



## **Bacteriological and Histopathological Studies on Pulmonary Lesions of Camels (*Camelus dromedarius*) in Sudan**

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### **Authors' contributions**

*This work was carried out in collaboration between all authors. Authors MEA and AMZ designed the study, wrote the protocol and histopathological studies. Author MEA collected samples, isolated and identify the bacteria and wrote the first draft of the manuscript. Authors MHS, AMA and MOH managed the literature searches and discussed the histopathological results. Authors IGI and HAMI revised the manuscript. All authors read and approved the final manuscript.*

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### **ABSTRACT**

**Aims:** To isolate and identify the aerobic bacteria associated with pulmonary lesions and study the histopathological changes.

**Study Design:** Forty-five sections of pneumonic lungs from one humped camels with different sex and ages ranging from (6 months -15 years) and originated from different states of the Sudan including Kassala, AlGadarif, Kordofan and Darfur, were subjected to bacteriological and histopathological studies.

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**Place and Duration of Study:** The study was undertaken in the Departments of Bacteriology and Pathology, Central Veterinary Research Laboratory, Ministry of Animal Resource and Fisheries, Khartoum, Sudan in 2015.

**Methodology:** The isolates were fully confirmed by full biochemical identification using conventional and automated techniques which were API kits and full automated system Vitek2 compact and the histopathological lesions were studied using H&E stain.

**Results:** Eighty bacterial isolates were recovered, they were: 15 (18.75%) *S. aureus*, 7 (8.75%) *S. epidermidis*, 5 (6.25%) *S. warner*, 1 (1.25%) *S. heamolyticus*, 6 (7.5%) *Str. pneumonia*, 5 (6.25%) *Str. pyogenes*, 1 (1.25%) *Str. suis* 11 (13.75) *E. coli*, 3 (3.75%) *Coryneulcerans*, 1 (1.25%) *C. amycolatum*, 1 (1.25%) *Actinomyces naeslandii*, *Actinomyces pyogenes*, 9 (11.25%) *K. pneumonia*, 7 (8.75%) *Ps. aeruginosa*, 2 (2.5%) *Aeromonas salmonicida*, 1 (1.25%) *Burkholderia cepacia*, 2 (2.5%) *Bacillus* and 1 (1, 25%) *Facklamia hominis*. The histopathological changes observed were emphysema with an incidence of 25 (55.6%), atelectasis in 24 (53.3%), hemorrhage in 20 (44.4%), edema in 19 (42.2%), inflammatory changes represented by fibrinous pneumonia in 25 (55.6%), Purulent bronchopneumonia in 13 (28.9%), spirasion pneumonia in 10 (22.2%), interstitial pneumonia 9 (20%) and abscesses in 4 (8.9%) and Tumors in 3 (6.7%).

**Conclusions:** Pneumonia in camel is complex multifactorial disease in which bacterial, viral, mycoplasma and fungal infections combine with other predisposing factors such as rearing systems, stress factors, climatic changes, and unhygienic conditions. Identification of the pneumonic pathogens in the present work cleared that *S. aureus*, was the most pneumonic bacteria isolated from lung tissue at rate of 18.75%.

**Keywords:** Camel; Sudan; bacteria; pathological lesions; lungs; pneumonia.

## 1. INTRODUCTION

Camel (*Camelus dromedarius*) is an important multipurpose livestock species. Uniquely adapted to harsh arid and semi-arid areas [1]. They play a vital economic role that can support the survival of millions of people. One-humped camel used mainly as a source of milk, meat and weber. In addition, camel is used as a mean of transportation and tourism [2]. Respiratory diseases of camel especially pneumonia continues to be a major problem commonly encountered in camel [2]. The disease is multifactorial in origin often involve a combination of infectious agents as well as environmental and managemental factors. Bad sanitation, stressful conditions, and an immunosuppressed state are often related to camel respiratory infections [3, 4]. Outbreaks occur in camels in different countries, causing economic losses represented in decrease animal productivity, cost of treatment, contaminated parts and sudden death [5,6]. Only few studies were found in the available literature on pathological and bacteriological affections of pneumonic lungs in camels [7], Therefore, the objective of this work was to study the bacterial etiology as well as histopathological findings of pneumonia in camels in Sudan.

## 2. MATERIALS AND METHODS

Forty-five samples of pneumonic lung tissues were collected from camel

(*Camelus dromedarius*) among different areas, ages and sex as mention above. Bacteriological and pathological studies were done according to standard methods [8,9].

### 2.1 Bacteriological Studies

A piece of lung tissue from each sample was cultivated under aseptic condition on media like blood agar, Brain heart infusion agar, MacConkey's agar, mannitol salt agar, EMB and nutrient agar, then incubated aerobically at 37°C for 24 hours. This was followed by morphological identification of the suspected colonies on the fermented media according to their staining reaction, shape, size and arrangement. The isolates were then fully confirmed by full biochemical identification using conventional and automated techniques which were API kits and full automated system Vitek2 compact.

### 2.2 Histopathological Studies

For histological sections, the collected lung samples were rapidly fixed in 10% formalin solution, paraffin embedded and sections were prepared and stained with hematoxylin and eosin according to Bancroft and Gamble [9].

## 3. RESULTS AND DISCUSSION

Table 1 shows the different species of bacteria that isolated from different cases, Table 2 shows

the various lesions found in the affected lungs and Table 3 show the bacteria isolated from affected lungs cases.

**Table 1. The bacterial species that isolated from affected lungs**

Bacterial isolates	Number of isolate	The percentage
<i>Staphylococcus spp</i>	28	35%
<i>S. aureus</i>	15	18.75%
<i>S. epidermedius</i>	7	8.75%
<i>S. warner</i>	5	6.25%
<i>S. heamolyticus</i>	1	1.25%
<i>Str. pneumoniae</i>	6	7.5%
<i>Str. pyogenes</i>	5	6.25%
<i>Str. suis</i>	1	1.25%
<i>E. coli</i>	11	13.75%
<i>Coryneulcerans</i>	3	3.75%
<i>C. amycolatum</i>	1	1.25%
<i>Actinomyces naeslandii</i>	1	1.25%
<i>Actinomyces pyogenes</i>	2	2.5%
<i>K. pneumoniae</i>	9	11.25%
<i>Ps. aeruginosa</i>	7	8.75%
<i>Aeromonas salmonicida</i>	2	2.5%
<i>Burkholderia cepacia</i>	1	1.25%
<i>Bacillus</i>	2	2.5%
<i>Facklamia hominis</i>	1	1.25%
Total	80	100%

**Table 2. Show the different lesions found in the affected lungs**

Lung lesions	The number of affected lungs
1- Emphysema	25 (55.6%)
2- Atalactasis	24 (53.3%)
3- Heamorrhge	20 (44.4%)
4- Odema	19 (42.2%)
<b>5- Pneumonia</b>	
5-a Fibrinous pneumonia	25 (55.6%)
5-b Purulent bronchopneumonia	13 (28.9%)
5-c Spirasion pneumonia	10 (22.2%)
5- d Interstitial pneumonia	9 (20%)
6- Abccesses	4 (8.9)
7- Tumors	3 (6.7%)
8-adhision	1 (2.2%)

Histopathological examination of lungs revealed thickening of the alveolar wall and interlobular septa, infiltration of inflammatory cells and exudation of edematous fluid into alveolar lumen (Fig. 1), emphysema, necrosis and sloughing of bronchiolar lining epithelium (Fig. 2), purulent core surrounded by connective tissues (Fig. 3),

hyperplasia of smooth muscle of bronchioles and blood vessels, blood vessels dilatation and hyperplasia of the bronchiolar lining epithelium (Fig. 4). Peribronchiolar fibrosis and atelectasis (Fig. 5) and fibrosis of lung parenchyma (Fig. 6).

Respiratory disorders are still serious problem facing camel rearing. The importance of respiratory diseases of camel comes from their prevalence, effect on productivity and for some extent their international spread [3,4]. The condition is a complex multifactorial disease in which bacterial, viral, mycoplasma and fungal infections combine with other predisposing factors such as rearing systems, stress factors, climatic changes, and unhygienic conditions [10].

A total of 80 bacterial isolates were collected from the 45 pneumonic lungs cultured. More than one bacterial species were isolated from 40 pneumonic lungs and no bacterial growth was obtained from 5 lungs. Failure of bacterial isolation in 5 lungs tissues with observable pneumonia may be due to suggestive of mycobacterial infection and in addition probable viral implication or other anaerobic bacteria.

Identification of the pneumonic pathogens in the present work cleared that *S. aureus*, was the most pneumonic bacteria isolated from lung tissue at rate of 18.75%. This result was higher than Al-Tarazi [7] who recorded 4%, and lower than Awol [11] who reported 21.1%. The presence of *S. aureus* in lung as a primary pathogen may represent predisposing factor for other pathogens to increase the rate of infection in animals, these results were in agreement with Taha, Sayed and Zaitoun [4,12].

In this study different types of pneumonia were noticed which varied from mild to severe fibrinous pneumonia, interstitial pneumonia, and suppurative inflammation was observed in most of the examined lungs. These results are consistent with the previous observations of Al-Tarazi and Awol [7,11] who demonstrated that acute and chronic suppurative, fibrinous, and interstitial bronchopneumonia were the predominant lesions in dromedary lungs.

In this investigation, the histopathological examination of the lungs revealed that, the recorded pulmonary lesions were classified into the following, lesions associated with changes in air contents such as emphysema and atelectasis, or inflammatory lesions as pneumonia (fibrinous

pneumonia, interstitial pneumonia and aspiration pneumonia), bronchitis or non inflammatory lesions represented by pulmonary fibrosis, hemorrhages, edema and tumors.

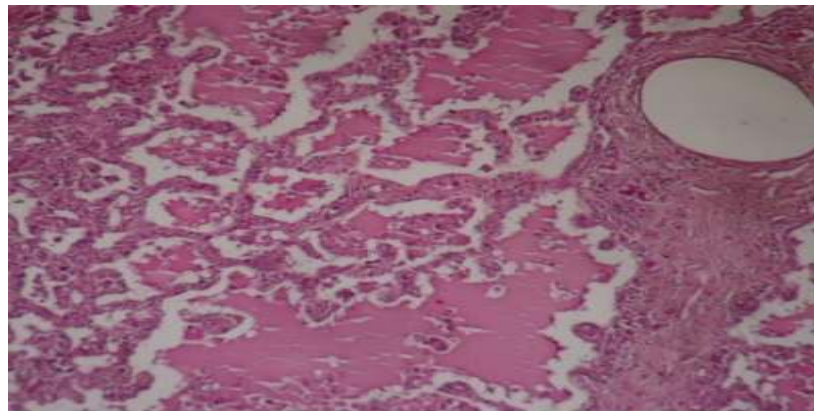
In the present study, pathological examination of lungs of 45 slaughtered camels revealed different pulmonary lesions. The incidence of lung lesions were nearly similar to those recorded by Al-Trazi [7] who recorded the incidence of lung

lesions among slaughtered camels in Northern Jordan and almahdi in Saudi Arabia Camels [13].

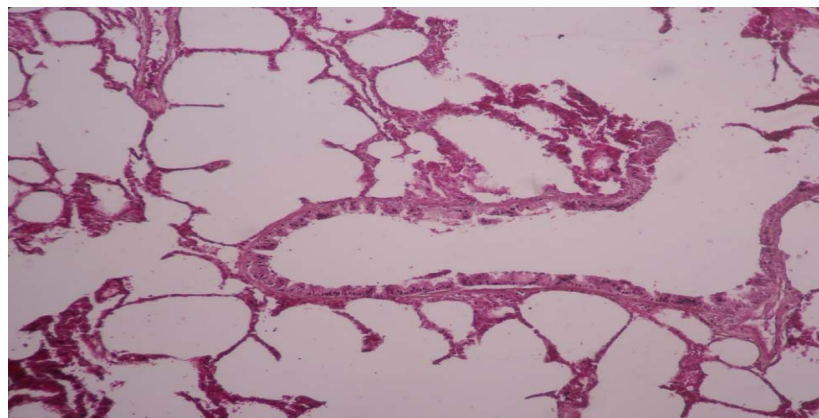
Regarding the alveolar emphysema, 25 cases were recorded in the present work and represented by 55.6% from the total lung lesions. This incidence was higher than that recorded by Al Mahdi [13] who found that, alveolar emphysema represented 20 % from total lungs lesions of slaughtered camels.

**Table 3. Show the bacteria isolated from pneumonic cases**

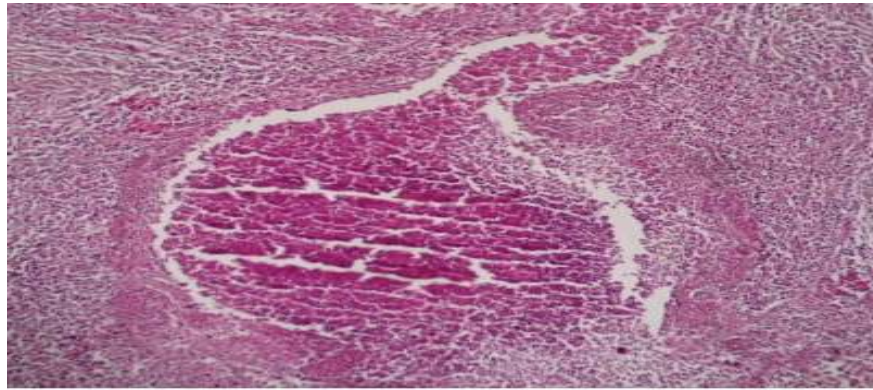
<b>Lung lesions</b>	<b>Bacteria isolated</b>
Fibrious pneumonia	<i>E. coli</i> , <i>Ps. aeruginosa</i> , <i>K. pneumoniae</i> , <i>Staph spp</i> , <i>Aeromonas salmonicida</i>
Purulent bronchopneumonia	<i>Staph spp</i> , <i>Strep spp</i> , <i>E. coli</i> , <i>Corynespp</i> , <i>Burkholderia cepacia</i> , <i>Aeromonas salmonicida</i>
Interstitial pneumonia Abscesses	<i>E. coli</i> , <i>sp. Aeruginosa</i> , <i>K. pneumoniae</i> , <i>Staph spp</i> , <i>Strep spp</i> , <i>Actinomyces spp</i> , <i>Corynespp</i> , <i>Facklamia hominis</i> , <i>Ps. aeruginosa</i>
Tumors	<i>S. suis</i> , <i>Staph spp</i> , <i>Bacillus spp</i>



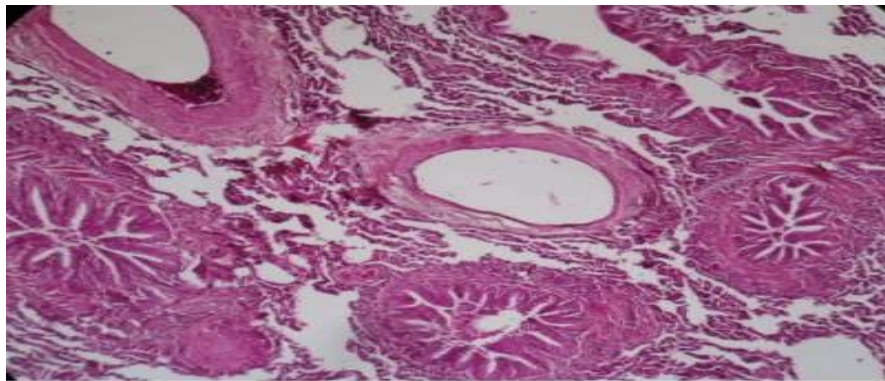
**Fig. 1. Lung section showing thickening of the alveolar septa (interstitial pneumonia), edema, infiltration of inflammatory cells, blood vessels dilatation and thickening of the interlobular septa**



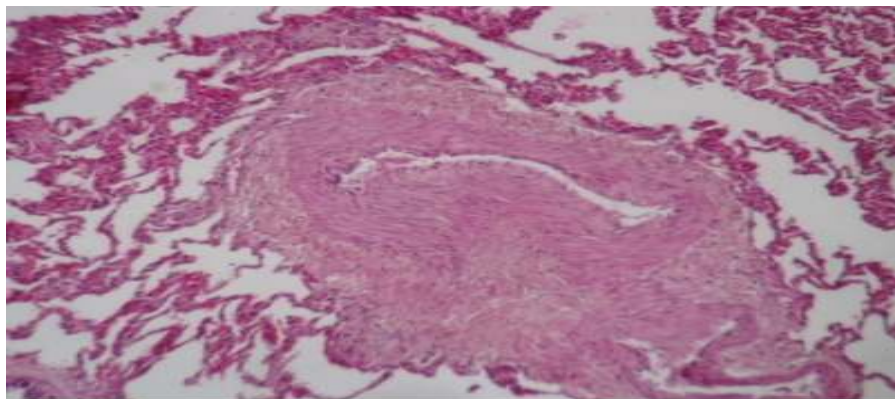
**Fig. 2. Lung section showing enlargement of airspace with evidence of loss or destruction of their walls (emphysema), necrosis and sloughing of the bronchiolar lining epithelium**



**Fig. 3. Lung section showing abscessation of pulmonary parenchyma**



**Fig. 4. Lung section showing dilatation of the blood vessels, hyperplasia of smooth muscle of bronchioles and blood vessels and hyperplasia of the bronchiolar lining epithelium**



**Fig. 5. Lung section showing narrowing of bronchiolar lumen by peribronchiolar fibrosis and alveolar collapse (atalectasis)**

Concerning atelectasis which detected in 24 (53.3%) and was accompanied with pulmonary fibrosis and alveolar emphysema. Histopathologically, the alveoli were collapsed, partially or completely compressed, free from air and slit like in appearance.

The interalveolar blood capillaries were dilated and engorged with blood. These results were in accordance with those reported by Bekele and Tigani [14,15].

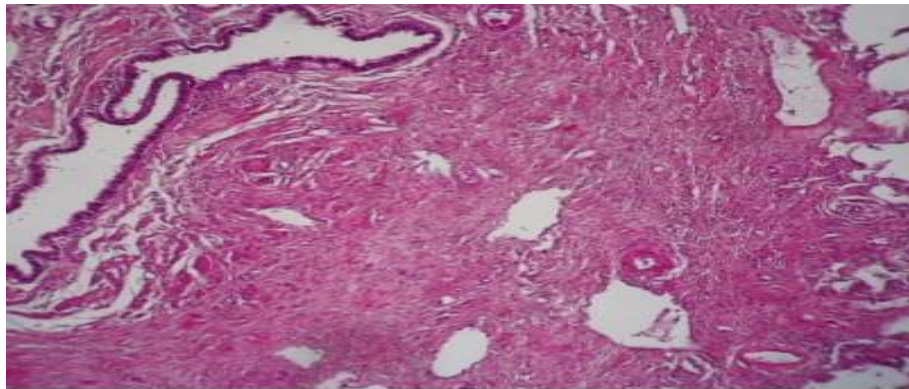
20 cases of pulmonary hemorrhages (44.4%) were detected in this study. Macroscopically,

multiple reddish patches of variable sizes were seen on pulmonary surfaces. In one case, large irregular sharply demarcated dark red area was noticed. Microscopically, severe congestion of pulmonary blood vessels and interalveolar capillaries together with the presence of multiple areas of hemorrhages. These results were in agreement with the result of Tigani [15].

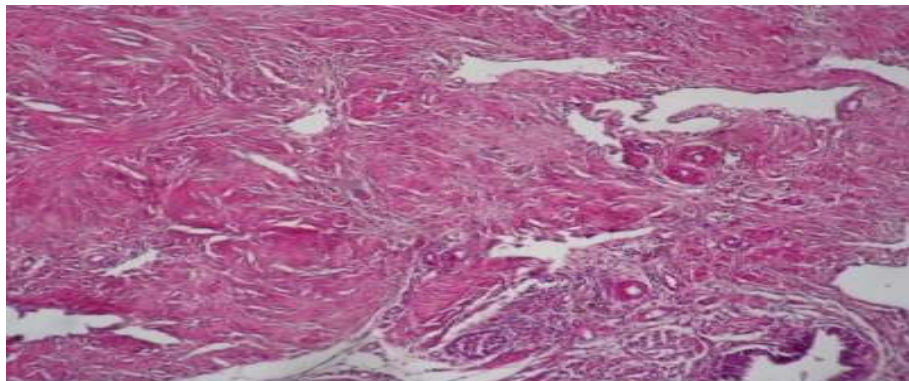
In the present work, 19 (42.2%) pulmonary edema were recorded. The gross examination of the lungs showed pale edematous areas. Microscopically, an accumulation of edematous fluid in the alveoli with congestion of the interalveolar capillaries was seen (Fig. 1).

Four (8.9%) lungs showed multiple abscesses (1-2 cm in diameter), from which *S. aureus*, hemolytic streptococci spp and *A. pyogenes* were isolated. Such types of abscesses in camel lungs were reported in Iraq, where five camels showed small localized abscesses in the lungs and *A. ovis* was isolated [16]. These results agree with what was mentioned in the literature

about causative agents of abscesses [17,18]. In this work, fibrinous pneumonia was found in 25 (55.6%), it was characterized grossly by presence of dark reddish, bluish and purple hepatized areas with emphysematous pale areas gave the lungs mosaic appearance. Microscopically, in areas of red hepatization, congestion and thrombosis of some blood vessels of the interlobular septa evidenced by large number of extravasated erythrocytes mixed with few lymphocytes and fibrin threads. Areas of gray hepatization represented by presence of fibrin threads mixed with inflammatory cells mostly lymphocytes with few macrophages and neutrophils. In some areas the alveoli contained plugs of condensed fibrin infiltrated by macrophages and few fibroblast cells as seen in (Figs. 5 and 6) In addition, also thickening of the interlobular septa and subpleural zone due to fibrinous deposits mixed with inflammatory cells particularly lymphocytes with focal areas of compensatory alveolar emphysema were noticed. These microscopic findings were in accordance with that recorded by Bhardwaj [19].



**Fig. 6. Lung section showing fibrosis of lung parenchyma**



**Fig 7. Lung section showing narrow veins lumen due to fibrosis (sclerosis)**

A total of 9 (20%) cases were observed of interstitial pneumonia, in which the lungs were grossly firm in consistency and pale pink in color with thickened interlobular septa. Microscopically, the alveolar septa were thickened by congested alveolar blood vessels and accumulation of edematous exudates containing leukocytes. In some areas the pneumocytes were proliferated leading to marked thickening of the alveolar septa (Fig. 1). Prominent thickening of the alveolar septa due to presence of homogenous, eosinophilic materials with fibrinous exudate, and few inflammatory cells mainly mononuclear cells and fibroblast cells giving the honeycomb appearance, in addition the alveoli contained mononuclear cells and few detached cells (Fig. 1).

In this study, 13 (28.9%) cases of purulent bronchitis and bronchiolitis were diagnosed. Grossly, thickening of the walls of bronchi and bronchioles with occlusion of their lumens with thick whitish mucous were seen. Microscopically, congestion and thrombosis of the peribronchial blood vessels, hyperplasia and desquamation of the lining epithelium of the bronchi and bronchioles with accumulation of desquamated cells mixed with inflammatory cells in their lumens were prevalent. Focal mononuclear inflammatory cells aggregation beside some bronchioles was noticed (Fig. 4) these microscopic pictures were also described by Al-Tarazi Zubair and [7,20].

#### 4. CONCLUSIONS

Pneumonia in camel is complex multifactorial disease in which bacterial, viral, mycoplasma and fungal infections combine with other predisposing factors such as rearing systems, stress factors, climatic changes, and unhygienic conditions.

Identification of the pneumonic pathogens in the present work cleared that *S. aureus*, was the most pneumonic bacteria isolated from lung tissue at rate of 18.75%.

Bacteria isolated from pneumonic camel lungs in this study produced clear pneumonic lesions in the pulmonary tissue, under stressful conditions, bad sanitation, and immunosuppression; they could predispose camels to other infections.

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#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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