesses in situations of inhalational pneumonia or as a result of pharyngeal abscessation, while *S aureus* lung abscesses can develop with tick pyaemia. Similar to this, *Actinobacillus lignieresii* can result in oral abscesses and lung diseases. After a lung abscess, pyaemia may develop and present itself suddenly (Bell, 2008; Mellua *et al.,* 2010).

2.4.7. Bronchitis and bronchiolitis

Grossly, in the mucosa of the bronchi and bronchioles, there was noticeable congestion in the acute case, whereas in the chronic cases, the bronchial lumenae were packed with of copious amounts of mucous exudate, and the bronchial walls were thickened. Microscopically, peribronchiolar lymphocyte infiltrations and blood vessels congested with mononuclear leukocytic cellular infiltrations were characteristics of bronchitis and bronchiolitis. Moreover, it is characterized by marked desquamation and hyperplasia of the bronchiole lining epithelium, along with a significant amount of exudate in the lumenae (El-Mashad *et al.,* 2020).

2.4.8. Pleurisy (pleural fibrosis)

Inflammation of the fibrinous adhesions between the parietal pleura and the lung surface is known as pleurisy. It is a disorder that is frequently related to pneumonia. Grossly, the pleura seemed velvety were thickened and opaque. The sub-pleural emphysema and areas of edoema and fibrous connective tissue proliferation caused the affected pleura to thicken microscopically. From the centre to the outside of the lesions, liquefactive necrosis, many neutrophils,

mononuclear inflammatory cells, and fibrous connective tissue were all seen (Mellau *et al.*, 2010).

2.4.9. Anthracosis and Melanosis

Melanosis is an abnormal accumulation of melanin pigments in various organs which causes dark or brownish-black pigmentation of the tissues and organs resulting from a disorder of pigment metabolism melanin is not harmful and can be consumed without causing injury. However, affected meat, because of its unsightly appearance, is not suitable for human consumption (Mellua *et al*., 2010).

Gross appearance; Depending on the quantity and location of the pigment in the damaged tissue, melanin gives tissues a black, brown, or red color. Anywhere in the lung, it manifests as tiny black spots (multifocal) of pigmented parenchyma in the airway, ranging in size from 1 mm to patches of 4–5 cm or bigger (locally extensive). They shouldn't be referred to as tumors because they are not space-occupying masses Microscopic appearance, Melanin can be recognized microscopically if thin histologie preparations are examined with a bright light. Individual melanin granules are very small brown bodies, uniform in size and spherical in shape. Most of the melanin is located intracellularly within melanoblasts or melanophores. These heavily pigmented cells appear as black or brown globular masses (melanophores) (Mellua *et al*., 2010).

Anthracosis: Long-term inhalation of carbon particles in both humans and animals results in anthracosis, which is the deposition of black particulate matter in the lung parenchyma.. The carbon particles are found as a black pigment in tissues particularly in the lungs and corresponding lymph nodes in animals raised in urban areas (Mellua *et al*., 2010).

2.5. **Diagnostic Methods**

Different diagnostic approaches are used globally to prevent and control deadly infectious respiratory infections of small ruminants. The diagnostic processes and tests used around the world combine both traditional and cutting-edge diagnostic techniques (AL Salihi and Yahia, 2018). General patient examination is the most crucial diagnostic technique for identifying respiratory illnesses. When a patient has a respiratory condition as their main complaint, taking an accurate and pertinent history is the first step in making a diagnosis (Jesse *et al*., 2018). They are challenging to distinguish by visual inspection alone, thus veterinary investigation is necessary to get accurate diagnosis (Bell, 2008).

Diagnosis of the causative agent of the respiratory disease can be obtained through further laboratory diagnostic work-up such as bacteriology culture, isolation and identification of the causative agent (Jess *et al*., 2018)**.** Bacterial culture is used to confirm the causal bacteria and Diagnosis is possible from laboratory examination of faces, though rarely required. For the detection of antigens and antibodies directly from samples, modern enzyme-linked immunosorbent assays (ELISAs) are primarily accessible for all clinical conditions with specificity and sensitivity. Similar to this, molecular diagnostic assays and microsatellites provide thorough support for epidemiological investigations, diagnosis, and treatment (Kumar *et al.*, 2014).

Pathology is a key tool in the diagnosis, understanding and control of infectious diseases and non infectious essential when studying their pathogenesis (Caswell and Callanan, 2014). A thorough post-mortem examination is probably the most useful tool for achieving a correct pathological diagnosis and the only way to identify the majority of lesions (Benavides *et al*., 2015). Histopathological examination can identify distinctive changes in the cells of the lung tissue (Kumar *et al.,* 2014). Postmortem findings followed by serological and molecular methods for the confirmation of etiological agents (AL Salihi and Yahia, 2018). Infection of ovine verminous pneumonia is often recognized at slaughter house meat inspection by gross pulmonary lesions (Ayana and Chane, 2013). The lung pathological examinations were considered a basis for comparison between sonographic findings and pathological changes (Kumar *et al.,* 2014).

Ultrasonographic examination of the lungs has the potential to identify abscesses close to the surface or in the pleural space Lung auscultation scoring method is a systematic approach that could be adopted to detect, identify and differentiate the degree of severity in ruminant pneumonia cases (Bell, 2008).

2.6. **Economic Importance of pulmonary Disease in sheep and goat**

Respiratory disorder in sheep and goat can have devastating effects on economic success in the industry by morbidity and mortality in sheep and goat, respiratory infections limit growth rate, compromise animal wellbeing, induce carcass condemnation, and cost to activated chemotherapeutic and vaccination programs (Mekibib *et al*., 2019; Mohammed *et al*., 2022). The annual direct financial loss from international and domestic markets due to organ and carcass condemnations at abattoir and indirect financial loose due to human factors either as a result of mishandling of animals during transport to the slaughterhouse or due to faulty slaughter operations in the abattoir (Jibat *et al*., 2008).

To mitigate the economic and public health impacts of diseases of small ruminants, proper disposal of offal, prohibition of backyard slaughter of small ruminants and construction of slaughter houses, better disease control strategies, adequate training of abattoir personnel on the slaughtering operation and regular deworming of stray and home dogs and Education of small ruminant traders how to transport the livestock from farm to markets recommendations are forwarded (Assefa *et al*., 2022).

3. MATERIALS AND METHODS

3.1. Description of Study Areas

The study was conducted at Dessie municipal abattoir. Dessie is located about 401km north east of Addis Ababa and located at latitude of 11°07´59.81¨N and a longitude of 39°37´ 59.83 E. It is the main town and the capital city of South Wollo zone. Its elevation is between 2400 and 2550 metres above sea level. There are 11.7°C and 24°C of mean minimum and maximum temperatures every year, respectively. The region has a bi-modal rainfall pattern with an annual rainfall of 1100- 1200 mm. The annual rainfall ranges 1100- 1200 mm and the area experiences a bi-modal rainfall patterns with a short rainy season which occurs from February to March and long rainy season which starts at the end of June to September (DFEDB, 2015). The abattoir receives animals that originated from South Wollo, North Wollo, Oromiya Special zone and Afar regions.

South Wollo is located in the south east corridor of the Amhara region in northern Ethiopia and it lies within 10°12′N, and 11°40′N latitude and 38°30′E and 40°05′E longitude (Mohammed, *et al*., 2018). The altitude of the area ranges from 1500 to 3500 meters above sea level (Rosell and Holmer, 2015). The mean annual temperature and rainfall of the area ranges from 14 to 20 °C and from 680 to 1200 mm, respectively. The main farming system is mixed farming and sheep are the predominant animal species kept in the area (Agidew and Singh 2018).

North Wollo zone, which is located in eastern part of Amhara regional state within 8º95’- 12º8’N longitude and 38º5’-40º20’E latitude. The altitude ranges from 700-4100 m asl (AbunaYosef Mountain) in Gidan district (in the western parts of the study area). The annual rainfall varies from 650 mm (low altitude) to 1200 mm (high altitude) with the maximum temperature of 25ºC in the low altitude and minimum temperature of 16ºC in the high altitude. The zone is divided into three main agro-ecological zones: High altitude (>2500 masl) 31.951%, mid altitude (1500–2500 masl) 57.493% and low altitude (Aragie and Genanu 2017).

Oromia Special zone is found in Amhara Regional State, north eastern Ethiopia. It is located at

325 km North of Addis Ababa, the capital city of Ethiopia. Currently, seven districts are found in this special zone including Dawa Chefa, Bati, Jile Timuga, Artuma Fursi, Dawa Harewa, Bati and Kemise towns. The geographical location of this zone is between 10˚01' N to 11˚ 25'N and 39˚ 41'E to 40˚24'E with elevation ranges from 1000 to 2500 meters above the sea level. The mean annual rainfall of the area ranges from 600 mm to 900 mm, and the minimum and maximum temperatures are 12˚C and 33˚C, respectively (Molla *et al.*, 2021).

The Afar National Regional State is characterized by an arid and semi-arid climate with the altitude ranges from 120 meter below sea level to 1500 meters above sea level. The area located in northeast of Ethiopia between 39°34′ and 42°28′ E longitude and 8°49′ and 14°30′ N latitude at about 621 kilometers away from Addis Ababa (MoARD, 2008).. The mean maximum temperature reaches up to 41 °C in June, and the mean minimum temperature ranges from 21 to 22 °C between November and December (Shiferaw *et al.*, 2019). The area has mean annual rainfall below 500 mm in the semi-arid western escarpments and decreasing to 150 mm in the arid zones to the east. The rainfall is low, erratic and bimodal, the long rain usually occurs in the months of mid-June to mid-September, while the short rains usually come in March and April (MoARD, 2008).

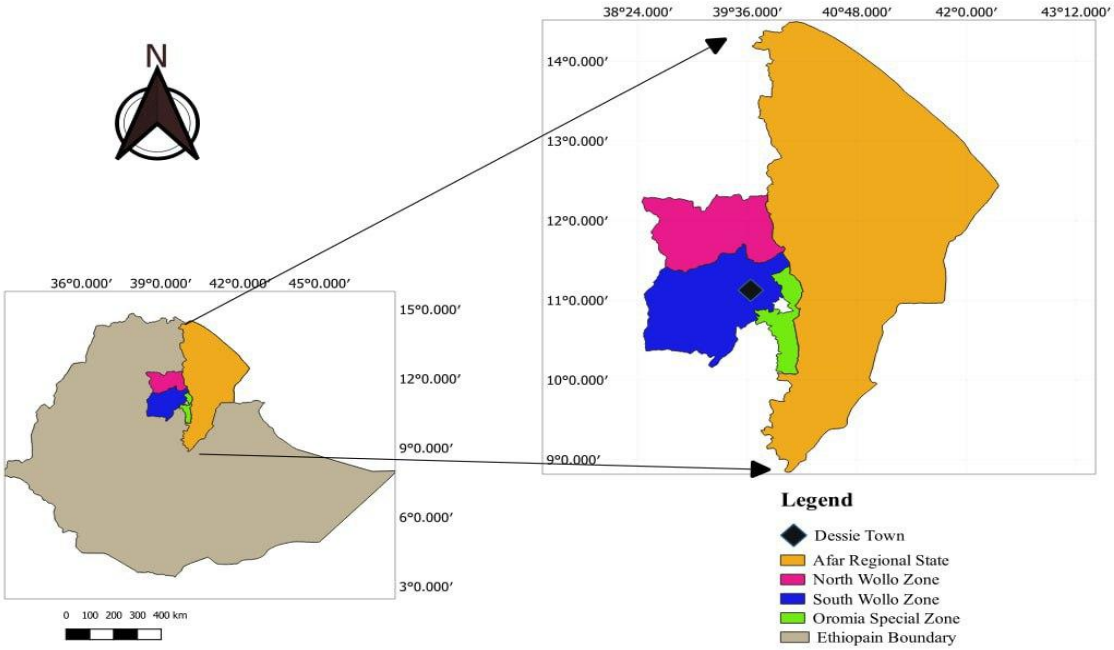


Figure 1: map of study area

3.2. Study Animals

The study animals were the sheep and goat brought for slaughter at Dessie municipal abattoirs. A total of 420 small ruminants (302 sheep and 118 goats) were included in the study. Animals brought to the abattoir were originated from the Oromiya Special Zone, South Wollo, and North Wollo and Afar regions. All the sheep and goats brought to the abattoir were males, indigenous breeds raised for meat production and managed under extensive production system either as part of a mixed crop–livestock production system or a pastoral system of production. Sheep and goats are usually kept mixed with other livestock species (cattle, camel, dog and donkeys) in communal grazing and watering areas.

3.4. Study Design, Sample Size Determination and Sampling Methods

A cross-sectional study design was conducted from December 2022 and May 2023. The total number of sheep and goat required for this study was calculated based on the formula given by Thrusfield (2005). By rule of thumb where there was no previous information regarding total pulmonary lesions in the study area 50% expected prevalence with 5% desired level of precision and 95% of confidence interval was considered.

$$N = (1.962 * Pexp(1-Pexp))/D2$$

Where;
N= Sample size
Pexp= expected prevalence
d= desired absolute precision

By substituting these values in the formula, 384 sheep and goat were included. But 420 sheep and goat were selected to increase the precision of the estimated prevalence of the lesions.

A Systematic random sampling method was implemented to collect the required sample for major pathological lesion characterization, and particularly sample from pneumonic lesion was

emphasized for isolation and identification of bacteria and parasite. Regular visits were made to the abattoir four days per week. Species, age and body condition score (BCS) of the study animals were meticulously recorded and considered as variable of interest. The body condition score of animals were grouped according to Yami *et al.* (2008). Accordingly, animals were grouped into medium (score 2 and 3) and good (score 4 and 5) (Annex I). In addition estimation of age was carried out by dentition according to Getenby (1996), subsequently, animals were grouped to <1years, 1-3 years and >3 years age group. Sheep and goat lungs were grossly inspected and having lesions were purposefully chosen for detailed histopathological investigation and pneumonic lesions were subjected to bacteriological and parasitological analysis.

3.5. **Sample Collection, Transportation and Storage**

A representative sample of lung tissues for each lesion types, including the active portion of the lesions and its surrounding normal tissue were cut into pieces of 2-3 cm in size and placed in a bottle containing 10% Neutral Buffered Formalin. Cotton was added to the top of the container containing the sample to prevent flotation and stored at room temperature for 24hrs. Then, Neutral Buffered Formalin was replaced and sample was stored at room temperature until process. Consequently, Sample was transported to Animal Health Institute (AHI), Sebeta for histopathological investigation. Lung samples exhibiting pneumonic lesion were aseptically collected, and then transported on cold chain to Wollo University School of Veterinary Medicine Microbiology Laboratory for bacterial isolation examination. After collection and transportation to the laboratory, the samples were processed immediately. For parasitological investigation, the lung was dissected for purpose of adult parasite collection. Furthermore, the bronchi and bronchioles were checked, and careful gross examination of the whole lung was performed to check parasitic nodules. Visible worms were removed from the dissected lungs and small cut of parasitic nodules was transferred to universal bottle containing 70% alcohol. Then, collected samples were transported to Wollo University School of Veterinary Medicine Parasitology Laboratory. All the samples were labeled properly with permanent pencil; including species, age, body condition and date of sampling.

3.6. Diagnostic Techniques

3.6.1. Gross Examination

Gross pathological examinations on lung were conducted by visualization, palpation, incisions, and dissection of bronchial tree. The lungs were inspected for changes in color and texture and distribution appearance of lesion and consistency. The presence of cyst or parasites, emphysema, pneumonia, atelectasis, and congestion were examined based on their gross characteristics. According to the standards for meat inspection, pathological lesions were distinguished and evaluated during routine gross examination (FAO, 2007). Although different lesions sometimes sat on the same lung only dominant lesion has been considered.

3.6.2. Histopathological Examination

Histopathogical examination of the lung tissue samples were carried out in accordance with the steps outlined in by Talukder (2007) (Annex III). The samples were first trimmed and fixed with by formalin, dehydrated by ascending degraded alcohol and cleaned with xylene. The samples then impregnated with molten paraffin wax, sectioned at 5μm thickness using the microtome, spread on warm water and attaching the tissue to microscopic glass slide. The slides were incubated at 60°C to molten paraffin wax. Hematoxylin and Eosin (H and E) stain was used to stain the sectioned tissue after it had been deparaffinized in three changes of xylene and rehydrated in decreasing graded alcohol. Stained slides were mounted by Distrene plasticizer/ Dibutyl phthalate xylene (DPX) and finally examined under microscope.

3.6.3. Parasitological Examination

Lung worm: The recovered parasites were kept in 70% alcohol and were transferred on to petridish and were examined under the stereomicroscope. The nodule formed by parasite were trimmed off and worms extracted from the tissue by gentle compressed of nodule between two glass slides, and incising the nodules and then carefully teasing the worm out of the tissue by scraping with blunt object and examined under light microscope. Identification were made

according to morphological characteristics of parasites (Chilton *et al*., 2006) (Annex IV).

Hydatid cyst: Grossly, lung was examined for degeneration, and calcification. The collected cysts were carefully incised by scalpel and scissors, and the contents were transferred into a sterile container. Then, the smear prepared from suspension was examined microscopically for the presence of protoscolices, in Hydatid fluid so as to classify cysts as fertile or infertile. Cysts which contained no protoscolices as well as heavily suppurative or calcified ones were considered unfertile. Fertile cysts (Fluid filled cyst with protoscolices) were also further subjected for viability test and classified as dead or alive. The viability of protoscolices was assessed by morphology, motility and presence of flame cells activity like peristaltic movement and when necessary, a drop of 0.1% aqueous eosin solution was added, and examined under a light microscope (living protoscolices did not take the dye, while dead one did). Then microscopic examination of the cyst fluid was conducted to look its characteristics (Abegaz and Mohammed, 2018) (Annex V).

3.6.4. Bacterial Isolation and Identification

Lung samples were aseptically collected and placed in sterile plastic bags within an ice box and were submitted to Wollo University Microbiological laboratory. The outer surface of the pneumonic lungs was first seared with a heated spatula followed by cutting and mincing of the inner surface of the lungs using sterile scissors and forceps. The cut inner surface of the lungs then inoculated into the tryptone soya broth, incubated at 37°C for 24 hours and growth were evaluated by turbidity. loopful of the broth culture was taken by agitating cultured broth sample wisely to aid mixing and streaked over an identified Petri dish containing blood agar base supplemented with 5% sterile sheep blood and immediately incubated aerobically at 37°C for 24 hrs. Colonies from the culture were subjected to Gram's staining to study staining reactions and cellular morphology under light microscope. Gram-negative, coccobacilli bacteria were again sub cultured with due care, on both blood and MacConkey agar plates and Gram-positive bacteria colony sub cultured to blood agar and manitol salt agar at 37°C for 24-48 hour to get pure cultures for further analysis. The pure cultures of single colony type from pure cultures on blood agar were transferred to nutrient agar for a series of primary and secondary biochemical

tests. Primary tests such as Grams staining, motility, catalase, oxidase and oxidative-fermentative (O-F) tests and secondary test; coagulase, Indole, methyl red, MR-VP test, TSI, citrate utilization tests and urease tests were conducted. Isolation and identification of Gram positive and Gram negative aerobic bacteria were performed as described by Quinn *et al*. (2011) (Annex VI). All bacteriological procedures were conducted in a level two biological safety cabinet.

3.7. Data Management and Analysis

Prevalence of pulmonary lesion was calculated as a percentage of the population screened (Thrusfield, 2005). Although different lesions sometimes sat on the same lung only dominant lesion has been considered. The data was entered, coded and scored in Microsoft Excel worksheet (Microsoft Corporation) and STATA version 14 was used for descriptive analysis of frequency and percentages of obtained results. The significant of difference of prevalence between different age groups, species and body conditions was determined using χ^2 test. The differences were regarded significant if p-value is <0.05. Data obtained were summarized as gross, histopathological lesions and bacterial and parasitic agents observed were presented as tables showing frequencies of isolation.

3.8. Ethical Clearance

The study considered direct observation of slaughter animals in the abattoir and took appropriate samples for further laboratory experiments and there are no procedures in the study that suffers animals/against animal welfare. Ethical approval was conducted by research ethical approval committee (VM/ERC/03/19/03/15/2023) of Addis Ababa University, College of Veterinary Medicine and Agriculture, Bishoftu, Ethiopia (Annex XV).

4. RESULTS

4.1. Prevalence of pulmonary Lesions

In present investigation 420 (319 sheep and 101 goat) were observed and examined. The overall abattoir prevalence of pulmonary lesion in sheep and goat was 328(78.1%). Pulmonary lesions were studied in relation to the species, age and body condition of animals. From 319 sheep and 101 examined goats, 265 (83.1%) sheep and 63 (62.4%) goats were showed gross lesion respectively.

In the current study nine different types of pulmonary lesions were observed based on gross characterstics and lobular distribution which are pneumonia 233 (55.5%), hydatidosis 18 (4.3%), Emphysema 30 (7.1%), pulmonary atelectasis 14 (3.33%), pulmonary congestion10 (2.83%), Pulmonary hemorrhage 13 (3.1%), Bronchitis and bronchiolitis 3 (0.71%), Melanosis 4 (0.95%) and Adhesion 3 (0.71%). Different lung lesions were studied in relation to the species, age and body condition of animals.

Pneumonia were stastically significant ($p<0.05$) pulmonary lesion with the species group of animals. Pneumonia, hydatidosis, and pulmonary emphysema were significantly different ($p<0.05$) between animal age groups, however they were not significantly ($p>0.05$) related to the animals' physical condition. All factors; species, age, and body condition of the animals, were not significantly ($p>0.05$) associated with pulmonary congestion, pulmonary haemorrhage, Bronchoectasis, adhesion, and Melanosis (Table1-3). Pneumonia, hydatidosis, pulmonary congestion and pulmonary hemorrhage were statically significant association with the lobe distribution of the lesion (Table 5).

Table 1: Prevalence of different gross pathological lesions in the lungs of sheep and goat examined at Dessie municipal abattoir.

gross lesion	Total prevalence	Ovine (n=319)	Caprine (n=101)	X^2	p-value
Pneumonia	233(55.5%)	191 (59.9%)	42 (41.6%)	10.3899	0.001*
Hydatid cyst	18 (4.3%)	15 (4.7%)	3 (3%)	0.5609	0.45
Emphysema	30 (7.1%)	24 (7.5%)	6 (5.9%)	0.289	0.59
Atelectasis	14 (3.3%)	9 (2.9%)	5 (5.1%)	1.079	0.299
Congestion	10 (2.4%)	7 (2.2%)	3 (3%)	0.198	0.656
Hemorrhage	13 (3.1%)	10 (3.1%)	3 (3%)	0.067	0.934
Bronchoectasis	3 (0.7%)	3 (0.9%)	0	1.18	0.27
Melanosis	4 (0.9%)	3 (0.9%)	1 (1%)	0.002	0.964
Adhesion	3 (0.7%)	3 (0.9%)	0	0.959	0.328
	328(78.1%)	**265(83.1%)**	**63(62.4%)**		

**stastically significance*

Table 2: Prevalence of gross pathological lesions in the lungs of sheep and goat with their perspective body condition examined at Dessie abattoir.

Lung lesions	Body condition				X^2	p-value
	Good (n=232)		Moderate(n=188)			
Pneumonia	118	50.8%	115	61.2%	4.467	0.035
Hydatid cyst	14	6%	4	2.1%	3.34	0.06
Emphysema	16	6.9%	14	7.4%	0.16	0.68
Atelectasis	10	4.3%	4	2.1%	1.24	0.2
Congestion	4	1.7%	6	3.2%	1.19	0.27
Hemorrhage	7	3%	6	3.2%	0.051	0.82
Bronchoectasis	2	0.8%	1	0.5%	0.117	0.73
Melanosis	3	1.3%	1	0.5%	0.117	0.732
Adhesion	2	0.8%	1	0.5%	0.539	0.55
Total	**176**	**75.8%**	**152**	**80.8%**		

Table 3: Prevalence of different gross pathological lesions in the lungs of sheep and goat with their perspective age examined at Dessie abattoir.

Lesions	**<1years (n=82)**		**1-3years (n=142)**		**>3 years (n=196)**		**X^2**	**P_value**
Pneumonia	39	47.5%	96	65.6%	98	50%	8.49	0.014*
Hydatid cyst	0	0	10	8.9%	8	4.1%	6.32	0.04*
Emphysema	13	15.8%	8	5.6%	9	4.6%	11.79	0.003
Atelectasis	4	4.9%	7	4.9%	3	1.5%	3.706	0.16
Congestion	2	2.4%	4	2.8%	4	2%	0.241	0.89
Hemorrhage	4	4.8%	6	4.2%	3	1.5%		
Bronchoectas	0	0	0	0	3	1.5%	3.453	0.178
Adhesion	0	0	1	0.7%	2	1%	1.669	0.434
Melanosis	0	0	1	0.7%	3	15%	1.575	0.455
	62	**75.6%**	**133**	**93.7%**	**133**	**67.8%**		

**stastically significance*

4.2. Pathological Pulmonary lesions with Gross and Microscopic Characterization

4.2.1. Pneumonia

Pneumonia was the most prevalent pulmonary lesion in the present study. Based on macroscopic characteristics including distribution, texture, color, and appearance of the affected lungs and microscopic appearance different forms of pneumonic lesions were identified. Verminous pneumonia was most frequently examined 134 (57.5%) followed by broncho pneumonia 61(26.2%).

Broncho pneumonia: Out of 233 pneumonic lesions, 61 were found to have broncho pneumonia, which was accompanied by neutrophilic exudates, cell debris, neutrophils, and macrophages in the alveolar spaces and lumens of the bronchioles and bronchi. In bronchopneumonia the cranioventral portion of the lungs are mainly affected. Based on its gross and histological features, it was categorized as either suppurative or fibrinous.

Suppurative bronchopneumonia: out of total pneumonic lesion 39 (16.7%) cases and 63.9% of bronchopneumonia were found to have suppurative bronchopneumonia. The lesions were red-brown to purple grey and had a firm and mainly involved the cranial lobes and distributed to the cranioventral aspects of the both right and left lungs, especially involving the right lung. The cut surface of the consolidated lobules was moist and purulent exudates leaked from bronchi and bronchioles (Figure 3a) and in some cases, the exudates were mucus and foamy exudates in bronchi and bronchioles (Figure 3b). Microscopically, heavy and severe neutrophilic infiltrations were present into the bronchus, bronchiole, alveoli (Figure 4d). In most case, the inflammatory process confined to the individual lobules and normal alveoli were seen adjacent to the alveoli filled with neutrophilic exudates. In few case presence heavy peribronchial lymphoid hyperplasia and the center of the nodule were less cellular or necrosis of central lymphocyte (Figure 3e).

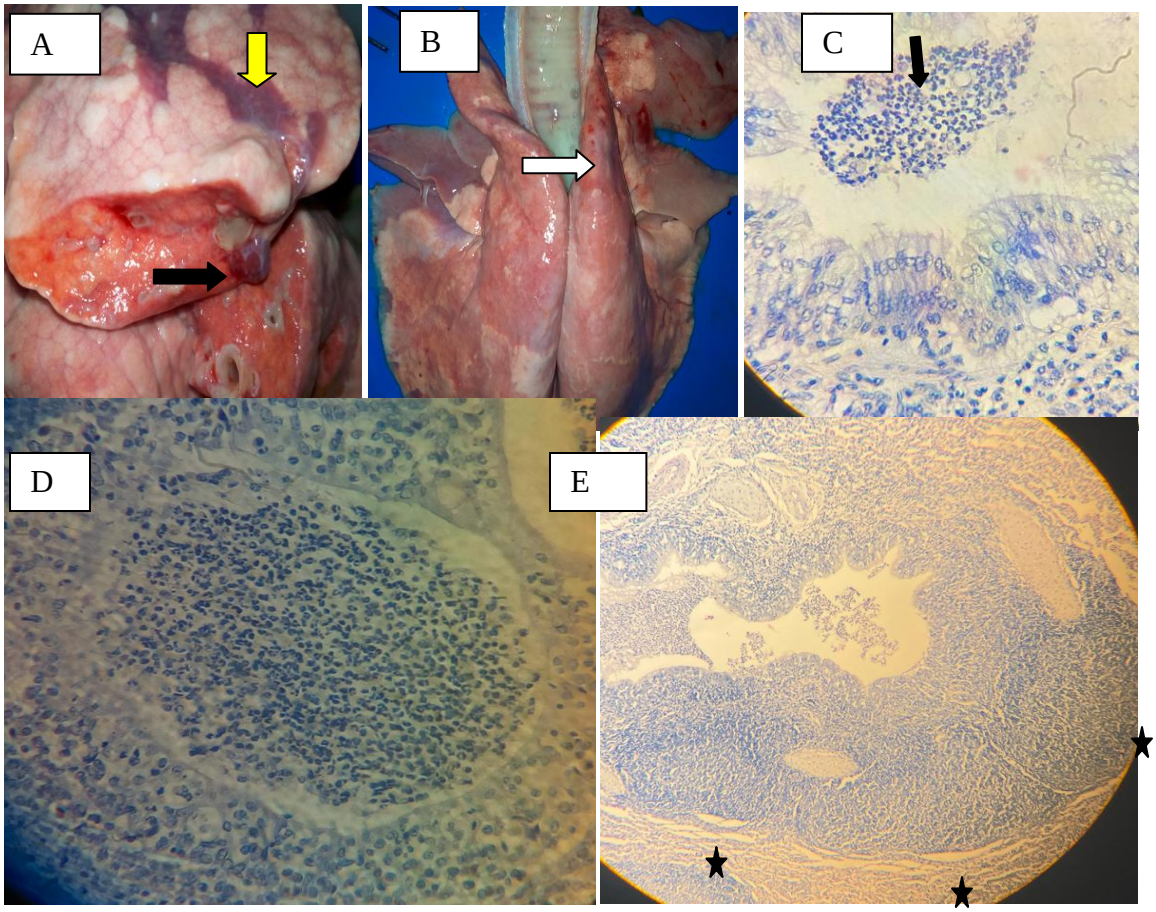


Figure 2: Gross and Microscopic picture of suppurative broncho pneumonia sheep lung. **A**) pus like grayish fluid from cut surface of right apical lobe, *Pasturella multocida* were isolated. **B**) Consolidated area on both cranial lobe and foamy fluid from bronchi. *Pseudomonas* and *Staphylococcus spp* were isolated. **C**) Acute suppurative bronchopneumonia, exudates plugged in the bronchioles dominated by neutrophil **D**) Excess neutrophilic infiltration within the bronchioles. **E**) Chronic suppurative bronchopneumonia with peribronchial lymphoid hyperplasia (asterisks).

Fibrinous bronchopneumonia: Fibrinous bronchopneumonia was examined from 22 pneumonic lungs. The lesions were mainly in the cranial lobes, especially the right lung, and included congestion, oedema and fibrin accumulation in addition to condensation (Figure 4a). A thin layer of fibrin usually covered the pleural surface accompanied with notable marbled appearance of the affected lung parenchyma (Figure 4b).

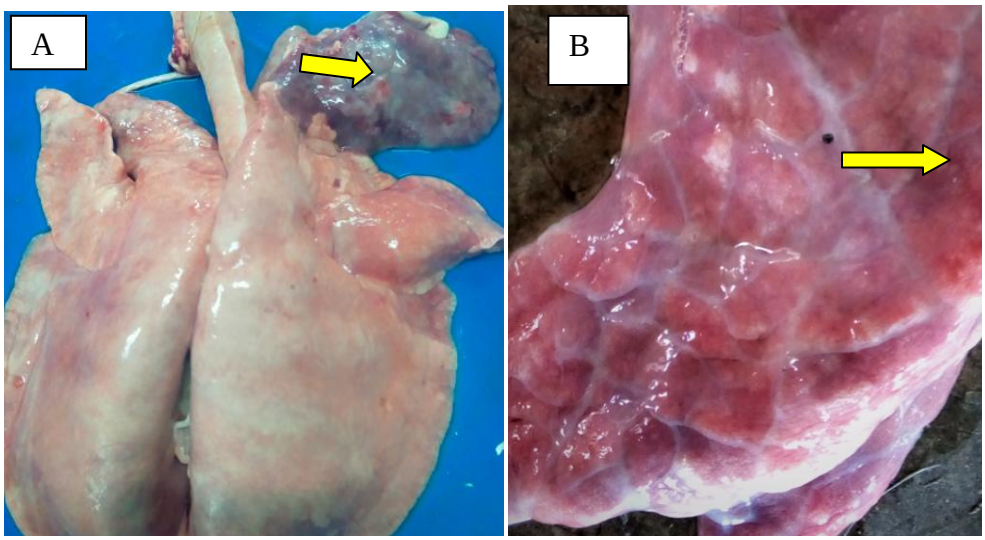


Figure 3: Gross and Microscopic picture of fibrinous bronchopneumonia. **A**) Consolidation of right cranial lobe with fibrin on the plural surface and *M. haemolytica* were isolated. **B**) Marbling appearance of cranial lobe of sheep.

Broncho interstitial pneumonia: the lesions were not remarkable on gross examination. Microscopically, the characteristic features of both suppurative bronchopneumonia and interstitial pneumonia were found admixed (Figure 5a&b). Infiltration of inflammatory cell in the alveoli lumen and bronchiole. Peribronchiolar lymphoid hyperplasia with formation of prominent well-defined lymphoid nodules and excessive plasma cell growth (Figure 5b).

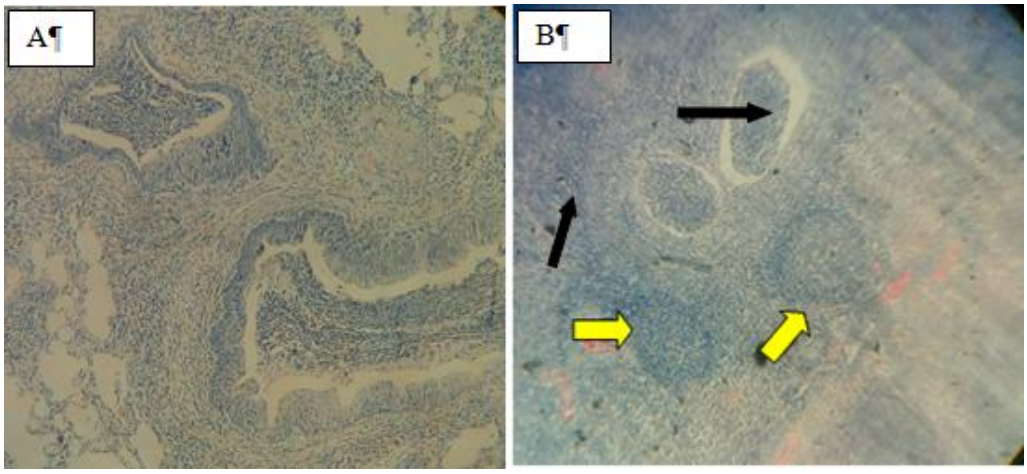
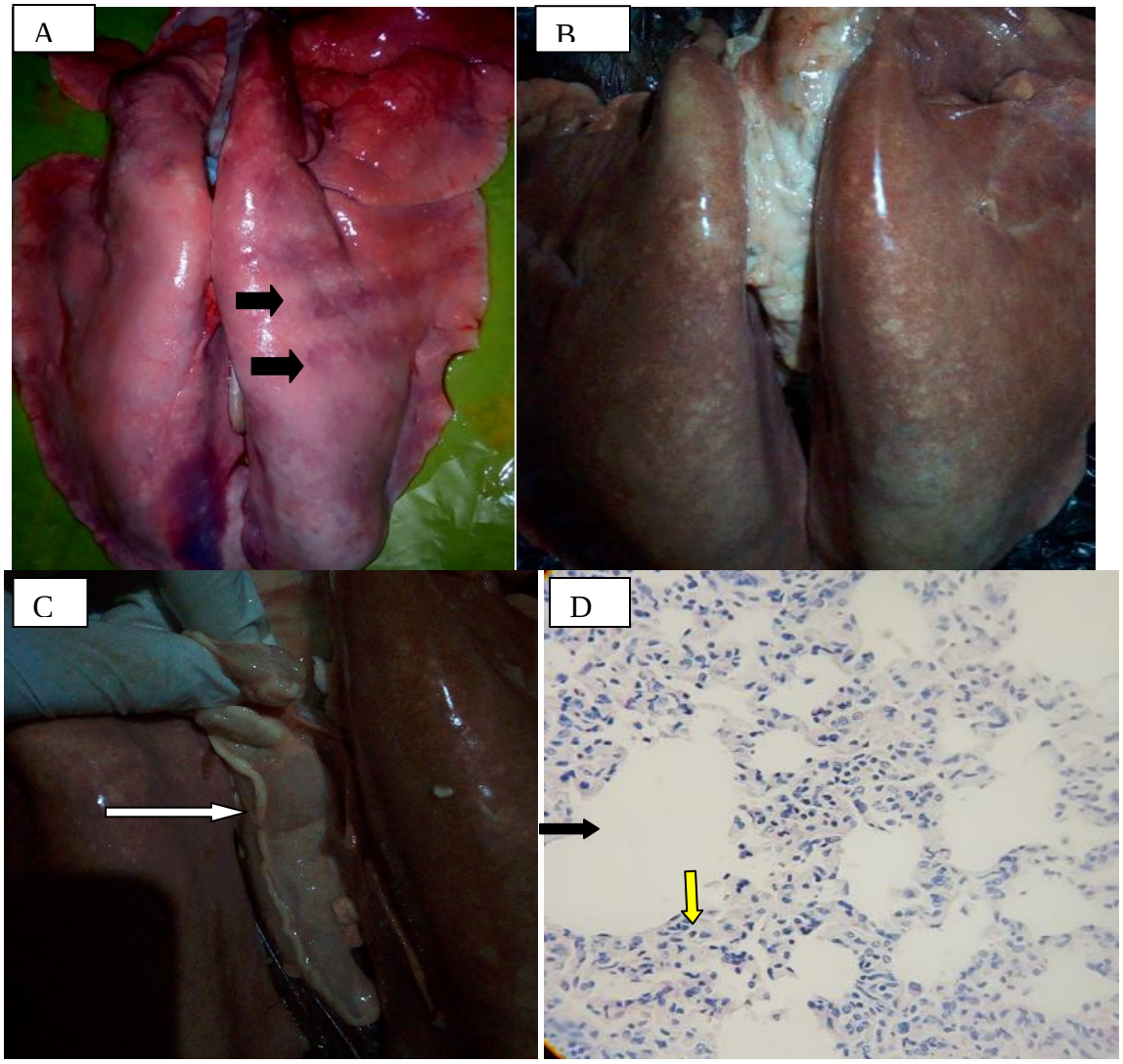


Figure 4: Microscopic picture of broncho interstitial pneumonia. **A**) Thickening of intra alveolar septa with prominent exudates in the alveoli and bronchiole. **B**) Infiltration of inflammatory cell in bronchiolar lumen and intestitium (black arrow), with granule formation (yellow arrows).

Interstitial pneumonia: grossly the lung was enlarged than normal, had a rubbery substance, looked meaty, and did not collapse when pressed (Figure 6b). The lungs appeared more "meaty" when cut. On the pleural surface of the diaphragmatic lobes, rib imprints in caudal lobe were detected (Figure 6a). Consistently, the mediastinal lymph nodes were enlarged (Figure 6c). Mostly lesions distributed throughout lungs and not restricted only in cranial or caudal lobe. Microscopically, they were characterized by lymphocytic infiltration in the interalveolar septa or interstitial tissue. The alveolar spaces and airways lacked exudates (Figure 6e). Alveolar and bronchial epithelial hyperplasia was seen. Numerous cases of interstitial pneumonia were found to have peribronchial and peribronchiolar lymphocyte proliferation, excessive plasma cell, and significant alveolar tissue necrosis.



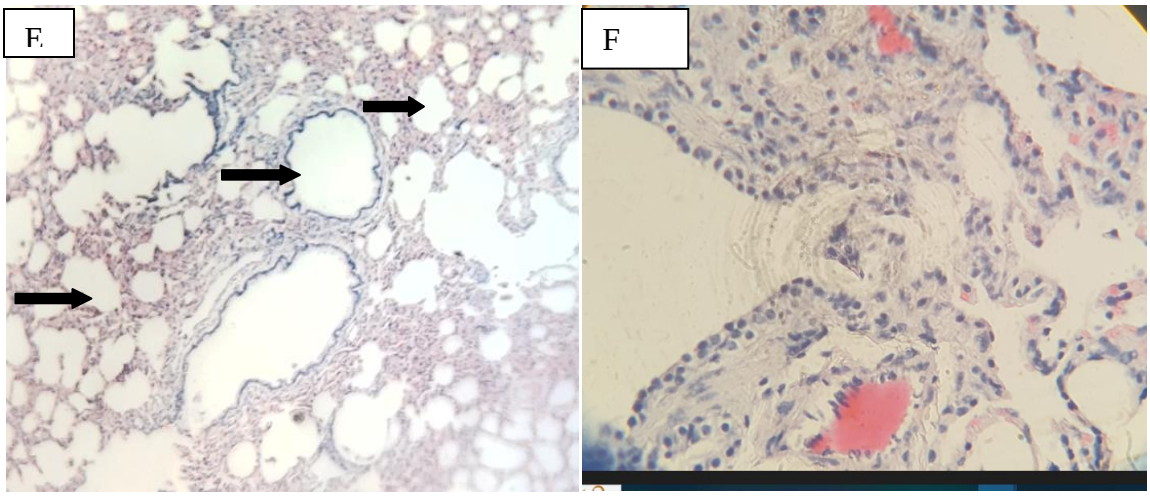


Figure 5: Gross and Microscopic picture of interstitial pneumonia gross and microscopic. **A**) Ribs imprint (arrow). **B**) Enlarged, ruby, meaty appearance and *E. coli* were isolated. **C**) Enlarged Mediastinal lymph nodes. **D**) Thickening of inter-alvelar septa mononuclear infiltration in the interstisium. **E**) Thickening of inter-alvelar septa no exudates on bronchioles. **F)** Thick inter-alvelar septa and engorged blood vessels with red blood cell.

Hemorrhagic interstitial pneumonia: it is type of interstitial pneumonia diagnosed microscopically. Grossly, remarkably red area was seen in the affected lungs. Leukocytic infiltration and RBC were observed in the alveoli and inter-alveolar septa (Figure 7a&b).

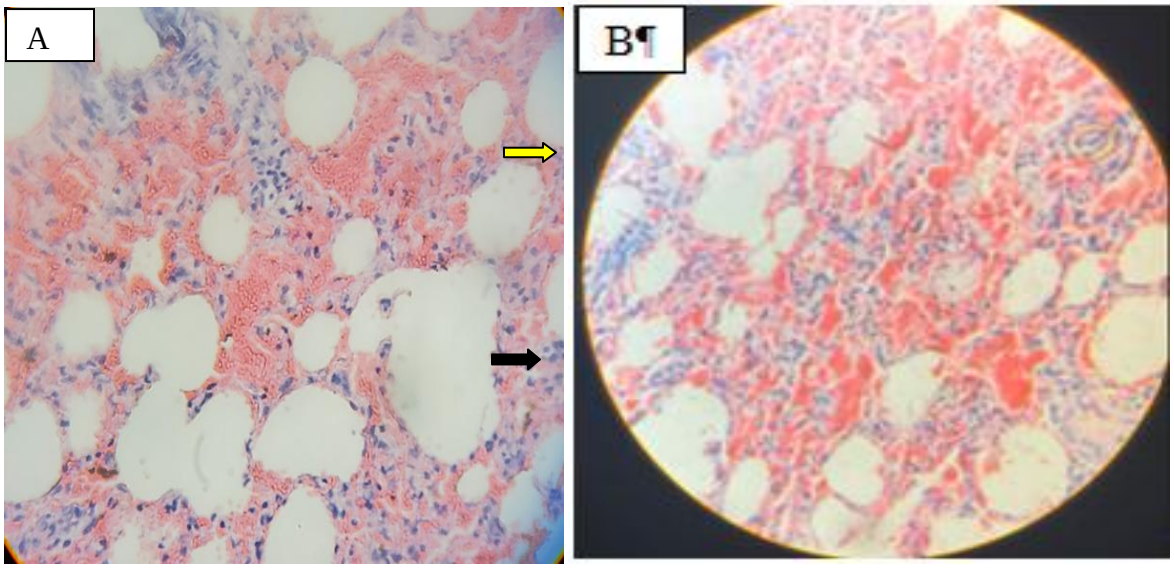


Figure 6: Microscopic picture of hemorrhagic interstitial pneumonia in sheep lung. **A&B**) Large

number of RBC was seen in alveoli and inter-alveolar septa (black arrow), leukocytic infiltration (yellow arrow) and thickening of interlobular septa.

Granulomatous pneumonia: Granulomatous pneumonia was characterized by formation of nodules which were diffusely or a multifocal distribution on the pleural surface. Microscopically, the granulomatous pneumonia was recognized by the presence of cellular granulomatous rim zone of layers consists of various inflammatory cells and few fibrosis in outer layer which were surrounding the central caseous necrotic area). Multifocal and locally extensive large granulomatous inflammatory lesions were observed. The centers of granuloma were made up of dead lymphocyte with nuclear rehexis (Figure 8 b&c). There were also swollen lymphocyte and lack nucleus vacuolated cytoplasm found when distant increase from the center (Figure 8d).

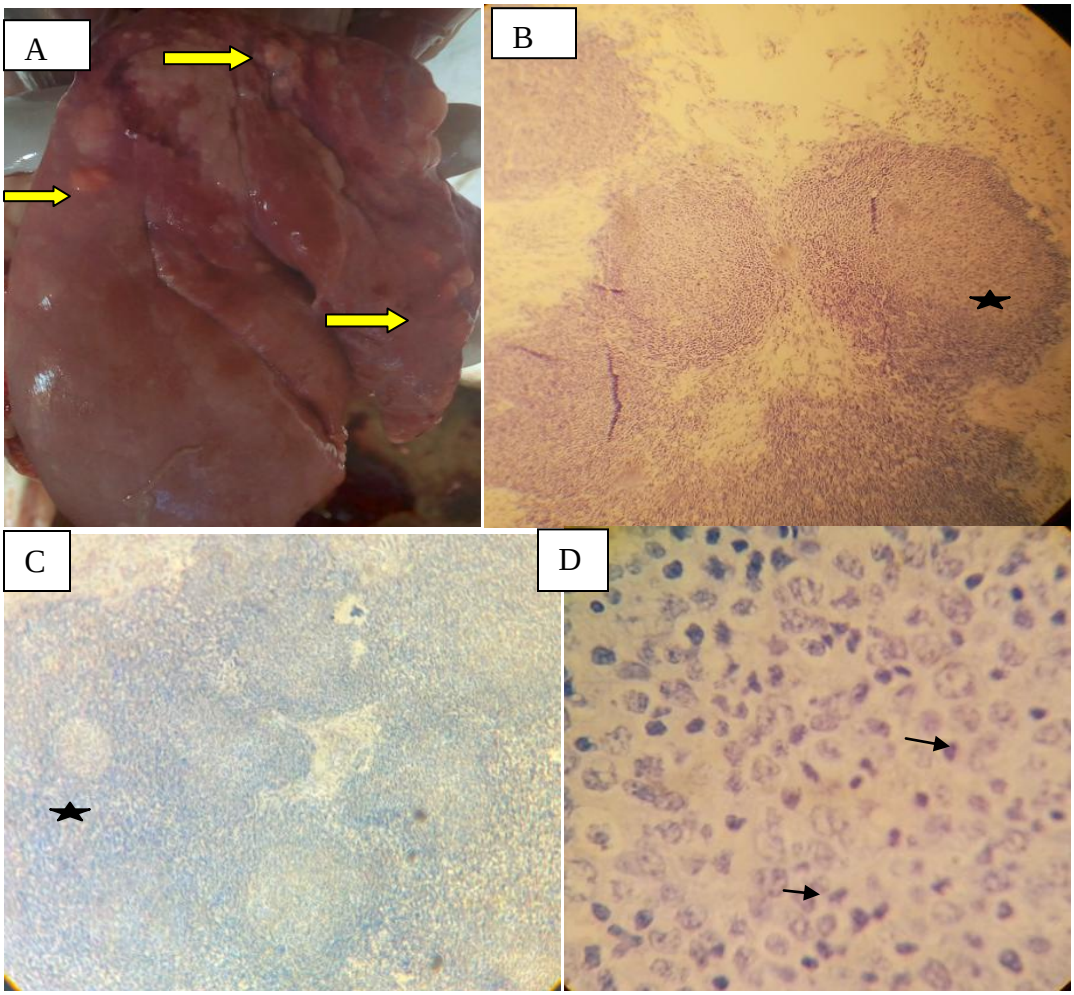


Figure 7: Gross and Microscopic picture of granulomatous pneumonia in sheep lung. **A**)

Multifocal, variable sized nodules diffusely disseminated on the surface of lungs. Granulomatous involves most of the lung lobe (yellow arrow). **B&C**) Granulomatous reaction consists of central area of necrosis (asterisks) surrounded by different inflammatory cells and fibrous connective tissue. **D)** Swollen and lack nucleus vacuolated cytoplasm cell.

Aspirational pneumonia: Grossly, the affected portions of the lungs were congested and somewhat meaty in consistency (Figure 9b). Blood plaque was scattered in sheep and goat lung with aspirated blood with no presence of inflammatory signs (Figure 9a).

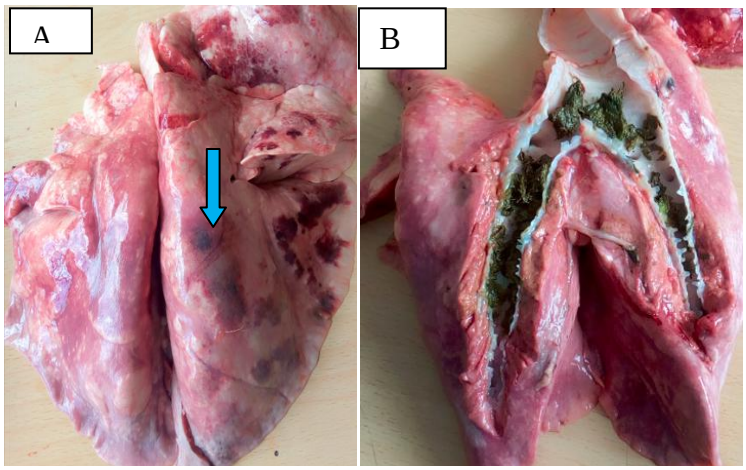
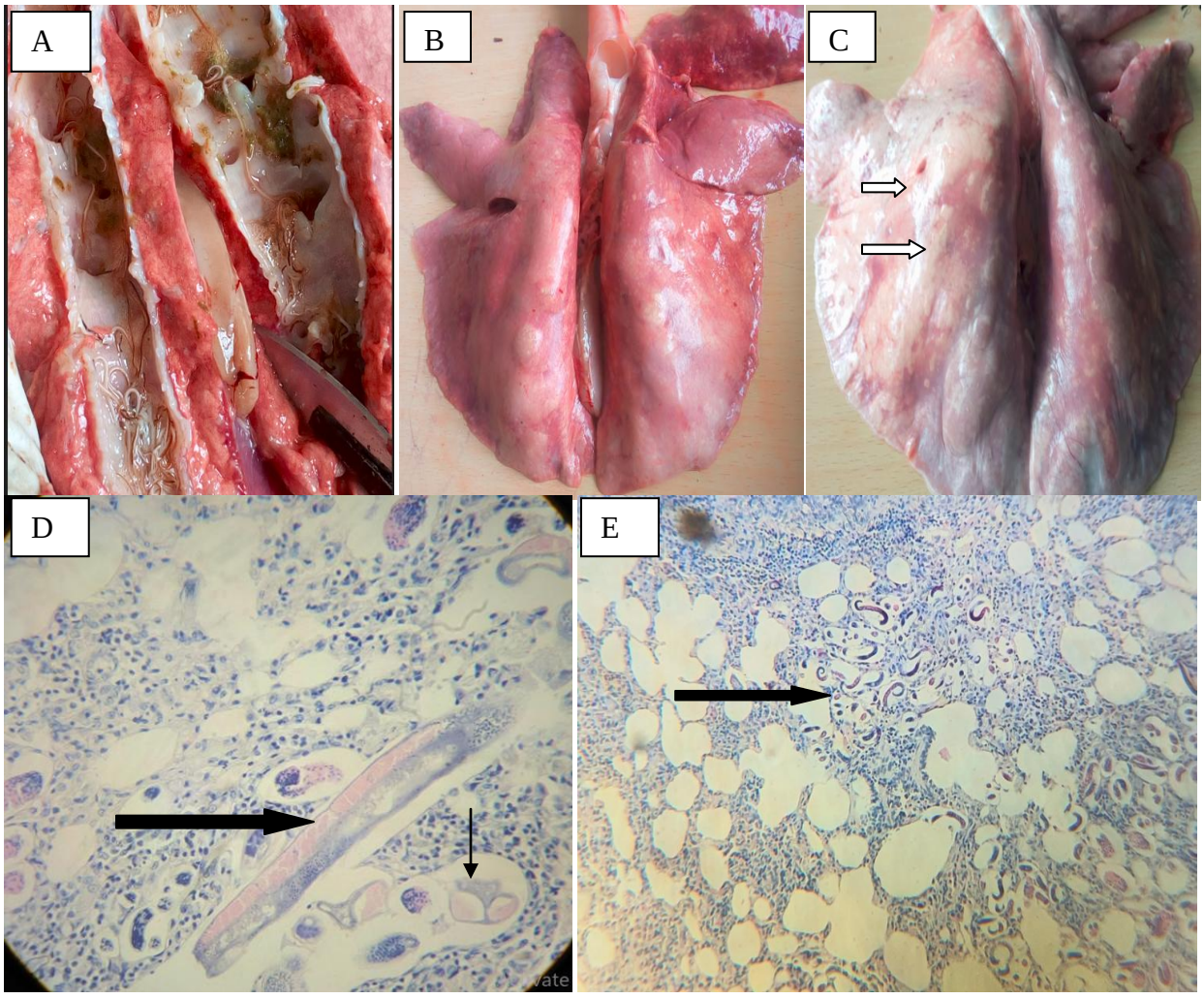


Figure 8: gross and microscopic picture of aspirational pneumonia in sheep lung. **A**) Aspirated blood plaque scattered on right lung particularly caudal lobe. **B**) Aspirated rumen content inside bronchi, consolidation of parenchyma sheep lung.

Verminous pneumonia: The lungs with parasitic pneumonic lesion may reveal raised emphysematous patches, depressed consolidated areas, or dirty white to irregular nodular lesions dispersed throughout the various lobes, particularly in the caudal lobe. There were lot of foamy froth that was blood-tinged and that contained several slender, creamy white worms as well as groups of worms that were often seen in the terminal bronchioles of the caudal lobes (Figure 10a). *Protostrongylus rufescens* infected bronchioles frequently have worms and exudates covering them (Figure 10b). The diaphragmatic lobes of infected lungs with *Mullerius capillaries* feature red, grey, or green nodules that vary in size and consistency. In some instances, they did not develop into nodules and instead appeared as hard, grey to black patches

that covered a significant portion of the lung surfaces. Verminous pneumonia was normally distributed mostly on the dorsal part of the caudal lobes of the lungs, with the disease occasionally extending onto the dorsal half of the cranial lobe (Figure 10c).

Microscopically, Cross section of a lung worm in the lumen of a bronchus, rupture of the alveolar walls, infiltration of neutrophils, macrophages, and giant cells, as well as eosinophils, the most noticeable inflammatory cell (Figure 10). Around the adult worms, eggs, and larvae of the parasites, particular alterations in the lung parenchyma and interstitium are demonstrated to progress. Multifocal granule with cross section of parasite cuticle at the center and inflammatory cell forming the next layer, presence of lymphocyte, plasma cell and giant cell (Figure 10g). Peribronchiolar lymphoid hyperplasia was observed.



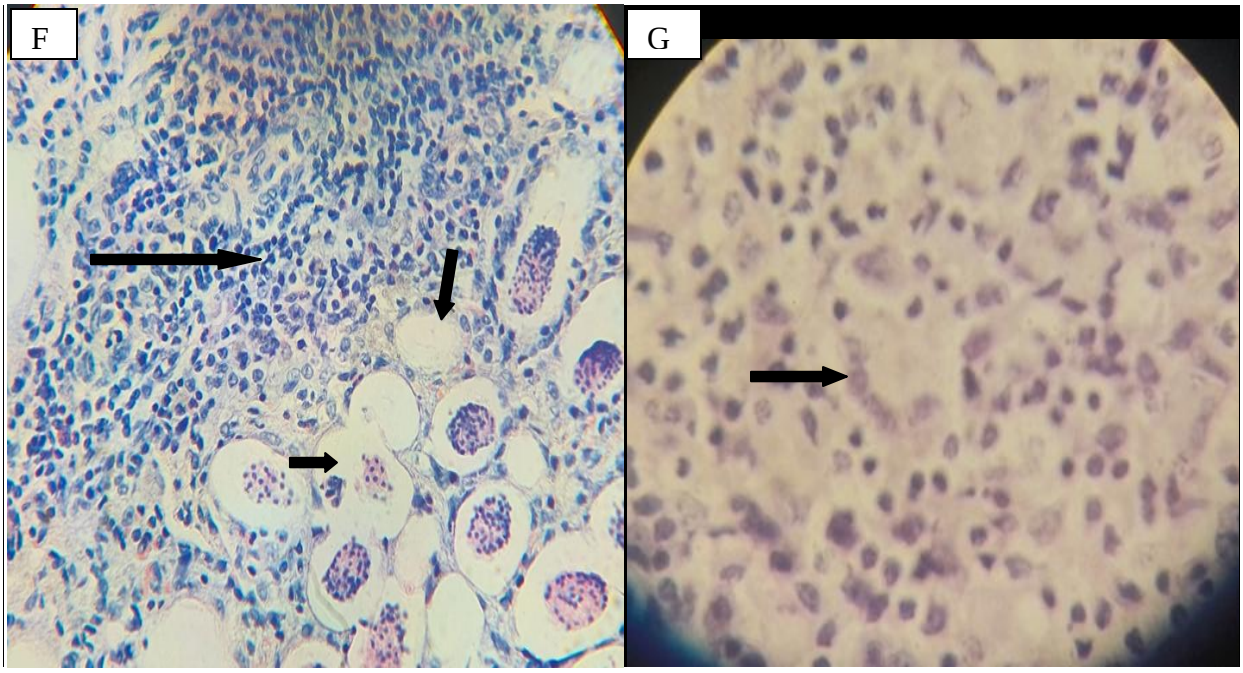


Figure 9: Gross and Microscopic picture of verminous pneumonia. Verminous pneumonia of sheep lung with nodule and *Protostrongylus rufescens* isolated. **A**) Mixed infection of adult *Dictyocaulus filaria* and *Protostrongylus rufescens* discovered on bronchi and bronchioles. **B**) *Protostrongylus rufescence* nodules on both right and left caudal lobe lung parenchyma. **C**) Grayish nodule of *Mulerius capillaries*, consolidated and emphysematous patches area on surface of caudal lobe. **D**) Eggs and larvae in various stages of development (arrow) and cut section of adult parasite (arrows). **E**) More prominent are the nodular accumulations of larva, accompanied by lymphocytic or granulomatous inflammation. **F**) Infiltration of mononuclear cells and eosinophils (large arrow) with presence egg parasite in the dilated alveolar lumen (small arrow) and thinking of interalveolar septa. **G**) Inflammatory cell including giant cell (arrow).

4.2.2. Hydatidosis

The lungs revealed single to multiple Hydatid cysts of varying sizes. These cysts were either confined mainly diaphragmatic lobe or to all over the lobes of the lung. The cysts were soft to touch and filled with clear translucent to slightly turbid fluid contents (Figure 11a). However, some cysts were appearing firm and contained inspissated contents (Figure 11c) and some were

calcified, gritty and hard to cut. The cysts were either fully embedded in the lung parenchyma (Figure 11b) or were partially embedded when they were visible from the lung surface. On aspiration of fluid, the cyst collapsed (Figure 11a arrow) and the cyst membrane, appearing creamy white, could be easily removed from the organ and its fluid contents were found clear to slightly turbid or semi-solid material (Figure 11b).

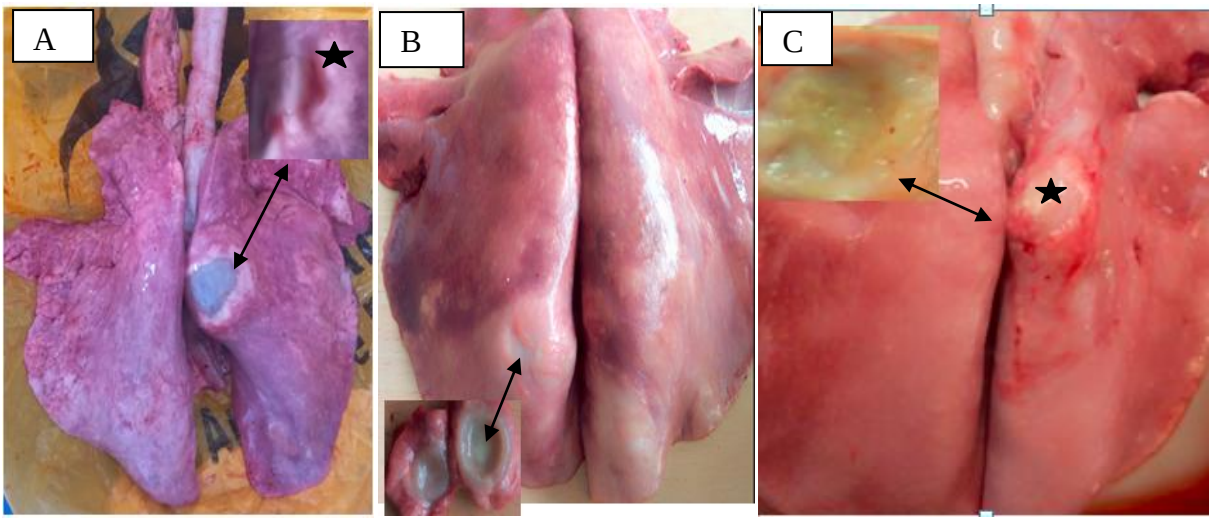


Figure 10: Gross and Microscopic picture cyst from of sheep and goat lung. **A**) Fertile cyst with fine fluid in goat lung, after fluid removed (*). **B**) Fully embedded sterile cyst in both right and left caudal lobe of sheep lung with semi solid fluid. **C**) Calcified cyst in left lung of goat lung(*).

4.2.3. Emphysema

The emphysematous lungs were large, dry, pale in colour, pearl-like shiny lesions with puffy and crepitate feel upon palpation and easily compressed by finger (Figure 12a). Sharply defined foci of pale and enlarged emphysematous areas involving one or more lobes of the lungs were observed, and they appeared to be slightly projecting from the surrounding areas (Figure 12b). The texture of these lungs was notably crepitous due to the accumulation of air in the pulmonary parenchyma. Microscopically, sections of lung revealed distended alveoli, ruptured inter-alveolar septa forming giant alveoli or bullae and the alveolar wall atrophic and very thin (Figure 12c&d).

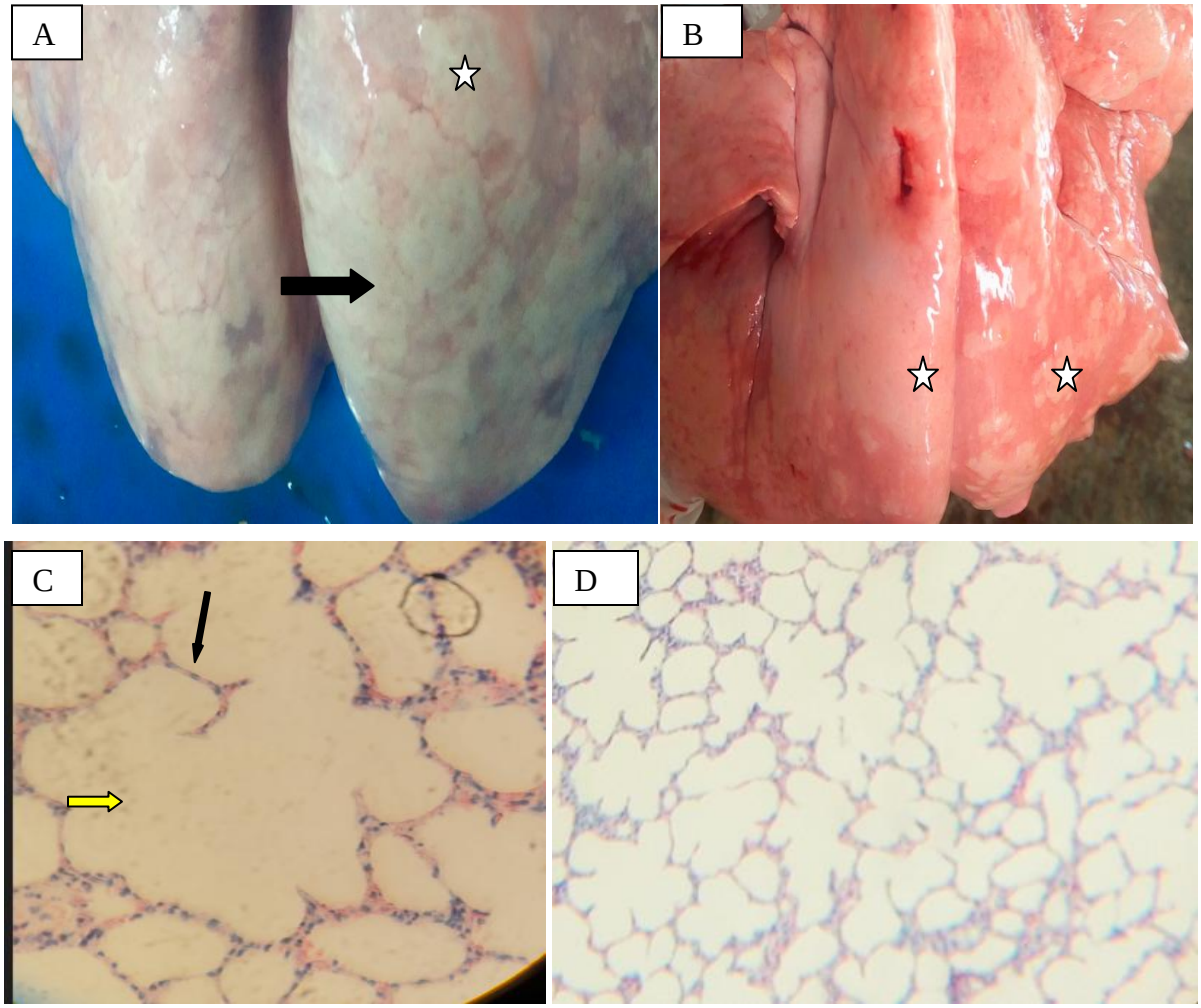


Figure 11: Gross and Microscopic picture of emphysematous in sheep lung. **A).** grossly enlarged emphysematous lung. **B)** Grossly multifocal emphysematous area on lung (asterisks). **C)** Enlarged alveoli space (yellow arrow), accompanied by destruction of alveolar walls (black arrow). **D**) Emphysematous lung at low magnification.

4.2.4. *Atelectasis*

Lungs were homogenously dark-red and depressed below the surface with firm texture in one or more lobes of the lungs, and the texture was fleshy or firmer and non-spongy (Figure 13) The cut sank into the water after the cut surface showed a smooth, dry surface. Microscopically, atelectasis areas revealed collapsed alveoli with the narrow lumen; alveolar walls appear parallel and close together and with emphysematous foci in the adjacent areas (Figure 13).

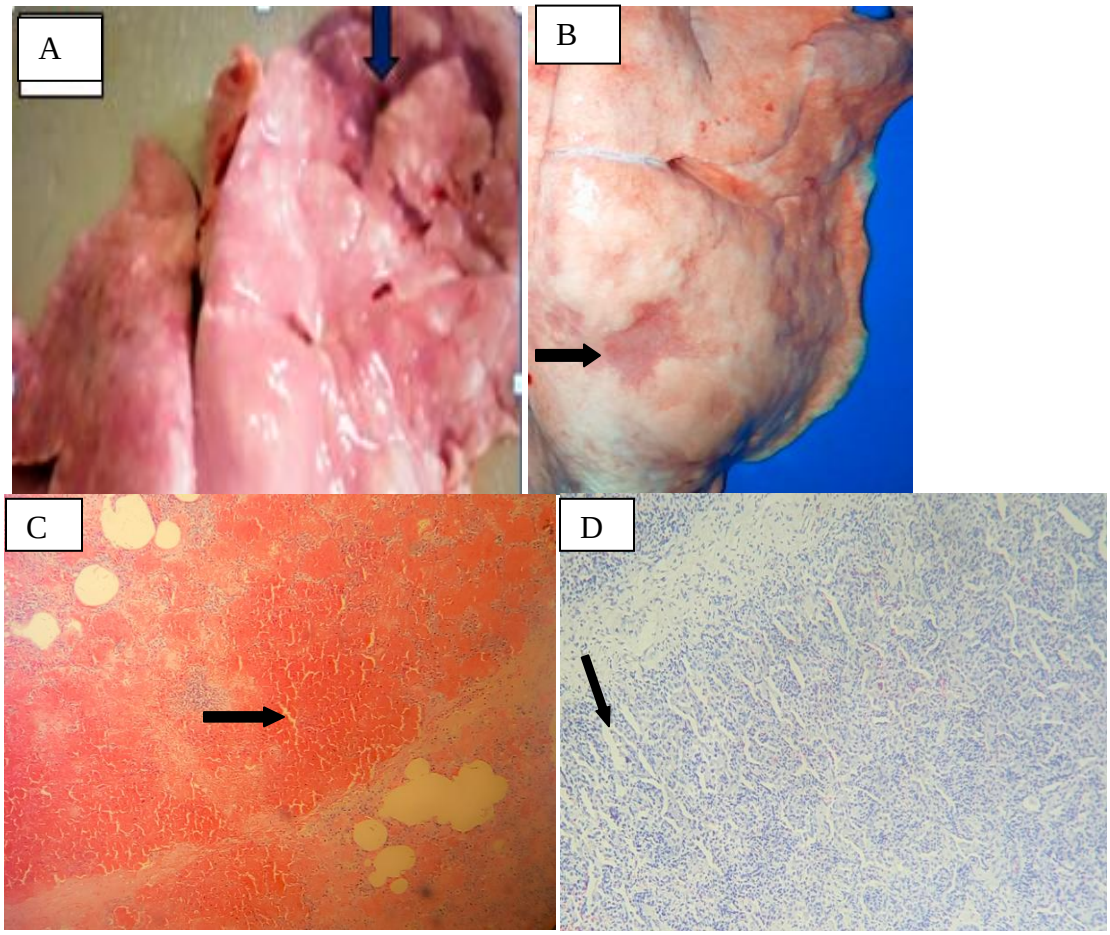


Figure 12: Gross and Microscopic picture of atelectasis in goat lung. **A**) Depressed area in the apical lobe. **B**) Depressed area in the caudal lobe. **C**) Alveolar wall lying in close apposition with slit like residual Lumina and having sharp angular ends. **D**) Massive infiltration of inflammatory cell in the interstitial and alveolar walls closes apposition and appears parallel.

4.2.5. *Pulmonary Congestion*

Pulmonary congestion was distributed into all lobes of the lung and in animals with good body condition. Microscopically, blood vessels were engorged and filled with blood (Figure 14).

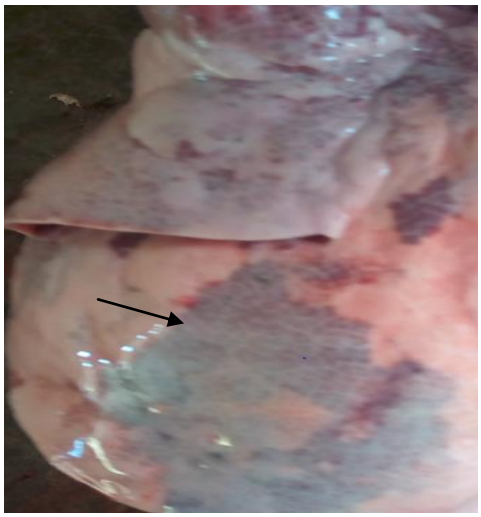
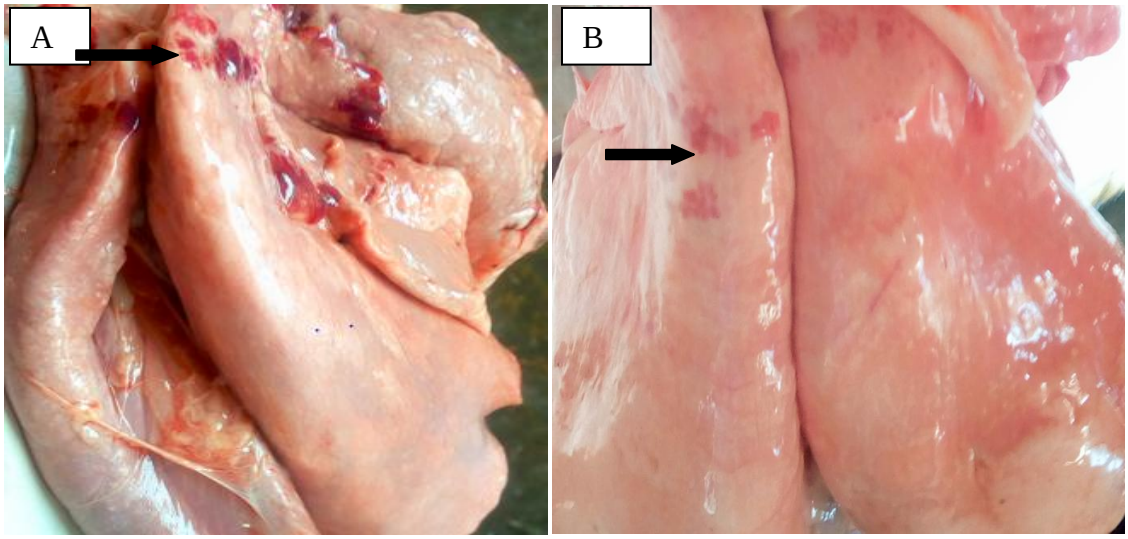


Figure 13: Gross and Microscopic picture of congestion sheep lung. Presence of a large brown color on plural surface.

4.2.6. *Pulmonary Hemorrhage*

The lungs showed dark red patches on the plura surfaces (figure 15). Microscopically, the presences of a large number of RBC out of the blood vessels were prominent. When seen under a microscope, it was clear that there were several RBCs present outside of the blood arteries.



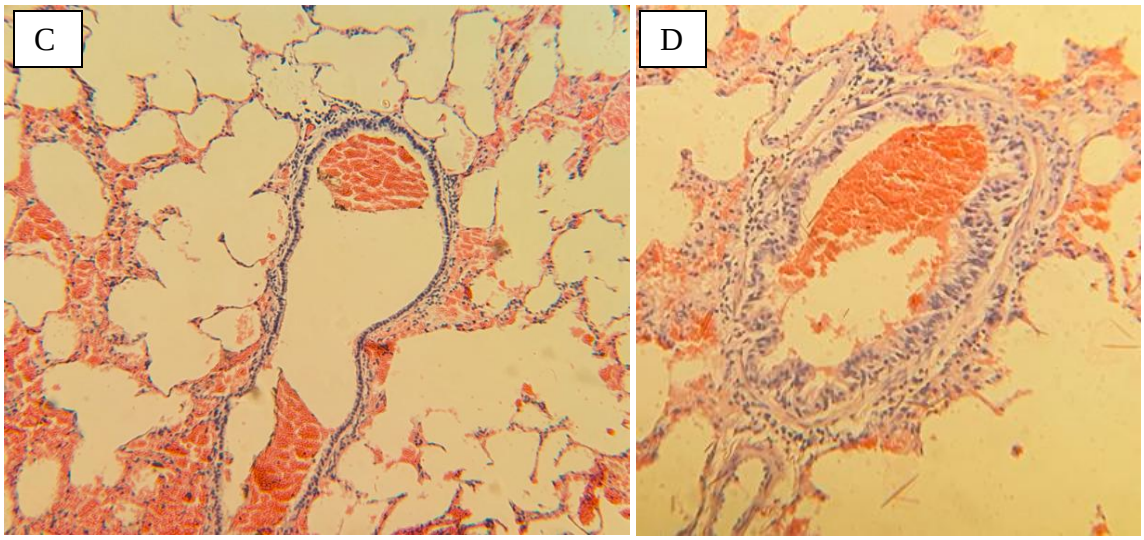


Figure 14: Gross and Microscopic picture of congestion and hemorrhage sheep lung. **A**) Dark red patches on the pulmonary surfaces dorsocranial lobe. **B**) Red patches on the pulmonary surfaces dorsocranial lobe (black arrow). **C**) Presence of a large number of RBC outside the blood vessel in the alveolar lumen or interstitial. **D**) Bronchiole filled with erythrocyte.

4.2.7. *Bronchitis and Bronchoelitis*

The lungs showed prominence of the pleural surface and a grayish-white discoloration. The cut surfaces of dilated bronchi and bronchiole are filled with abundant amount of mucous exudates (Figure 16). Microscopically, characterized by hyperplasia of bronchiole and desquamation of lining epithelium.

4.2.8. *Adhesion (pleuritis)*

The pleura were inflamed, and fibrinous adhesions the lung surface and parietal pleura which lining coastal or diaphragm (Figure 17).

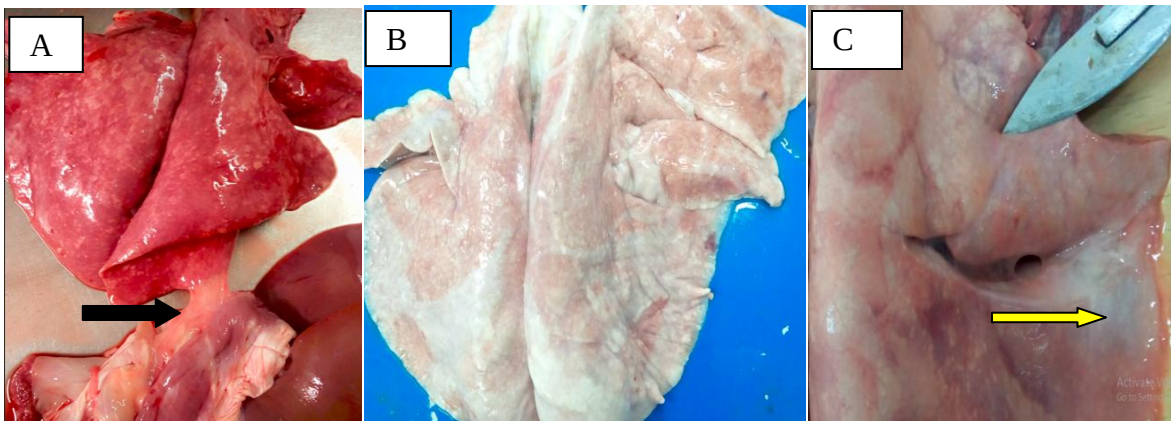


Figure 15: Gross picture of pleuritis/adhesion goat and sheep lung. A) Goat lung adhesion to thoracic cavity (diaphragm) of goat lung. B) Fibrin loosely adheres with the pleura surface and cloudiness in pleura of affected lungs. C) Adhesion between lobes (arrow) sheep lung.

4.2.9. *Melanosis*

Grossly, the lungs were usually speckled with fine sub pleural black foci. Microscopically, fine black granules were observed within the lung parenchyma and alveolar (Figure 18).

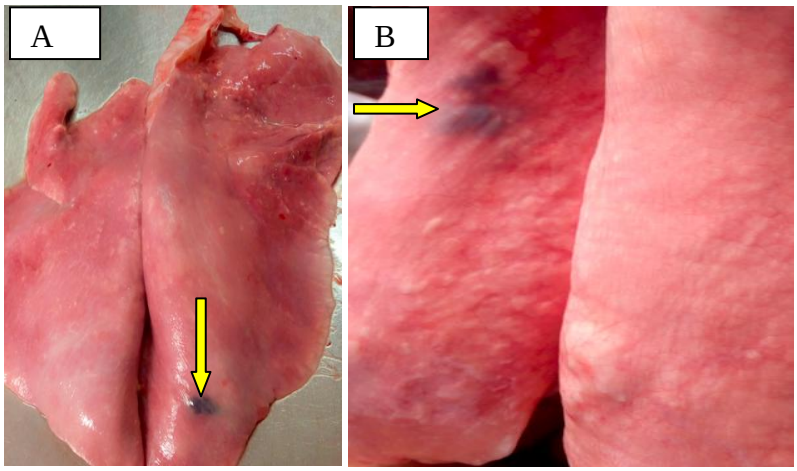


Figure 16: Gross picture of Melanosis in sheep and goat lung. **A**) Black area on right caudal lobe of goat lung. **B**) Black area in the left lung parenchyma of sheep.

Table 4: Lobular distribution of different pulmonary lesion.

Gross lesion	RCC	CrL	RM	RCdL	LCdL	CdL	Diffuse	p- value
Pneumonia	20	5	4	24	7	39	9	0.04
Hydatid cyst	-	-	-	10	2	6	-	0.027
Emphysema	4	2	1	3	1	14	5	0.26
Atelectasis	3	1	-	-	4	4	-	0.28
Congestion	-	-	-	6	2	-	2	0.046
Hemorrhage	-	2	7	-	4	-	-	0.06
Bronchoectasis	-	-	-	-	-	1	2	0.217
Adhesion	1	-	-	1	-	-	1	0.79
Melanosis	-	-	-	1	2	1	-	0.301

RCC: right crania cranial, CrL: cranial lobe (both right and left), RM: right middle, RCdL: right caudal lobe, LCdL: left caudal lobe, CdL: caudal lobe, diffuse: distributed more than two lobe in multifocal or diffuse feature.

4.3. Parasite Identification and Examination

4.3.1. *Lung worm identification and examination*

The three lung worms (*Dictyocaulus filarial, Protostrongylus rufescens,* and *Mullerius capillary*) were detected in verminous pneumonia**.** Out of 134 possitive lung worm infection during postmortem *Dictyocaulus filarial* (48%) is the most predominant lungworm species, followed by *Mulleries capillaries* (23%) and *Protostrongylus refescens* (17.2%) is the least frequent and mixed infection 16(11.8%) either with two or three (Figure 19).

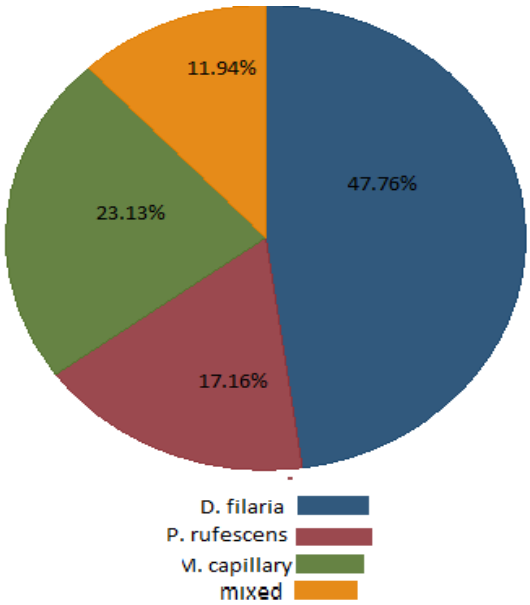


Figure 17: lung worm species that identified from verminous pneumonia.

Smears from the cut surface usually revealed segments of adult worm, ova at different stages of development and larvae (Annex VII). Large lungworms were sometimes found in the larger bronchioles and branches of bronchi as a mixed infestation (Figure 19). Adult worm of *Dictyocaulus flaria* which is slender, threadlike, white and found mostly in bronchi of the caudal lung lobes associated with an excess of mucous (catarrhal bronchitis) are easily observable. They have a whitish to grayish color. Posterior end of an adult male lung worm *Dictyocaulus filaria* has short bursa having short, stout, dark brown spicules "boot-shaped" as indicated in (Annex VII**).** *Mulerius capillaris* are medium-sized and thin worms (hence their common name hairworms), while adult *Protostrongylus rufescens* are slender, reddish to brownish color worms (Annex VII).

The occurrence of verminous pneumonia in both species was statistically significant difference (P =0.04) with 25.24% in sheep and 6.67% in goat and there were highly associate (p=0.002) with age group age group 1_3 years and age >3 years were high occurrence than age group <1 years. Lungworm infections were assessed in relation to body condition, and it was shown that infection was not significantly associated (p>0.05) (Table 6).

There were significant (p=0.000) association of lung worm species and animal species and age however, no significant (p>0.000) difference of the occurrence of different lung worm species between body condition of the animal (Table 6).

Table 5: Association Lungworm species with Species, age and body condition of animal.

Lung worm species	**Species**		**Age**			**Body condition**	
	Sheep	Goat	<1 year	1_3 year	>3 year	Modera	Good
D. filaria	56	9	30	23	12	34	31
P. rufescens	23	0	4	4	15	15	8
M. capillary	18	13	5	12	14	17	14
Co_ infection	10	6	1	10	5	10	6
P-value	106	28	40	49	45	75	59
	0.000		**0.000**			**1.5956**	

4.3.2. Hydatid cyst examination

From the total 18 cysts collected and examined, 11(61.1%) were fertile (81.8%) viable and 18.2% non viable) and 7(38.9%) (57.1% sterile and 42.8% calcified) were unfertile. cyst fertility or viability were observed on both sheep and goat and no no-viable cyst were observed in goat (Table 7).

Table 6: prevalence of Hydatid cyst with fertility test

Species	No of cyst examined in %	Fertile		Unfertile	
		Viable	non viable	Sterile	Calcified
Sheep	14(77.8%)	8(44.4%)	2(11.1%)	3(16.7%)	1(5.5%)
Goat	4(22.2%)	1(5.5%)	0	1(5.5%)	2(11.1%)
total	18(100%)	9(81.8%)	2(18.2%)	4(22.23%)	3(16.7%)

4.4. Bacterial Isolation

The bacteria isolation was conducted on 99 lungs with pneumonia except verminous pneumonia. In general, a total of 102 bacteria were isolated from 85 pneumonic lungs. However, Bacteria were not isolated from the 14 pneumonic lesions. There were 9 species type of bacteria isolated. These include; *E. coli* 21(20.6%), *Klebsiella Pneumoniae* 13(12.7%), *Mannheimia haemolytica* 11(10.8%), *Streptococcus species* 5(4.9%), *Staphylococcus aurous* 8(7.8%) and *Pasteurella multocida* 3(2.9%), *Pseudomonas* (6.9%), *Proteus* 2(1.9%), *Cornybacterium* 1(1%) and in 14(13.7%) pneumonic lung bacteria occurred as co-infections. Gram negative bacteria were dominant over Gram positive in sheep and goat in the study area (Figure 20).

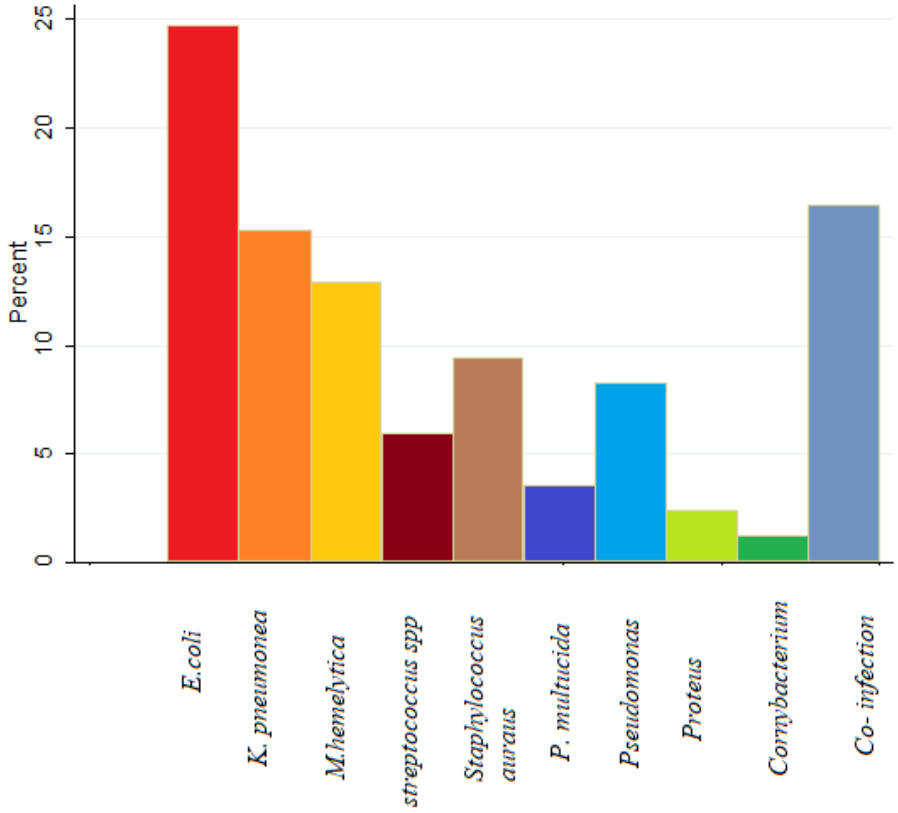


Figure 18: Frequency of aerobic bacterial species isolated from Caprine and ovine pneumonic lungs in Dessie municipal abattoir.

68 bacteria out of 98 were isolated from broncho pneumonia, 25 from interstitial pneumonia, 3 from granulomatous pneumonia and 2 bacteria isolated from aspirational pneumonia. Bacteria were not isolated from the other 14 pneumonic lesions. *E. coli* and *Mannheimia haemolytica* were the most frequently isolated bacteria from lungs with pneumonic lesions of both bronchopneumonia and interstitial pneumonia.

5. DISSCUSION

The overall prevalence of lung lesion of sheep and goat in Dessie municipal abattoirs in the current was 78.1%. The present study was higher than the previous study reported by different researcher in Ethiopia; Mandefro *et al*. (2015); Assefa *et al.* (2017) and Denebo and Tafere (2022) had reported variable prevalence of pulmonary lesions in both sheep and goat as 37.4%, 29.18%, 62.5% respectively in different areas of the country. The current finding suggesting that the pulmonary lesion is an important cause of organ condemnation and thereby economic losses. The variation might be due to managing system, geographical location, metrological situations and age.

Pneumonia is one of the major complex condition involving interaction between the host (i.e. immunological and physiological), multiple agents (e.g. bacterial, viral, mycoplasma) and environmental factors (Hashemnia *et al*., 2019) and causes pathological changes in lungs hence leads to decreased productivity and growth performances, serious financial losses, mortality of sheep and goats. In the current gross and histopathological study unveiled that more than 55.5% of sheep and goats slaughtered at Dessie municipal abattoir were affected with different forms of pneumonia. In agreement with the present finding, previous investigations also reported pneumonia as the commonest type of lung lesion in sheep and goats (Mandefro *et al*., (2015); Assefa *et al.*, 2017; Hashemnia *et al*., 2019). A number of factors may explain the high prevalence include variability in the sampling season, geographical locations and stress factors such as long travel before being slaughter at abattoir, poor housing and overcrowding result opportunistic bacteria like *Pasteurella* species and parasite to attack the lungs.

Different types of pneumonia were observed based on their gross and microscopic feature and confirmed that pneumonia continues to be one of the most important causes of sheep morbidity and mortality in Ethiopia. Hemorrhagic interstitial pneumonia, broncho interstitial pneumonia, acute interstitial pneumonia, suppurative and fibrinous broncho pneumonia were diagnosed Histopathologically and this suggest the histopathology is better sensitive and specific for diagnosis of lungs potentially helped to classify the various forms of pneumonia (Mekbib *et al.*, 2019).

Bronchopneumonia (61%) was the most frequent next to verminous pneumonia from those pneumonic lesions. This is in agreement with report of Mekbib *et al*. (2019), Mishra *et al*. (2018) and disagreed with the report of Mohamed *et al.* (2022) report interstitial pneumonia were frequent pneumonia. The gross and microscopic lesions observed with bronchopneumonia were in agreement with the description of Yesuf (2012), Singh *et al*. (2017) and Mekbib *et al*. (2019). This type of pneumonic lesion associated with pneumonic pasteurellosis and other respiratory bacteria as these diseases are characterized by fibrinous and suppurative bronchopneumonia patterns. In cases of bronchopneumonia, the cranioventral consolidation of the right side lungs in the majority of the cases, this was in agreement with the observation of earlier workers Kumar *et al*. (2014) and Singh *et al*. (2017). This distribution may due to the topographic location which gives tendency to the gravitational sedimentation and deposition of the exudates and infectious pathogens. It is reported that the lesion on bronchopneumonia starts at the bronchiolar-alveolar junction and then the inflammatory lesions can spread downward to the lower portion of the alveoli and upward to the bronchi (Zachary, 2017).

The microscopic appearance of bronchointerstitial pneumonia include; thickening of intraalveolar septa with infiltration of inflammatory cell in the bronchi, bronchiole and alveoli. These were in consistent with the reports of Mekibib *et al*. (2019). Bronchointerstitial pneumonia happens when viral agents affect the lungs as the primary etiology of interstitial pneumonia with further invasion of the infected lungs by bacteria (Mekibib *et al*., 2019). The frequency of interstitial pneumonia in the present study was observed in 21 cases (9%) and this were in agreement of the report of. the gross lesions were enlarged, rubbery, meaty, and distributed throughout the lungs and microscopic lesion; lymphocytic infiltration and macrophages in the alveolar lumina and thickening of alveolar septa. the description were in accordance with the description of Mekibib *et al*. (2019) and Mohammed *et al*. (2022).

The occurrence of aspirational pneumonia was observed in 11 cases (4.7%) out of 234 pneumonic lesions. The gross and microscopic features detected in this study were consistent with the previous reports Mekibib *et al*. (2019). The gross and microscopic lesions of aspiration pneumonia are greatly variable and usually depend on the physical and chemical nature of the aspirated material. Apart from the inherent inflammatory and necrotic nature of the aspirated

fluid, most bacteria from the nasopharynx was fushed down the respiratory tree and reach the lungs by gravitational drainage and cause lesions ranging from bronchopneumonia to granulomatous in type (Fentahun *et al*., 2017; Mekbib *et al*., 2019).

The present study revealed the association between the type of pneumonia and the isolated aerobic bacteria in sheep. *E. coli* was the most frequently isolated bacteria from lungs with pneumonic lesions of sheep and goat in the stud area and in agreement with the previous study of Yimer and Asseged (2007) at Dessie municipal abattoir and Tijjiani *et al*. (2012) while, disagreed with Addisu *et al*. (2017) at Addis Ababa enterprise abattoir who report 3.3%. The total isolation rate of *M. haemolytica* were higher than *Pasteurella multocida* agreed with the report of Demissie *et al*. (2014), Addisu *et al*. (2017) and Marru *et al.* (2013). Mixed infections of bacterial agent were detected since the respiratory air pathways act as a reservoir for potentially pathogenic micro-organisms which develop into pneumonia following stress, decline of hygiene measures or climatic conditions. No bacteria could be isolated from 14(%) sampled pneumonic lungs in the present study and this supported by the findings of Yimer and Asseged (2007) in Dessie, Azizi *et al*. (2013) in South western Iran. This may due to antibiotic therapy before slaughter or may due to the etiological agent.

Among the total sheep and goat examined, the overall prevalence of verminous pneumonia 31.9% was recorded and the most frequent lesion (57.5%) from those observed pneumonic lesion in the current study. The current finding was in accordance with the findings of Addis *et al*., (2011) from Gonder Ethiopia, Ayana and chanie (2013) from Bahirdar, Mulate and Mammo, (2016) in south Wollo, who report (57.55%) and (45.7%) respectively and was higher than observed by other workers in other parts of country such as Dar *et al*. (2012); Mekonnen *et al*. (2011). This may be attributed to the diagnosis method and sample size researcher used to determine prevalence, geographical variation; the climate of area, altitude, intermediate hosts and favorable ecological conditions such as rain fall, humidity, temperature, and marshy area for grazing, sheep and goat management system for survival of the larvae of lungworms or intermediate host, nutritional status and season of the study (Dar *et al*., 2012; Kebede *et al*., 2014).

The prevalence of lungworm infection were higher in sheep than goat in the present study this may due to the sample size and the grazing system of sheep exposed to parasite and Goats with their browsing behavior consume uncontaminated matter with parasite larvae, so being less exposed to infective larvae. With regard to age, the overall prevalence of lungworm infection was higher in adult (>1year) than (< 1year). The report present study were in agreement with Ayana and chanie, (2013) in Bahirdar however, disagreed with the findings of Radostitis *et al*. (2007) and Mekonnen *et al.* (2011) who reported that young sheep were found to be infected more than adults. This may related to adults could be carrier of infection and highest prevalence may be attributed to higher prevalence in adults (Ayana and Chanie, 2013).

Dictyocaulus filaria was the most frequent lungworm species than *Mullerius capillaries* and *Protostrongylus rufescence*. The present findings were not in agreement with Mulate and Mammo, (2016) in south Wollo and Basaznew *et al*. (2012) in and around Dessie zuria. The variation might be season of sample collection, methods followed to detect the parasitic larvae and in the life cycle of the lungworm species. For instance, *Dictyocaulus filaria* has a direct life cycle and takes less time to reach the infective stage, and after ingestion, larvae can appear in feces within 5 weeks (Adem, 2016). Whereas *P. rufescens* and *Mullerius capillaries* have an indirect life cycle that requires longer time and a wet or rainy season to complete their complex life cycle. The combinations of the two or three of them were present together in one lung in the study area.

Gross appearances of lungs with verminous pneumonia include presence of adult parasite in bronchi and bronchiole, formation of different size and color and appearance of nodule in the lung parenchyma. The affected lung also show different lesion such as atelectasis, emphysema and congestion. Microscopically, cross section of lung worm in the lumen of bronchi, bronchiole and alveoli, rupture of the alveolar walls, neutrophil, macrophage, and eosinophils infiltration, hyperplasia of the connective tissue and smooth muscle cells were observed. Similar findings were described by Adem (2016), Ayana and chanie (2013), Dar *et al*. (2012) and others.

The overall prevalence of Hydatid cyst in the present study was 4.3%. This value was in accordance with Hashim export abattoir 5.7% but, slightly lower prevalence than 10.2% in

ELFORA export abattoir Denebo and Tafere (2022). These differences may be associated agro-ecological and socio-cultural factors, the contact between large numbers of stray dogs are important factor that have been responsible for high prevalence of hydatidosis elsewhere (Abegaz and Mohammed, 2018). In the present study Sheep were more infected with hydatid cyst than goat and in agreement with Abegaz and Mohammed, (2018); Denebo and Tafere (2022). The nature of the pasture and grazing patterns of animals; sheep was feeding graze while goats were feed browser so sheep become potential consumers of the eggs from contaminated pastures (Nyero *et al*., 2015; Abegaz and Mohammed, 2018). In the present study With regard to cyst fertility, fertile cysts were higher than unfertile cyst; this may related with lung has relatively softer constancy which allows easier development of the pressure cysts and fertility of Hydatid cyst. Cyst fertility were higher in sheep than goat this suggest indicators of the importance of sheep as a potential source of infection than goats for the final hosts. The gross of pulmonary hydatidosis characterization in the current study were consistent with the previous reports Nyero *et al*. (2015); Ibrahim and Gameel (2014).

In the present study abnormal inflation included emphysema and atelectasis were observed. The overall prevalence for pulmonary emphysema in present study was 7.1%. the result were in agreement with Gill *et al*. (2022) who report 7.1% however, slightly lower than the report of Denebo and Tafere (2022) in bishoftu. Emphysema was the second prevalent lesion next to pneumonia. Pulmonary emphysema in animals is normally secondary to some respiratory disease conditions and due to a well-developed interlobular septa and lack of collateral ventilation in sheep is susceptible to interstitial emphysema (Mallua *et al*., 2010). In the current study overall prevalence of pulmonary atelectasis was 3.33%. The result was in agreement with the report of Gill *et al*. (2022). The gross and microscopic features of pulmonary emphysema and pulmonary atelectasis detected in the current study were in line with the reports of Mallua *et al*. (2010), Gill *et al*. (2022).

The overall prevalence of pulmonary congestion and hemorrhage was 2.38% and 3.1% respectively. The prevalence of hemorrhage was in agreement with the report 3.7% by Singh *et al*. (2017). Pulmonary congestion was distributed into all lobes of the lung and in animals with good body condition. This may be due to poor pre-slaughter treatments, improper stunning and

improper bleeding. Without a known cause, this is almost always the result of blood pooling in the lungs after being forced out of the muscles by rigor mortis. The gross and microscopic features of pulmonary hemorrhage and pulmonary congestion detected in the current study were consistent with the previous reports El_Mashad *et al*. (2020).

In the present study the prevalence of Bronchoectasis and bronchioleitis was 3(0.7%). Prevalence of Bronchoectasis and bronchioleitis was found only in sheep and no this case were observed in goat. Grossly, the lungs showed prominence of the pleural surface and a grayish-white discoloration. Pleurisy was recorded contributing prevalence 0.7% of total lungs examined. The pleurisy in this study is significantly lower than what was reported in Mohammed *et al*. (2022).

The total prevalence of pulmonary Melanosis among the samples examined was 0.95%. The present prevalence was in line with the prevalence 0.25% reported by Mohammed *et al*. (2022).

6. CONCLUSION AND RECOMANDATION

We deduced from recent observations that pulmonary lesions are relatively common in sheep and goat. Pulmonary lesion can considered an important sheep and goat disease affected on anatomy and leading to economic losses. Although the sheep and goats are that apparently healthy slaughtered were found certain pathological lesions or being infected with different diseases in large percentage. The gross observations could be used to identify subclinical pneumonia in slaughtered sheep and goats however histopathological examination was a more accurate to detect pulmonary lesion and particularly to classify pneumonia.

In line with this conclusion the following recommendation were forwarded

- Histopathology should be employed routinely as an ancillary test in the major abattoirs, regional veterinary laboratories and universities to generate additional epidemiological data for a better disease control and prevention measures.
- Further study on cause of respiratory organs disorders, isolation and identification of both aerobic and anaerobic bacteria, viruses and fungi, also required to formulate feasible and cost-effective control strategies.
- Verminous pneumonia caused by different species of lung worm is highly prevalent and economically important so, appropriate control and prevention measures should be taken.

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8. APPENDICES

Annex I: Body condition scoring system (Yami *et al.*, 2008).

Score of 1 (very poor body condition)

- The spinous processes are prominent and sharp. The transverse process are also sharp, the fingers pass easily under the ends, and it is possible to feel between each process. The eye muscle areas are shallow with no fat cover.
- Ribs are clearly visible.
- Sternal fat is easily grasped and moved from side to side.

Body condition score 2 (poor body condition)

- The spinous processes feel prominent but smooth, and individual processes can be felt only as fine corrugations. The transverse processes are smooth and rounded, and it is possible to pass the fingers under the ends with a little pressure. The eye muscle areas are of moderate depth, but have little fat cover.
- Some ribs can be seen. There is a small amount of fat cover. Ribs are still felt.
- Sternal fat is wider and thicker but can still be grasped and moved slightly from side to side.

Body condition scoring 3 (good body condition)

- The spinous processes are detected only as small elevations; they are smooth and rounded and individual bones can be felt only with pressure. The transverse processes are smooth and well covered, and firm pressure is required to feel over the ends. The eye muscle areas are full, and have a moderate degree of fat cover.
- Ribs are barely seen; an even layer of fat covers them. Spaces between ribs are felt using pressure.
- Sternal fat is wide and thick. It can still be grasped but has very little movement.

Body condition score 4 (fat)

- The spinous processes can just be detected with pressure as a hard line between the fat covered eye muscle areas. The ends of the transverse processes cannot be felt. The eye muscle areas are full, and have a thick covering of fat.
- Ribs are not seen.
- Sternal fat is difficult to grasp and cannot be moved from side to side.

Score of 5 (very fat)

- The spinous processes can't be detected even with firm pressure, and there is a depression between the layers of fat in the position where the spinous processes would normally be felt. The transverse processes cannot be detected.
- The eye muscle areas are very full with thick fat cover. There may be large deposits of fat over the rump and tail.
- Ribs are not visible and are covered with excessive fat.
- Sternal fat extends and covers the sternum. It cannot be grasped.

Annex II: Gross pathological lesion recording formats

No	date	Sample code	species	age	Body condition	Gross lesion description	Type of lesion	Sample taken	Remark
1									
2									
3									
3									
4									
5									

Annex III: Histopathological Technique Procedures (Takulder, 2007)

1. Fixation of tissue by 10% neutral buffered formaldehyde

2. Trimming part of the tissue in a way that the lesion we require be included or not missed and to fit standard histological processing tissue cassettes (5mm thickness).

3. Tissue specimen processing: fixation of tissue by formalin, dehydrating tissue by increasing alcohols concentration, clearing of tissue by xylene, and impregnation of tissue by paraffin wax.

- Fixation by formalin two times: Formalin-I for 2hr and Formalin-II 2hr
- dehydrating by different concentration of Alcohol: 70% Alcohol for 1hr, 95% Alcohol for 1hr and three times by concentrated alcohol; 100% Alcohol-I 1hr → 100% Alcohol-II 2hrs →100% Alcohol-III 2hrs]
- clearing by Xylene three times 1:30hrs for each
- Impregnation with Paraffin wax-I for 2hrs and Paraffin wax-II for 3hrs.

4. Embedding/ Blocking of processed tissue: impregnated tissue is placed in a mould with their labels and then fresh melted wax (54-60oc) is poured and allowed to settle and solidify.

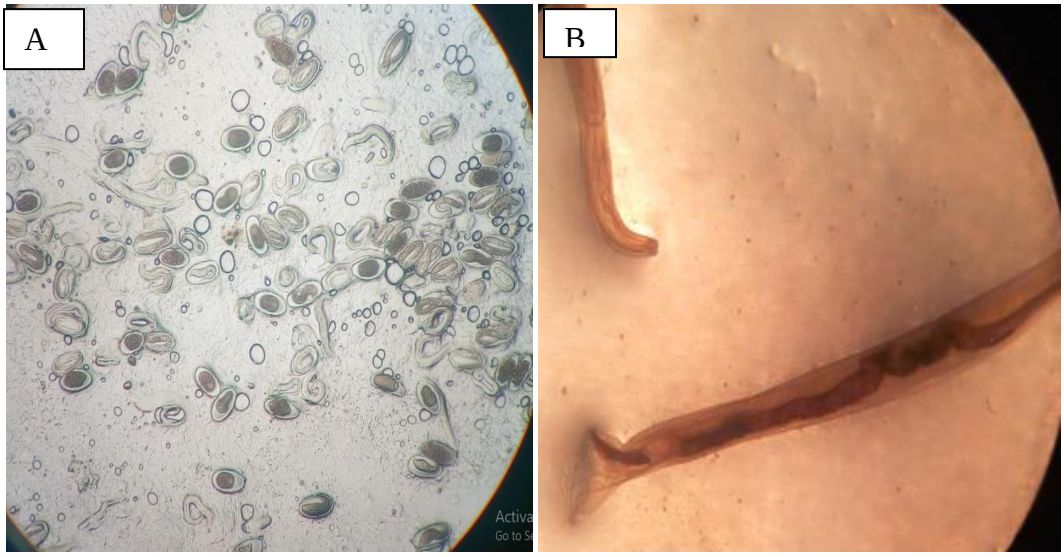
5. Sectioning: sectioning of tissue in 3-5 micron thickness and put on warm water bath to straighten the ribbon, and then adhere on the surface of frost ended and clear slide. Later label and put the slide in incubator at 60^0 c overnight.

Staining Procedure:

1. Deparaffinize slides in 3 changes of xylene (xylene-I, xylene-II and xylene-III) for 3 minutes each.
2. Hydrate slides in 100% alcohol and 95% alcohol, 2 changes for 3 minutes each, and rinse in distilled water until ripples disappear from slides.
3. Place in Hematoxylin for 8 - 15 minutes.
4. Rinse in tap water until water runs clear
5. Decolorize in 1% acid alcohol, 3 - 6 quick dips. Check differentiation microscopically: Nuclei should be distinct; Cytoplasm should be uncolored.
6. Rinse in tap water until ripples disappear from slides.

7. Dip in bluing agent, 3 - 5 long dips.
8. Wash in lake-warm tap water for 5 minutes (37-40°C.)
9. Stain in Eosin for 30 seconds - 2 minutes.
10. Dehydrate in 95% alcohol and 100% alcohol, 3 changes each for 2 minutes.
11. Clear in 3 changes of xylene for 2 minutes each.
12. Mount cover glass with Canada balsam or Deapistix (DPX)
13. Examination of the prepared slides under microscope at low to high magnification power (4x, 10x, 40x and 100x) and finally the photomicrographs taken for documentation of every histopathological l

Annex IV: Morphology of lung worm identified and recovered from lung parasitic pneumonia of sheep and goat.



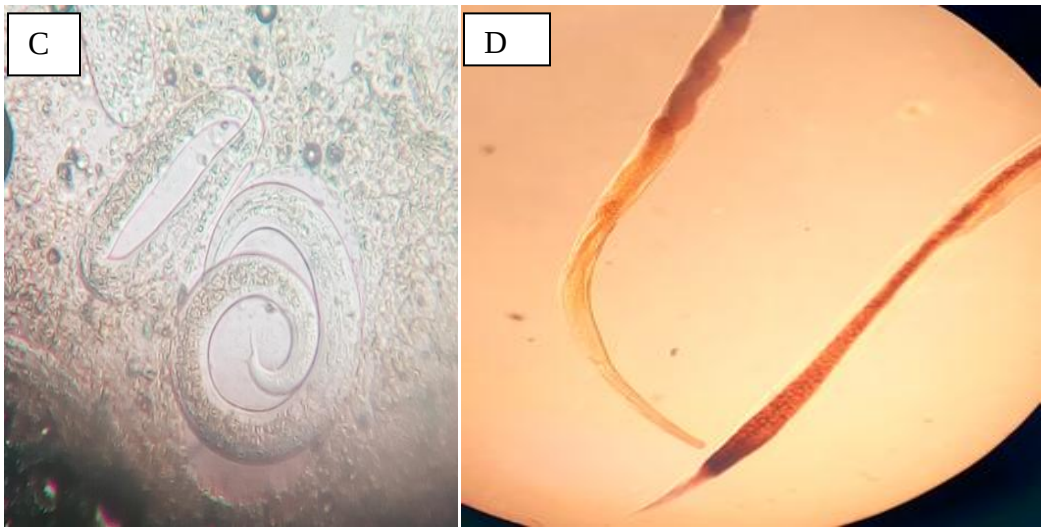
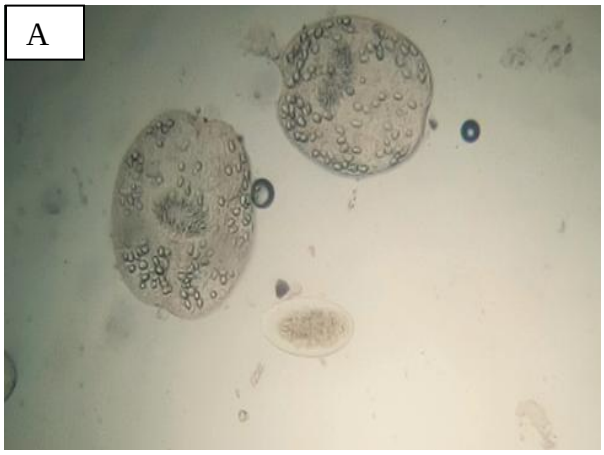


Figure 19: identification of three type of lung worm parasite at egg, larvae or adult stage; a), smear from nodule and egg with larvae observed with light microscope. b), anterior and Posterior end of an adult *Dictyocaulus filaria* with short bursa having a short, stout, dark brown, "boot-shaped" spicules with stereomicroscope. C), larvae of *Mulerius capillary* d), adult parasite of *protostrongylus rufescence*

Annex V: Procedures for cyst fertilility and viability: (Macpherson *et al.,* 1985).

- ✓ Obtain/collect non-degenerated hadatid cyst from infected organs of slaughtered animals.
- ✓ Take the cysts to laboratory in cool box.
- ✓ Aspirate hydatyid fluid from the cyst by a sterile 18 gauge needle and transfer to a test tube.
- ✓ The protoscolices allowed to sediment in the fluid for 20-30 minutes which indicates fertility and the supernatant discarded.
- ✓ Confirm the fertility of the cyst by microscope examination of sediment protoscoliices.
- ✓ A drop of sediment contained protoscolices on microscopic glass slide and cover with the
- ✓ Cover slip; observe for amoeboid like peristaltic movements with high power objective.
- ✓ For clear vision a drope of 0.1% aqueous eosine solution added to equal volume of protoscolices in Hydatid fluid on the microscopic slide with the principle that viable protoscolices should completely or partially exclude the day while the did ones take it up.

- ✓ Furthermore, infertile cysts where further classified as sterile and calcified. Sterile Hydatid cysts were characterized by their smooth inner lining usually with slightly turbid fluid in its content while typical calcified cysts produce a gritty sound feeling up on incision.



Protoscolices of fertile cyst from goat lung

Annex VI: Methods used to identify different bacteria (Quinine, 2011)

Media preparation

- ✓ Measure the powder media by sensitive balance
- ✓ Add into sterile flask
- ✓ Measure the distilled water with graduate cylinder, add into the flask, and cover with aluminum foil
- ✓ Mix with temperature mixer
- ✓ Adjust and clean the number of Petri plates required
- ✓ Enter the media into the autoclave at 121 0c for 15 minutes and petri plates into hot air oven for sterilization
- ✓ Put the flask media into the water bath to reduce the temperature to 45-50oc
- ✓ Then, dispatch the media into the sterilized Petri plates in biosafety cabinet to avoid contamination

- ✓ Incubate overnight to check media contamination
- ✓ After checking the contamination, inoculate the sample onto the prepared media or put the media in refrigerator up to two weeks

Tryptone Soya Broth

Preparation: Suspend 30 grams of the medium in one litter of distilled water. Mix well and Heat slightly until complete dissolution of the medium if necessary. Dispense in tubes and sterilize by autoclaving at 121°C for 15 minutes.

Gram Staining

Procedure:

- ✓ Prepare a smear on a clean slide from colonies of fresh culture and heat gently to fix by passing the slide above the flame of the Bunsen burner
- ✓ Applying a primary stain (crystal violet) for 60 second to a heat-fixed smear of a bacterial culture. Then rinse the slide gently with tap water to remove excess stain
- ✓ Addition of iodide which remain for 1 minute. Gram's Iodine combines with crystal violet to form di-iodine complex. Then wash off with tape water.
- ✓ Rapid decolourization with ethanol for only 15-30 second. until colour ceases to run out. Then wash off withtape water.
- ✓ Counterstaining with safranin for 60 seconds. wash briefly with water then air dry or driedwith bloating paper.
- ✓ Finally, view under the microscope with oil immersion objective lens to observe cell morphology and gram reaction

Catalase Test

Procedure: a loop of bacterial growth is taken from nutrient agar medium. Then the bacterial cell placed on a clean microscopic slide and a drop of 3% hydrogen peroxide is added. An effectiveness of oxygen gas, within a few seconds, indicates a positive reaction.

Indole Test

Principle: Organisms those possess the enzyme tryptophanase can break down the amino acid tryptophan to indole. When indole reacts with para-dimethylaminobenzaldehye (Kovac's reagent) a pink -colored complex is produced. Tryptophan is plentiful in most media, but growth on blood agar or chocolate agar produces the best effects.

Procedure: Take loopful of inoculum by touching the 3-5 representative colonies with inoculating loop from pure colonies and inoculate Tryptone soya broth tube. Incubate the tube at 37°C for 24 hours and cap left loosen to aerate the tube. After incubation, add 5-10 drops (0.5ml) of Kovac's reagent to the culture broth and agitate gently. Then observe the tube for color change within 5 minutes.

Methyl Red Test Solution

Test Principle: Some organisms produce acid from the metabolism of glucose in a sufficient quantity to produce a pH of 4.4 in the media. These acids are not further metabolized and are said to be stable acids. At a pH of 4.4 or less the pH indicator methyl red is a bright cherry red. While also some organisms initially produce acid from glucose metabolism but further metabolize the acid produced to neutral end products, such as acetoin, and 2, 3-butanediol. Initially the pH may drop to 4.4 but the neutral end products raise the pH so the methyl red test will be negative. Acetoin and 2, 3-butanediol under alkaline conditions will react with alpha-naphthol (1-naphthol) to produce a mahogany red color.

Procedure: The tested organism was inoculated into glucose phosphate medium (MR-VP medium) then incubated at 37°C for 48 hours. Two drops of methyl red reagent were added, shaken well and examined. Appearance of red colour indicated a positive reaction, whereas orange or yellow colour indicated a negative reaction.

Simmons Citrate Agar

Principle: Citrate contains carbon. If an organism can use citrate as its only source of carbon the citrate in the media will be metabolized. Bromthymol blue is incorporated into the media as an indicator. Under alkaline conditions this indicator turns from green to blue. The utilization of citrate in the media releases alkaline bicarbonate ions that cause the media pH to increase above 7.4 causes the media blue.

Procedure**:** Take loopful of inoculums by touching the center of 3-5 representative colonies with inoculating loop and streak it onto the surface of a Citrate slant. Incubate the tube aerobically at 35°C with cap left loosen for 22 hours. After 22 hrs incubation observe the tube for growth and color change.

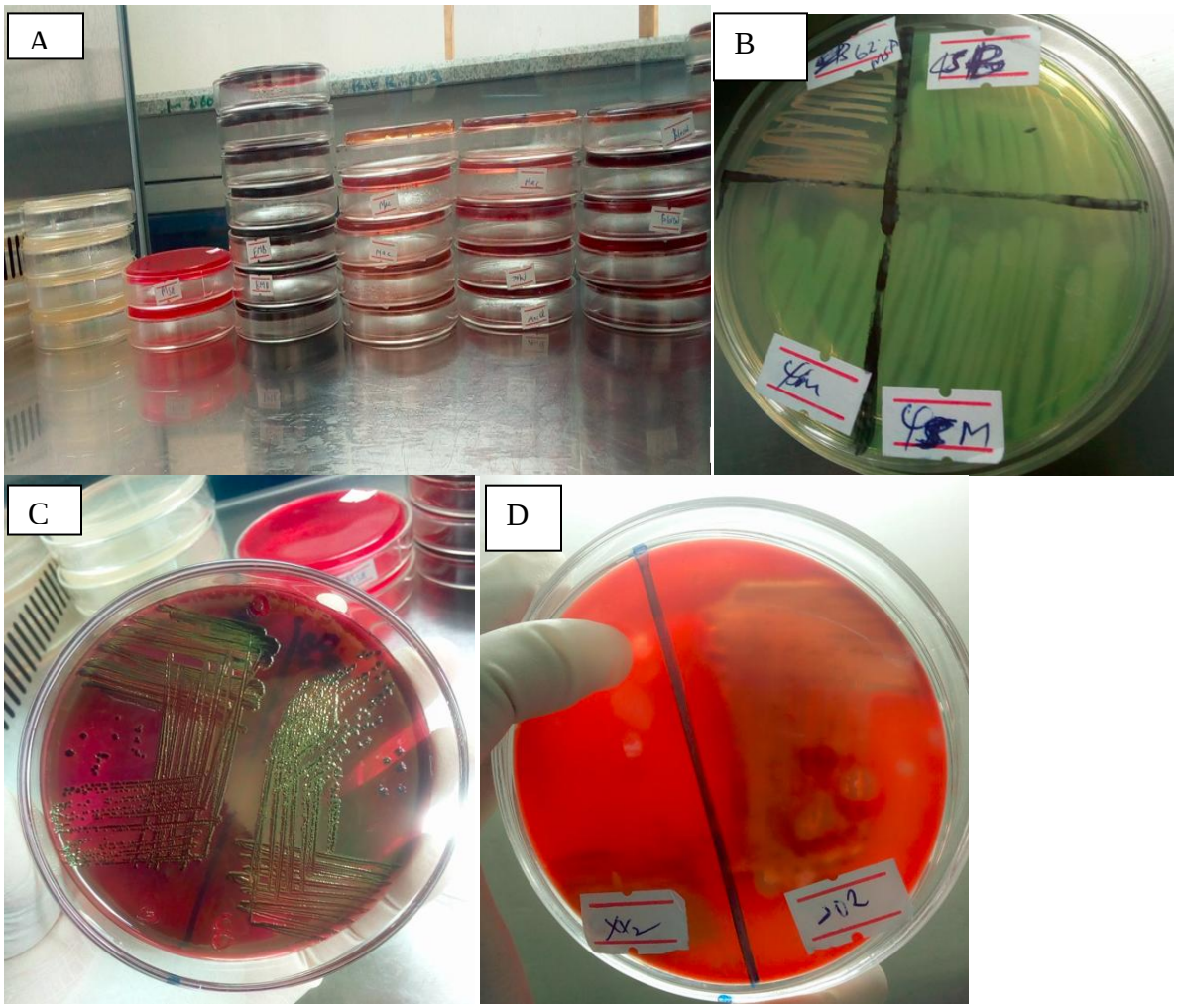
Triple Sugar Iron Agar

Procedure*:* By sterile inoculating loop touching the center of colony from isolated pure colony take loop full of inoculum. Streak the inoculum back and forth on TSI agar in tube along the surface of the slant. Incubate the tube with the cap loosened at 35 °C for 22 hours. yellow/yellow tube and remains that way due to the large amount of acid produced in the reaction.

Motility Medium

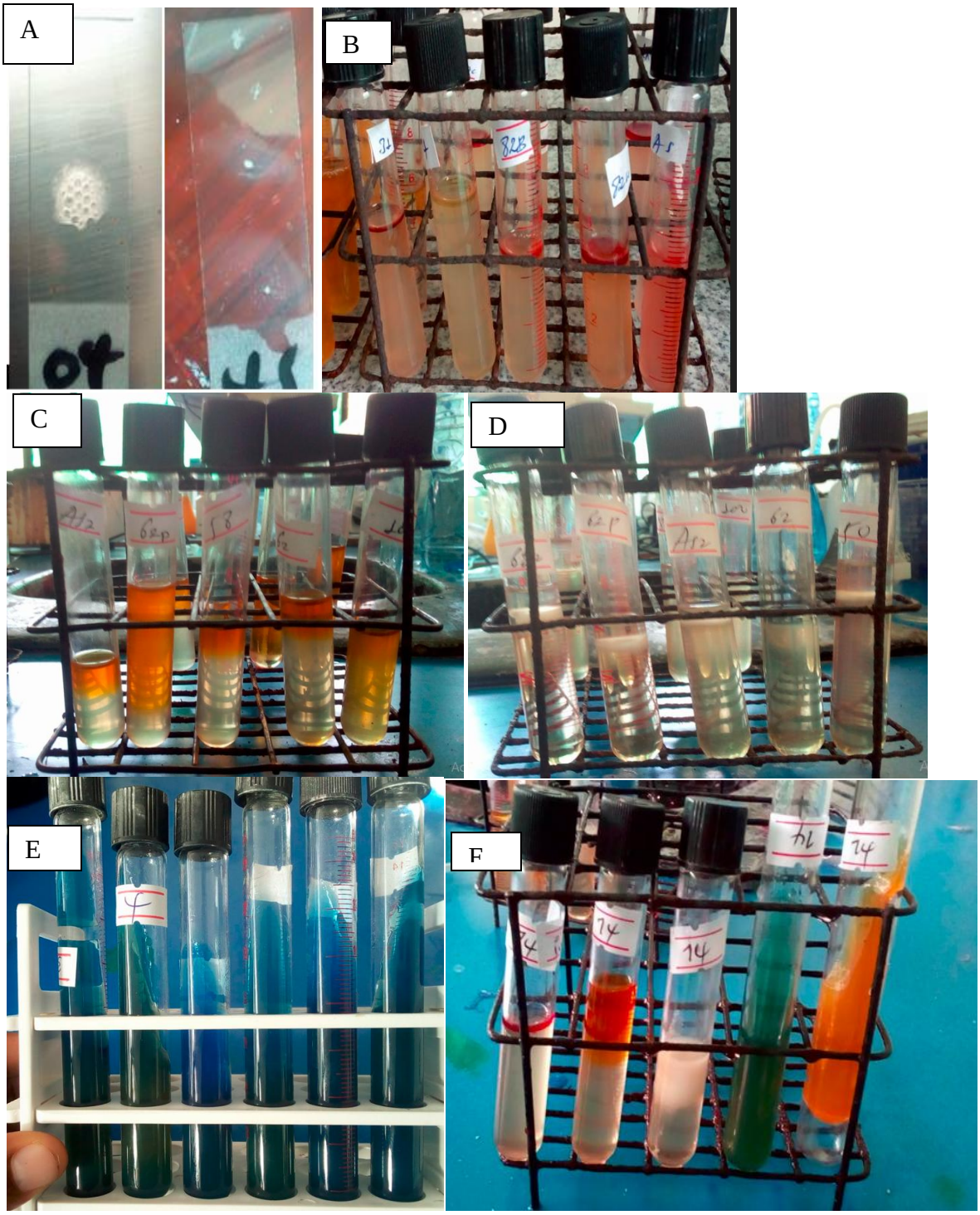
Test procedure and principle: The tube of motility medium was stabbed by inoculums to depth of about five mm and incubated at 37°C. Motile organism migrated through the medium, which become turbid while the growth of non-motile organisms was confined to the stab inoculums

Annex VII: Picture of colony characterization and biochemical test



A) Different prepared agar media. **B**) *Pseudomonas argons* on nutrient agar. C) E. coli on EMB agar. **D**) hemolysis on blood agar, no hemolysis on left side(xx2) and Beta hemolysis on right side(102)

Annex VIII: Picture of colony characterization and biochemical test



A) Catalase test. Catalase possitive left side (04) and negative right side (46). **B**) Indole test. **C**) MR test. D) VP test. **E**) Simmons citrate test. **F**) E. coli on IMViC and TSI test.