Histopathology of Multiple viral infections in lung of camel (Camelus Dromedaries) in Sudan

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Abstract: Pneumonia is the most common respiratory disease of camels. It's defined as an inflammation of the lung. It can be caused by direct infection with viruses, bacteria and fungi, aspiration or inhalation. 45 lungs were collected from camels of different ages, from different parts of the Sudan. These lungs showed Histopathological lesions. One of them was found positive for parainfluenza 3, adenovirus, respiratory syncytial virus (RSV) and BVD as a mixed infection. No bacteria isolated from this sample but the above four type of viral antibodies were detected using Fluorescence antibody technique (FAT). Complete histopathological pictures for these viruses were observed. FAT was found to be more sensitive diagnostic methods for detection of virus than Eliza.

Keywords: bacteria, FAT, histopathology, pneumonia, virus.

I. Introduction
Camels are the most capable animal species in utilizing marginal areas and in survival and production under harsh environmental conditions [1, 2]. The camel is well adapted to the climatic extremes and is well appreciated for its significance in the pastoral economy [3] Camels can live in areas that are inhospitable to other domestic animals and therefore have important feature in the capacity of humans to survive and using these drier regions [4]

Camels are important animals reared mainly for meat, milk and transport; Camels are mainly raised by migratory pastoralist in Africa, where the majority of camels are kept (approximately 11.5 million animals represent two thirds of the world’s camel population; over 80% of which are kept in Eastern African [2]. Pneumonia is the most common respiratory disease of camels. It's defined as an inflammation of the lung .It can be caused by direct infection with viruses, bacteria and fungi, aspiration or inhalation. [5]. Viruses encountered in respiratory infections in camels are parainfluenza 3, influenza virus A and B, adenovirus, respiratory syncytial virus (RSV) and infectious bovine rhinotracheitis (IBR) [6]. This study was conducted with an attempt to isolate and characterize bacterial and viral causes of respiratory infections from one lung during this survey that showing severe pneumonic lesions.

II. Materials And Methods

Isolation of bacteria
Different types of media were used for isolation of bacteria from the specimen. Those were: nutrient agar, blood agar and MacConkey agar. The identification of bacteria was done using API kits and full automated system Vitek 2 compact.

Procedures for isolation of bacteria
The surface of the lung sample was cauterized with a red hot scalpel blade for decontamination. A deep incision was made in the lung surface using sterile scalpel blade; a sterile swab was dipped into the incised area and streaked onto sheep blood agar plate. From the incised area a piece of sample was cut and put in brain heart infusion broth in a bijou bottle. The cultures were incubated aerobically at 37° C for 24 hr. Any plate that did not show growth within 24hr was incubated for five days and examined daily to ensure bacterial growth before considering it negative.

Histopathological method
Tissue specimen collected for histopathological examination were fixed in 10%formalin solution, processed by standard paraffin embedding technique; microtectomy of the embedded tissue to 5-6 micron thick sections was carried out. The sections were placed onto glass slides, dried and stained with hematoxylin and eosin (H&E) .

Direct immunofluorescence technique
Lung tissue was impressed in slide by forceps and was left to dry. Then the tissue was fixed by acetone overnight at -20°C or for 15 minutes at room temperature. Then diluted fluorescein-labeled conjugate 1:20 in PBS was added to the fixed slide. The slide was incubated for 1 hour at room temperature and then rinsed with PBS and was left to dry. After dryness one drop of mounting solution (glycerol/PBS: 9 vol/1 vol) was added to the slide and then covered with cover slip and was examined under fluorescent microscope.
III. Results

No bacterium was isolated even after five days incubation.

Histological examination of lung section reveals acidophilic homogenous or faintly granulomar materials filling alveoli, interstitial tissues and alveolar septa (fig 1) and thickening of alveolar septa and there capillaries by infiltration of mononuclear cells (lymphocyte, plasma cells and macrophages ) and red blood cells and in some areas fibroblast. This causes atelectasis of alveoli. Such histological pictures indicate interstitial pneumonia (Fig1), proliferation, bronchiolitis and bronchopneumonia with severe infiltration of mononuclear and macrophages cell inside bronchioles with focally coagulative necrosis often present. The bronchiolar epithelium is generally normal but in some areas hyperplasia (Fig2). Accumulation of mononuclear cells in form of lymphoid follicles surrounded by fibrous tissues were seen in some sections (Fig3). The epithelium of some bronchioles has grown into alveoli and replaced the alveolar epithelium. Also there were subsequent metaplasia and overgrowth of alveolar epithelium, sloughing of epithelium cells inside alveolar lumen. The adjoining lung tissues exhibits signs of interstitial pneumonia (Fig 4).

Fig 1:
This section of lung shows oedema and interstitial pneumonia.
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Fig 2:
This section of lung shows bronchiol filled by mononuclear cells and tissue depress surrounded by fibrous tissues.

Fig 3:
This section of lung shows lymphoid follicle like character surrounded by fibrous tissues.
Four different types of viral antibodies were detected using Direct immunofluorescence technique and these were: parainfluenza 3 (PI3), adenovirus, respiratory syncytial virus (RSV) and BVD.
IV. Discussion

No bacterium was isolated from this pneumonic lung; this may refer to heavy doses of antibiotics that used by the owners of animal in Sudan.

The histopathological findings in this study indicates typical histological pictures of viral infection caused by para influenza 3 which causes consolidation, pseudoepithelization of alveoli hyperplasia of bronchiolar epithelium. Adeno virus infection resulted in accumulation of fluid in perivascular and peribronchial pneumonia with cytomegaly of epithelium cells. Respiratory syncytial virus causes interstitial pneumonia and pulmonary adenomatosis where thin alveolar cells replaced columnar or cuboidal cells and bronchiolar proliferation [9].

Parainfluenza virus 3 is one of the viruses known to cause respiratory infection. According to [12] Parainfluenza-3 was found as primary responsible agent of the camel respiratory disease outbreak in Ethiopia. Antibodies to PI3 were detected in this sample, this is agree with Intisar, 2010 who test 495 camel sera and found that The highest seroprevalence was observed in Central (92.6%), then in Eastern (92.2%) and Central to South Sudan (82.5%); the lowest prevalence was found in Northern Sudan (64.8%).

Respiratory syncytial virus is one of the main viruses associated with respiratory infections in various animal species[13,14,15] also antibodies to RSV were detected in this sample. The role of Paramyxoviruses (PI3 and RSV) in causation of respiratory infections in camels in Sudan is poorly studied (Intisar, 2010). She found that the highest prevalence was observed in Western (33.5%) then Central (31.6%) and Eastern Sudan (23.5%). BVD and adenovirus antigen were reported for the first time in sudan by Intisar using FAT, in the current study antibodies of BVD and adenovirus were detected in this sample. FAT was found to be more sensitive diagnostic methods for detection of virus [9], and direct immunofluorescence technique was used to confirm the ELISA positive antigens for BHV-1, BVDV, RSV, PI3 and adenovirus [8]. In this study lesions associated with pneumonia were emphysema, congestion, hemorrhages, edema, abscess, pleurisy, bronchitis and bronchiolitis, this agree with [10,11].

V. Conclusion

Respiratory diseases are the major threats to the camel population in Sudan. The bacteria and viruses causes pneumonia must be detect. Histopathological picture more severe in mixed viral infection than individual one. Antibodies of the four viruses were detected in this study; this may pay attention to the importance of this combined infection and to the close association of them with each other and to the severe complications of animal health situation if camels are exposed to mixed infection. FAT was found to be more sensitive diagnostic methods for detection of virus

References

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