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Pathological and Bacteriological Studies with Viral Detection from Pulmonary Lesions of Dromedary Camels Slaughtered at Akaki Abattoir, Ethiopia

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Abstract: A total of 207 camels were inspected for any respiratory disorders of which 25.6% had one or more gross lesions. Of 53 lungs with lesions 30.1%, 22.6%, 20.8%, 9.4% respectively, had interstitial pneumonia, pulmonary emphysema, bronchopneumonia and atelectasis. 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 distributions significantly were varied (P<0.05) among different age groups, lung lobes and between right and left lungs. Microscopically interstitial pneumonia was characterized by thickening of intra alveolar septae; congested interstitial capillaries and interstitial infiltrations by lymphocytes. Suppurative bronchopneumonia was characterized by bronchiolar wall and peribronchiolar infiltration by neutrophils. A total of 70 bacterial isolates were detected from 50 lung tissues with different lesions. *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. Twenty five lungs were processed for viral culture on VERO cell monolayer and 40% of them exhibited cytopathic effect that were characterized by rounding, sloughing and aggregation of infected VERO cells. Large number of apparently health camels had lung lesions on post mortem. The majority of viruses detected were from lungs with interstitial pneumonia and bacteria from bronchopneumonia.It could be concluded that the large number of microorganisms isolated might be the real cause of lesion. However, further study should be conducted to clearly elucidate causal relation.

Key words: Bacteria · Camel · Ethiopia · Lung · Pulmonary lesion · Virus

INTRODUCTION

The dromedary camel (*Camelus dromedarius*) is an important multipurpose livestock species uniquely adapted to arid and semi-arid environments [1]. Ethiopia takes the third place in Africa next to Somalia and Sudan by owning 3 million dromedary camels [2]. Ethiopian camels are an important source of milk, meat, draught power and transportation for the pastoralists. There are a number of economically important diseases that affect camels [3]. Respiratory diseases are among the emerging problems of camels that are causing considerable loss in production and death [4]. Scarce research showed that Ethiopian camels may be either carriers of, susceptible to or suffering from the vast arrays of infectious and parasitic diseases [5]. The objectives of the present study were to describe the most common forms of respiratory diseases in dromedary camels, pathological characteristics of these diseases with isolation and characterization of microorganisms from lesions.

MATERIALS AND METHODS

Study Area: The study was conducted from December, 2014 to April, 2015 at Addis Ababa Akaki Abattoir. Akaki is located at 27 km South of Addis Ababa [6]. Camels that were slaughtered at this abattoir during this study were mainly originated from Borana and Kereyu (Fentale) areas

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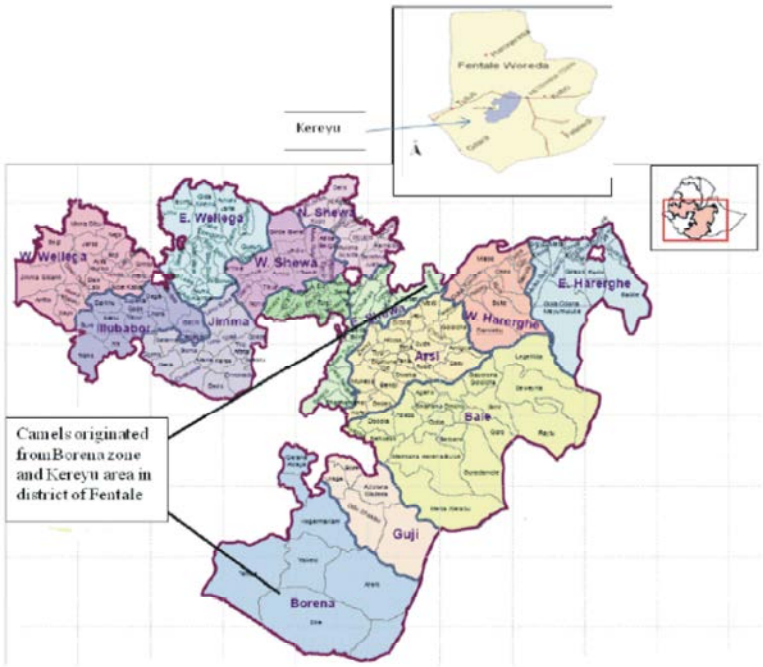

Fig. 1: The origins (Borana and Kereyu) of camels slaughtered at Addis Ababa Akaki Abattoir from map of Oromia region [8].

of Ethiopia. Borana is located at approximately 600 km South of Addis Ababa (Figure 1). The altitude ranges from 970 meter above sea level. The climate of Borana is semiarid [6]. The Kereyu Pastoral area is located at about 250 km East of Addis Ababa at an altitude of 930 meter above sea level. The prevailing climate in Kereyu area is arid. The district is affected by recurrent droughts due to disrupted rainfall patterns [7]. It falls in a semi-arid zone and receives an annual rainfall ranging from 400 to 700 mm. Temperature ranges from 29 to 38°C [6].

Study Animals: The study was conducted on 207 camels that were slaughtered at Addis Ababa Akaki Abattoir. The study animals comprised of 157 female and 50 male camels, their age ranged from 7 to 10 years and all of them were 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.

Study Design and Sampling: Non probability sampling [9] with a purposive inclusion of the study animals was conducted. Ante-mortem examination was done on 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.

Sampling Techniques and Sample 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*. [10]. Post-slaughter examination was conducted using the routine methods namely visual examination, thorough palpation and incision [11]. The respiratory tracts (From the laryngeal cartilage up to the lungs) 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^3 of the lung tissue with gross lesion was collected into 10% neutral buffered formalin [12] and then transported to laboratory.

According to Quinn *et al*. [13] about 4cm^3 tissues, particularly with active lesion at the boundary was collected aseptically into the sterile screw capped universal bottles, for bacterial isolation. About 6 gm of lung tissue with lesion was aseptically/carefully collected into labeled sterile glass tube with phosphate buffered saline solution (PBS) with antibiotics for viral cultivation [14]. Fresh tissue samples were freezed at -85°C until testing [14].

Sample Processing

Histopathology: After proper fixation, 30 representative lung tissues with different lesion types were trimmed and dehydrated in ascending grades of ethyl alcohol, cleared in 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 [15].

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 [16]. After proper incubation, growth characteristics were examined [17]. 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 [16, 18].

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) [14].

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%.

RESULTS

Pathological Findings and Their Frequencies: From the 207 lungs examined, 53 (25.6%) had one or more gross lesions of which 26.4% were from male camels and 73.6% were from female camels. The distribution of gross lesions did not show significant difference ($P>0.05$) by sex of camels (Table 1). However, the difference was statistically significant ($P<0.05$) when age and lung lobes were considered as factor. The details of factors considered, lesion distribution over lung lobes (Caudal, cranial and middle) and lung part (Right and/or left) were shown on Tables (2-4).

Interstitial Pneumonia: The details of the lesions types, frequency or percentage by camel's age & sex were shown in Table (4). Camels of 7 years old were most commonly affected by interstitial pneumonia (7.2%) than camels of 8-9 (1%). However, interstitial pneumonia was not detected from camels of 10 years old and above (Table 4).

Interstitial Pneumonia: 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 (Fig. 1a). Three of these lungs showed characteristic indicator of interstitial pneumonia, the rib imprint on their surfaces (Fig. 1b).

Microscopically, 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 (Fig. 2a). In four lung tissue sections, the alveolar lumen contained eosinophilic edema fluid and thick eosinophilic hyaline membranes in the alveoli. There were infiltrated neutrophils in the interstitium, which indicate acute interstitial pneumonia (Fig. 2b).

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

	Sex				
Lung lobes	Male	Female	Total	P-value	χ^2- value
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%)		

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

	Age groups					
Lung lobes	7 years	8-9 years	10 years	Total	P-value	χ^2- value
Cranial	7 (3.4%)	7 (3.4%)	0 (0.0%)	14 (6.8%)	0.007	17.586
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%)		
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

	Lung lobes						
Sides of lungs	Cranial	Caudal	All lobes	No lesion	Total	P-value	χ^2-value
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: Frequency and percentage of pulmonary lesions types by age and sex

		Sex		Age group (Years)		
Pulmonary lesions	Proportion	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%)

Pulmonary Emphysema: Pulmonary emphysema showed significant ($P<0.05$) variation 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. When incised, the size (Volume) appeared decreasing with no exudate coming out. Seven lungs had large focal emphysematous bullae (Air filled pockets) (Fig. 3a), 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 crepitating on palpation (Fig. 3b). Microscopically, the emphysematous lungs revealed distended alveoli (Fig. 4a) some of which contained some edema fluid. Many alveoli were ruptured and formed large empty spaces (Fig. 4b).

Bronchopneumonia: Grossly lungs affected by bronchopneumonia were characterized by irregular consolidations especially at cranio-ventral regions (Fig. 5). Consolidated lungs had variable appearance which ranged from dark red to grey and the affected areas

Table 5: Association of bacterial species isolated and type of pulmonary lesion

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

Note: χ^2= 117.930; 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 edema).

were hard and firmer on palpation. Microscopically, there were peribronchiolar and bronchiolar infiltration with inflammatory cells particularly neutrophils. There were accumulations of exudates in the lumen of bronchioles and the alveoli (Fig. 6a). 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 (Fig. 6b), which indicate chronic suppurative bronchopneumonia.

Pulmonary Atelectasis: Grossly, those atelectatic parts looked dark red in color, were depressed, had fleshy texture and were non-spongy. In three lungs with atelectasis there was emphysema surrounding the adjacent regions (Figure 7). Microscopically, the alveoli were collapsed, look slit like and the alveolar walls appeared parallel and close together. The alveoli adjacent to the atelectatic alveoli were distended and emphysematous (Fig. 8).

Pulmonary Hemorrhage: From the examined lungs, 3.7% had hemorrhages in their cranial lobes. Grossly, in the affected lungs, the cranial lobes were distended (Emphysematous) and on their surfaces, there were multifocal and patchy hemorrhages (Fig 9a). When the affected regions were incised the tissues revealed hyperemic parynchyma with oozing of foamy red fluid (Fig. 9 b). Microscopically, the main features detected in the affected regions of the lungs were intra-alveolar and minor intra-bronchiolar hemorrhages (Fig. 10 a), inflammatory cells in air spaces, mild bronchiolitis and the alveoli were filled with acidophilic edema fluid (Fig. 10 b).

Hydatid Cysts: Female camels (3.7%) with age range of 8-9 years old had multiple hydatid cysts with different sizes. Grossly, the majority of the hydatid cysts were small and detected in the parynchyma of the lung up on palpation or up on careful inspection (Fig. 11a). The larger cysts were easily visible on the surface of the lungs. Clear watery fluid flowed out when those cysts were sectioned and the walls of the cysts were made of white-thick capsule. Microscopically, lung tissues adjacent to the cyst capsule revealed compressed alveolar septa and narrow bronchiolar spaces with intense inflammatory cells infiltration. The fibrous connective tissue capsule of the cysts revealed eosinophilic outer fibrous layer and faint eosinophilic inner germinal layer that contained calcium casts (Fig. 11b).

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 of which 37 (52.9 %) were Gram positive and 33 (47.1%) were Gram negative.

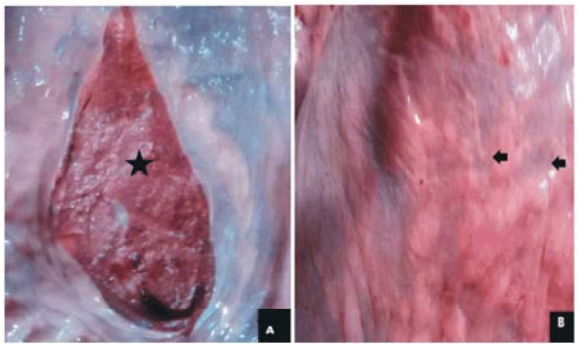

Fig. 1: Lung with 'meaty' appearance on cut surface (Star) (A) and rib imprints (Arrows) on the surface (B), both of which are characteristic indicator of interstitial pneumonia

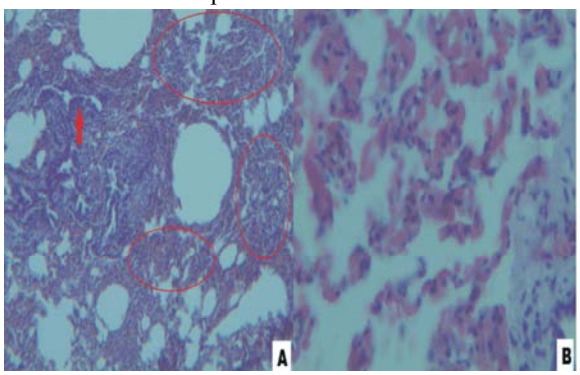

Fig. 2: Interstitial pneumonia. Note excessively thickened alveolar septa (Red circles) and narrow slit like (Arrow) alveoli (A) from chronic case and relatively slightly thickened septa but with eosinophilic exudate (Hyaline) from acute case (B) (H&E stain, 40X)

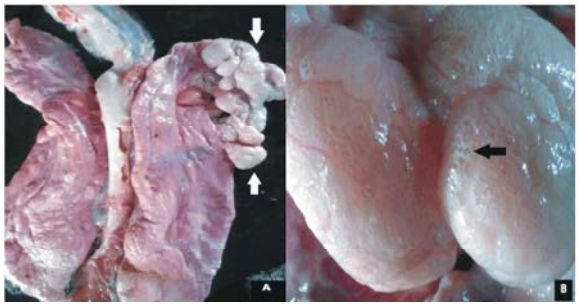

Fig. 3: Note the characteristic bullous alveolar emphysema (A) of the right cranial lobe with multiple air pockets (White arrows) and air bubbles (B) clearly visible (Black arrow)

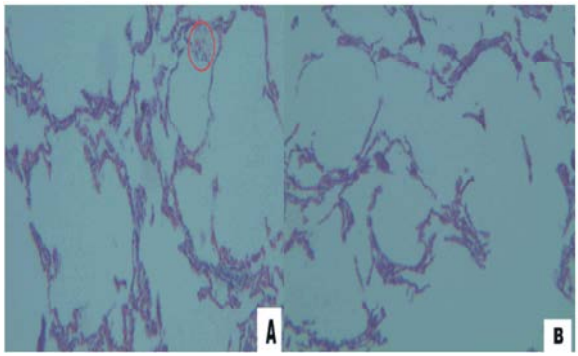

Fig. 4: Note the distended alveoli (A) and large spaces resulted from ruptured alveoli due to over distention (B) (H & E stain, 40X)

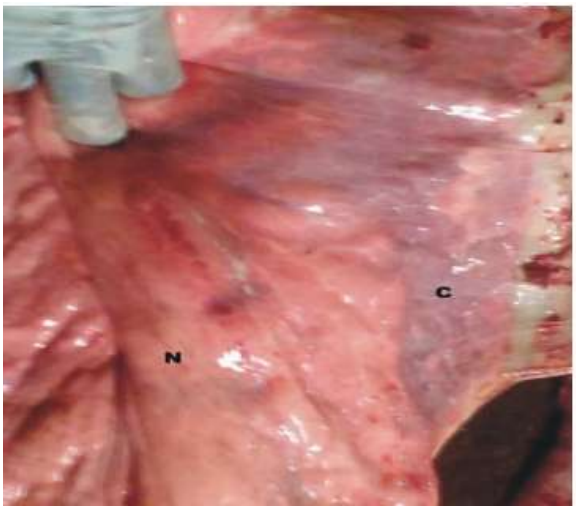

Fig. 5: Note the dark red and consolidated (C) lesion of bronchopneumonia over cranioventral regions. The dorsal part is normal (N) and spongy

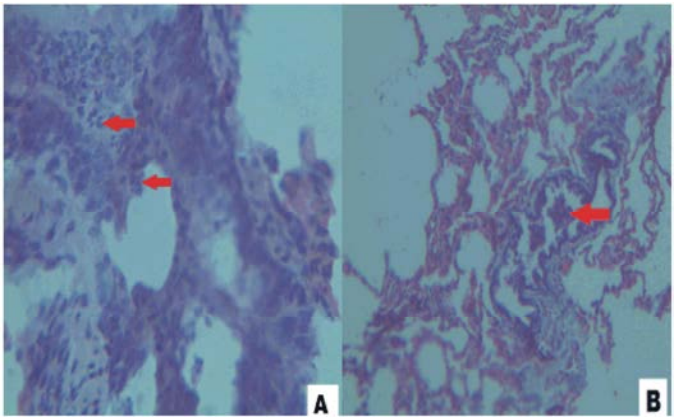

Fig. 6: Note the neutrophilic exudates with characteristic nuclei (Red arrow) in alveoli (A) from acute bronchopneumonia and the mononuclear cells in the bronchiolar lumen (B) from chronic bronchopneumonia(H& E stain, 40X)

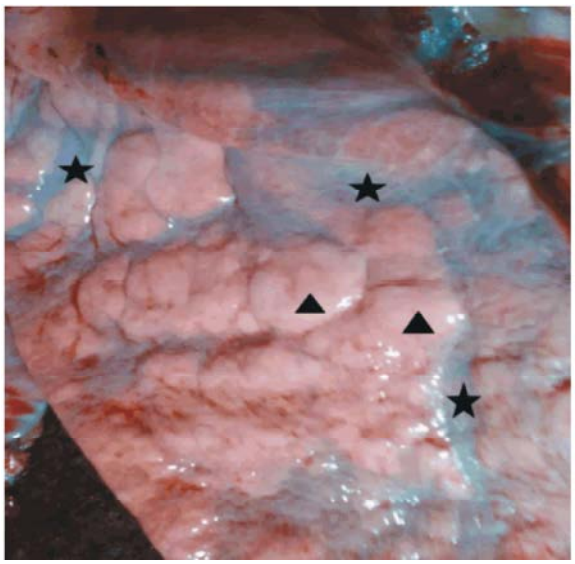

Fig. 7: Note the atelectatic regions (Stars) were deep adjacent to the emphysematous tissues (Arrow heads)

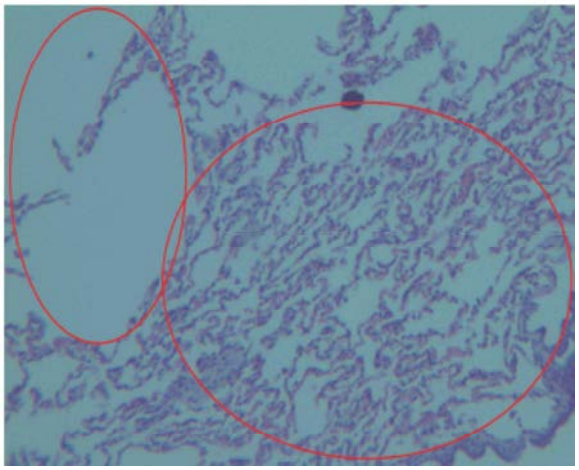

Fig. 8: Microscopic lesion of atelectatic lung, the alveoli were compressed at atelectatic region (Large circlular inset) and ruptured at adjacent emphysematous region (Small circlular inset) (H & E stain, 40X)

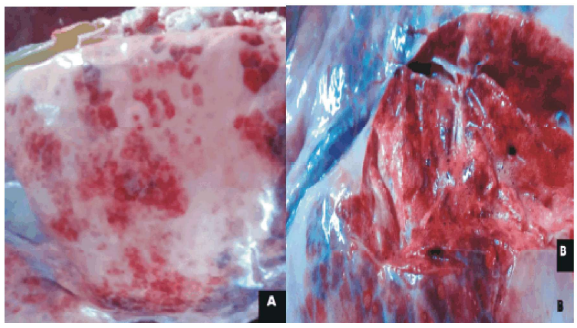

Fig. 9: Patchy haemorrhages on the surface of cranial lobe of lung (A). Hyperemic parenchyma and frothy fluid of hemorrhagic cranial lobe on cut (B)

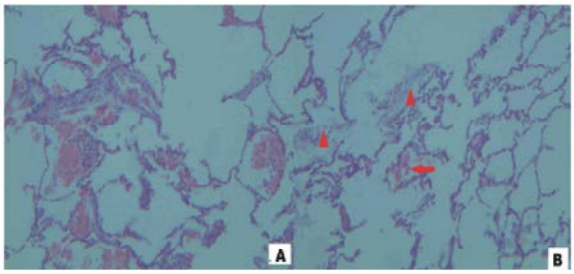

Fig. 10: Intra-alveolar haemorrhage (A) and inflammatory cells (Arrow) and acidophilic edema fluid free (Arrow heads) in alveoli (B) (H & E stain, 40X)

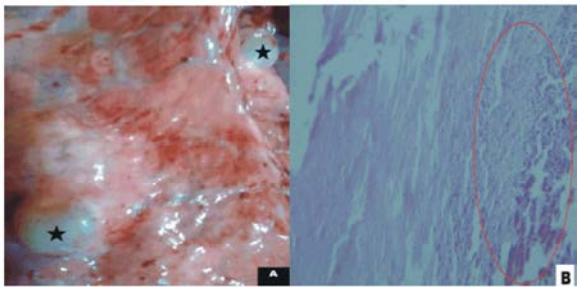

Fig. 11: Transparent fluid filled hydatid cysts (Astrix) (A) and compressed alveoli (Circular inset) near the cysts. Note the eosinophilic stained fibrous connective tissue of capsule (on the left of the circular inset) and calcium deposits

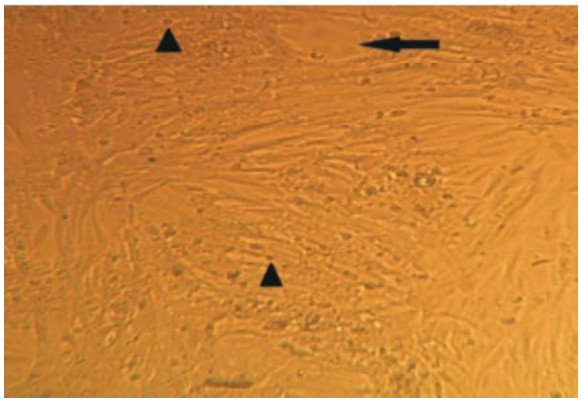

Fig. 12: Note the CPE characterized by cellular shrinkage and aggregation (Arrow heads) and sloughing of infected cells (Arrow).(10X)

From the isolated bacteria, *Rhodococcus equi* was the most frequent isolate (20%) and it was frequently isolated from lung with pulmonary atelectasis and pulmonary emphysema followed by *Corynebacterium* spp. *Aeromonas hydrophila* and *Actinobacillus* spp. were frequently isolated from lungs with interstitial pneumonia. *Coagulase negative staphylococci*, *Aeromonas hydrophila, Actinobacillus* spp and *Bacillus* spp. were bacterial spp frequently isolated from lung with bronchopneumonia.

Viral Detection: Twenty five lungs with different gross lesions were cultured for virus detection of which 40% exhibited cytopathic changes (CPE) on VERO cell monolayer which indicates the presence of virus. Lungs with interstitial pneumonia were the most frequently (50%) positive for CPE that were characterized by rounding and sloughing of infected VERO cells (Fig. 12) followed by lungs with bronchopneumonia (30%).

DISCUSSION

We found significant number of camels looking healthy had one or more pulmonary lesions during postmortem examination. We also found there was variation in number of camels with pulmonary lesions at the same abattoir but at different year [18] and at different abattoirs in Ethiopia [19]. 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 or there might be other factors. Stress factors, sanitation and climatic conditions, which vary from place to place, are the major predisposing factors to respiratory infections of camels [20].

Interstitial pneumonia and pulmonary emphysema were the most frequent lesions encountered in this study. Interstitial pneumonia was also isolated frequently from camels in Jordan [21] and Egypt [22]. Using specific pathogen free VERO cell monolayer we detected viral induced cytopathic effect frequently from lungs with interstitial pneumonia. The most probable cause of large number of interstitial pneumonia in these camels here in Ethiopia and elsewhere as well might be viral infection. However, this will give gap for another detail study that should include isolation, further characterization of viral agents and experimental reproduction of identical lesions. Gelagay *et al*. [14] isolated, Respiratory syncytial virus, Adenovirus, Peste Des Petits Ruminants Virus (PPRV) and Parainfluenza viruses from camels lungs with lesions using universal degenerate oligonucleotide primed– polymerase chain reaction (DOP-PCR) and conventional polymerase chain reaction (PCR) techniques. Of the large number of bacteria isolated in this study, the majority was from lesions with bronchopneumonia. Camel's respiratory disorder 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 to produce the disease [23].

It can be concluded that, from previous as well as from this study pulmonary diseases were prevalent in camels. This domestic animal with uniquely adaptation to the hot and arid environment and contributing a lot to Ethiopian pastoralists need good health management. Further study on various infectious and parasitic diseases that disproportionate camel's potential should be conducted and measures to reduce disease should be implemented.

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