

Thesis Ref. No. \_\_\_\_\_

**LESION CHARACTERIZATION, BACTERIAL ISOLATION AND VIRAL  
DETECTION FROM RESPIRATORY TRACT OF DROMEDARY CAMELS  
SLAUGHTERED AT ADDIS ABABA AKAKI MUNICIPAL ABATTOIR, ETHIOPIA**

**MSc. Thesis**



**By**

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Department of Pathology and Parasitology**

**June, 2015  
Bishoftu, Ethiopia**

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

By

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June, 2015

Bishoftu, Ethiopia

**Addis Ababa University**  
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As members of the Examining Board of the final MSc open defense, we certify that we have read and evaluated the Thesis prepared by: Kibruyesfa Bayou entitled: Lesion characterization, bacterial isolation and viral detection from respiratory tract of dromedary camels slaughtered at Addis Ababa Akaki Municipal Abattoir, Ethiopia and recommend that it be accepted as fulfilling the thesis requirement for the degree of: Masters of Science in Tropical Veterinary Pathology.

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## **DEDICATION**

*This MSc thesis paper is dedicated to my father Bayou Tegegn who died in 2002 E.C. and to my elder brother Wondwosen Bayou who died in 2004 E.C.*

## **STATEMENT OF AUTHOR**

First, I declare that this thesis is my original work and that all sources of material used for this thesis have been duly acknowledged. This thesis 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/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 the award of any academic degree, diploma, or certificate.

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## **LIST OF ABBREVIATIONS**

AGID	Agar-gel immunodiffusion
CPE	Cytopathic effect
cm	centimetre
CSA	Central Statistics Agency

cm <sup>3</sup>	centimetre cube
ELISA	Enzyme Linked Immunosorbant Assay
FAO	Food and Agriculture Organization
gm	gram
H&E	Hematoxyline and Eosine stain
hr	hour
Km	Kilo metre
mm	millimetre
MDBK	Madin-Darby Bovine Kidney
min	minute
MRVP	Methyl Red Voges-Proskauer
masl	meter above sea level
ml	millilitre
NAHDIC	National Animal Health Disease Investigation Centre
NVI	National Veterinary Institute

**LIST OF ABBREVIATIONS (Continued)**

PBS	Phosphate Buffered Saline solution
RBC	Red blood cell
rpm	revolution per minute
RT-PCR	Reveres Transcriptase Polymerase Chain Reaction

SPSS	Statistical Package for Social Sciences
Spp	Species
TB	Tuberculosis
$\chi^2$	Chi-square
%	Percent
°C	Degree centigrade
°F	Degree faharanite

## **ABSTRACT**

*This study was carried out with the aim of lesions characterization, bacterial isolation and identification and viral detection from lesions of respiratory system of dromedary camels slaughtered at Addis Ababa Akaki Abattoir from December 2014 to April 2015. A total of 207 camels were inspected during the study period. Of the lungs inspected, 53 (25.6%) had one or more gross lesions. Interstitial pneumonia 17 (8.2%), pulmonary emphysema 12 (5.8%), bronchopneumonia 11 (5.3%) and atelectasis 5 (2.4%) were among the most*

frequently detected pulmonary lesions. The difference of gross lesions frequency and distribution was not statistically significant ( $P>0.05$ ) between male and female camels. However, gross lesions frequency and distribution significantly varied ( $P<0.05$ ) among different age groups, lung lobes and between right and left lungs. Histologically interstitial pneumonia was characterized by thickening of intra alveolar septae; congested interstitial capillaries and interstitial infiltrations by lymphocytes and other mononuclear cells and suppurative bronchopneumonia by bronchiolar wall and peribronchiolar infiltration with inflammatory cells particularly neutrophils. Whereas, distended alveoli and collapsed alveoli were seen in tissue sections of pulmonary emphysema and atelectasis, respectively. From 53 lungs with gross lesions bacterial isolation and identification was made. A total of 70 bacterial isolates were detected on sheep blood agar plates from 50 (94.3%) of the processed lung tissues. *Rhodococcus equi* (20%), *Aeromonas hydrophila* (12.9%), Coagulase negative staphylococci (11.4%), *Actinobacillus* spp. (10%), *Bacillus* spp. (10%), *Corynebacterium* spp. (8.6%) *Escherichia coli* (7.1%) and *Pasteurella* spp. (5.7%) were among the major bacterial isolates. Besides, 25 lung samples with gross lesions were processed for viral propagation in tissue culture; 10/25 (40%) of them exhibited morphologic alterations (CPE) on VERO cell monolayer. The presence of virus in the samples was evidenced by rounding, sloughing and aggregation of infected VERO cells. The result of this study highlights that pulmonary lesions could be detected from camels that apparently looked healthy harbouring a number of pathogenic organisms in the lesions. Hence, further study should be conducted on the camels' respiratory diseases and lesions.

**Key words:** Bacteria, Camel, Ethiopia, Histopathology, Pulmonary lesion, Virus.

## 1. INTRODUCTION

Thirty-four percent of the land surface distributed worldwide is deserts and semi-deserts (Roger, 2006). The Ethiopian low lands comprise 61% of the national land area (Coppock, 1994). The dromedary camel (*Camelus dromedarius*) is an important multipurpose livestock species uniquely adapted to these harsh environments (Abdelhakim and Youcef, 2012). There are 25.89 million dromedary camels in the world with 80% of them in Africa. Ethiopia takes the third place in Africa next to Somalia and Sudan (FAO, 2011) in possessing 3 million dromedary camels (Bekele *et al.*, 2002). Ethiopia camels are kept in arid and semi-arid lowlands such as Borana, Somalia and Afar regions (Teshome *et al.*, 2003). They serve as an important source of milk, meat, draught power and transportation for the pastoralists. Despite the versatile uses in the pastoralists' areas, camel has been largely neglected by international agencies and local governments as regards to improvement in its health and productivity (Bekele, 1999).

Camels were formerly considered resistant to most of the diseases commonly affecting livestock; but as more research was conducted, camels were found to be susceptible to a large number of pathogenic agents (Abbas and Omer, 2006). Scarce research reports on disease reveals that camels may be either carriers of, susceptible to or suffering from a vast array of infectious and parasitic diseases (Richard, 1979; Demeke, 1998). There are a number of economically important diseases that affect camels (Dia, 2006).

Respiratory diseases are among the emerging problems of camels that are causing considerable loss in production and death (Zubair *et al.*, 2004). A variety of bacterial, viral, fungal and parasitic microorganisms have been associated with outbreaks of respiratory disease among camels (Dioli and Stimmelmarty, 1992; Intisar *et al.*, 2010). In eastern low lands of Ethiopia, respiratory diseases have been a major threat to the camel population with the whole camel population affected during the camel respiratory disease outbreak in 1995. It was characterized by a highly contagious nature with high rate of morbidity (over 90%) and a variable rate of mortality (Bekele, 1999; Roger *et al.*, 2001).

Pulmonary lesions have been reported to cause decreased productivity and huge economic loss to animal owners (Zubair *et al.*, 2004; Kane *et al.*, 2005), because it can interfere with pulmonary functions especially oxygenation of blood and supply of oxygen (Anosa, 1983), and it provides basis for the clinical, prognostic and diagnostic decisions of various types of diseases in domestic animals (Egbe-Nwiyi *et al.*, 2000; Fatihu *et al.*, 2000).

Camel respiratory problem has received little consideration, even though it is an emerging disease in Ethiopia causing considerable loss of production and deaths (Rufael, 1996; Bekele, 1999). The respiratory diseases are still flaring up in various parts of the country and reports are coming year after year urging for an intervention measures (Fekadu and Esayas, 2010). The respiratory infection is mainly due to primary or secondary bacterial infections (Egbe-Nwiyi *et al.*, 2000; Fatihu *et al.*, 2000).

Several authors have reported high antibody titers against numerous respiratory tract viruses (Roger *et al.*, 2001). The pathological and epidemiological significance of these findings is not clear (Bekele, 2010). Further studies have been recommended on the epidemiology of the disease and the identification of the responsible pathogens and its serotypes to be involved for the development of vaccines (Fekadu and Esayas, 2010).

Only few studies were conducted on the pathological lesions of the respiratory tracts of camels. Despite all efforts done so far for the identification of the possible causes of the disease, there was no substantial result obtained. Yet there is a need to conduct further pathological studies on the lesions of the lungs and associated tissues to identify the causes of respiratory diseases in camels in order to design better diseases control strategies. Therefore, the objectives of the study were:

- To characterize the gross and microscopic lesions of the respiratory tract of camels that were slaughtered at Addis Ababa Akaki Abattoir.
- To isolate and characterize bacteria and to detect the presence of viruses from the gross lesions of respiratory tracts of the camels.

## **2. LITRATURE REVIEW**

### **2.1. Socio-economic importance of the camel**

Camels play diverse roles in livelihood of the poor pastoralists, including the building of assets, insurance against unexpected events; have spiritual and social values, traction and movement of goods, food supply income generation in Ethiopian pastoralists and very recently it plays pronounced role in the export revenue of the country in both live animal and carcass export (SOS Sahel-Ethiopia, 2007; Ali *et al.*, 2004). Pastoralists own all the 3 million camel populations in Ethiopia supporting more than 10 million pastoralists (Bekele *et al.*, 2002). The camels have been bred owing to the extraordinary power to withstand thirst and hunger for long duration in the most inhospitable ecological conditions (Al-Dahash and Sassi, 2009).

Camels play an important role in the local economy of the Somali community and camels are essential to the subsistence of the Somali pastoralists. The importance of the camel for the Somalis, however, arises primarily from its provision of milk and meat within the subsistence economy, and its use as a beast of burden for transporting milk to the market, water from wells, and household belongings when the families move to new areas. Besides its economic importance, the camel has a social and cultural importance for the Somalis (Abokor, 1993). Camels are used for the payment of bride wealth and compensation of injured parties in tribal feuds (Hussein, 1993). Camels also act as a linking factor between different lineage groups and promote group solidarity (Farah, 2004).

In Borana high milk production (45%) was reported to be the primary purpose of camel production in the area followed by transportation (26%), income generation (16%) and meat production. However, their contribution as means of draught power seems to be insignificant (Bekele, 2010).

Afar pastoralists have to cover long distances to neighboring Amhara and Tigray Regions, especially during the dry season and even more in periods of drought. Having to cover long

distances with cattle in northern Afar always bears the risk that part of the herd perishes due to water or grazing shortage. But camels are primary stock and status indicators and represent the pastoral capital wealth of the Afar society and are essentially raised and kept for this reason (ANRS, 2010).

## **2.2. Impacts of respiratory diseases in camel production**

Among the numerous diseases, respiratory disorders are the major threats to the camel population of Ethiopia (Roger *et al.*, 2000; Roger *et al.*, 2001). Their economic impact is considerable because they result in high morbidity and mortality (Fassi-Fehri, 1987). They are among the emerging problems of camels that are causing considerable loss in production and death (Zubair *et al.*, 2004; Kane *et al.*, 2005). However, in Ethiopia, few studies were conducted on the extent of respiratory problems of camels compared with other species of livestock (Bekele, 1999). Agents for the respiratory infection of camels may represent risk to camels, other livestock and even human population (Bardonnnet *et al.*, 2002; Teshome *et al.*, 2003).

Pneumonia is among the most important and commonly encountered disease of the camel. Despite low mortality and morbidity rates, the recovery period is quite long having negative impact on overall productivity. More fatal and highly contagious camel disease with respiratory syndrome has also occurred as a large scale outbreak during wet season (April to May) of 2007 in Borana areas. The disease resulted in disastrous losses causing 18% morbidity and over 50% mortality in affected herds (Bekele, 2010). Bekele (1999) described the outbreak of pneumonia in camels that involved camel herds from the Ogaden and Afar regions to Borana and southern part of Ethiopia causing up to 30% morbidity and 6.4% mortality.

## 2.3. Major upper respiratory tract diseases of camels and their pathology

### 2.3.1. *Nasal myiasis (nasal bots)*

Probably the most important of the flies, which causes myiasis is the nasal bot, *Cephalopina titillator*, a fly belonging to the family Oestridae of the order Diptera. The adult is short lived and rarely seen. The larvae, which hatch from eggs laid by the female fly in the camel's nostrils, are widespread, numerous and almost universally present in camel sinuses (Wilson, 1998). The maggots are white and about 35 mm long (LPPS, 2005). Later the emerging larvae migrate to the naso-pharynx. After completing their development, the larvae are usually removed by sneezing. The characteristic symptoms are bleeding from the nose, nasal discharge due to swelling and secondary infection of the respiratory tract and respiratory distress (Wilson, 1998).

#### Pathology

According to a study conducted by Bekele (1999), *Cephalopsis titillator* larvae were found lodged in the chambers of the pharynx in all the autopsied camels. The fly deposits the larvae of *C. titillator* in the nostrils which migrate to the nasal cavity of dromedaries causing irritations, heavy congestion, hemorrhages and meningitis (Moallin, 2009).

### 2.3.2. *Aspergillosis*

*Aspergillus fumigatus* is associated with respiratory system infections in livestock. It is an opportunistic fungus and has been recorded in alpacas and dromedaries (Gareis and Wernery, 1994). Genus *Aspergillus* is a wide spread fungi around the world. Among its types; *A. fumigatus* is the most pathogenic type causing diseases in human and animals. Elevated pathogenic manifestations of *A. fumigatus* belong to several factors; it's ability to grow faster than other types in a wide range of temperature (20-50) °C and it is highly sporelating fungus (Lacey, 1996).

The clinical signs displayed by affected animals were dyspnea, dullness and progressive weight loss (Fahad, 2014). This infection may be developed from the embedding of spores on the naso-pharynx mucosa and lower respiratory passages, and the granulomatous lesions are some what slow to develop and so not seen in acute cases, hence the dissemination of the fungus from the pulmonary lesions can occur (Jubb *et al.*, 1993).

## Pathology

The most striking necropsy findings were hemorrhages in most organs. Oedema and hemorrhages were present in the throat, submandibular, pharyngeal and laryngeal regions. Hemorrhages were always observed on the mucosa of the trachea and accompanied by frothy exudates. The surface of the lungs displayed large numbers of petechial and ecchymotic hemorrhages. Lymph nodes of the thoracic cavity were oedematous and hemorrhagic. Associated lymph nodes were severely congested and oedematous (Fahad, 2014).

Histopathological changes were numerous foci of necrosis in the lungs and liver (Severo *et al.*, 1989). The tracheal and bronchial mucosa revealed fibrinonecrotic membrane associated with vascular thrombosis of submucosal blood vessels. This finding was accompanied by lymphocytic and histolytic aggregations. Alternative areas of necrosis could be seen everywhere in lung tissue. In the submandibular, bronchial and mesenteric lymph nodes the subscapular sinuses showed marked lymphocytic aggregations associated with prominent vascular congestion (Fahad, 2014).

## **2.4. Major lower respiratory tract diseases of camels and their pathology**

Respiratory infections are quite common in camels and occur in two forms: acute and chronic forms. The chronic form is characterized with coughing while the acute form with nasal discharge. The most important and severe form is the acute respiratory infection, which has occurred in the form outbreak during the major wet season (Bekele, 2010). A number of microbial agents are involved as a primary or secondary infection of camel respiratory disease (Fekadu and Esayas, 2010). As it is mentioned above, pneumonia is among the most important and commonly encountered disease of the camel. Despite low mortality and morbidity rates, the recovery period is quite long having negative impact on overall productivity (Bekele, 2010).

### *2.4.1. Pneumonia*

Diseases of the respiratory tract, particularly pneumonia, were among the most important and commonly encountered diseases of camels (Abbas and Omer, 2005). Pneumonia or inflammation of the lungs can be caused by bacteria, viruses, foreign bodies or parasites (Al-Tarazi, 2001; Awol *et al.*, 2011). Outbreaks occur in camel, cattle, buffaloes and small ruminants in various countries (Schwartz, 1992). Housing of camels in unsheltered pens (Chauhan *et al.*, 1986; Abbas *et al.*, 2002) as well as the long trips undertaken by camels during the rainy season were major predisposing factors (Schwartz and Dioli, 1992; Agab and Abbas, 1999).

Pneumonias in domestic animals can be classified based on texture, distribution, appearance and exudation into four morphologically distinct types: bronchopneumonia, interstitial pneumonia, embolic pneumonia, and granulomatous pneumonia. By using this classification, it is possible to predict with some degree of certainty the likely causes (virus, bacteria, fungi, parasites) and routes of entry (aerogenous versus hematogenous). However, overlapping of these four types of pneumonias is possible, and sometimes two morphologic types may be present in the same lung (McGavin and Zachary, 2010).

#### 2.4.1.1. Bronchopneumonia

Bronchopneumonia refers to a particular type of pneumonia in which injury and the inflammatory process take place primarily in the bronchial, bronchiolar, and alveolar lumens (McGavin and Zachary, 2010). Bronchopneumonia is usually caused by bacterial or fungal infection (Jubb *et al.*, 2005). Bronchopneumonia is undoubtedly the most common type of pneumonia seen in domestic animals and is, with few exceptions, characterized by cranioventral consolidation of the lungs. The reason why bronchopneumonias in animals are almost always restricted to the cranioventral portions of the lungs is not 'well understood' (McGavin and Zachary, 2010).

#### Pathology

Lobular bronchopneumonia is generally less severe and more slowly progressive, and the angular outlines of individual lobules are visible at the margin of affected and normal lung, which have well developed interlobular septa, but the junction between affected and normal lung is less distinct. In contrast, in lobar pneumonia, the entire pulmonary lobe is consolidated. This is usually a fulminating bronchopneumonia, in many such cases, the pulmonary parenchymal lesions are accompanied by pleuritis; which may vary from a dull granular appearance to a spectacular coating of fibrin with large pockets of edema (Jubb *et al.*, 2005). In cases of fibrinous pneumonia, grossly, the lung appeared pale red, dark brownish-red, or grey in color (marbling) and hard in texture resembling liver (hepatized) (Khaled *et al.*, 2007).

Histologically, the nidus of inflammation in bronchopneumonia is in the bronchiolar-alveolar junction. In early bronchopneumonia, bronchioles and immediately adjacent alveoli are filled with neutrophils and sometimes cell debris, mucus, fibrin and macrophages, and the wall of the bronchiole may be edematous and infiltrated by neutrophils. By the time the bronchopneumonia is clinically apparent, the exudate has usually filled the bronchioles and alveoli, and it is often impossible to appreciate that the lesion is centered on the terminal airways. The bronchiolar epithelium in mild cases is usually normal; extensive bronchiolar

necrosis suggests an underlying viral infection. Suppurative or lymphoplasmacytic inflammation may be visible in the bronchi. The alveoli in mild or early lesions are atelectatic and edematous, but this is obscured by the suppurative infiltrate in severe lesions (Jubb *et al.*, 2005).

Specifically, suppurative bronchopneumonia, characterized by the presence of suppurative exudates in the lumen of bronchioles and peribronchiolar tissues with partial replacement of the bronchiolar wall. Pus appeared to be accumulated focally as multiple differently sized abscesses with necrosed centers surrounded by larger numbers of inflammatory cells (Gamal *et al.*, 2014).

In the early stages of fibrinous pneumonia, the interalveolar capillaries were dilated and engorged with blood. A transition stage which is the stage between stage of red and grey hepatization was observed. Advanced cases of this type of pneumonia was characterized by infiltration of a large number of polymorph nuclear leucocytes that may obliterate the alveolar lumens; many of these cells were disintegrated (stage of grey hepatization). The exudate may become organized into fibrous connective tissue or liquified by lysis of fibrin and leucocytes (stage of resolution) (Khaled *et al.*, 2007).

#### 2.4.1.2. Interstitial pneumonia

Interstitial pneumonia refers to a particular type of pneumonia in which injury and the inflammatory process take place primarily in any of the three layers of the alveolar walls (endothelium, basement membrane, and alveolar epithelium) and the contiguous bronchiolar interstitium. Most interstitial pneumonias in animals are infectious and caused by viruses, bacteria or parasites. In addition, pneumoconiosis can also cause interstitial pneumonia (Jubb *et al.*, 1993). This type of pneumonia is perhaps the most difficult to diagnose at necropsy and generally requires microscopic confirmation (McGavin and Zachary, 2010). The most commonly identified form of interstitial pneumonia is diffuse alveolar damage, which represents diffuse injury to type I pneumocytes or endothelial cells in the alveolar

septa, and results in formation of hyaline membranes, proliferation of type II pneumocytes, and interstitial fibrosis (Jubb *et al.*, 2005).

## Pathology

Gross lesions are distributed widely throughout the lungs, often with greater involvement of dorsocaudal regions. This pattern is in sharp contrast to the cranioventral distribution of lesions in most cases of bronchopneumonia. Histologically, most causes of diffuse alveolar damage follow a stereotyped pattern of response, with an acute exudative phase, subacute proliferative phase, and chronic fibrosing phase. In the acute exudative phase, the alveolar septa are congested and the alveoli contain protein-rich edema fluid with delicate interlacing fibrin strands and variable numbers of neutrophils and macrophages. However, the characteristic lesion is the presence of hyaline membranes, which are aggregates of fibrin, other serum proteins, and cell debris (Jubb *et al.*, 2005).

Lungs showed the signs of acute interstitial pneumonia characterized by the presence of edema and leucocytic cellular infiltration with congestion in peri-alveolar capillaries resulting in thickening of the interalveolar septa. Thickening in the interstitial tissues due to fibrous tissue proliferation infiltrated with lymphocytes and macrophages indicates chronic interstitial pneumonia (Gamal *et al.*, 2014).

Interstitial histiocytic pneumonia with increased proliferation of macrophages; the cells were large, polyhedral or rounded and had abundant cytoplasm and vesicular nucleus. Proliferation of this type of cells leads to increased thickening of alveolar septa and many were found in the alveolar lumen. The alveolar epithelial cells tend to become smaller and may be desquamated. The alveolar and bronchial lumens in these cases were filled with masses of cells either originating from epithelial lining or related to clusters of proliferating macrophages. Focal area of granulomatous reaction was observed with accumulation of a large number of macrophages which undergo caseous necrosis (Khaled *et al.*, 2007).

#### 2.4.2. Caseous lymphadenitis (*Corynebacterium pseudotuberculosis* infection)

Caseous lymphadenitis is a chronic disease in adult sheep and is worldwide in distribution. The disease is also of economic concern in adult camels (Hawari, 2008). Caseous lymphadenitis in dromedary camels is caused by *Corynebacterium pseudotuberculosis* (Hawari, 2008) and *Corynebacterium ulcerans* (Tejedor *et al.*, 2004). Other bacteria, including *Corynebacterium renali*, *Corynebacterium equi* and *Staphylococcus aureus* are isolated from abscesses (Hawari, 2008). The majority of cases are confined to the externally placed carcass lymph nodes (Tejedor *et al.*, 2004). Multiple large abscesses are also found in the internal organs, particularly the lungs (Hawari, 2008).

In Saudi Arabia (Radwan *et al.*, 1989), isolated *C. pseudotuberculosis* strain from eight camels with caseous lymphadenitis. Postmortem revealed emaciation and presence of multiple external and internal abscesses particularly in the lungs and liver. In United Arab Emirates (Afzal *et al.*, 1996) isolated *C. pseudotuberculosis* from eleven cases of lymphadenitis in camel. In Ethiopia, Domenech *et al.* (1977) reported a chronic condition locally called ‘mala’ in adult camels which resembled caseous lymphadenitis in small ruminants. In certain herds up to 15% of adult camels were found affected at one time. These camels showed progressive weakness, difficult breathing, paleness of mucous membranes, diarrhea, loss of appetite, emaciation and depression (Azmi, 2008).

#### Pathology

Multiple large abscesses were found in the lungs. The abscesses were encapsulated by a relatively thick layer of necrotic and fibrous tissues. They contained odorless, non-granular and non-calcified thin creamy yellowish white pus (and sometimes tinged with blood) (Azmi, 2008). *Corynebacterium* and mixed bacterial infections leading to formation of grayish white patches on the lung tissue with scanty amount of greenish white pus occluding the bronchi. Microscopically, suppurative bronchopneumonia was detected (Nagi *et al.*, 1997).

#### 2.4.3. *Pasteurellosis (hemorrhagic septicemia)*

Nasopharynx of apparently normal camels contains *Pasteurella multocida* as commensals, which reached there either through inhalation or during drinking. Stress factors like under nourishment, unsanitary environmental conditions and changes in the macro and micro climate lower the local immunity of the respiratory tract and the existing organisms like *P. multocida* get an opportunity to manifest the effects of their presence (Fraz, 2011). Although *Pasteurella multocida* is not a common respiratory tract pathogen among dromedary (Al-Tarazi, 2001) yet hemorrhagic septicemia is regarded as one of the five important camel diseases and economic losses incurred by it (Mochabo *et al.*, 2006). The disease was characterized by pyrexia (up to 107.4°F), severe dyspnea due to choking of nasal cavity with thick gummy material and abortion during late gestation (Khan *et al.*, 2011).

#### Pathology

Febrile carcasses showed congestion of all visceral organs and petechial hemorrhages on the serosal surfaces through out the body. Serosanguineous fluid was present in the thoracic and abdominal cavities. There was tracheal congestion, pneumonia with emphysema of borders of lobes and occlusion of nasal passage with thick gummy mucous. These findings indicated septicemia (Fraz, 2011).

#### 2.4.4. *Tuberculosis*

Tuberculosis is a serious chronic infectious disease of humans and animals worldwide (Thoen *et al.*, 2009; Ndukum *et al.*, 2010). Tuberculosis is rare among camels kept under nomadic conditions. It manifests itself particularly in the lungs and lymph nodes or other organs, with granulomas known as tubercles. The two most important members of the genus *Mycobacterium* are *Mycobacterium tuberculosis* and *Mycobacterium bovis* and atypical mycobacteria including *Mycobacterium kansasii*, *Mycobacterium aquae*, *Mycobacterium fortuitum* and *Mycobacterium smegmatis* have been isolated in the dromedary camel as causative agents of camel tuberculosis (Kinne *et al.*, 2006).

Recent studies in Ethiopian abattoirs reported isolation of *M. bovis* (Mamo *et al.*, 2011) and *M. tuberculosis* from tissue lesions (Zerom *et al.*, 2012). In camels, the difference in prevalence between male and female based on macroscopic tuberculous lesions was not statistically significant (Mammo *et al.*, 2009).

## Pathology

The organs most frequently affected in dromedary camels are the lungs, the bronchial and mediastinal lymph nodes and the pleura (Kinne *et al.*, 2006; Pate *et al.*, 2006). The lungs and mediastinal lymph nodes are the main target (Wernery *et al.*, 2007). One or both lungs are consolidated with solid abscesses of different shapes and sizes (Kinne *et al.*, 2006). The lesions appear more frequently in the apical and cardiac lobes of both lungs than in the diaphragmatic lobes (Mamo *et al.*, 2011). Areas of mineralization, solid abscesses and granulomatous lesions have been observed in the lungs (Alvarez *et al.*, 2012). Caseous foci in lung lymph nodes have also been described in camels infected with *Mycobacterium pinnipedii* (Huard *et al.*, 2006). Microscopical examination revealed granulomatous reaction with the presence of Langhans giant cells and caseated and calcified center (Nagi *et al.*, 1997). Some Ziehl-Neelsen staining of centrifuged sediments of the lesions smears demonstrated acid fast bacteria (AFB) (Ashenafi *et al.*, 2014).

### 2.4.5. Hydatidosis

Cystic echinococcosis (hydatidosis) is one of the most important parasitic zoonotic diseases in the world (Borji *et al.*, 2011). Both cystic hydatidosis (CE) caused by *Echinococcus granulosus* and alveolar echinococcosis (AE) caused by *Echinococcus multilocularis* have been reported in several countries (Omer *et al.*, 2010). All agents of CE fall under the name *E. granulosus* (Almeida *et al.*, 2007). Hydatidosis is of considerable economic and public health importance (Borji *et al.*, 2011).

It is one of the most important parasitic infections in livestock, particularly dromedary camels (Njoroge *et al.*, 2002; Mellau *et al.*, 2011).

The life cycle of *E. granulosus* involves domestic carnivores (dogs) and wild carnivores (jackals, hyenas, foxes and wolves) as definitive hosts. They are infested by the ingestion of offal containing the larval forms (hydatid cysts) with viable protoscoleces producing the adult stage in the intestine (Fathi *et al.*, 2011).

The camel has attracted much interest as an intermediate host (Fathi *et al.*, 2011; Ibrahim, 2010) because they seem to be an important reservoir for human infection (Eckert *et al.*, 1989). In Africa and Asia hydatid cysts are frequently found in camels (Wernery and Kaaden, 2002). The high prevalence of camel hydatidosis affecting different organs indicates the seriousness of this disease particularly in the area of the origin of these animals that require an immediate control intervention. From among affected organs, 40.39% of all the hydatid cysts found in lungs of camels (Bulto *et al.*, 2013).

### Pathology

Most hydatid cysts reside in the lung parenchyma. Grossly, hydatid cyst(s) were filled with fluid or semisolid contents. The cysts were embedded in the lung with no predilection with respect to the anatomical structure. The size of the cyst may reach several centimeters in diameter and their cross section revealed a thickened fleshy wall (Bekele, 2008).

Microscopically, embedded cysts were surrounded by compressed alveolar septa and fibrous connective tissue capsule. The wall of the cyst consisted externally of more or less a cellular homogenous laminated cuticular structure investing a layer of epithelial cells, the germinal layer. Fertile cysts contained protoscoleces in different stages of development. The cyst contents were generally clear but occasionally contained desintegrated materials. Mature protoscolces, probably originated from ruptured cysts, were found lying free in the alveolar lumens. Accumulations of mononuclear inflammatory cells focally surrounded the cyst; giant cells may occur in the lesion. The fibroblastic layer that surrounded the cyst may undergo calcification become metaplastic into chondrocyte (Khaled *et al.*, 2007).

#### 2.4.6. *Dictyocauliasis (verminous pneumonia)*

Small white round worms (*Dictyocaulus filaria* and *Dictyocaulus viviparus*) that are 5-10 cm long and live in the bronchi. They infect camels, sheep, goat, buffalo and cattle (LPPS, 2005). *Dictyocaulus filaria* is the nematode which is encountered most frequently in the respiratory tract of camels, having been found in Africa (Dakkak and Ouhelli, 1987). Sick animals cough out the worm eggs onto pasture and into drinking water. Camels become infected by feeding on egg infested pasture or infested water. It is more frequent after the rainy season. The main symptoms of the disease include: Coughing, open mouth breathing in later stages, reluctant to move, yellowish discharge from nostrils, the lips become itchy and camel rubs them on trees or walls, the eggs and worm larvae can be seen through microscope in the nasal discharge and in faeces (LPPS, 2005).

#### Pathology

Pathologically, dictyocauliasis is accompanied by an abundance of white and foamy mucus, perhaps mixed with pus. The nematodes become intertwined and form balls held together by mucus (Dakkak and Ouhelli, 1987).

#### 2.4.7. *Pulmonary leiomyoma*

Although neoplastic conditions are infrequently reported in camels (Ramadan, 1994), it is supposed that Camelids are susceptible to all the various tumor types that affect domestic animals (Bryant *et al.*, 2007). The classification of lung neoplasm is slightly more difficult due to the possibilities of epithelium and mesenchymal metaplasia and occurrence of the intermediate or mixed forms. Leiomyoma is a benign tumor of smooth muscle cell primarily arising from the musculature tubular and hollow organs, such as the gastrointestinal, urinary, and genital tracts (Mobini and Kufuor-Mensah, 1987). Leiomyoma is a rare disease though to be derived from uterine leiomyoma. Despite leiomyoma is histological benign tumor, it has the possibility to metastasize to distant sites such as to the lungs (Funakoshi *et al.*, 2004).

## Pathology

During post-slaughter inspection of dromedary camel (*Camelus dromedarius*), two cases of leiomyoma were described in an eight and ten years old male camels. The neoplastic masses involved in the visceral pleura of one camel and lung tissue of another. Histopathologic features revealed proliferated neoplastic cells, resembling the smooth muscle cells, in the pleura and adjacent pulmonary tissue. The tumor masses appeared as circumscribed area of interlacing bundles of smooth muscle cells arranged in various directions and surrounded by connective tissue capsule. Moreover, the adjacent alveoli exhibited areas of atelectasis and emphysema (Gamal and Shawky, 2013).

Bronchoalveolar adenocarcinoma was seen in the lung tissue in which tumour, the cells form sheats and glands, secrete mucin. The cells are tall, well-differentiated with basely located nuclei (Khaled *et al.*, 2007).

## 2.5. Common lower respiratory tract lesions of camels

Lung suffers from various pathological changes (Azwai *et al.*, 1996; Kinne *et al.*, 1998). Lungs showed features of acute bronchitis and bronchiolitis. In chronic stage, hyperplasia of the bronchial lining epithelium, peribronchial lymphocytic aggregation and fibrosis of the bronchial wall that may cover the bronchial lumen leading to bronchiolitis obliterans and lymphocytic, interstitial and bronchoalveolar pneumonia. Hydrothorax, adhesion of the lung to the thoracic cavity, with a thick layer of white fibrin in between, red and grey hepatization of the cut surface of the lung, and emphysema in the borders of the lung lobes were observed (Khaled *et al.*, 2007). According to Bekele (1999), for the different lesions there was no significant difference in distribution among male and female camels.

According to Awol *et al.* (2011), from all grossly examined lungs 77.5% possessed one or more types of gross lesion. The lungs were affected with conditions like hydatidosis, emphysema, atelectasis, aspiration of blood, pneumoconiosis, pulmonary edema and congestion. Pneumonia is one of the most important lesions (Azwai *et al.*, 1996; Kinne *et al.*, 1998). Other common lesions were pulmonary fibrosis (50.0%), pulmonary abscess (3.9%) and parasitic bronchopneumonia (0.96%). Among different lobes, the highest being seen in the caudal lobe (Teklu, 2008).

### 2.5.1. *Pneumoconiosis*

Pneumoconiosis is lung disease ensuing from inhalation and retention of inorganic dusts (Jubb *et al.*, 2005). In camels, pneumoconiosis in the form of deposition of fine dust particles and their phagocytosis by epithelium of alveolar septa and macrophages and their adherence to the internal surface of alveoli was observed rarely. This particle initiated a chronic reaction and induced metaplasia of the alveolar epithelium. Lung fibrosis and replacement of active pulmonary tissue by inactive fibrous connective tissue appeared to be the end result of many inflammatory reactions in their late stages (Khaled *et al.*, 2007).

## Pathology

Miliary lesions are distributed throughout the lung, and consist of extensive areas of fibrosis containing multifocal granulomatous infiltrates, often with necrosis and mineralization in the centers. Birefringent, eosinophilic or brown crystals are indistinctly visible in the cytoplasm of the macrophage (Jubb *et al.*, 2005). By using masson's trichrome stain that the collagen fiber appeared green while elastic fibers stained pink color. The alveoli were obliterated and the blood vessels were sclerosed with subsidence of all features of acute inflammation (Khaled *et al.*, 2007).

### 2.5.2. Pulmonary emphysema

Lung emphysema is an abnormal and permanent enlargement of air spaces distal to terminal bronchioles with destruction of their alveolar walls. Primary lung emphysema is rare in animals but extremely important in humans (Lopez, 2012). Emphysema may also be compensatory especially in alveoli occurring neighbouring of collapsed lung tissue (Anderson, 1983). Emphysema may result from exposure to dust particles that led to chronic bronchitis, post-inflammatory weakness and dilatation of bronchioles (Khaled *et al.*, 2007).

Most common types in domestic animals are: *Alveolar emphysema* which is difficult to evaluate in mild cases. It may require inserting a plug in the trachea during the necropsy and rapidly perfusing the lungs with fixative. *Interstitial emphysema* is rather common in bovines and characterized by distention of interlobular septa and pleura with gas. It is presumably caused by violent respiratory movements. *Bullous emphysema* is a large focal accumulation (pocket) of air (bullae) in the lung. Large bullae form when there is rupture of pulmonary tissue (Lopez, 2012). In camels emphysematous lungs were seen in the abattoir during postmortem examination (Awol *et al.*, 2011; Khaled *et al.*, 2007).

## Pathology

Emphysematous lungs are grossly voluminous, pale and puffy. When the lesion is diffuse, the lungs fill the thoracic cavity and may bear imprints of the ribs. The enlarged air spaces are often visible as small vesicles, and in severe cases coalescence of air spaces can produce large air-filled bullae. Emphysematous bullae occasionally rupture to cause fatal pneumothorax. Ruptured bullae are notoriously inconspicuous, and their detection is facilitated by submerging the lung in water or saline before inflating it with air, or by filling the trachea with saline to detect pleural leaks. Enlargement and coalescence of air spaces are apparent histologically (Jubb *et al.*, 2005).

### 2.5.3. Pulmonary atelectasis

Lung atelectasis is the failure of alveoli to open or the alveoli are collapsed and thus do not have air (Chauhan, 2010). In camels the most common causes of atelectasis in the lungs examined were blockage of a bronchus under the pressure of the parasitic cyst from outside or a significant amount of inflammatory exudate in the bronchial lumens (Khaled *et al.*, 2007).

## Pathology

Atelectatic lung is homogeneously dark-red and sunken relative to aerated lung, and the texture is fleshy or more firm and non-spongy than normal lung (Jubb *et al.*, 2005). In many cases of the camels, the lung tissue showed the presence of the features of pressure collapse (atelectasis) which was especially common in the neighborhood of parasitic (hydatid) cyst(s) and emphysematous tissue (Khaled *et al.*, 2007). Microscopically, simple atelectasis appears as slightly congested alveolar walls lying in close apposition with slit-like residual lumina having sharp angular ends. Atelectatic alveoli often contain scant edema fluid and excess alveolar macrophages. The edema may result from hypoxic damage, hypoxic vasoconstriction, or reduced surfactant activity (Jubb *et al.*, 2005).

#### 2.5.4. Pulmonary oedema

Pulmonary edema is a frequent complication of many diseases and is therefore one of the most commonly encountered pulmonary abnormalities. If severe, pulmonary edema has a catastrophic effect on lung function by reducing pulmonary compliance, blocking ventilation of the alveoli, obstructing gas exchange across the alveolar septa, and reducing the surface area of the air-liquid interface in the alveoli. In addition, proteins present in the edema fluid interfere with surfactant function, further reducing compliance and contributing to pulmonary dysfunction. Edema of the lung was, in many respects, similar to edema of other tissues, and is governed by the permeability of the vascular wall and by Starling forces-the balance of hydrostatic and osmotic pressures between the intravascular and interstitial compartments (Jubb *et al.*, 2005). Pulmonary oedema was commonly found in the collected lung specimens of camels (Awol *et al.*, 2011; Khaled *et al.*, 2007).

#### Pathology

On gross examination, edematous lungs are wet, heavy and do not collapse completely when the thorax is opened, and fluid oozes from the cut surface. Edema is prominent in the pleura and the pulmonary interstitium, and may form shallow pools in the hilus of the lung or the mediastinum (Jubb *et al.*, 2005). In the lungs of camels, oedema was associated with vascular dilatation and hemorrhages that varied from excessive extravascular accumulation of pale eosinophilic, homogenous fluid extending to the pulmonary interalveolar interstitium (Khaled *et al.*, 2007).

Histologically, edema fluid is acidophilic, homogeneous or faintly granular material filling alveoli except for occasional discrete holes that represent trapped air bubbles. The same material is often present in interstitial tissue and lymphatics around vessels and airways and in interlobular septa and subpleural zones in those species where these are well developed. Chronic edema is accompanied by a diffuse increase in the number of alveolar macrophages, and in heart failure these may contain phagocytosed erythrocytes or hemosiderin (Jubb *et al.*, 2005).

#### 2.5.5. Pulmonary hemorrhage

Pulmonary hemorrhages vary from petechiation to massive filling of large regions by blood. They occur frequently in the hemorrhagic diatheses, septicemias, disseminated intravascular coagulation and severe congestion. They can also be caused by vasculitis, infarction, ruptured aneurysms, trauma, hemangiosarcoma, tumors that have undergone necrosis, or drug reactions. Aspiration of blood is frequent at slaughter, and has a characteristic pattern of multiple, small, bright-red foci with feathery or indistinct borders. Abscesses that erode large blood vessels may cause massive hemorrhage. Affected animals may be found dead with blood flowing from the nares (Jubb *et al.*, 2005).

#### Pathology

The hemorrhage occurs in the dorsocaudal portions of both caudal lobes, and appears as red, brown or grey discoloration of the lung or as patchy, blue-brown subpleural foci. It is usually possible to distinguish hemorrhage from congestion based on the gross appearance: hemorrhages are usually multifocal or patchy, whereas congestion is diffuse within the affected region of the lung (Lopez, 2012).

Histologically, the main features in affected regions are hemosiderin-laden macrophages in airspaces and in the interstitium, alveolar septal fibrosis, and mild bronchiolitis and bronchiolar fibrosis. These changes are the result of intra-alveolar hemorrhage rather than the cause, and can be created by intrapulmonary injection of autologous blood (Jubb *et al.*, 2005).

#### 2.5.6. Hyperemia and congestion

Hyperemia is an active process that is part of acute inflammation, whereas congestion is the passive process resulting from decreased outflow of venous blood, as occurs in congestive heart failure. In the early acute stages of pneumonia, the lungs appear notably red,

and microscopically, blood vessels and capillaries are engorged with blood from hyperemia (McGavin and Zachary, 2010).

Pulmonary congestion is most frequently caused by heart failure, which results in stagnation of blood in pulmonary vessels, leading to edema and egression of erythrocytes into the alveolar spaces. It can progress to lung edema and intra-alveolar hemorrhagewith erythrophagocytosis ("heart failure cells"). "Wet and heavy lungs" have red patchy discoloration (Lopez, 2012).

### Pathology

As with any other foreign particle, erythrocytes in alveolar spaces are rapidly phagocytosed by pulmonary alveolar macrophages. When extravasation of erythrocytes is severe, large numbers of macrophages with brown cytoplasm may accumulate in the bronchoalveolar spaces. The brown cytoplasm is the result of accumulation of considerable amounts of hemosiderin; these macrophages filled with iron pigment (siderophages) are generally referred to as heart failure cells. The lungs of animals with chronic heart failure usually have a patchy red appearance with foci of brown discoloration because of accumulated hemosiderin (McGavin and Zachary, 2010).

#### *2.5.7. Pulmonary calcification ("calcinosis")*

Calcification is the deposition of calcium phosphates and calcium carbonates in soft tissues other than bones and teeth. It may be classified as dystrophic and metastatic calcification (Chahuan, 2010). Metastatic calcification of the lungs occurs in some hypercalcemic states, generally secondary to hypervitaminosis D or from ingestion of toxic (hypercalcemic) plants, such as *Solanum malacoxylon* that contain vitamin D analogs (McGavin and Zachary, 2010). Dystrophic calcification is characterized by the deposits of calcium salts in necrosed tissue of any organ. It can occur due to parasitic infections or tuberculosis lesions (Chahuan, 2010).

## Pathology

Calcified lungs may fail to collapse when the thoracic cavity is opened and have a characteristic "gritty" texture (McGavin and Zachary, 2010). Organ becomes hard, nodular. Gray-white deposits in necrosed tissue looking like honey comb. Gritty sound on cutting. Microscopically, lesions vary from calcification of the alveolar basement membranes to heterotopic ossification of the lungs. Irregular deposits of calcium salts in necrosed tissue and calcium takes black/purplish colour on H&E staining (Chahuan, 2010).

## 2.6. Common bacterial isolates from pneumonic lungs of camels

A number of studies proved that different bacterial agents were isolated from lung tissue samples of camels. According to the findings of Al-Rawashdeh *et al.* (2000) indicated that 10% of slaughtered camels had bacterial pneumonia among which *Mannheimia haemolytica* was found the most isolates (56%) from lung tissue, whereas 6.66% was reported by Al-Tarazi (2001). *Mannheimia haemolytica* and *Pseudomonas aeruginosa* were the most frequent isolates from cases of chronic proliferative bronchopneumonia and chronic pleuropneumonia, while *Escherichia coli* and *Klebsiella* spp. were the most frequent isolates from cases of interstitial pneumonia (Al-Tarazi, 2001).

On the other hand, different *Mycoplasma* species were isolated from lungs of camels with lesions. There is very little information in the literature with regards to the role played by mycoplasmas in the aetiology of pneumonia in camels (Wernery and Kaaden, 2002). Elfaki *et al.* (2002) isolated *Mycoplasma arginini* along with several other bacteria from 8.8% of camel lungs with lesions suggestive of chronic interstitial pneumonia. The pathogenic role of this mycoplasmal isolate was not confirmed by other methods, and its involvement in respiratory tract disease of camels remains doubtful (Bekele, 2010).

In Ethiopia few works have been done on camel respiratory diseases and *Mannheimia haemolytica* in the lung tissue seems to be low in number. However, in most active respiratory disease problem *M. haemolytica* is the predominant isolates (Samuel, 2008). Shemsedin (2002) reported that 8.7% of the total bacterial isolates from lung tissue were *Pasteurella haemolytica* (Fekadu and Esayas, 2010). On the other hand, a total of 54 bacterial species were isolated and identified from 50 of the pneumonic lung samples. Among which the main ones included were *coagulase negative staphylococci* (21.1%), *Streptococcus* spp. (19.3%), *Escherichia coli* (17.5%), *Francisella tularensis* (5.3%), *Flavobacterium* spp. (5.3%), *Rhodococcus equi* (5.3%) and *Bordetella bronchoseptica* (3.5%) (Awol *et al.*, 2011).

## 2.7. Common viral isolates from respiratory infections of camels

Camels are subjected to infection by various types of viruses (Kinne *et al.*, 1998). Virus isolation in tissue culture is one of the standard techniques in virological work although it is time consuming for some viruses. Anderson (1999) stated that even when diagnosis of peste des petits ruminants virus (PPRV) has been carried out by rapid technique like ELISA or AGID, for further studies the virus should always be isolated from field samples in tissue culture. Taylor (1979) reported that PPRV may be isolated in primary lamb kidney cells or VERO cell tissue cultures. CPE of PPRV was described by many authors (El Hag and Taylor, 1984; Abu El Zain *et al.*, 1990). Abraham *et al.* (2005) have recorded PPRV antibodies in 3% of Ethiopian camels. However, a clinical disease in camels due to PPRV had not yet been established.

Several authors have reported high antibody titers against numerous respiratory tract viruses (Roger *et al.*, 2001). *Respiratory syncytial virus (RSV)* is one of the well-known causes of respiratory infection in human and various animal species (Murphy *et al.*, 1999). Intisar *et al.* (2010) confirmed RSV infection from the lung specimens of Sudan camels by cell culture, RT-PCR and serological methods (Intisar *et al.*, 2013). Gelagay *et al.* (2013) indicated also the involvement of RSV and *Adenovirus* as causative agents for camel respiratory disease in Ethiopia.

*Parainfluenza virus 3 (PIV3)* is one of the viruses known to cause respiratory infection. According to Kebede and Gelaye (2010), PIV3 was found as the most frequent isolated virus of the camel respiratory disease outbreak in Ethiopia. The detection and isolation of PIV3 using haemo-agglutination inhibition method from imported Djiboutian camels in Egypt were reported for the first time by Nawal *et al.* (2003). Similarly, Shaker (2003) reported about isolation of PIV3 from camel lungs in Egypt. Intisar *et al.* (2009) isolated PIV3 from camel lung specimens in MDBK cell cultures observing the typical CPE of PIV3.

## **2.8. Physiological particularities of the respiratory system of the Dromedary**

In camels the nasal system is characterized by a full nasal cavity and well developed nasal glands. The sinus is subdivided in several furrows. Nostrils are able to close completely, thus avoiding draining of mucous membrane, and maintain wet atmosphere, which limits the water losses in the upper respiratory tracts. Such anatomy allows the dromedary recover water at expiration by nasal way. The diaphragm is powerful and partly ossified. The respiratory frequency varies from 9 to 2 cycles/min and respiration is markedly abdominal (Faye, 1997).

Under severe heat stress, the camel does not pant. The respiratory rate decreases in the dehydrated dromedary with an increase of the partial blood pressure of carbon dioxide and reduction in that of oxygen. The nasal passageways are cooled by the inhaled air that flows across the surfaces in the nose. Although this process is basic, it is important in the camel ability to retain water that would normally be lost to evaporation under high temperatures (Yagil, 1985; Wilson, 1989).

When the camel is dehydrated, the nasal passages exhibit hygroscopic characteristics. A camel's hygroscopic surface will absorb water from air that passes by it. The process that allows the camel to uptake water vapor is very similar to the process of nasal heat exchange. However, the difference is that water vapor is given off and then absorbed instead of heat. When dry inhaled air passes over the nasal passages, water is given off, and during exhalation, water is taken up by the then dry nasal passages (Schmidt-Nielsen *et al.*, 1981).

### **3. MATERIALS AND METHODS**

#### **3.1. Study area**

The study was conducted from December, 2014 to April, 2015 at Addis Ababa Akaki Abattoir. Akaki is located at 8° 9' N Latitude and 38° 8' E longitude, 27 km South of Addis Ababa (NMSA, 2000). Camels that were slaughtered at this abattoir during this study were originated from the Menjar, Borana and Metehara areas of Ethiopia.

Menjar Shenkora is one of the weredas in the Amahara Region of Ethiopia, located at the southern end of the Semien Shewa Zone. Menjar Shenkora is bordered on the east, south and west by the Oromia Region and on the northwest by Hagera Mariam. Based on the 2007 national census conducted by the CSA of Ethiopia, this wereda has a total population of 128,879 and the wereda is known with its scattered bushes, shrubs and acacia trees. The area receives 800-1000mm annual rainfall (LPAR, 2007).

Borana is located at approximately 600 km South of Addis Ababa between 03037' 23.8" to 050 02' 52.4" North and 370 56' 49.4" to 390 01' 101" East, in the Southern part of Ethiopia (Figure 1). The altitude ranges from 970 masl in the south bordering Kenya to 1693 masl in the Northeast. It borders republic of Kenya to the south, Somali Regional State to the east and, Southern Nation and Nationalities Regional State to the west and Gujji Zone to the North. The climate of Borana is semiarid. Animal husbandry in this region is characterized by extensive pastoral productions system and seasonal mobility to relatively better areas where green fodder is available. As aridity increases, the principal stock shifts gradually from cattle combined with small stock to camels combined with small stock, with a relative degree of the social and cultural values accounting for differences (Demeke, 1998).

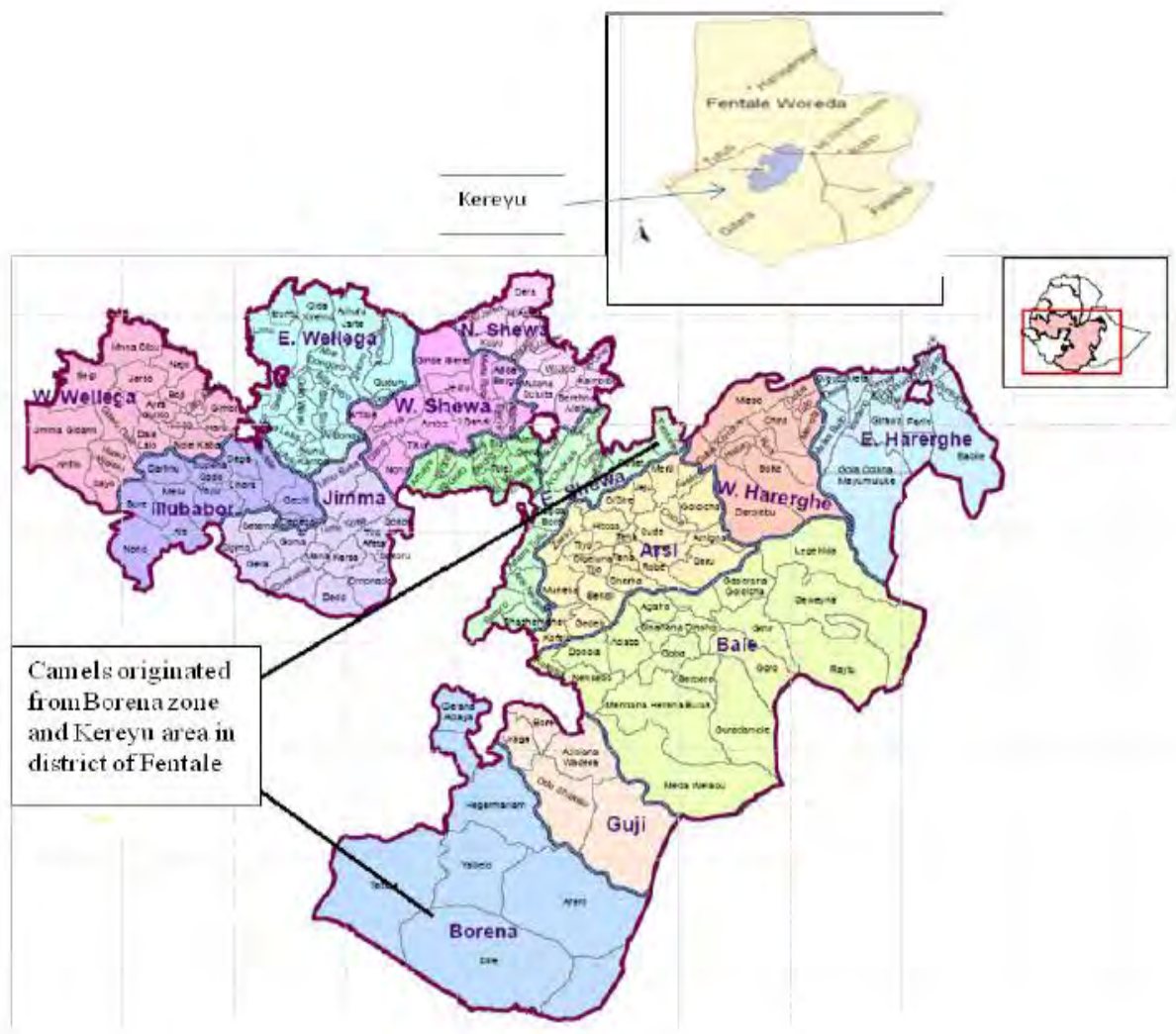


Figure 1: Zones of Oromia regional state of Ethiopia, showing main origins (Borana and Kereyu) of camels slaughtered at Addis Ababa Akaki Abattoir (Aboma *et al.*, 2015).

The Kereyu Pastoral area is located at about 250 km East of Addis Ababa at an altitude of 930 masl. The prevailing climate in Kereyu area is arid (NMSA, 1999). Pastoralism and agro-pastoralism are the main livelihood systems in the area. Fentale mountain, Awash, Kesem, Burka, Lake Beseka rivers and Metahara town are located in East Shoa zone of Oromia Region (Figure 1). Shoats, cattle and camels are the livestock species found in the area (Abule, 2004). The district is affected by recurrent droughts due to disrupted rainfall patterns (CSA, 2000). It falls in a semi-arid area, and receives an annual rainfall ranging from 400 to 700 mm. Temperature ranges from 29 to 38°C (Abule, 2004).

### **3.2. Study animals**

The study was conducted on 207 camels that were slaughtered at Addis Ababa Akaki Abattoir. Of which 201 camels were originated from Borana, 4 camels were from Metahara and the rest 2 camels were from Menjar areas of Ethiopia. The study animals comprised of 157 female and 50 male camels, their age ranged from 7 to 10 years (Table 5) and all of them appeared healthy during pre-slaughter inspection. The camels were transported from their origin to the abattoir by trucks and kept at lairage for 3 to 4 days.

### **3.3. Study design and sample size**

Non probability sampling with a purposive inclusion of the study animals was conducted. Ante-mortem examination was done to all camels at the lairage during the visit day. After slaughter, all camels with visible gross respiratory lesions were sampled. Accordingly, 53 camels with gross lesions in their respiratory system were sampled from a total of 207 camels examined by ante mortem during the 60 days of visits to the abattoir.

### **3.4. Sampling techniques and transportations**

Before slaughter, all camels at the lairage were physically examined for any abnormalities shortly prior to slaughter. Inspection of the camels was made while at rest or while in motion for any obvious sign of disease. Origin, sex and age (based on dentitions) were recorded according to Bello *et al.* (2013) (Annex 9). Post-slaughter examination was conducted using the routine methods namely visual examination, thorough palpation and incision (Taiwo, 2005). The respiratory tracts (from the laryngeal cartilage up to the lungs and associated lymph nodes) were removed and taken to one corner of the abattoir for gross pathological examination. Gross lesions were recorded and photographed.

A total of 53 lung tissues with visible gross lesions were sampled separately for histopathology, bacterial isolation and viral detection. For histopathology, about 1 cm<sup>3</sup> of the lung tissue with gross lesion was collected into 10% neutral buffered formalin (Sheehan and Harpachak, 1980) and then transported to the NAHDIC.

According to Quinn *et al.* (2002), about 4cm<sup>3</sup> tissue, particularly with active lesion at the boundary was collected aseptically using sterile forceps, scissors and scalpel blade and placed into the sterile screw capped universal bottles, labeled and transported to the NAHDIC on ice box containing ice packs for bacterial isolation.

About 6 gm of lung tissue with lesion was aseptically/carefully collected using sterile forceps, scissors and scalpel blade into labeled sterile glass tube with phosphate buffered saline solution (PBS) with antibiotics and the tube was placed in an ice box containing ice packs and transported to the NVI for viral cultivation. Fresh tissue samples were freezed at -85°C until testing (Gelagay *et al.*, 2013).

### **3.5. Sample processing**

#### *3.5.1. Histopathology*

After proper fixation, 30 representative lung tissues with different lesion types were trimmed and dehydrated in ascending grades of ethyl alcohol, cleared in xylene, imprgnated by molten paraffin wax and embedded. Thin sections of the tissues, about 4-5 micro metres in thickness, were prepared and stained with Haematoxylin and Eosin (Kul and Yildiz, 2010).

#### *3.5.2. Bacterial culturing techniques*

The surface of lung tissues with gross lesions were seared by hot scalpel blade, cuts were made using sterile scalpel blade and forceps and pieces of tissue were taken from the inner part of cuts and minced. The minced tissues were inoculated into tryptic soya broth and

incubated at 37°C for 24 hrs. After 24 hrs, loopful of the broth were streaked onto blood agar with 5-7 % sheep blood agar and incubated at 37°C for 24 to 48 hrs depending on the growth of bacteria (Quinn *et al.*, 1999).

After proper incubation, growth characteristics were examined. Colony color, size, presence or absence of hemolysis, pigment production, colony edges like round or smooth and colony shape like raised or flat were recorded. Smears were made from representative colonies, gram stained and bacterial morphologies were recorded. Cultures which showed different bacterial colonies (mixed colonies) were sub-cultured on blood agar and nutrient agar and further incubated for 24 hrs to get pure bacterial colony. Further characterizations and identification of the grown bacteria were done using the conventional biochemical tests including catalase, oxidase, fermentative-oxidative (OF), motility, growth on mackonkey agar, coagulase, aesculin hydrolysis, indole, urease, mannitol, citrate, glucose, sucrose, lactose, maltose, hydrogen sulphide gas and Methyl red/MRVP tests (Quinn *et al.*, 1999; Betty *et al.*, 2007) (Annex 8). Respective results were recorded using the format developed (Annex 3).

### 3.5.3. *Viral culturing techniques*

Twenty five lung tissue samples with lesions were processed and grown on monolayer of VERO cell in flasks. Briefly, 1 gm of each sample of lung tissue was washed three times using sterile PBS on Petri dish, and then washed tissue was transferred to sterile mortar and cut into small pieces using scissor and minced by sterile scalpel blade. The minced tissues were ground and homogenized using pestle. Nine ml of PBS was added to the prepared lung tissues and well mixed. The homogenized tissues were transferred to test tube and centrifuged at 3400 rpm for 10 min and 0.5 ml of the supernatant was inoculated on the confluent VERO cells and incubated at 37°C for 1 hr. Following incubation, the inoculated cell lines were washed using PBS and 10ml complete Glasgow Minimum Essential Medium (GMEM) was added and incubated at 37°C to follow-up the development of cytopathogenic effect (CPE) (Gelagay *et al.*, 2013).

### **3.6. Data analysis**

All generated data were entered in Microsoft Excel 2010. The data were analyzed using Statistical Package for Social Sciences (SPSS) software version 20 and descriptive statistics like percentage and frequency distribution were used to determine the proportion of the gross and histopathological lesions, bacterial isolates and viral detection. chi-square test was used to study if there is association for the occurrence of lesions in different lung lobes, age groups, sex and also to study association between lesions and bacterial isolation. The significance level was set at 95%.

## 4. RESULTS

### 4.1. Pathological changes encountered and frequencies

During the postmortem examination of the respiratory tracts, 3 camels had white-foamy fluid in conducting portion of upper respiratory tracts starting from laryngeal cartilage to the endings of the bronchi. Ten camels were observed with freely crawling *Cephalopina titillator* larvae coming out through their nostrils soon after slaughter (Figure 2).

From the 207 lungs examined, 53 (25.6%) had one or more gross lesions. The lesions were observed in the lungs of 14 (6.8%) male and 39 (18.8%) female camels. The distribution of gross lesions did not show significantly difference ( $P>0.05$ ) between male and female camels (Table 1). However, distribution of gross lesions varied significantly ( $P<0.05$ ) among different age groups, lung lobes and between right and left lungs (Tables 2, 3 and 4). The right lung and the caudal lobes were the most affected regions. Interstitial pneumonia 17 (8.2%), pulmonary emphysema 12 (5.8%), bronchopneumonia 11 (5.3%) and pulmonary atelectasis 5 (2.4%) were among the more frequent lesions observed in this study (Table 5).

Table 1: Distribution of pulmonary lesions in different lung lobes of male and female camels

Lung lobes	Sex		Total	P-value	$\chi^2$ - value
	Male	Female			
Cranial	3 (1.4%)	11 (5.3%)	14 (6.8%)	0.880	0.670
Caudal	6 (2.9%)	19 (9.2%)	25 (12.1%)		
All lobes	5 (2.4%)	9 (4.3%)	14 (6.8%)		
No lesion	36 (17.4%)	118 (57.0%)	154 (74.4%)		
Total	50 (24.2%)	157 (75.8%)	207 (100.0%)		

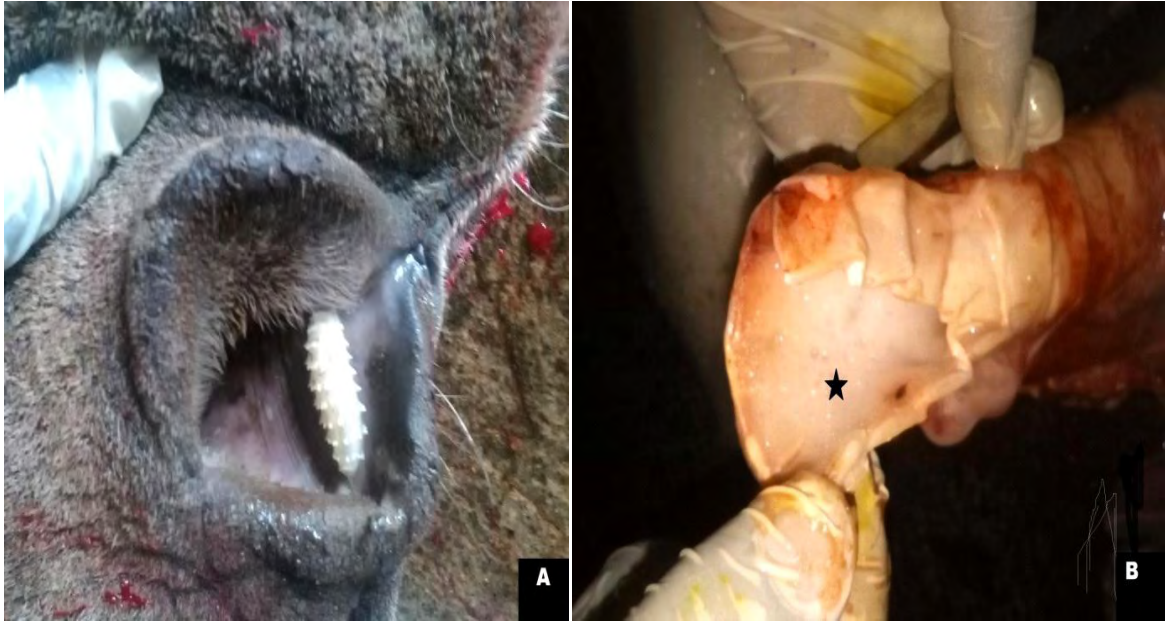


Figure 2: A slaughtered camel with white and actively crawling *Cephalopina titillator* larvae coming out through its nostril (A). Laryngeal cartilage filled with foamy fluid (shown by star) (B).

Table 2: Pulmonary lesions distribution in lung lobes of different camel age groups

Lung lobes	Age groups			Total	P-value	$\chi^2$ - value
	7 years	8-9 years	10 years			
Cranial	7 (3.4%)	7 (3.4%)	0 (0.0%)	14 (6.8%)		
Caudal	9 (4.3%)	16 (7.7%)	0 (0.0%)	25 (12.1%)		
All lobes	6 (2.9%)	7 (3.4%)	1 (0.5%)	14 (6.8%)	0.007	17.586
No lesion	58 (28.0%)	55 (26.6%)	41 (19.8%)	154 (74.4%)		
Total	80 (38.6%)	85 (41.1%)	42 (20.3%)	207 (100.0%)		

Table 3: Association of pulmonary lesions occurrence between right and left lungs in different lobes

Sides of lungs	Lung lobes					P-value	$\chi^2$ -value
	Cranial	Caudal	All lobes	No lesion	Total		
Right lung	8 (3.9%)	11 (5.3%)	5 (2.4%)	0 (0.0%)	24 (11.6%)	0.001	213.746
Left lung	3 (1.4%)	6 (2.9%)	5 (2.4%)	0 (0.0%)	14 (6.8%)		
Both	3 (1.4%)	8 (3.9%)	4 (1.9%)	0 (0.0%)	15 (7.2%)		
None	0 (0.0%)	0 (0.0%)	0 (0.0%)	154 (74.4%)	154 (74.4%)		
Total	14 (6.8%)	25 (12.1%)	14 (6.8%)	154 (74.4%)	207 (100.0%)		

Table 4: Distribution of pulmonary lesions in different lung lobes

Types of pulmonary lesions	Lung lobes				Total	P-value	$\chi^2$ -value
	Cranial	Caudal	All lobes	No lesion			
Interstitial pneumonia	2 (1.0%)	11 (5.3%)	4 (1.9%)	0 (0.0%)	17 (8.2%)	0.000	283.268
Pulmonary emphysema	8 (3.9%)	2 (1.0%)	2 (1.0%)	0 (0.0%)	12 (5.8%)		
Bronchopneumonia	2 (1.0%)	6 (2.9%)	3 (1.4%)	0 (0.0%)	11 (5.3%)		
Pulmonary atelectasis	1 (0.5%)	2 (1.0%)	2 (1.0%)	0 (0.0%)	5 (2.4%)		
Calcified nodules	0 (0.0%)	1 (0.5%)	1 (0.5%)	0 (0.0%)	2 (1.0%)		
Pulmonary haemorrhage	1 (0.5%)	0 (0.0%)	1 (0.5%)	0 (0.0%)	2 (1.0%)		
Hydatid cysts	0 (0.0%)	2 (1.0%)	0 (0.0%)	0 (0.0%)	2 (1.0%)		
Pulmonary oedema	0 (0.0%)	1 (0.5%)	1 (0.5%)	0 (0.0%)	2 (1.0%)		
Total	14 (6.8%)	25 (12.1%)	14 (6.8%)	154 (74.4%)	207 (100.0%)		

Table 5: Frequency and percentage of pulmonary lesions between different age groups and sexes

Pulmonary lesions	Proportion	Sex		Age group (years)		
		Male	Female	7	8-9	10
Interstitial pneumonia	17 ( 8.2% )	3 (1.5%)	14 (6.8%)	15 (7.2%)	2 (1.0%)	0 (0.0%)
Pulmonary emphysema	12 ( 5.8% )	7 (3.4%)	5 (2.4%)	4 (1.9%)	8 (3.7%)	0 (0.0%)
Bronchopneumonia	11 ( 5.3% )	3 (1.4%)	8 (3.9%)	1 (0.5%)	9 (4.4%)	1 (0.5%)
Pulmonary atelectasis	5 ( 2.4% )	0 (0.0%)	5 (2.4%)	1 (0.5%)	4 (1.93%)	0 (0.0%)
Calcified nodules	2 (1.0%)	0 (0.0%)	2 (1.0%)	0 (0.0%)	2 (1.0%)	0 (0.0%)
Pulmonary haemorrhages	2 (1.0%)	0 (0.0%)	2 (1.0%)	1 (0.5%)	1 (0.5%)	0 (0.0%)
Hydatid cysts	2 (1.0%)	0 (0.0%)	2 (1.0%)	0 (0.0%)	2 (1.0%)	0 (0.0%)
Pulmonary oedema	2 (1.0%)	2 (1.0%)	0 (0.0%)	0 (0.0%)	2 (1.0%)	0 (0.0%)
Total	53 (25.6%)	15 (7.2%)	38 (18.3%)	22 (10.6%)	30 (14.5%)	1 (0.5%)

#### 4.1.1. Interstitial pneumonia

Interstitial pneumonia was observed in 17/207 (8.2%) lungs. Camels of 7 years old were the most commonly affected with frequency and percentage of 15/207 (7.2%) while camels of 8-9 and 10 years old had low frequency and percentage of 2/207 (1.0%) and 0 (0.0%), respectively (Table 5). Grossly, lungs with interstitial pneumonia had diffused lesion distribution that widely affected dorsocaudal regions of the lungs. Notably, the affected lungs had dark-red color, elastic and rubbery in consistency and they were meaty in appearance (Figure 3A). Three of these lungs showed characteristic indicator of interstitial pneumonia, the ribimprint on their surfaces (Figure 3B).

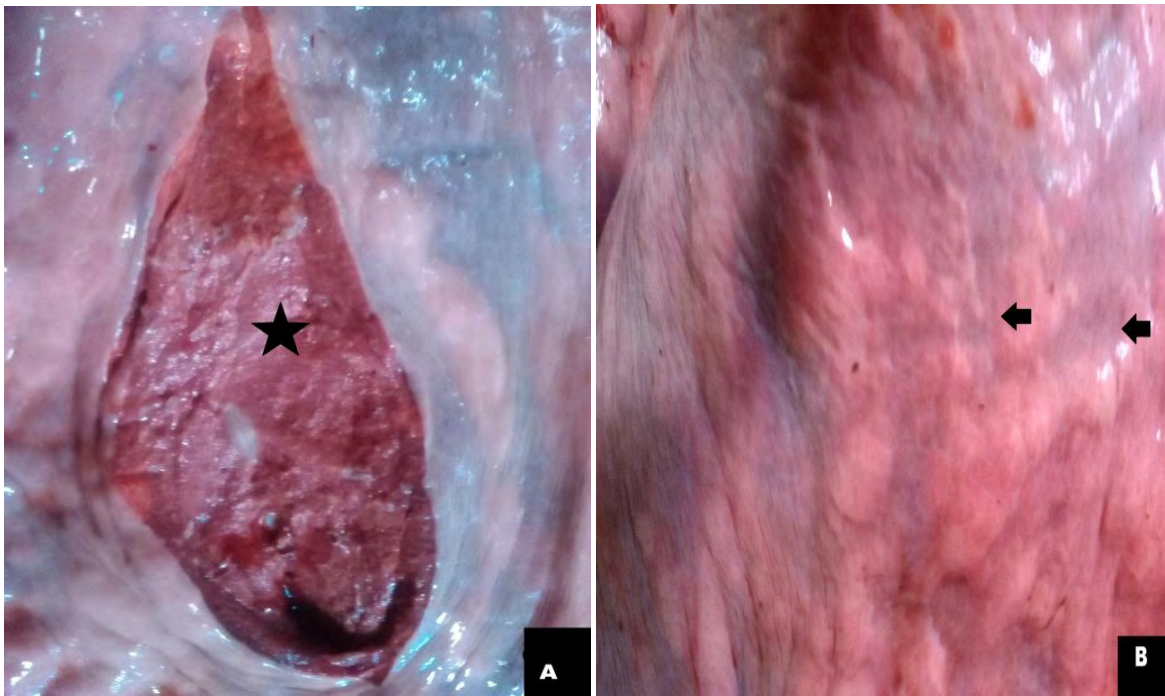


Figure 3: Lung with ‘meaty’ appearance on cut surface (star) (A) and rib imprints (arrows) on the surface (B), both of which are characteristic indicator of interstitial pneumonia.

Histologically, lungs with interstitial pneumonias showed thickened interstitial tissues. In three lung tissue sections, the capillaries of the alveolar septae were congested and there were cellular proliferations and interstitial infiltrations with mononuclear cells which indicate chronic interstitial pneumonia (Figure 4). In four lung tissue sections, the alveolar lumen contained eosinophilic edema fluid and thick eosinophilic hyaline membranes lined in the alveoli. There were infiltrated neutrophils in the interstitium, which indicate acute interstitial pneumonia (Figure 5).

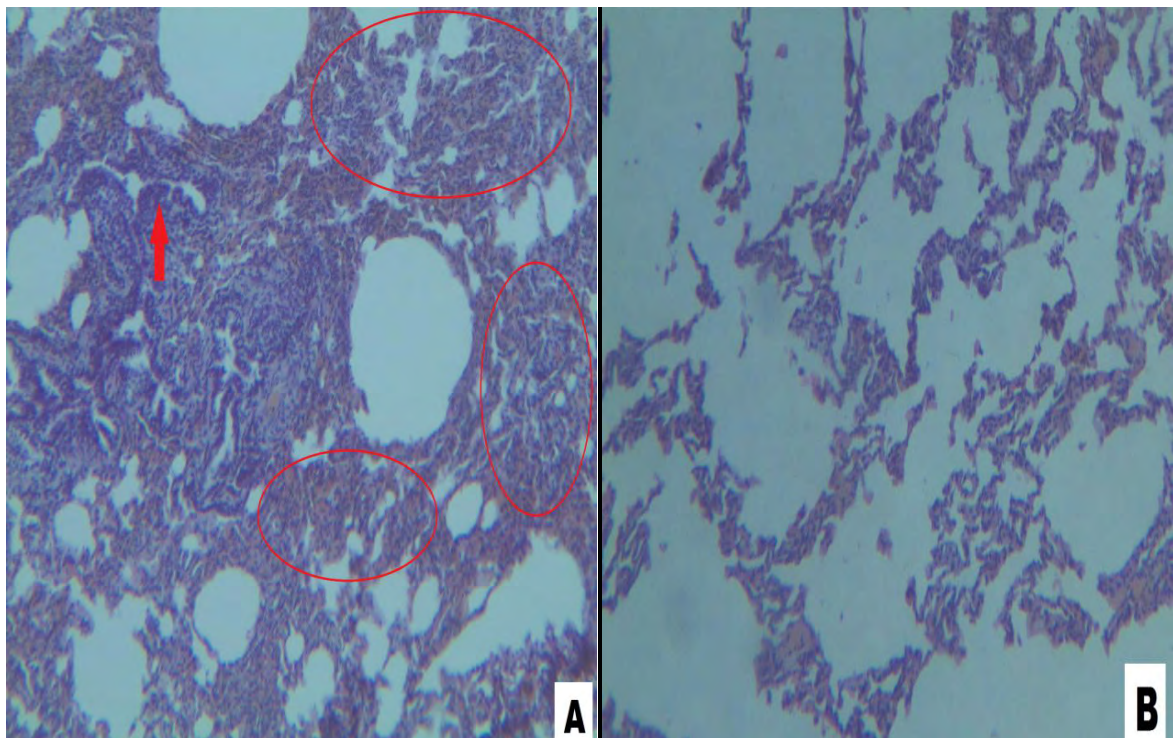


Figure 1: Thick alveolar septae with cellular proliferation and interstitial infiltrations of lymphocytes and macrophages and with narrow bronchiolar lumen (arrow) (A). Thick and congested alveolar septae due to interstitial pneumonia (B) (H&E, X10).

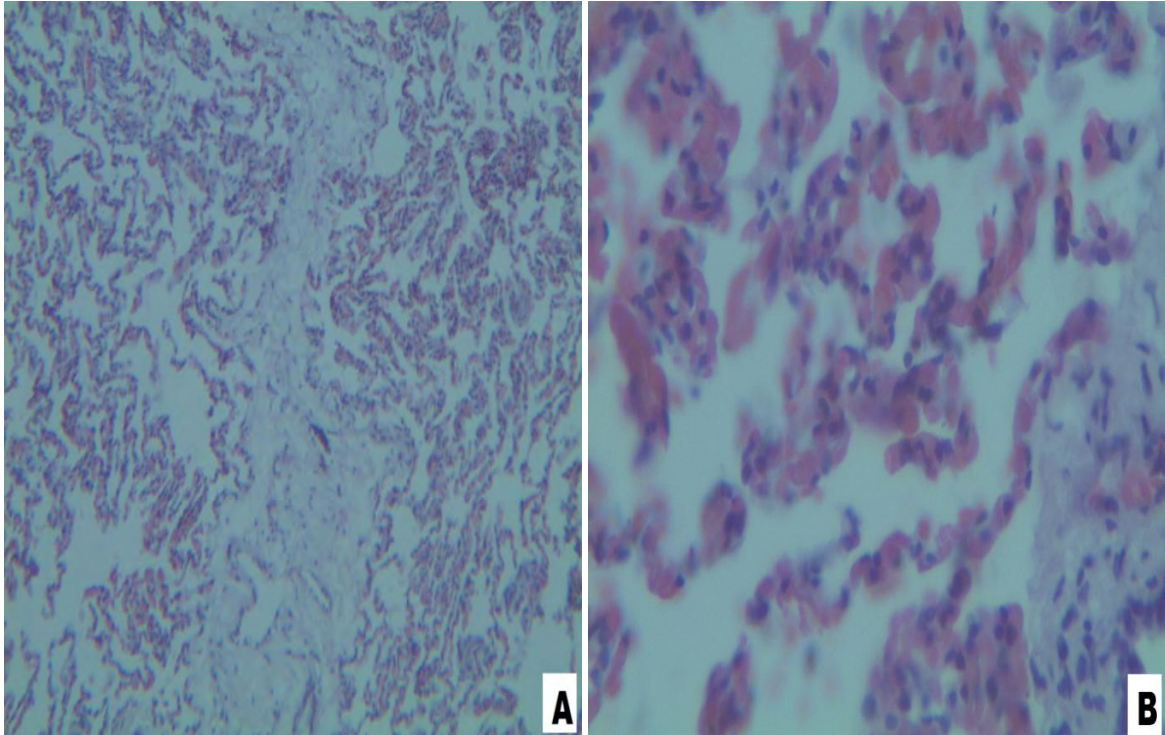


Figure 2: Thick alveolar septae, congested alveolar capillaries, edema fluid in central areas (A) and in left-down areas (B) (H&E, X10 and X40 respectively).

#### *4.1.2. Pulmonary emphysema*

There were 12/207 (5.8%) lungs observed with emphysema, of which, 8 lungs were from the camels of 8-9 years old (Table 5). Gross lesions including pulmonary emphysema varied significantly ( $P < 0.05$ ) among different age groups. Three lungs showed generalized emphysema which grossly looked relatively large in size, pale in color and were puffy when pressed with fingers (Figure 6A). When incised, the size (volume) appeared decreasing with no exudate coming out. Seven lungs had large focal emphysematous bullae (air filled pockets) (Figure 6B), three of them had cranioventral consolidation in some regions of their cranial lobes. Cranial lobes of two lungs were divided into bullous alveolar emphysema, with air bubbles on the affected surface and they were criptus on palpation (Figure 7).

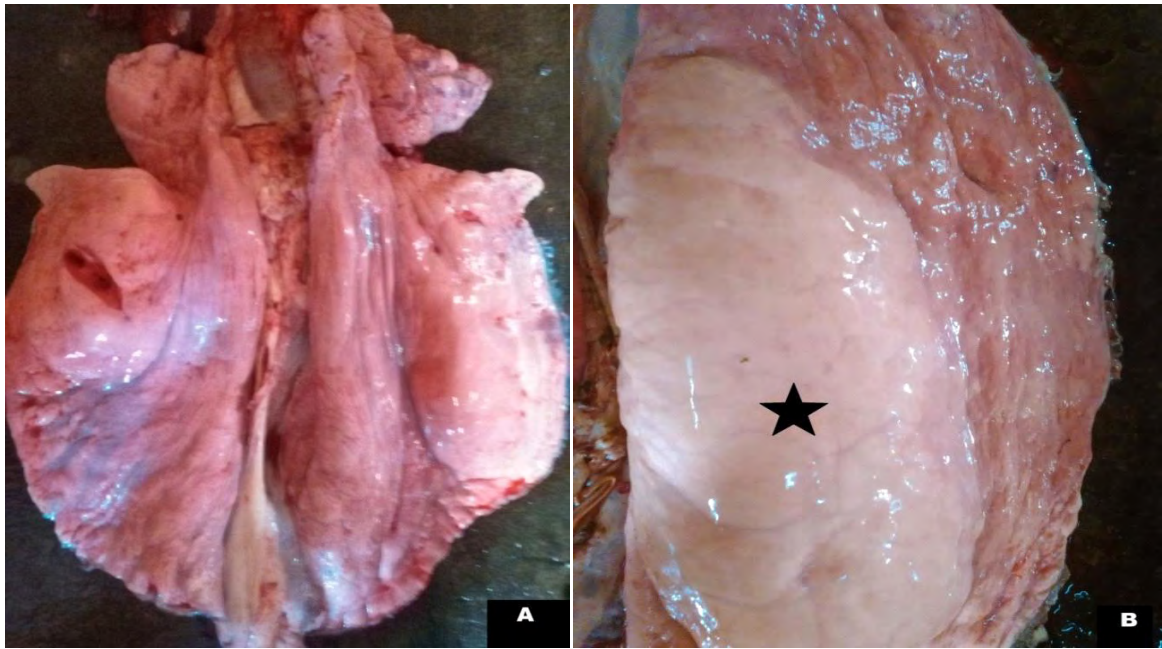


Figure 6: Distended and pale lung with diffused emphysema (A) and lung with unilateral focal emphysema (star) (B).

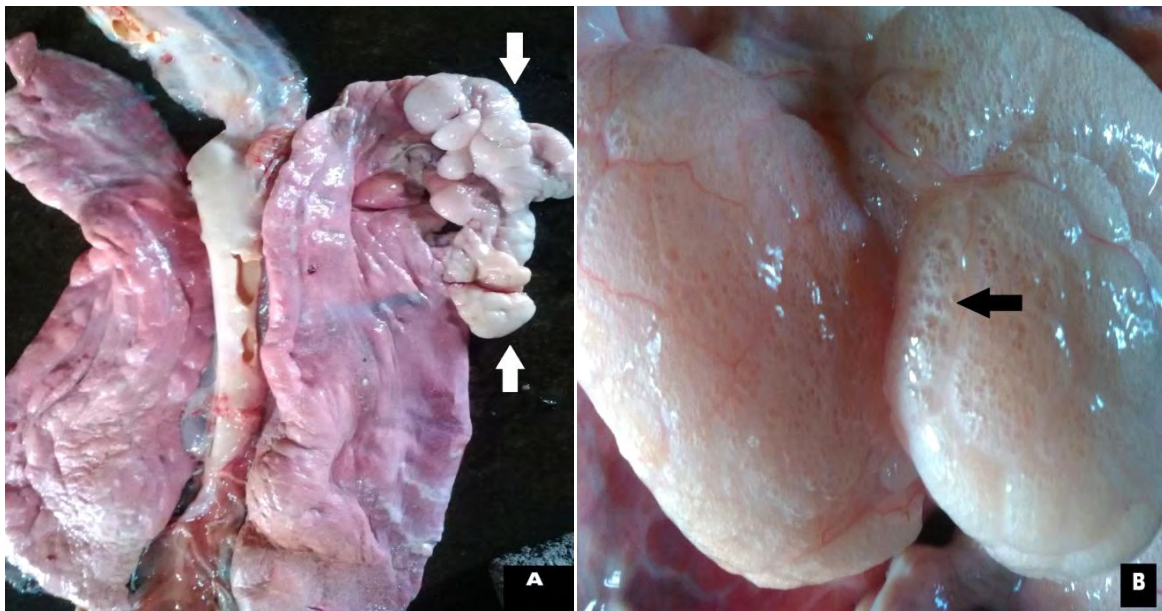


Figure 7: Bullous alveolar emphysema of the right cranial lobe of camel lung with multiple air pockets (white arrows) (A) and visible air bubbles (black arrow) (B).

Histologically, the emphysematous lungs revealed distended alveoli (Figure 8A) some of which contained some edema fluid. Many alveoli were ruptured and formed large empty spaces (Figure 8B).

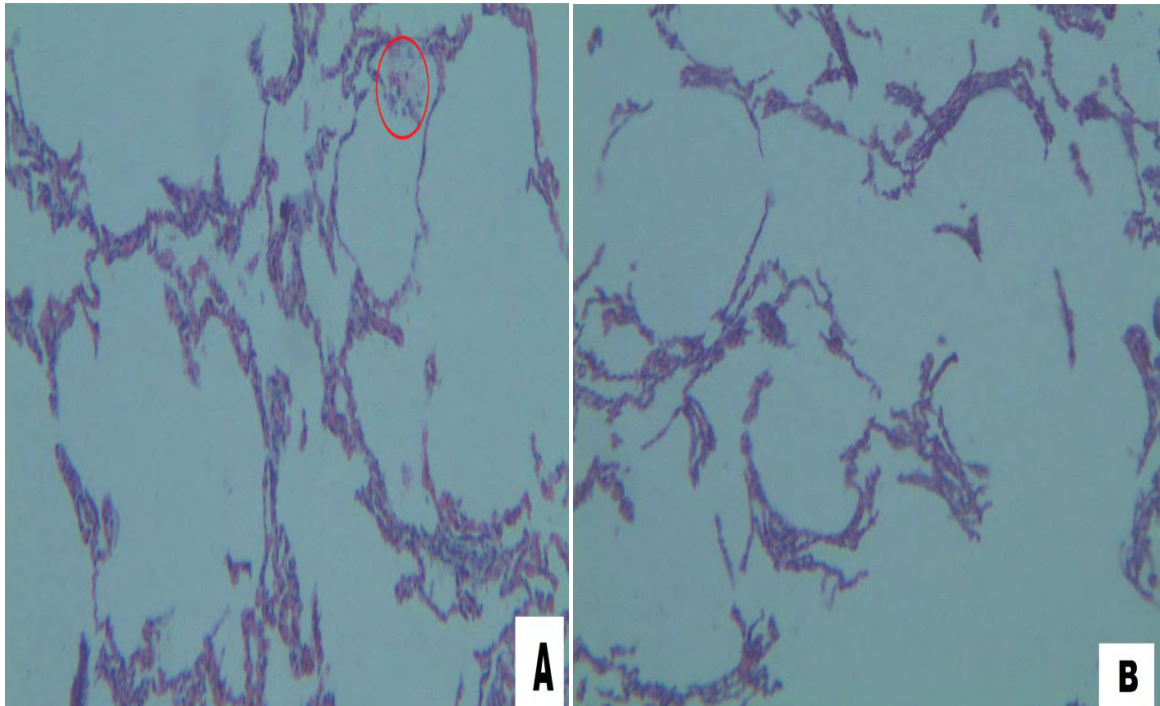


Figure 8: Distended alveoli (A) and large spaces resulted from ruptured alveoli due to over distention of the alveoli by excess air (B) (H&E, X10).

#### 4.1.3. Bronchopneumonia

A total of 11/207 (5.3%) lungs had bronchopneumonia and 9 of them were from the camels of 8-9 years old. As part of the gross lesions observed, distribution of bronchopneumonia varied significantly ( $P < 0.05$ ) among different age groups and lung lobes (Tables 2, 4 and 5). Grossly affected lungs were characterised by irregular consolidations of their cranio-ventral regions (Figures 9 and 10A). Consolidated lungs had variable appearance from dark-red to grey (Figure 10B) and the affected areas were hard and firmer on palpation.

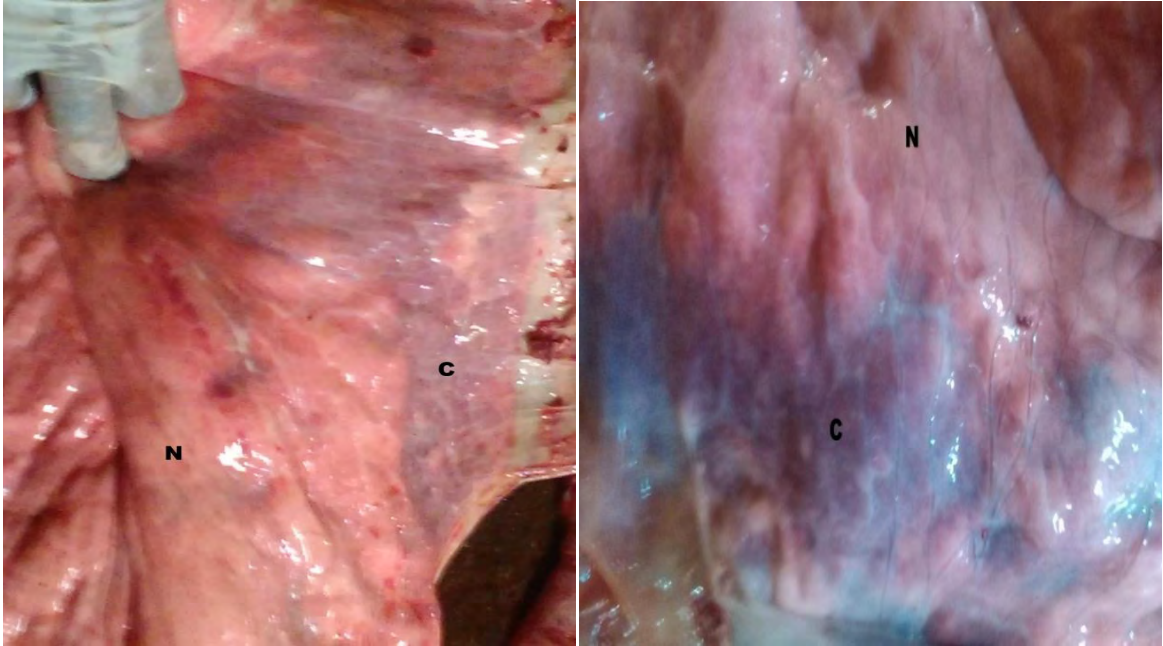


Figure 3: Dark-red and consolidated cranioventral regions (C) with relatively normal dorsal areas of the lungs (N) of both pictures.

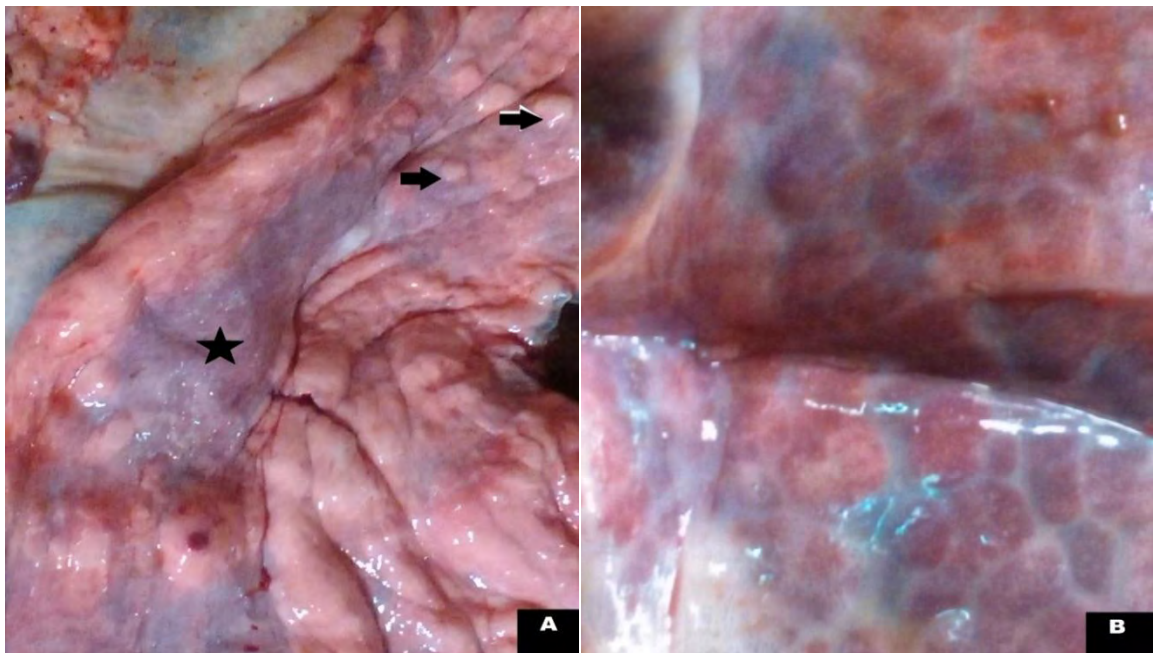


Figure 4: Consolidation of the right lung in its ventral portion of the cranial lobe (star) with pale nodules (arrows) (A) and with variable appearance from dark-red to grey (B).

Histologically, there were peribronchiolar and bronchiolar infiltration with inflammatory cells particularly neutrophils in the interstitium and lumen of alveoli and accumulation of fluid in the lumen of bronchioles and to some extent in the alveoli of four lung tissue sections (Figures 11 and 12A). These indicate the presence of acute suppurative bronchopneumonia. Three lung samples with bronchopneumonia revealed desquamation of bronchiolar epithelium, peribronchiolar, bronchiolar and alveolar lumen infiltration with mononuclear cells and hyperplasia of the lymphoid tissues (Figure 13), which indicate chronic suppurative bronchopneumonia.

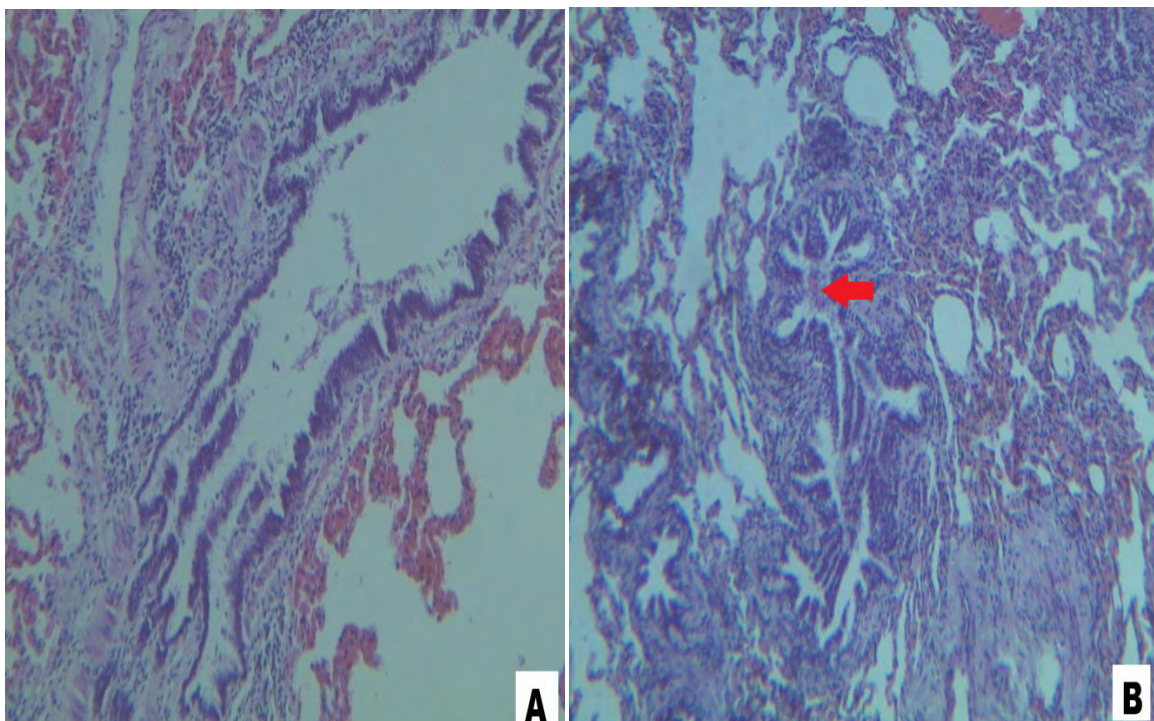


Figure 5: Peribronchoilar infiltrations of lymphocytes with some edema fluid and some infiltrations of inflammatory cells inside the bronchiole (A). Edema fluid inside the bronchiole (arrow) and in the lumen of many alveoli (B) (H&E, X10).

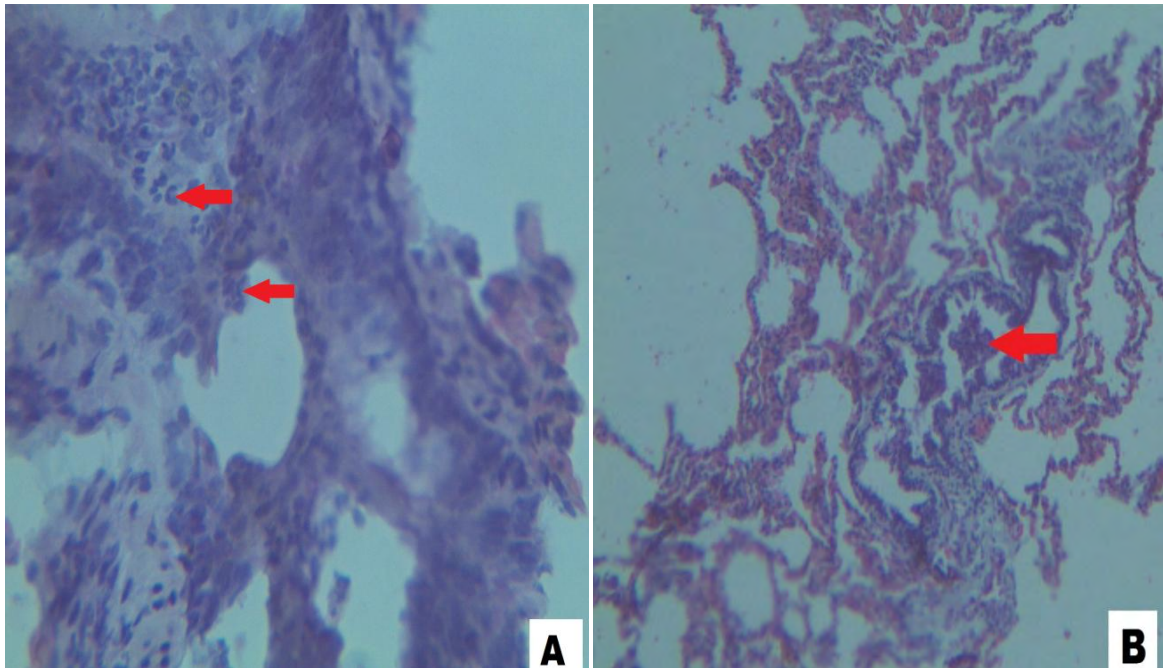


Figure 6: Neutrophilic infiltration in the interstitium and lumen of alveoli (arrows) (A) and mononuclear cells in the bronchiolar lumen (arrow) (B) (H&E, X10).

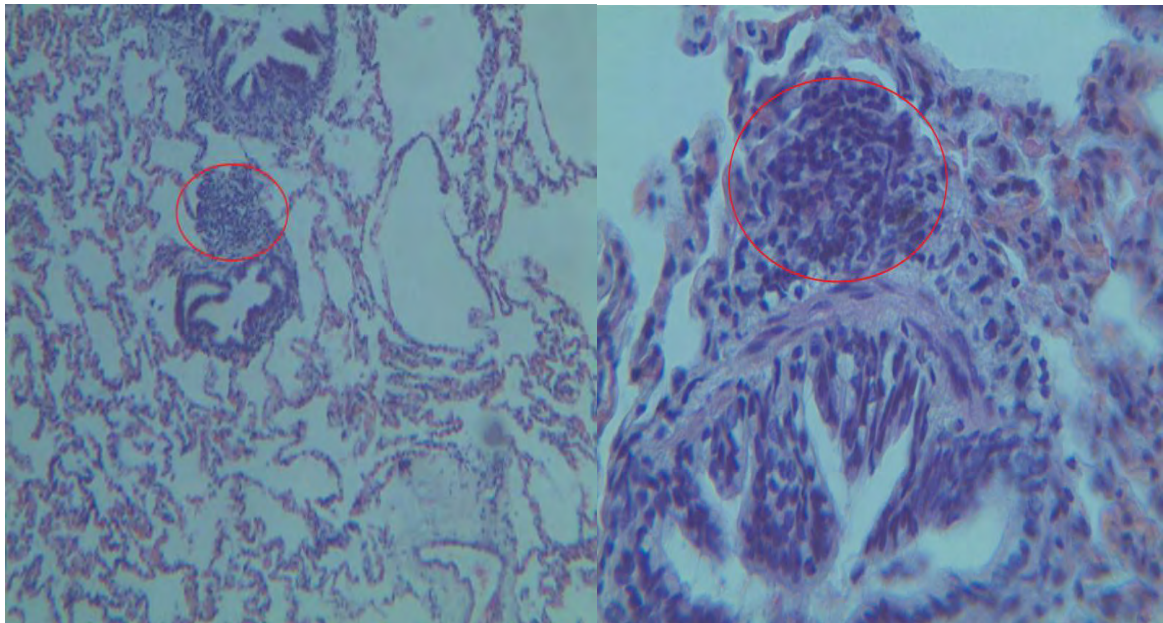


Figure 7: Proliferation of lymphocytes forming cuffing (H&E, X10 and X40 respectively).

#### 4.1.4. Pulmonary atelectasis

A total of 5/207 (2.4%) lungs of female camels had atelectasis, 4 (1.93%) of them were from the camels aged 8-9 years and 1 (0.5%) from 7years old (Table 5). Grossly, those affected lung tissues looked dark-red in color, their surfaces were depressed relatively with that of the normal lung tissues; these lungs had fleshy texture and they were non-spongy. In three lungs of the 8-9 years old camels the atelectatic lung tissues were surrounded by adjacent emphysematous tissues (Figure 14).

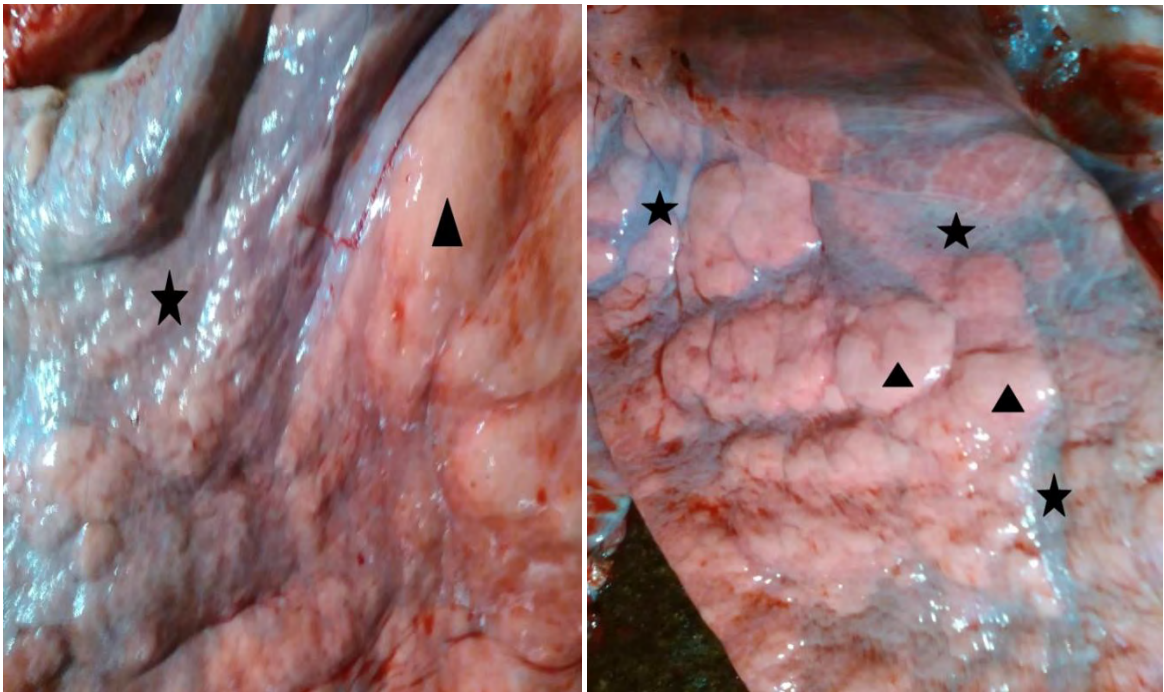


Figure 8: Atelectatic regions of camel lungs (stars) adjacent to the emphysematous tissues (arrow heads) of both pictures.

In histologically examined slides, the alveoli were seen collapsed or slitlike and the alveolar walls appeared parallel and close together and adjacent to the atelectatic alveoli distended-emphysematous alveoli were seen (Figure 15).

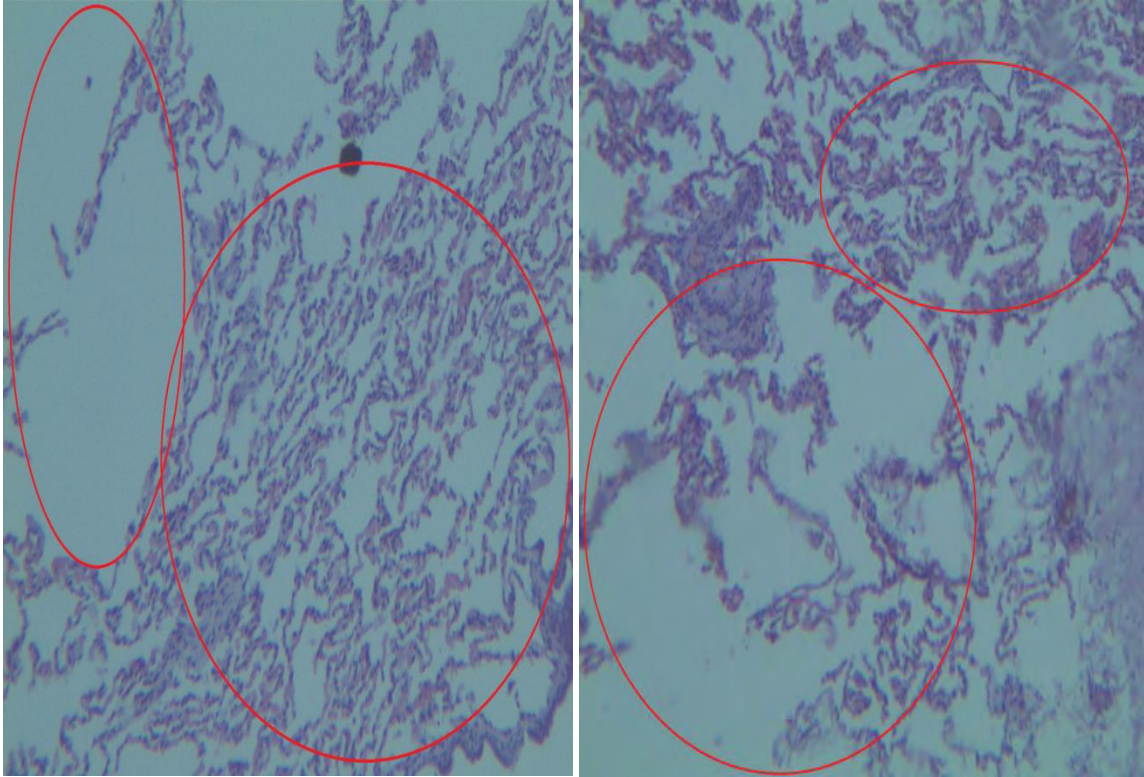


Figure 9: Collapsed alveoli adjacent to the distended ones (H&E, X10) of both pictures.

#### *4.1.5. Calcified nodules (white-hard nodules)*

In this study, 2/207 (0.96%) lungs had multiple calcified nodules with different sizes. All of these lesions were found in female camels of 8-9 years old (Table 5). Grossly, lungs had hard-white nodules, seen partly on the surface and partly rooted in the parynchymal tissues of the lung during palpation of those affected areas (Figure 16). When they were sectioned, ‘gritting’ sounds were heard and inside there were caseated materials and white capsule (Figure 17).

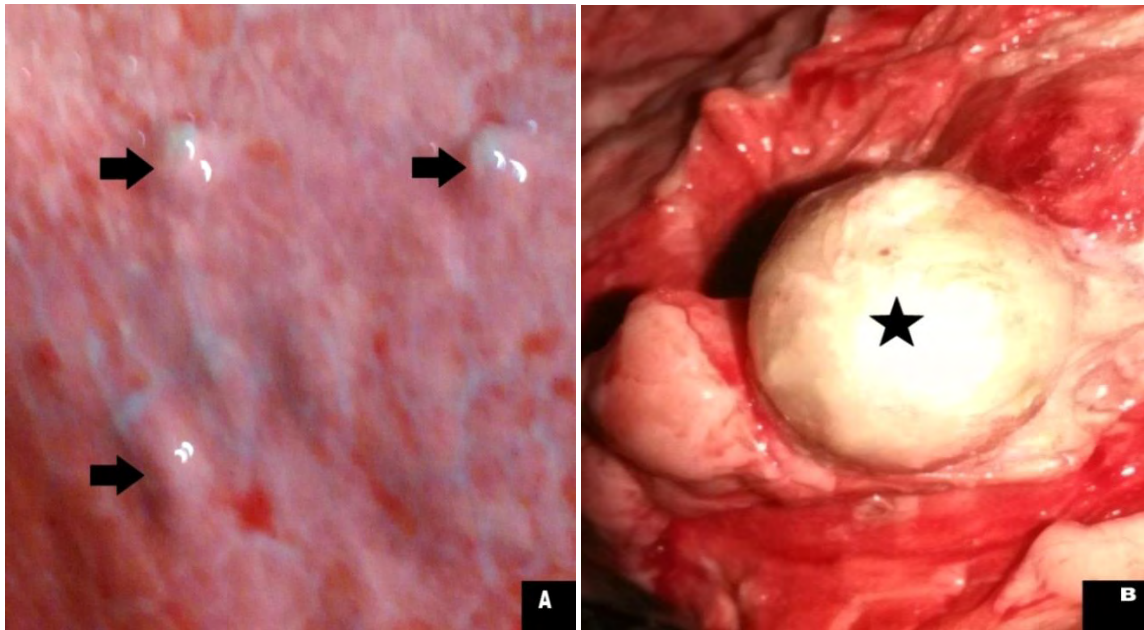


Figure 16: Multiple calcified nodules on the surface of lung tissues (arrows) (A). White hard nodule from the internal tissues of a lung (star) (B).

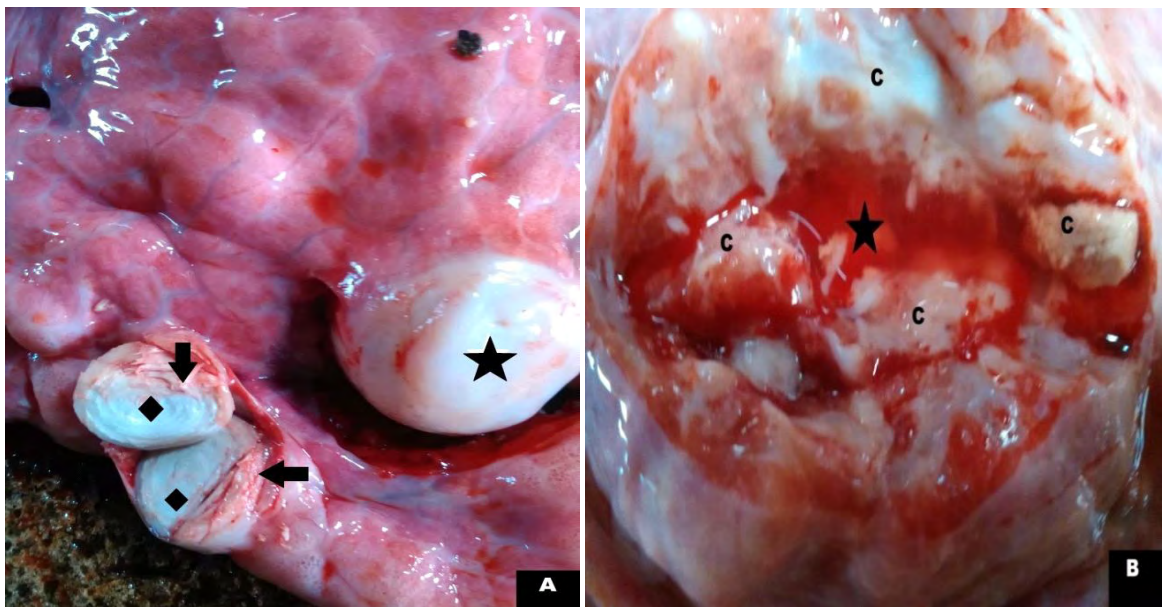


Figure 17: White-hard nodules in the cranial lobe (star) and cross-sections of the two nodules with white-solid mass (diamonds) and white capsules (arrows) (A). A cross-section of another nodule with calcified (caseated) substance (C) and blood (star) (B).

Histologically tissues taken from the adjacent areas of the nodules (capsule) and lung tissue revealed irregular deposits of calcium salts in necrosed capsular tissues (Figure 18).

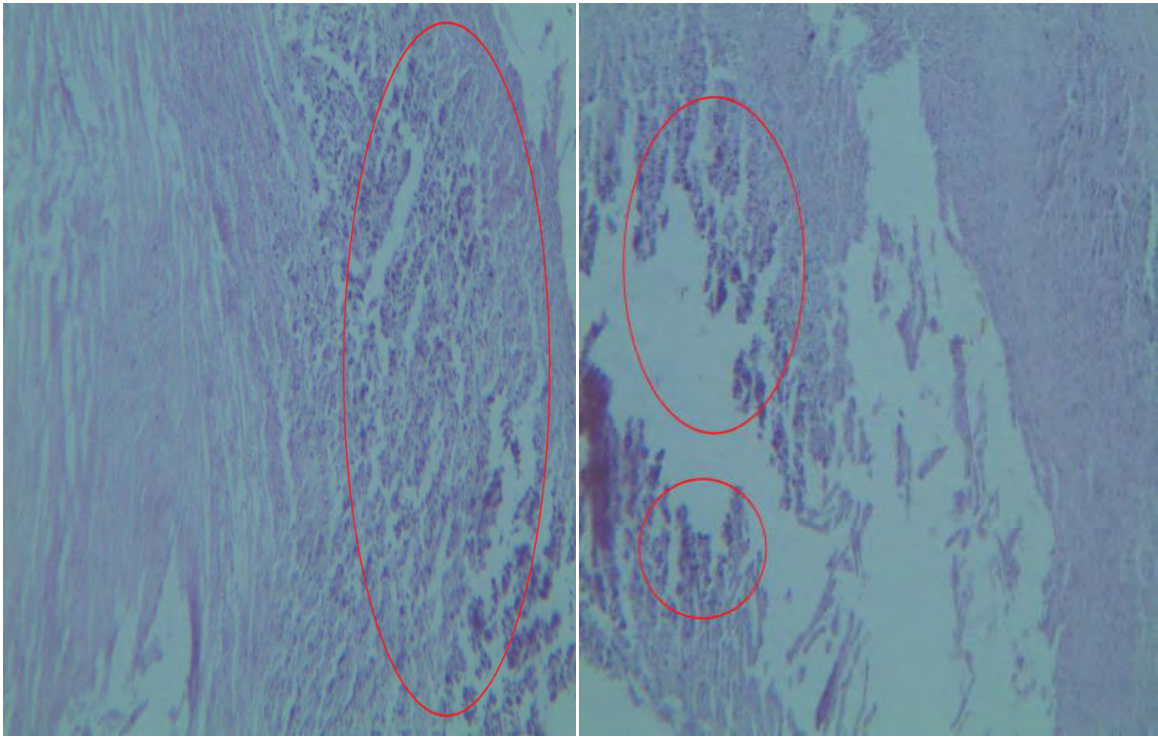


Figure 18: Irregular deposits of calcium salts (blackish/purple) in necrosed capsular tissues (H&E, X10) of both pictures.

#### *4.1.6. Pulmonary haemorrhage*

From the examined lungs, 2/207 (0.96%) lungs of the female camels had haemorrhages in their cranial lobes (Table 5). Grossly, in both sides of the affected lungs, the cranial lobes were distended (emphysematous), and on their surfaces, there were multifocal and patchy hemorrhages. Up on incision of the affected regions, the tissues revealed hyperemic parynchyma and oozing of foamy fluid (Figures 19 and 20).

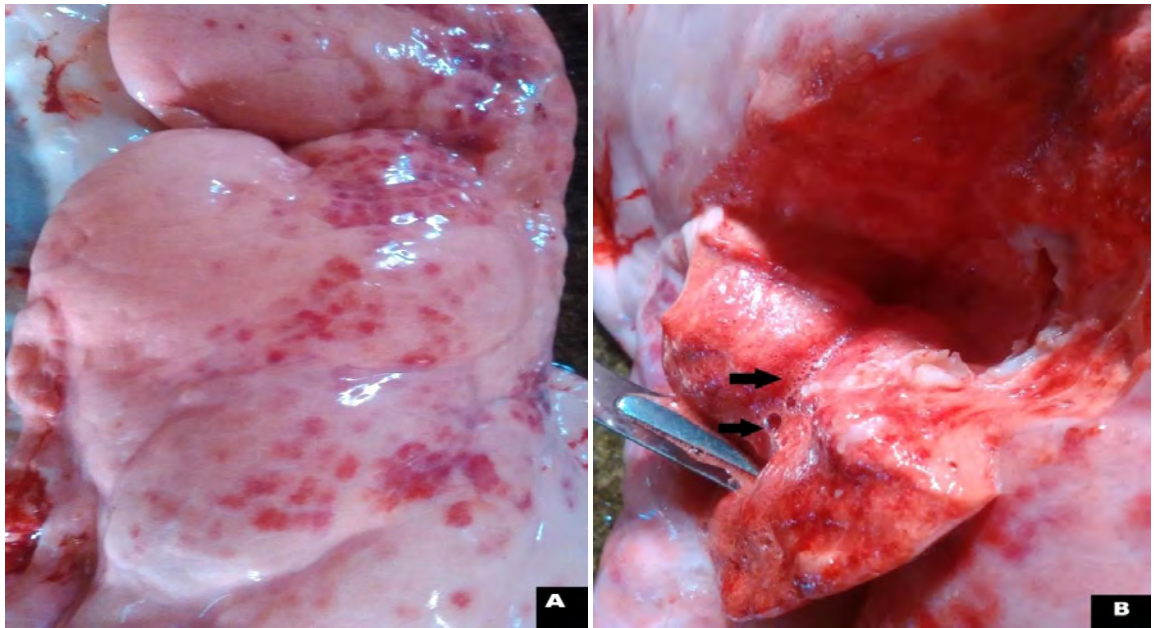


Figure 19: Multifocal haemorrhages on the surface of emphysematous right cranial lobe of affected lung (A). Incised region of the affected lung showing hyperemic parenchyma and oozing of foamy fluid (arrows) (B).

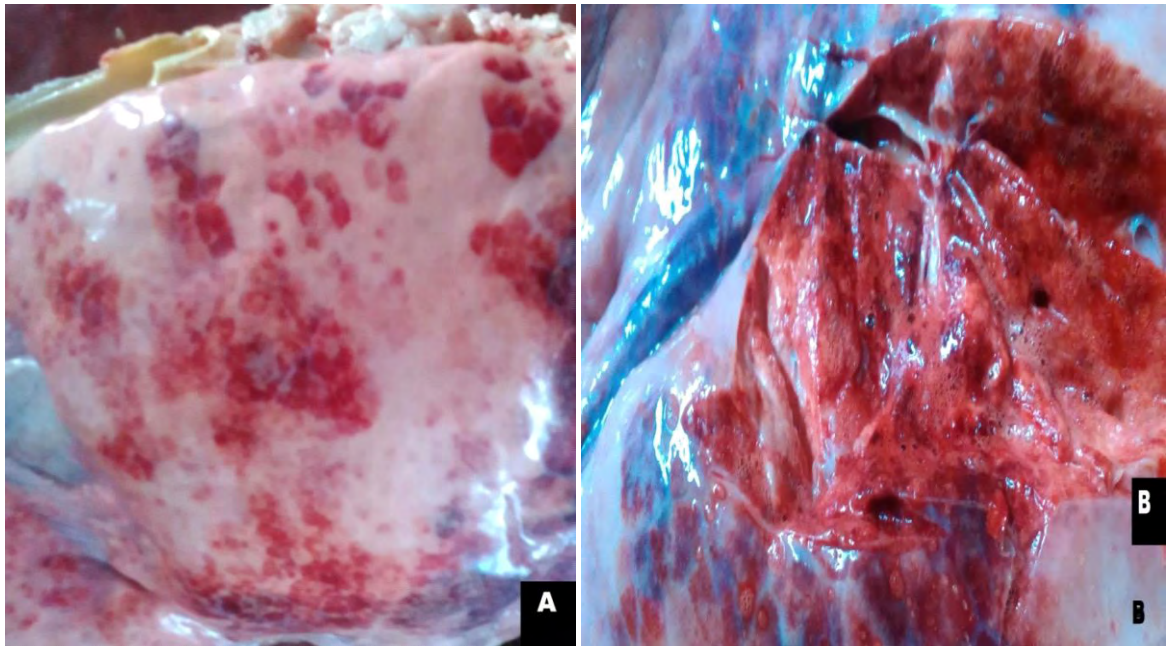


Figure 20: Patchy haemorrhages on the surface of cranial lobe of lung (A). Hyperemic parenchyma and frothy fluid of haemorrhagic cranial lobe (B).

Histologically, the main features detected in the affected regions of the lungs were intra-alveolar and minor intra-bronchiolar hemorrhages, inflammatory cells in airspaces, mild bronchiolitis and the alveoli were filled with acidophilic edema fluid (Figures 21 and 22).

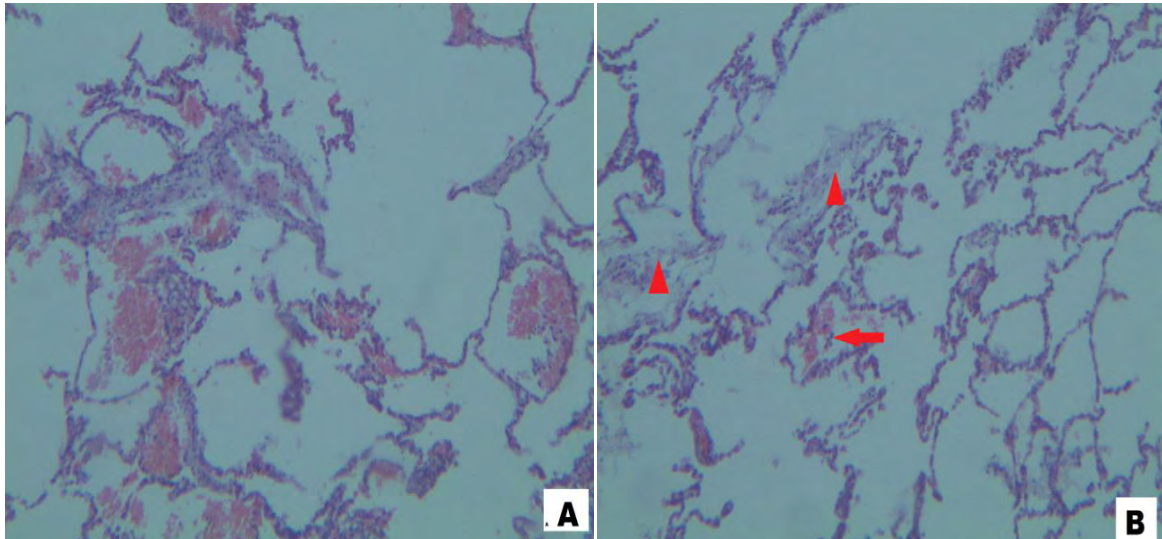


Figure 21: Intra-alveolar haemorrhage (A and B) and inflammatory cells (arrow) and acidophilic edema fluid (arrow heads) in alveoli (B) (H&E, X10).

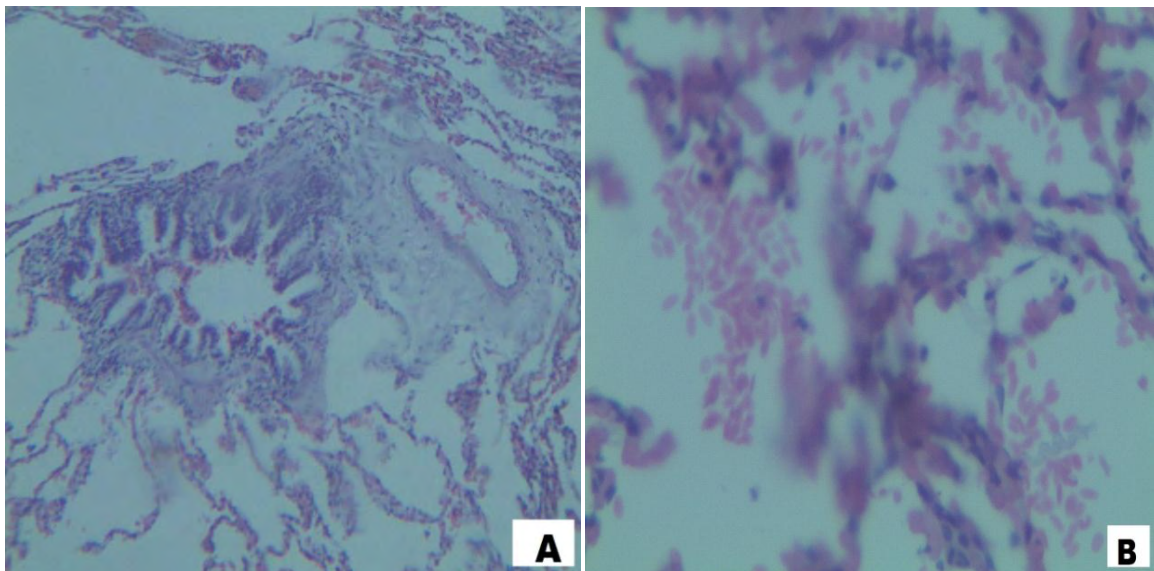


Figure 22: Minor haemorrhages in the bronchiolar lumen (A) (H&E, X10) and red blood cells in the alveolar lumen (B) (H&E, X40).

#### 4.1.7. Hydatid cysts

Two (0.96%) lungs of the female camels with age range of 8-9 years old had multiple hydatid cysts (fluid filled cysts) with different sizes (Table 5). Grossly, those fluid filled hydatid cysts were detected in the parynchyma of the lung up on palpation and in some regions the cysts were found on the surface of the affected lungs (Figure 23A). Clear watery fluid flowed out when those cysts were sectioned and white-thick capsules were revealed (Figure 23B).

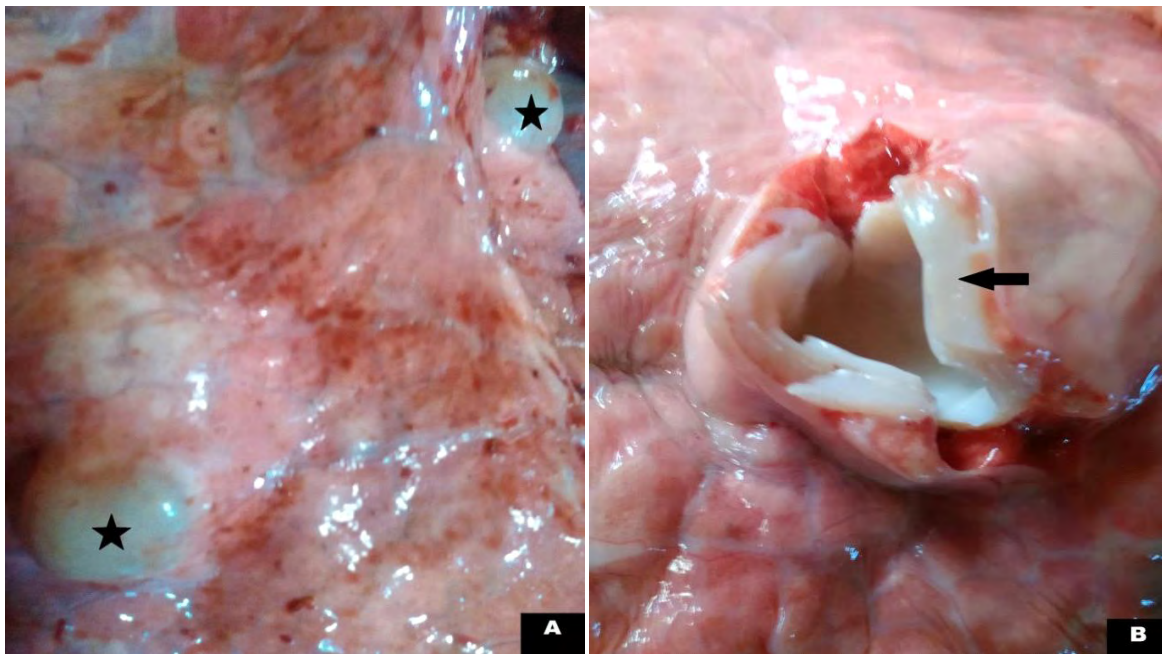


Figure 23: Hydatid cysts of camel lung (stars) (A). Opened hydatid cyst showing white-thick capsule (arrow) (B).

Histologically, lung tissues adjacent to the cyst capsule revealed compressed alveolar septa and narrow bronchiolar spaces with intense inflammatory cells infiltration (Figure 24A). The fibrous connective tissue capsule of the cysts revealed eosinophilic outer fibrous layer and faint eosinophilic inner germinal layer and contained calcium casts (Figure 24B).

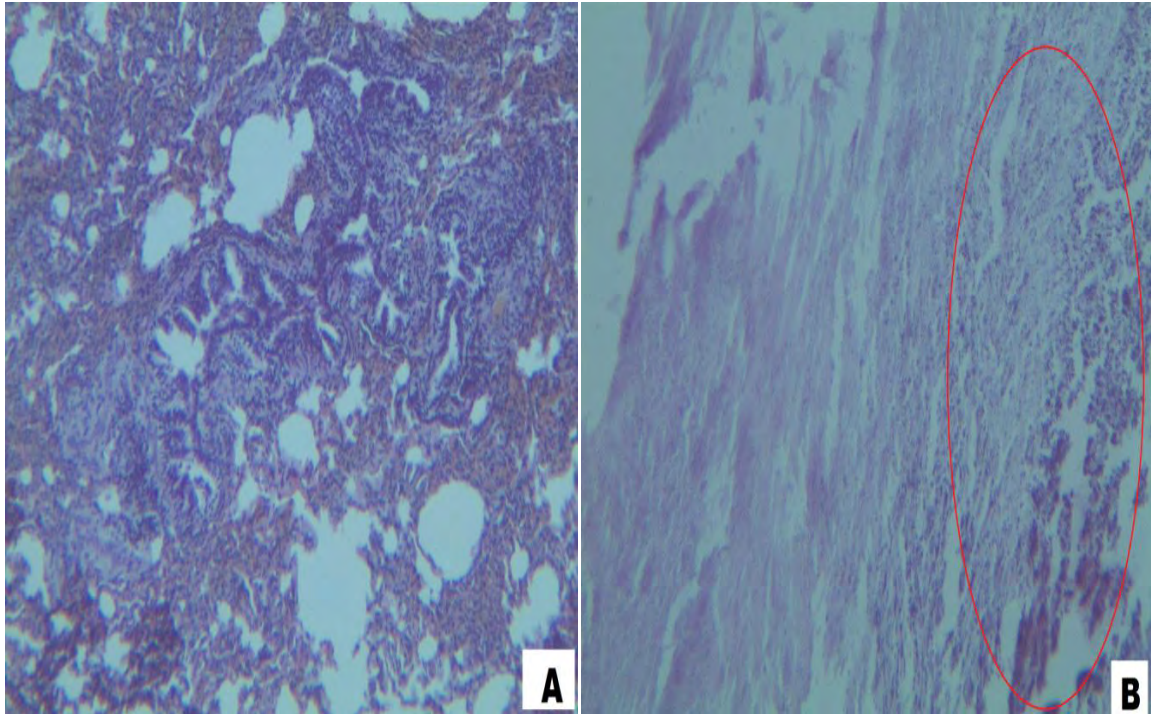


Figure 24: Narrow bronchiolar spaces and compressed alveolar septa in many areas with intense inflammatory cells infiltration (A) (H&E, X10). The fibrous connective tissue capsule of the cysts with eosinophilic outer fibrous layer and faint eosinophilic inner germinal layer and contained calcium casts (B) (H&E, X40).

#### 4.1.8. Pulmonary oedema

Two lungs (0.96%) of the male camels were found with pulmonary oedema (Table 5). Grossly, the affected lungs were distended (non-collapsed when thorax was opened), wet, relatively heavy, the interlobular septae were notably distended and foamy fluid was coming out when the tissues were incised (Figure 25). The internal walls of the laryngeal areas, trachea and bronchi were filled with the foamy fluid when they were opened. One of the two lungs had nodules coalescing together forming larger fibrous nodule in the caudal areas of the right lung. The nodules were pale-white, bulged slightly from the surrounding tissues and they were moderately firm with discrete borders (Figure 26).

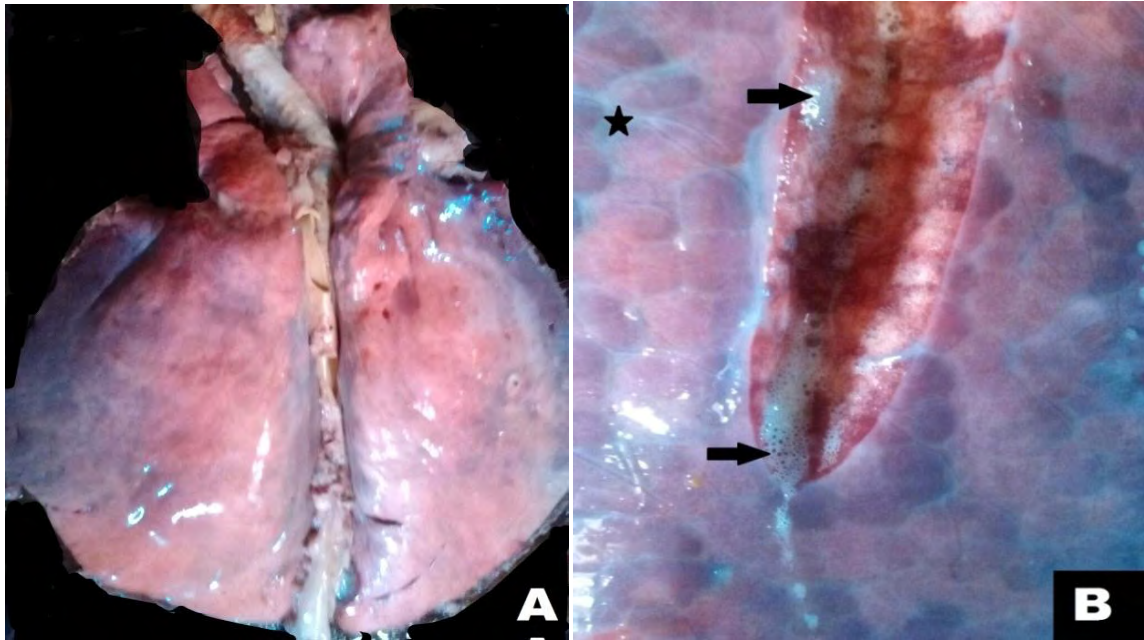


Figure 25: Distended, heavy and wet lung (A). Foamy fluid oozed out from edematous lung (arrows) and distended interlobular septae (star) (B).

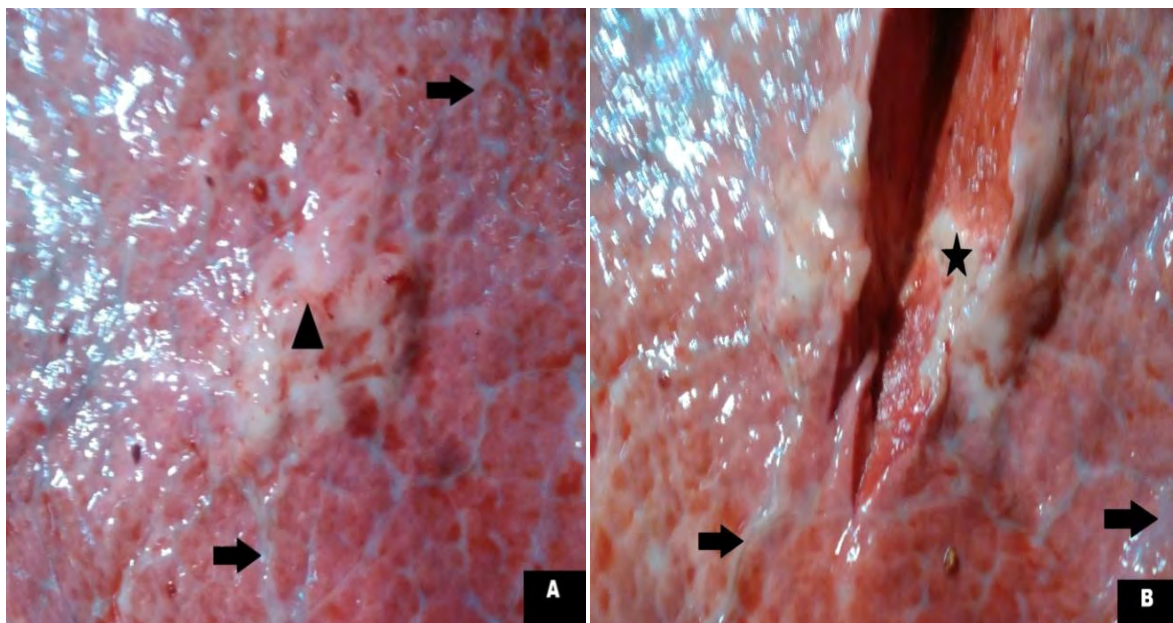


Figure 26: Whitish nodule (arrow head) and distended interlobular septae due to edema (arrows) (A). Fibrous tissue (star) in the parenchyma of the lung and distended interlobular septae due to edema (arrows) (B).

Histologically, the alveoli were filled with acidophilic edema fluid and neighboring alveolar septae were congested and condensed and also rarely emphysematous alveoli were seen in the examined tissue sections (Figure 27).

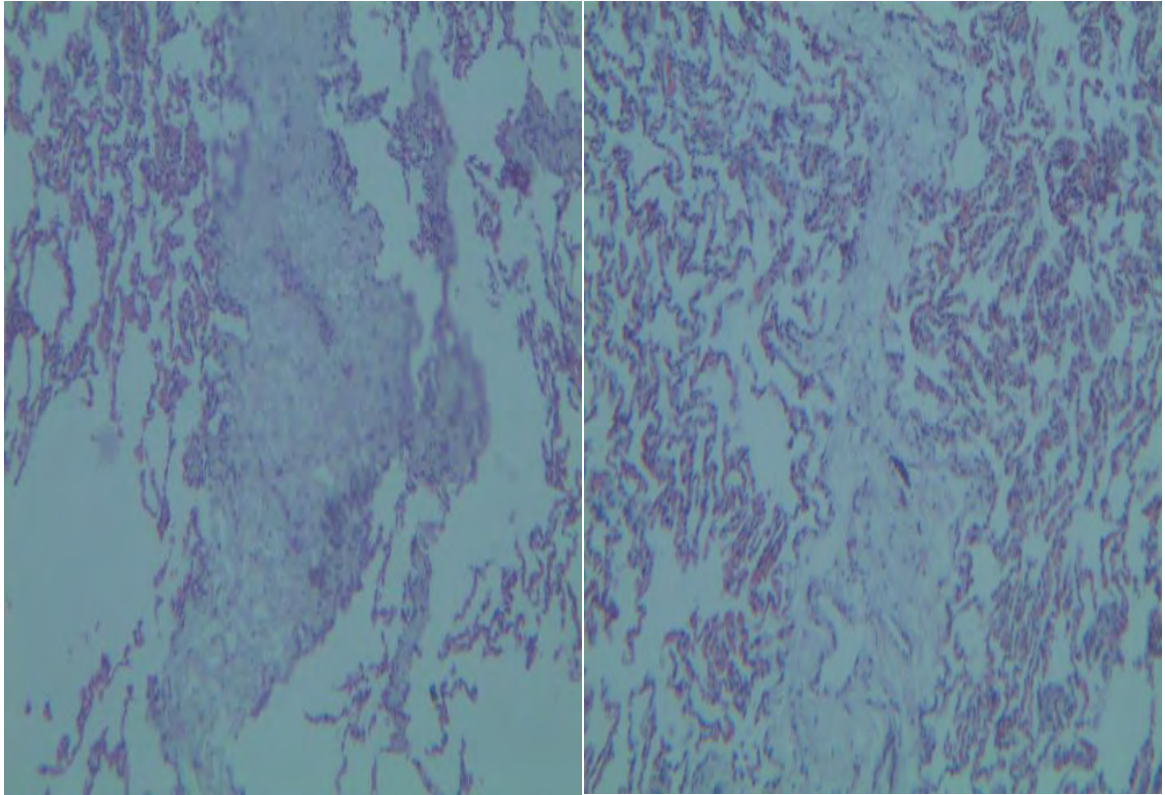


Figure 10: Acidophilic edema fluid in alveoli (central areas) and condensed alveolar septae in the left and right sides of the fluid (H&E, X10) of both pictures.

## 4.2. Bacterial isolates

All of the 53 lungs of camels with gross lesions were sampled and processed for aerobic bacterial isolation, of which, 50 (94.3%) lungs revealed growth of bacteria. A total of 70 bacterial isolates were detected on sheep blood agar plates (Table 6), 37 (52.9 %) of the isolates observed were Gram positive and 33 (47.1%) were Gram negative. More than one bacterial species were isolated from 17 lungs, while from 33 lungs only single bacterial species were isolated. There was no bacterial growth obtained from 3 (5.7%) lungs (Table 7).

Among the gross lesions, lung samples with interstitial pneumonia, bronchopneumonia and pulmonary emphysema were the most frequent lesions from which 49 (63.6%) of the isolated bacteria were detected. From the isolated bacteria, *Rhodococcus equi* was the most frequent isolate (Table 6). *Aeromonas hydrophila* and *Actinobacillus* spp. were the most frequent isolates from lungs with interstitial pneumonia. *Rhodococcus equi* were the most frequent isolates from lungs with pulmonary emphysema followed by *Corynebacterium* spp. *Coagulase negative staphylococci*, *Aeromonas hydrophila* and *Bacillus* spp. were the frequent bacteria isolated next to *Actinobacillus* spp. from lung with bronchopneumonia. *Rhodococcus equi* was the most frequent isolate from lung samples with pulmonary atelectasis, whereas lung samples of the rest types of lesions revealed single bacterial isolate of different bacterial spp. (Table 8).

Table 6: Types and percentage of the bacterial isolates from the camel lungs with clear gross lesions.

Bacterial isolates	Frequency	Percentage (%)
<i>Rhodococcus equi</i>	14	20
<i>Aeromonas hydrophila</i>	9	12.9
<i>Coagulase negative staphylococci</i>	8	11.4
<i>Actinobacillus spp.</i>	7	10
<i>Bacillus spp.</i>	7	10
<i>Corynebacterium spp.</i>	6	8.6
<i>Esherchia coli</i>	5	7.1
<i>Pasteurella spp.</i>	4	5.7
<i>Arcanobacterium pyogens</i>	2	2.6
<i>Mannheimia haemolytica</i>	2	2.9
<i>Bordetella spp.</i>	1	1.4
<i>Edwardsiella tarda</i>	1	1.4
<i>Enterobacter aerogenes</i>	1	1.4
<i>Klebsiella pneumoniae</i>	1	1.4
<i>Plesiomonas shigelloides</i>	1	1.4
<i>Proteus spp.</i>	1	1.4
Total	70	100

Table 7 : Types of lung lesions and isolation rate of bacteria

Types of lung lesions	Number of lungs affected by the lesion	Bacteria	
		Positive (%)	Negative (%)
Interstitial pneumonia	17	17 (100)	0 (0)
Pulmonary emphysema	12	10 (83.3)	2 (16.7)
Bronchopneumonia	11	11(100)	0 (0)
Pulmonary atelectasis	5	4 (80)	1 (20)
Calcified nodules	2	2 (100)	0 (0)
Pulmonary haemorrhages	2	2 (100)	0 (0)
Hydatid cysts	2	2 (100)	0 (0)
Pulmonary oedema	2	2 (100)	0 (0)
Total	53	50 (94.3)	3 (5.7)

Table 8: Association of bacterial species isolated from each type of pulmonary lesion

Bacterial isolates	Types of pulmonary lesions							
	IP	PE	BP	PA	CN	PH	HC	PED
<i>Rhodococcus equi</i>	2	6	-	3	1	-	1	1
<i>Aeromonas hydrophila</i>	5	-	2	-	-	1	1	-
<i>Coagulase negative staphylococci</i>	2	2	2	1	-	-	1	-
<i>Actinobacillus</i> spp.	4	-	3	-	-	-	-	-
<i>Bacillus</i> spp.	2	1	2	1	-	-	-	1
<i>Corynebacterium</i> spp.	1	3	1	-	1	-	-	-
<i>Esherchia coli</i>	2	-	-	1	1	1	-	-
<i>Pasteurella</i> spp.	2	-	1	-	-	1	-	-
<i>Arcanobacterium pyogenes</i>	-	-	1	-	-	1	-	-
<i>Mannheimia haemolytica</i>	1	1	-	-	-	-	-	-
<i>Bordetella</i> spp.	1	-	-	-	-	-	-	-
<i>Edwardsiella tarda</i>	-	-	-	1	-	-	-	-
<i>Enterobacter aerogenes</i>	-	1	-	-	-	-	-	-
<i>Klebsiella pneumoniae</i>	-	1	-	-	-	-	-	-
<i>Plesiomonas shigelloides</i>	-	-	-	-	-	-	-	1
<i>Proteus</i> spp.	-	-	-	-	-	-	1	-
Total	22	15	12	7	3	4	4	3

**Note:**  $\chi^2$ -value is 117.930 and P-value is 0.183. IP (Interstitial pneumonia), PE (Pulmonary emphysema), BP (Bronchopneumonia), PA (Pulmonary atelectasis), CN (Calcified nodules), PH (Pulmonary haemorrhages), HC (Hydatid cysts) and PED (Pulmonary oedema).

### 4.3. Viral detection

From the 25 (12.1% of the 207 lungs) lungs with different gross lesions cultured for virus detection, 10/25 (40%) exhibited cytopathic changes (CPE) on VERO cell monolayer. Ten viral cell cultures were performed from the lungs with interstitial pneumonia, 6 from bronchopneumonia and 7 and 2 from pulmonary emphysema and pulmonary haemorrhage respectively. Five of the 25 (20%) lungs with interstitial pneumonia were positive for viral growth as evidenced by rounding and sloughing of infected VERO cells (Figure 29). The most dominant and frequently observed type of CPE in 1/25 (4%) lung with bronchopneumonia and in 3/25 (12%) and 1/25 (4%) lungs with pulmonary emphysema and pulmonary haemorrhage respectively was the aggregation of infected cells (Figure 30). The duration of the CPE development in all positive samples ranged from 5-10 days of the inoculations.

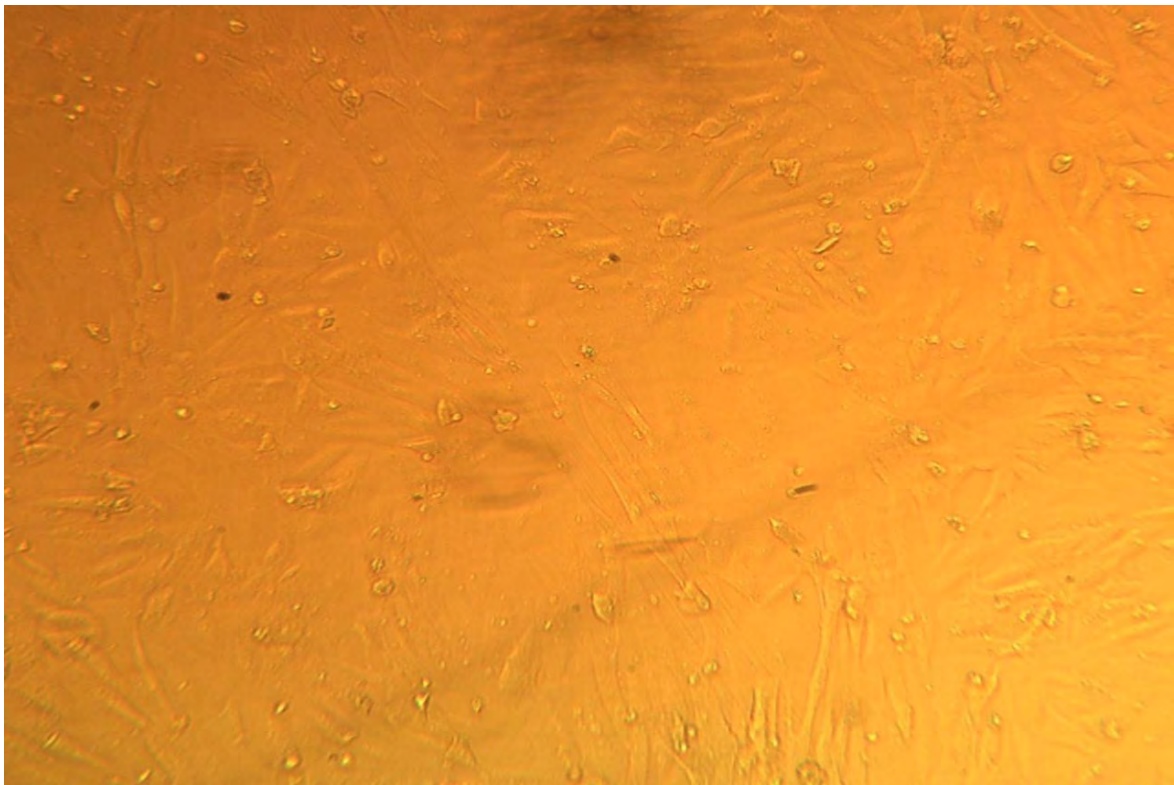


Figure 28: Uninfected VERO cells used as control.

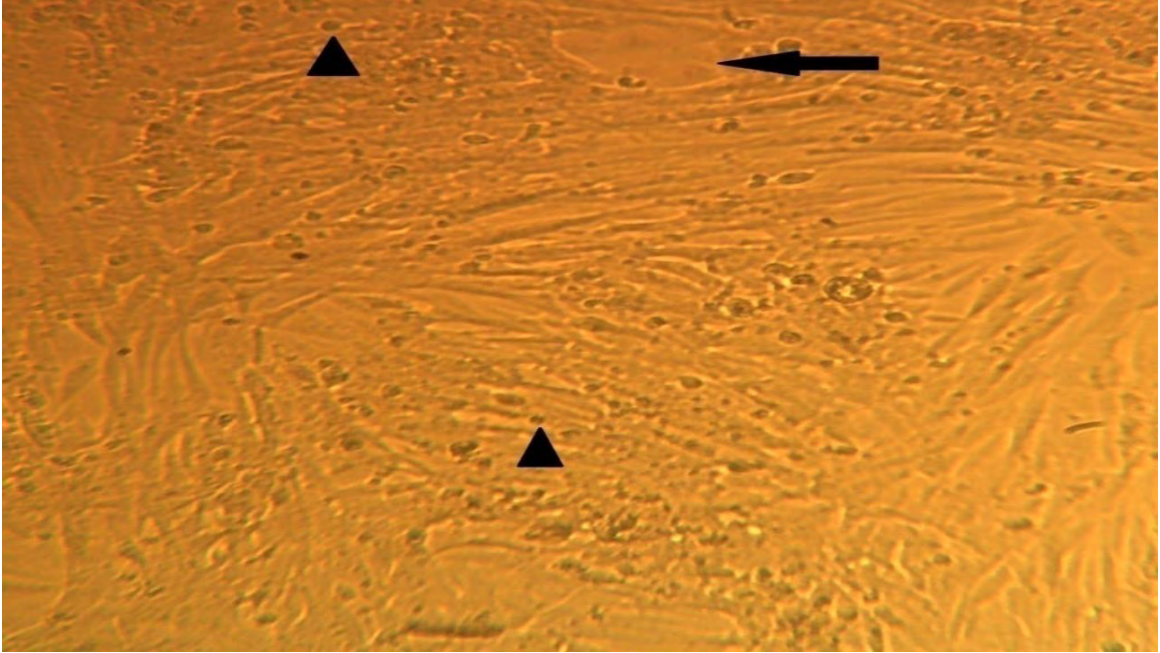


Figure 29: Cytopathic effect induced in infected VERO cells. Note: round cells (arrow heads) and sloughing of infected cells (arrow) (5 days post inoculation).

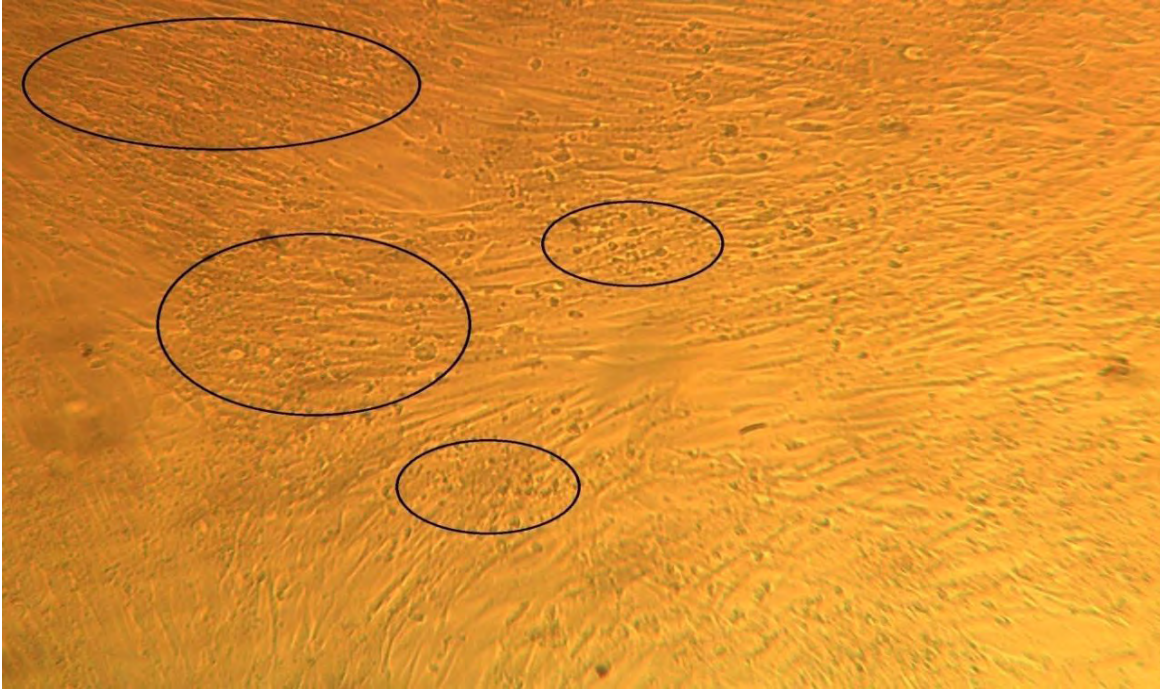


Figure 30: Aggregation of infected VERO cells (6 days post inoculation).

## 5. DISCUSSION

Many apparently healthy camels slaughtered in abattoirs were reported to have one or more pulmonary lesions during postmortem examination (Bekele, 2008; Awol *et al.*, 2011). In this study 25.6% of the examined lungs had one or more lesions which lower than 77.5% and 98% reported by Awol *et al.* (2011) and Teklu (2008) from Ethiopia, respectively and 64% reported by Abubaker *et al.* (2011) from Nigeria. The difference in occurrence of pulmonary lesions could be due to variation in sample size or due to variation among geographical areas from where the camels are originated.

Among the gross pathological lesions, interstitial pneumonia and bronchopneumonia together accounts for 13.5%, and individually 8.2% and 5.3%, respectively. This is lower than reports from Jordan and Egypt with 10.2% and 12% of pneumonic lungs, respectively (Al-Tarazi, 2001; Mahmoud *et al.*, 1988).

The types of pneumonias frequently detected in this study were suppurative bronchopneumonia and interstitial pneumonia in their acute and chronic forms. These results are consistent with the previous observations of Awol *et al.* (2011) and Al-Tarazi (2001) who reported observation of acute and chronic interstitial pneumonia, and acute and chronic suppurative bronchopneumonia from dromedary lungs. Gamal *et al.* (2014) also showed acute and chronic interstitial pneumonia in camels of Egypt.

Histopathological examination of lungs with interstitial pneumonia commonly revealed congestion of capillaries of the alveolar septae, infiltrations of interstitium by mononuclear cells especially lymphocytes which is a characteristics of chronic form of interstitial pneumonia. Some lesions shown acute interstitial pneumonia characterized by eosinophilic edema fluid in their alveolar lumen and thick eosinophilic hyaline membranes lined their alveoli along with infiltration of neutrophils in the interstitium. Similar findings were recorded by Gamal *et al.* (2014). Histopathological examination of lungs with grossly bronchopneumonic lesion revealed infiltration of the bronchiolar wall and the peribronchi with inflammatory cells involving neutrophils. The lumen of the airways were

filled with exudates having cellular debris and neutrophils, indicating acute bronchopneumonia.

Three lung samples with bronchopneumonia revealed debris of desquamated bronchiolar epithelium, peribronchiolar and bronchiolar infiltration with lymphocytes and macrophages and hyperplasia of the lymphoid tissues, which indicates chronic suppurative bronchopneumonia. The present findings are in agreement with the results reported by Taha *et al.* (2007).

In the present study, pulmonary emphysema (5.8%) was the second most frequent lesion identified next to interstitial pneumonia. This was in agreement with previous report by Awol *et al.* (2011) in dromedary camels of Ethiopia. A study conducted in Nigeria also indicated 2.5% emphysema (Abubaker *et al.*, 2011). Histopathological features of emphysema in the current study were over distension of some alveoli and rupture of many alveoli leaving large empty spaces. However, Abubaker *et al.* (2011) indicated alveolar distention with few alveoli containing some edema fluid. In chronic emphysema alveolar walls may become atrophic and rupture and the affected lung may lose its elasticity (van Dijk *et al.*, 2007). Emphysema may result from partial obstruction of the airways and subsequent air trapping in the distal alveoli or from exposure to dust particles that led to chronic bronchitis, post-inflammatory weakness and dilatation of bronchioles. It may also be a compensatory mechanism (reaction) especially in alveoli occurring neighbouring of collapsed lung tissue (Anderson, 1983).

In present study, 5 (2.4%) lungs had atelectatic tissues. According to Abubaker *et al.* (2011), there were 9.2% atelectatic lungs with collapsed alveoli and narrow respiratory bronchioles which is similar with the histopathological features observed in this study.

Two (1%) of the lungs observed in the present study showed multiple calcified nodules with different sizes ‘gritting’ sounds on cut which was a characteristic irregular deposits of calcium salts in necrosed tissues. This was consistent with many literatures for pulmonary calcification (Chahuan, 2010; McGavin and Zachary, 2010).

Dystrophic calcification is characterized by the deposits of calcium salts in necrosed tissue of any organ as happens in lesions due to parasitic infections or tuberculosis lesions (Chahuan, 2010).

Pulmonary hemorrhages vary from petechiation to massive filling of large regions with blood (Jubb *et al.*, 2005). The hemorrhage occurs in the dorsocaudal portions of both caudal lobes, and appears as red, brown or grey discoloration or as patchy, blue-brown subpleural foci (Lopez, 2012). Such gross lesion patterns were observed in 2 (0.96%) of the lungs in this study. Abubaker *et al.* (2011) indicated that 3/500 (0.6%) camel lungs had pulmonary hemorrhages with diffuse haemorrhages in the alveolar spaces and congestion which is similar to the current study.

In the present study, only 2/207 (1%) lungs of female camels, had hydatid cysts. In Egypt, the prevalence ranged from 4.3% to 8.2% in 400,159 camels imported from Sudan (Dyab *et al.*, 2005). From Ethiopia, a study conducted by Bulto *et al.* (2013) in camels indicated that 40.39% of all the hydatid cysts were found in lungs of female camels. In contrast to the current findings Abubaker *et al.* (2011) indicated that hydatid cysts were more common in male camels. Pulmonary complications of echinococcus cysts consisted of atelectasis and focal interstitial pneumonia of the lung parenchyma surrounding the cysts. This finding is in agreement with that reported by Sakamoto and Guti-errez (2005). These are due to the compression of neighbouring pulmonary tissues, that is concurred alveolar septae and bronchiolar spaces with intense inflammatory cells infiltration.

In the current study, the typical pulmonary edema was seen in 2 (1%) of the lungs, which grossly characterized by distended, wet and relatively heavy lungs with foamy fluid. Histologically the edema is characterized by acidophilic amorphous appearance in the alveoli with inter alveolar congestion. This is in agreement with reports (van Dijk *et al.*, 2007; McGavin and Zachary, 2010).

In this study, different types of aerobic bacteria were isolated from different types of pulmonary lesions but no bacterial growth was observed in 3/53 (5.7%) lungs having visible gross lesions. Bacterial isolation rate from pneumonic lungs was lower than that of Al-Tarazi (2001), Zubair *et al.* (2004) and Kane *et al.* (2005) but it was higher than that of Awol *et al.* (2011). As stated by Abubaker *et al.* (2011) and Awol *et al.* (2011), this variation in the isolation rate of bacteria could be due to variation in sample size, sample collection and processing and/or variation in geographical areas. The pathogenesis of pneumonia is complex and multifactorial. In the present study, failure of bacteriological isolation in the lung tissue samples might be due to involvement of other organisms involved in respiratory problems such as Mycoplasma, viruses, parasites, fungi or other anaerobic bacteria, which were not considered for the isolation due to resource problem.

*Micrococcus* spp., *Staphylococcus* spp. and *Proteus* spp. were reported as frequently isolated from milder cases of pneumonia (Abubaker *et al.*, 2011). *Arcanobacterium pyogenes*, *Mannheimia haemolytica* and *Enterobacter aerogenes* were also among the bacterial species isolated from pneumonic camel lungs by AL-Tarazi (2001). Whereas in Ethiopia, *Coagulase Negative Staphylococci*, *Escherchia coli*, *Francisella tularensis*, *Flavobacteria* spp., *Rhodococcus equi*, *Bordetella bronchoseptica*, *Aeromonas hydrophila* were among the bacterial species isolated from pneumonic camel lungs by Awol *et al.* (2011).

In the present study, *Rhodococcus equi* was the most frequently isolated bacteria (20%), which was higher than the rate reported by Awol *et al.* (2011) (5.3%); of which 42.9% of the *Rhodococcus equi* isolates were from lungs with emphysema. *Rhodococcus equi*, formerly known as *Corynebacterium equi*, is an important cause of morbidity and mortality in foals around the world. It causes two major forms of disease, one involves the intestine and the other affects the respiratory tract, resulting in a severe and often fatal bronchopneumonia (McGavin and Zachary, 2010). It inhabits soil and also the intestinal tracts of animals. It is generally acquired by inhalation of dust contaminated with *Rhodococcus equi* (Quinn *et al.*, 2002).

In the current study, *Aeromonas hydrophila* was the second common isolated bacteria at a rate of 12.9%. Five (55%) the bacteria were isolated from the lungs grossly with interstitial pneumonia. Awol *et al.* (2011) reported 3.5% in pneumonic lungs. *Aeromonas hydrophila* is an opportunistic pathogen of fish, reptile and rarely mammals (Quinn *et al.*, 2002). They are wide spread in fresh water, sewage and soil (Quinn *et al.*, 1999). The camels might acquire it from contaminated drink water (Awol *et al.*, 2011).

Coagulase negative *staphylococci* (CNS) were the third common bacteria isolated at a rate of 11.42%. The isolation rate is lower than Tigani *et al.* (2006) and Awol *et al.* (2011) who recovered CNS at a rate of 27.9% and 21.1%, respectively but almost four times higher than the results of Al-Doughaym *et al.* (1999) and Al-Tarazi (2001) who recovered 3.6% and 4% CNS from pneumonic lungs of camel, respectively. *Staphylococcus* species occur as commensals on the skin and mucous membranes and also as environmental contaminants. Staphylococcus infections are opportunistic and associated with trauma, immunosuppression, concurrent infections and other stress factors (Quinn *et al.*, 2002). As indicated also by Awol *et al.* (2011), isolation of staphylococci from the lungs of camel may be attributed to the stress of transportation and confinement. The camels were exposed to dusty conditions for prolonged periods (3 to 4 days) in the lairage without sufficient feed and water.

*Actinobacillus* spp. were the fourth commonly isolated bacteria in this study with a rate of 10%. There were no previous reports of *Actinobacillus* spp. isolated from the lung lesions of camels. The *Actinobacilli* are commensals on the mucous membranes in their hosts. Most of these spp. are distributed World wide and from those species, *Actinobacillus pleuropneumoniae* causes severe fibrinous pleuropneumonia, pleuritis, pulmonary sequestration and abscessation in pigs (Quinn *et al.*, 1999). Further and detail study is required to assess the relationship of these organisms and lesions of camels' respiratory system.

*Bacillus* spp. were isolated at rate of 10% in this study, which is higher than the results reported by Al-Tarazi (2001), who recovered at rate of 5.3% from pneumonic lungs of

camels but it is lower than the rate of isolation by Tigani *et al.* (2006) and Tarek *et al.* (2012) who recovered at rate of 12.8% and 21.4%, respectively. Most of the numerous *Bacillus* spp. are saprophytes widely distributed in air, soil and water (Quinn *et al.*, 1999).

*Corynebacterium* spp. were isolated at a rate of 8.6%, which is higher than the results reported by Tarek *et al.* (2012) and lower than that of Al-Tarazi (2001), who recovered at rate of 5.7% and 13.9%, respectively. In this study, these organisms were isolated from the camel lungs with pneumonia, emphysema and calcified nodules. Kane *et al.* (2005) reported that, this pathogen was involved in pneumonia of camels under condition of stress, poor sanitation and immunosuppression.

*Escherichia coli* was isolated at a rate of 7.1% in this study, which is lower than the results reported by Awol *et al.* (2011) and that of Al-Tarazi (2001), who recovered at a rate of 26.7% and 17.5%, respectively, but higher than the results of Al-Doughaym *et al.* (1999) and Zubair *et al.* (2004) with the 6.2% and 3% recovery rate, respectively. The high isolation rate of *E. coli* correlates with the natural habitat of *E. coli*, where it can survive in fecal particles, dust and water for weeks and months (Quinn *et al.*, 1999).

*Pasteurella* spp. and *Mannheimia haemolytica* were isolated at rate of 5.7% and 2.9% respectively in this study. This was higher than the results reported by Tigani *et al.* (2006) and Awol *et al.* (2011), who recovered at rate of 1.8% and 1%, respectively, but it is lower than 6.6% recovery rate reported by Al-Tarazi (2001). *Pasturella* spp. are reported to involve as a primary or secondary agent in pneumonia of cattle, sheep, goats, donkeys and horses following stressful conditions (Quinn *et al.*, 2002; Radostits *et al.*, 2007).

*Arcanobacterium pyogenes* (formerly *Actinomyces pyogenes*) were isolated at a rate of 2.6% in this study. This is lower than the result reported by Al-Tarazi (2001) from lung abscesses of camels, but it is higher than that of Tigani *et al.* (2006) and Tarek *et al.* (2012) with recovery rates of 6.6%, 1% and 1.4%, respectively. *Arcanobacterium pyogenes* cause suppurative pneumonia in cattle, sheep and pig (Quinn *et al.*, 1999). In

this study, *Arcanobacterium pyogenes* were isolated from the lungs with bronchopneumonia and pulmonary haemorrhage.

*Bordetella* spp., *Edwardsiella tarda*, *Enterobacter aerogenes*, *Klebsiella pneumoniae*, *Plesiomonas shigelloides* and *Proteus* spp. were isolated at rate of 1.4% each. This was lower than the result reported by Al-Tarazi (2001) for *Klebsiella pneumoniae*, *Enterobacter aerogenes* and *Proteus* spp.; and by Awol *et al.* (2011) for *Bordetella* spp., but it is higher than the result reported by Mohamed *et al.* (2014) for *Klebsiella pneumoniae*.

There were no previous reports of *Edwardsiella tarda* and *Plesiomonas shigelloides* isolation from camels suffering from respiratory diseases. *Edwardsiella tarda* which is a member of *Enterobacteriaceae* and is widely distributed throughout the environment, whereas, *Plesiomonas shigelloides* (previously *Aeromonas shigelloides*) is present in fresh water with limited distribution. It has been reported as a cause of gastroenteritis in man and the problem occurs mainly in tropical and subtropical regions (Quinn *et al.*, 1999). The exposure of camels to dusty conditions and poor sanitation for prolonged periods (3 to 4 days) in the lairage and probability of being contaminated with human waste may be accounted for infection and thus the isolation of these organisms from their lungs.

Virus isolation in tissue culture is the gold standard for the confirmation of virus infection and routinely used as diagnostic tool. In this study, it was possible to see cytopathic effect on 10/25 (40%) of the lung samples processed for virus propagation. This is lower than the results of Gelagay *et al.* (2013) who reported 27/35 (77%) CPE in pneumonic lungs. The same author also reported 30.5% acute and chronically interstitial pneumonia in camels, which is an indication of involvement of viral infections. This in agreement with the present finding, where, among the samples which developed typical CPE, 5/25 (20%) were sourced from lungs showing a lesion of interstitial pneumonia.

## 6. CONCLUSION AND RECOMMENDATIONS

Pulmonary lesions were detected from a significant number of camels that apparently looked healthy in this study. A number of pathogenic organisms were isolated from these pulmonary lesions and these pathogens might be directly or indirectly associated with the lesions. On an attempt made to detect virus from lesions, cytopathic effects indicative of viral growth were seen especially from those lungs with interstitial pneumonias. From the result it might be said that in addition to posing economic impact in distribution of diseases, apparently healthy camels with pulmonary lesions might carry potentially pathogenic agents and might pose a serious threat to humans and animals.

Therefore, based on the above conclusion the following recommendations are forwarded.

- Further study should be conducted on epidemiology, lesions and correlations with probable causes of camels respiratory diseases to set effective preventive measures to reduce the impact of the diseases in camels.
- The significance of the isolated bacteria in producing camel respiratory diseases need further investigation to establish cause-effect relationship in clinically sick camels.
- Continuous and special training need to be provided to stakeholders about camel diseases and their pathology.
- Further characterization of the viral agents from the pulmonary tissues which showed CPE should be done. Similarly, further characterization of the bacterial isolates to the species and strain level should be conducted.

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## 8. ANNEXES

**Annex 1:** Format used for recording pre-and post-slaughter examination results of camels at the abattoir

<b>Date</b>	<b>N<sub>o</sub></b>	<b>Origin of camels</b>	<b>Sex</b>	<b>Age (yrs.)</b>	<b>A.M. Exam. Results</b>	<b>P.M. Exam. Results (any gross respiratory tracts lesion with the respective of lung sides and lung lobes)</b>

**Note:** **Date** is date of examination, **A.M.** ( *Antemortem*), **P.M.**(*Postmortem*), **Yrs.**(*Years*), **Exam.** (*Examination*), **N<sub>o</sub>** is *Order of camels examined* (1, 2, 3....) and **N<sub>o</sub>** was used as animal-tissue sample code.

**Annex 2:** Format used for recording histopathological examination results

Tissue sample code on the microscopic slide	Gross lesion results at the abattoir	Histopathological results/descriptions

**Annex 3:** Format used for recording bacteriological examination results

Tissue Sample code	Bacterial colony (morphological characteristics)	Gram-staining results	Biochemical tests conducted and their results	Bacterial isolates	
				G+ves	G-ves

**Note:** **G+ves:** *Gram positive bacteria* and **G-ves:** *Gram negative bacteria*

**Annex 4:** Format used for recording viral cell culture results

<b>Tissue code</b>	<b>Gross and microscopic lesions of the sample</b>	<b>Any morphologic alterations (CPE) on the VERO cells</b>

**Annex 5:** Format used for recording summary of the results

<b>Tissue code</b>	<b>Sex</b>	<b>Age</b>	<b>Gross lesions</b>	<b>Histopathological results</b>	<b>Bacterial isolates</b>		<b>Viral culture results</b>
					<b>G+ves</b>	<b>G-ves</b>	<b>Any type of cytopathic effect (CPE)</b>

**Note:** **G+ves:** *Gram positive bacteria* and **G-ves:** *Gram negative bacteria*

**Annex 6:** Procedure for histopathological examination of tissue samples (Kul and Yildiz, 2010)

**1. Fixation of tissue** by 10% formaldehyde

**2. Trimming tissue** to fit in to standard histological processing tissue cassettes (5mm thickness)

**3. Tissue processing:** dehydration, clearing and impregnating

- ❖ Dehydrating tissue by using increasing strength of alcohol; e.g. 50%, 70%, 90% and 100%. Dehydration with 70% alcohol for 1hour, 90% alcohol I for 1 hr, 90% alcohol II for 2 hr. and

100% alcohol I	1 hour
100% alcohol II	2 hour
100% alcohol III	2 hours

- ❖ Clearing of tissue by Xylene

Xylene I	2 hours
Xylene II	2 hours

- ❖ Impregnation tissue with Paraffin wax

Paraffin wax I	1 hour
Paraffin wax II	1 hour
Paraffin wax III	1 hour

**4. Embedding or Blocking:** Impregnated tissues are placed in a mould with their labels and then fresh melted wax (54-60C<sup>o</sup>) is poured in it and allowed to settle and solidify.

**5. Sectioning:** sectioning of tissue in to 4-5 micron thickness and adhere on the surface of clear slide.

**6. Staining:** Automatic or manual staining with H&E to give colour for sectioned tissue.

### **Tissue Staining Procedure:**

- ❖ Put the sections fixed on slides in xylene for 3 minutes.
- ❖ Then transfer to absolute alcohol for 3 minutes.
- ❖ Transfer to 80% alcohol for 2 minutes.
- ❖ Place in 50% alcohol for 2 minutes.
- ❖ Wash the slide in running tap water for 1 minute and put in Harris's *Haematoxylin* for 5-7 minutes.
- ❖ Wash in running tap water for 30 seconds
- ❖ Wash excess dye in 1% acid alcohol by continuous agitation for 15 seconds.
- ❖ Wash in running tap water for 30 seconds.
- ❖ 2-3 dips in ammonia water solution until tissues attain a blue colour.
- ❖ Wash in running tap water for 30 seconds.
- ❖ Counter stain with *eosin* for 3-5 minutes.
- ❖ Wash in running tap water for 30 seconds.
- ❖ Dehydrate by keeping in increasing concentration of alcohol (2-3 minutes in 50%, 70%, 95% and absolute alcohol).
- ❖ Clear it in xylene and mount with DPX or Canada balsam.

**7. Microscopic examination:** stained slide is examined under microscope.

**Annex 7:** Procedures of Gram staining (Collins *et al.*, 2004)

### **Staining procedures**

After making and fixing smear, stain it with the crystal violet solution for 1 min. Wash with tap water. Stain with the iodine solution for 1 min. Decolourize with 95% ethanol until no more stain comes away. Wash with tap water. Stain with the safranin solution for 2 min. Wash and dry. Examine the slide after adding oil immersion.

**Annex 8:** Procedures of different biochemical tests for bacterial isolation (Queen *et al.*, 1999; Betty *et al.*, 2007)

## **A. Catalase test**

### **Principle**

The enzyme catalase mediates the breakdown of hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) into oxygen and water. The presence of the enzyme in a bacterial isolate is evident when a small inoculum is introduced into hydrogen peroxide (30% for the slide test), and the rapid elaboration of oxygen bubbles occurs. The lack of catalase is evident by a lack of or weak bubble production.

### **Method**

1. Use a loop or sterile wooden stick to transfer a small amount of colony growth to the surface of a clean, dry glass slide.
2. Place a drop of 30% hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) onto the medium.
3. Observe for the evolution of oxygen bubbles.

### **Expected results**

Positive: Copious bubbles produced and Negative: No or few bubbles produced

**Quality control:** Positive: *Staphylococcus aureus* and Negative: *Streptococcus pyogenes*

## **B. Oxidase test**

### **Principle**

To determine the presence of bacterial cytochrome oxidase using the oxidation of the substrate tetramethyl-p-phenylenediamine dihydrochloride to indophenol, a dark purple-colored end product. A positive test (presence of oxidase) is indicated by the development of a dark purple color. No color development indicates a negative test and the absence of the enzyme.

## **Method**

1. Moisten filter paper with the substrate (1% tetramethyl-p-phenylenediamine dihydrochloride) or select a commercially available paper disk that has been impregnated with the substrate.
2. Use a platinum wire or wooden stick to remove a small portion of a bacterial colony (preferably not more than 24 hours old) from the agar surface and rub the sample on the filter paper or commercial disk. **Note:** Nickel-base alloy wires containing chromium and iron (nichrome) used to rub the colony paste onto the filter paper may cause falsepositive results.
3. Observe inoculated area of paper or disk for a color change to deep blue or purple within 10 seconds (timing is critical).

## **Expected results**

Positive: Development of a dark purple color within 10 seconds and Negative: Absence of color.

**Quality control:** Positive: *Neisseria gonorrhoeae* and Negative: *Escherichia coli*.

## **C. Motility Test**

### **Principle**

The motility test is used to detect the presence of flagella by bacteria, In the tube test, semisolid motility medium is inoculated in a straight line down through the center of a tube. Motile organisms will migrate out from the line of inoculation, causing visible turbidity throughout the tube. Nonmotile organisms will grow only along the line of inoculation. Other substrates which allow simultaneous testing of other biochemical reactions that aid in the identification of microorganisms may be added to the medium.

### **Method**

1. Diffuse growth outward away from stab line or turbidity of the media (with or without TTC (triphenyltetrazolium chloride, a colorless vital dye ) is a positive test.

2. A clear tube (the same as the uninoculated media) with growth only along the line of inoculation indicates that the organism is nonmotile.
3. In media with TTC, the red color forms in the area of bacterial growth. Motile organisms produce a pink color that diffuses from the stab line. Organisms that are nonmotile produce a pinkish red pigment that is confined to the stab line

### **Expected results**

Positive: Motile organisms will spread out into the medium from the site of inoculation.

Negative: Nonmotile organisms remain at the site of inoculation.

**Quality control:** Positive: *Escherichia coli*. Negative: *Klebsiella pneumoniae*

## **D. Oxidation/Fermentation (OF) test**

### **Principle**

This test is used to determine whether an organism uses carbohydrate substrates to produce acid byproducts. Nonfermentative bacteria are routinely tested for their ability to produce acid from six carbohydrates (glucose, xylose, mannitol, lactose, sucrose, and maltose). In addition to the six tubes containing carbohydrates, a control tube containing the OF base without carbohydrate is also inoculated. OF glucose is used to determine whether an organism ferments or oxidizes glucose. If no reaction occurs in either the TSI or OF glucose, the organism is considered a nonglucose utilizer.

### **Method**

1. To determine whether acid is produced from carbohydrates, inoculate agar deeps, each containing a single carbohydrate, with bacterial growth from an 18 to 24-hour culture by stabbing a needle 4 to 5 times into the medium to a depth of 1 cm. Note: Two tubes of OF dextrose are usually inoculated; one is overlaid with either sterile melted petrolatum or sterile paraffin oil to detect fermentation.
2. Incubate the tubes at 35-37°C in ambient air for up to 7 days. Note: If screwcap tubes are used, loosen the caps during incubation to allow for air exchange. Otherwise, the control tube and tubes containing carbohydrates that are not oxidized might not become alkaline.

## Expected results

**Positive:** Acid production (A) is indicated by the color indicator changing to yellow in the carbohydrate-containing deep. Weak-positive (Aw): Weak acid formation can be detected by comparing the tube containing the medium with carbohydrate with the inoculated tube containing medium with no carbohydrate. Most bacteria that can grow in the OF base produce an alkaline reaction in the control tube. If the color of the medium in a tube containing carbohydrate remains about the same as it was before the medium was inoculated and if the inoculated medium in the control tube becomes a deeper red (i.e., becomes alkaline), the culture being tested is considered weakly positive, assuming the amount of growth is about the same in both tubes.

**Negative:** Red or alkaline (K) color in the deep with carbohydrate equal to the color of the inoculated control tube.

**No change (NC) or neutral (N):** There is growth in the media, but neither the carbohydrate-containing media nor the control base turn alkaline (red). Note: If the organism does not grow at all in the OF medium, mark the reaction as no growth (NG).

## Quality control

**Note:** Appropriate organisms depend on which carbohydrate has been added to the basal medium. Glucose is used as an example. **Fermenter:** *Escherichia coli*. **Oxidizer:** *Pseudomonas aeruginosa*. **Nonutilizer:** *Alcaligenes faecalis*

## E. Coagulative test

### Principle

This test is used to differentiate *Staphylococcus aureus* (positive) from coagulase-negative staphylococci (negative). *S. aureus* produces two forms of coagulase: bound and free. Bound coagulase, or “clumping factor,” is bound to the bacterial cell wall and reacts directly with fibrinogen. This results in an alteration of fibrinogen so that it precipitates on the staphylococcal cell, causing the cells to clump when a bacterial suspension is mixed with plasma. The presence of bound coagulase correlates well with free coagulase, an extracellular protein enzyme that causes the formation of a clot when *S. aureus* colonies are incubated with plasma. The clotting

mechanism involves activation of a plasma coagulase-reacting factor (CRF), which is a modified or derived thrombin molecule, to form a coagulase-CRF complex. This complex in turn reacts with fibrinogen to produce the fibrin clot.

## **Methods**

### **A. Slide test**

1. Place a drop of coagulase plasma (preferably rabbit plasma with EDTA) on a clean, dry glass slide.
2. Place a drop of distilled water or saline next to the drop of plasma as a control.
3. With a loop, straight wire, or wooden stick, emulsify a portion of the isolated colony being tested in each drop, inoculating the water or saline first. Try to create a smooth suspension.
4. Mix well with a wooden applicator stick.
5. Rock the slide gently for 5 to 10 seconds.

### **Expected results**

**Positive:** Macroscopic clumping in 10 seconds or less in coagulated plasma drop and no clumping in saline or water drop and **Negative:** No clumping in either drop. **Note:** All negative slide tests must be confirmed using the tube test. Equivocal: Clumping in both drops indicates that the organism autoagglutinates and is unsuitable for the slide coagulase test.

### **B. Tube test**

1. Emulsify several colonies in 0.5 mL of rabbit plasma (with EDTA) to give a milky suspension.
2. Incubate tube at 35° C in ambient air for 4 hours.
3. Check for clot formation. **Note:** Tests can be positive at 4 hours and then revert to negative after 24 hours.
4. If negative at 4 hours, incubate at room temperature overnight and check again for clot formation.

### **Expected results**

Positive: Clot of any size and Negative: No clot.

**Quality control:** Positive: *Staphylococcus aureus* and Negative: *Staphylococcus epidermidis*.

## **F. Fermentation media**

### **Principle**

These media are used to determine the ability of an organism to ferment a specific carbohydrate that is incorporated in a basal medium, there by producing acid with or without visible gas. A medium for *Enterobacteriaceae* and *coryneforms* and a medium for *streptococci* and *enterococci* are described.

### **Method**

A. Peptone medium with Andrade's indicator (for enterics and coryneforms)

1. Inoculate each tube with 1 drop of an 18- to 24-hour brain-heart infusion broth culture.
2. Incubate at 35°C for up to 7 days in ambient air. Note: Tubes are only held 4 days for the organisms belonging to the *Enterobacteriaceae* family.
3. Examine the tubes for acid (indicated by a pink color) and gas production.
4. Tubes must show growth for the test to be valid. If after 24 hours of incubation there is no growth in the fermentation tubes or control, add 1 to 2 drops of sterile rabbit serum per 5 mL of fermentation broth to each tube.

### **Expected results**

Positive: Indicator change to pink with or without gas formation in Durham tube.

Negative: Growth, but no change in color. Medium remains clear to strawcolored

B. Heart infusion broth with bromcresol purple indicator (for streptococci and enterococci)

1. Inoculate each tube with 2 drops of an 18- to 24-hour brain-heart infusion broth culture.
2. Incubate 4 days at 35°C in ambient air.
3. Observe daily for a change of the bromcresol purple indicator from purple to yellow (acid).

### **Expected results**

Positive: Indicator change to yellow

Negative: Growth, but no change in color. Medium remains purple

## Quality control

**Note:** Appropriate organisms depend on which carbohydrate has been added to the basal medium. An example is given for each type of medium.

A. Peptone medium with Andrade's indicator

*Dextrose:*

Positive, with gas: *Escherichia coli*. Positive, no gas: *Shigella flexneri*

Negative: *Pseudomonas aeruginosa*

B. Heart infusion broth with bromcresol purple indicator

*Sorbitol:*

Positive: *Streptococcus mutans*. Negative: *Streptococcus mitis*

## G. Citrate test

### Principle

This test is used to determine the ability of an organism to utilize sodium citrate as its only carbon source and inorganic ammonium salts as its only nitrogen source. Bacteria that can grow on this medium turn the bromthymol blue indicator from green to blue.

### Method

1. Inoculate Simmons citrate agar lightly on the slant by touching the tip of a needle to a colony that is 18 to 24 hours old. **Note:** There is no need to stab into the butt of the tube. Do not inoculate from a broth culture, because the inoculum will be too heavy. 2. Incubate at 35° to 37° C for up to 7 days. 3. Observe for development of blue color, denoting alkalinization.

### Expected results

Positive: Growth on the medium, with or without a change in the color of the indicator. The color change of the indicator is due to acid or alkali production by the test organism as it grows on the medium. Growth usually results in the bromthymol blue indicator, turning from green to blue and Negative: Absence of growth.

**Quality control:** Positive: *Klebsiella pneumoniae* and Negative: *Escherichia coli*.

## **H. Esculin hydrolysis test**

### **Principle**

This test is used to determine whether an organism is able to hydrolyze the glycoside esculin.

### **Method**

1. Inoculate the medium with 1 drop of a 24-hour broth culture.
2. Incubate at 35° C for up to 7 days.
3. Examine the slants for blackening and under the ultraviolet rays of a Wood's lamp for esculin hydrolysis.

### **Expected results**

Positive: Blackened medium which would also show a loss of fluorescence under the Wood's Lamp and Negative: No blackening and no loss of fluorescence under Wood's lamp, or slight blackening with no loss of fluorescence under Wood's lamp.

**Quality control:** Positive: *Klebsiella pneumoniae* and Negative: *Shigella flexneri*.

## **I. Indole test**

### **Principle**

The test is used to determine the ability of an organism to split tryptophan to form the compound indole.

### **Method**

#### **A. Enterobacteriaceae**

1. Inoculate tryptophane broth with 1 drop from a 24-hour brain-heart infusion broth culture.
2. Incubate at 35° C in ambient air for 48 hours.
3. Add 0.5 mL of Kovac's reagent to the broth culture.

#### **B. Other gram-negative bacilli**

1. Inoculate tryptophane broth with 1 drop of a 24-hour broth culture.

2. Incubate at 35° C in ambient air for 48 hours.
3. Add 1 mL of xylene to the culture.
4. Shake mixture vigorously to extract the indole and allow to stand until the xylene forms a layer on top of the aqueous phase.
5. Add 0.5 mL of Ehrlich's reagent down the side of the tube.

### **Expected results**

Positive: Pink- to wine-colored ring after addition of appropriate reagent and Negative: No color change after the addition of the appropriate reagent.

### **Quality control**

- A. Kovac's method Positive: *Escherichia coli* and Negative: *Klebsiella pneumoniae*
- B. Ehrlich's method Positive: *Elizabethkingia meningoseptica* and Negative: CDC group EO2

## **J. Methyl red/(MRVP) test**

### **Principle**

This test is used to determine the ability of an organism to produce and maintain stable acid end products from glucose fermentation, to overcome the buffering capacity of the system, and to determine the ability of some organisms to produce neutral end products (e.g., acetyl-methylcarbinol or acetoin) from glucose fermentation.

### **Method**

1. Inoculate MRVP broth with 1 drop from a 24-hour brain-heart infusion broth culture.
2. Incubate at 35° to 37° C for a minimum of 48 hours in ambient air. Tests should not be made with cultures incubated less than 48 hours, because the end products build up to detectable levels over time. If results are equivocal at 48 hours, repeat the tests with cultures incubated at 35° to 37°C for 4 to 5 days in ambient air; in such instances, duplicate tests should be incubated at 25° C.
3. Split broth into aliquots for MR test and VP test.

### **A. MR (methyl red) test**

1. Add 5 or 6 drops of methyl red reagent per 5 mL of broth.
2. Read reaction immediately.

### **Expected results**

Positive: Bright red color indicative of mixed acid fermentation, Weakly positive: Red-orange color and Negative: Yellow color.

### **B. VP (Voges-Proskauer) test (Barritt's method) for gram-negative rods**

1. Add 0.6 mL (6 drops) of solution A ( $\alpha$ -naphthol) and 0.2 mL (2 drops) of solution B (KOH) to 1 mL of MRVP broth.
2. Shake well after addition of each reagent.
3. Observe for 5 minutes.

### **Expected results**

Positive: Red color indicative of acetoin production and Negative: Yellow color.

### **C. VP (Voges-Proskauer) test (Coblentz method) for streptococci**

1. Use 24-hour growth from blood agar plate to heavily inoculate 2 mL of MRVP broth.
2. After 6 hours of incubation at 35° C in ambient air, add 1.2 mL (12 drops) of solution A ( $\alpha$ -naphthol) and 0.4 mL (4 drops) solution B (40% KOH with creatine).
3. Shake the tube and incubate at room temperature for 30 minutes.

### **Quality control**

- A. Methyl red Positive: *Escherichia coli* and Negative: *Enterobacter cloacae*
- B. VP (Barritt's method) Positive: *Enterobacter cloacae* and Negative: *Escherichia coli*
- C. VP (Coblentz method) Positive: *Streptococcus mutans* and Negative: *Streptococcus mitis*

### **K. Urea hydrolysis test**

#### **Principle**

This test is used to determine the ability of an organism to produce the enzyme urease, which hydrolyzes urea. Hydrolysis of urea produces ammonia and CO<sub>2</sub>. The formation of ammonia alkalizes the medium, and the pH shift is detected by the color change of phenol red from light orange at pH 6.8 to magenta at pH 8.1.

## Method

1. Streak the surface of a urea agar slant with a portion of a well-isolated colony or inoculate slant with 1 to 2 drops from an overnight brainheart infusion broth culture.
2. Leave the cap on loosely and incubate tube at 35° C in ambient air for 48 hours to 7 days.

## Expected results

Positive: Change in color of slant from light orange to magenta and Negative: No color change (agar slant and butt remain light orange).

**Quality control:** Positive: *Proteus vulgaris* and Negative: *Escherichia coli*.

**Annex 9:** Useful parametres for age estimation in camel up to 20 years (Bello *et al.*, 2013)

Parametres use in aging camel	Central incisors (years)	Lateral incisors (years)	Corner incisors (years)	Canine (years)
Eruption of permanent tooth	5	6	7	7 and half
Wearing of permanent tooth	9	10	11	12
Neck of tooth visible above gum line	13	14	15	----
Square table with increase gap	16	17	18	20

