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BACTERIOLOGICAL AND HISTOPATHOLOGICAL STUDIES ON PULMONARY ABSCESSES IN CAMELS (*Camelus dromedarius)* SLAUGHTERED AT NYALA SLAUGHTERHOUSE, SOUTH DARFOUR STATE, SUDAN

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المستخلص

أجريت هذه الدراسة في مسلخ نيالا بولاية جنوب دارفور في الفترة ما بين مارس 2009 الي مايو 2010 حيث تم فحص عدد 712 من الابل نحرت للاستهلاك الانساني وأظهرت النتائج اصابة 95 حيواناً(67 من االنوق و28 من الجمال) بالخراجات الرئوية بمعدل اصابة بلغت 13.3%. جمعت 95 عينة تمت معاملتها للفحص البكتيرى والفحص النسيجى المرضى.

أوضحت الاختبارات البكتيرية وجود نمو في 70 عينة (73.7 %), منها 10 عينات أظهرت نمو أكثر من بكتيريا في العينة الواحدة. وقد تم عزل 82 نوعاً من البكتيريا, شملت البكتيريا العنقودية، السبحية, المكورات الداخلية، الانتروباكتر، الكورانيوباكتريم، الباستريلا والاكتينومياسس.

احتوت معظم الخراجات الرئوية علي البكتيريا العنقودية, السبحية والكورانيوباكتريم والاركانوباكتريم والسدوموناس.

تم عزل الأنواع الاتية من خراجات الرئة بالجمال لاول مرة بالسودان:- الاركانوباكتريم بيوجينس, الراديوكوكس اكوي والاستربتوكوكس أبارس.

أظهرت الدراسة النسيجية المريضة وجود حالات نخروالتهابات رئوية ونفاخ وخزب بالاضافة الي احتقان في الأنسجة الرئوية. وقد عزلت العنقودية والكلبسيلا من التهاب الرئة الخلالي الحاد والخزب, الرئوي أما السبحية والباستورلا والسدوموناس فقد عزلت من التهاب الرئة الخلالي المزمن والتهاب القصبات الرئوية.

أوضحت هذه الدراسة وجود نسبة عالية للاصابة بالخراجات الرئوية وعليه نوصي بالعمل علي تحاشي هذه الاصابة حتي نضمن وصول لحوم سليمة للاستهلاك الانساني.

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Abstract

A total of 712 camels were presented for slaughter at Nyala slaughterhouse during March 2009 to May 2010. Ninety five camels revealed presence of abscess lesions in their lungs with a prevalence rate of 13.3%. Ninety five specimens were collected from the lung abscesses and processed for bacterial isolation and histopathological examination. Bacterial growth was observed in 70 (73.7%) samples, 60 (85.7%) of them showed pure growth, while 10 (14.3%) showed mixed growth on cultured media. Total of 82 bacterial isolates were *Staphylococcus* spp. 27(32.9%), *Streptococcus* spp. 18(21.9%), *Corynebacterium* spp. 10(12.2%), *Pseudomonas* spp. 5(6.0%), *Arcanobacterium pyogenes* 4(4.9%), *Proteus* spp. 4(4.9%), *Micrococcus* spp. 3(3.8%), *Pasteurella multocida* 3(3.8%), *Enterococcus* spp. 2(2.4%), *Enterobacter aerogenes* 2(2.4%), *Klebsiella pneumoniae* subsp.*pneumoniae* 2(2.4%), *Bacillus cereus* 1(1.2%), *Rhodococcus equi* 1(1.2%). In 10 samples, more than one species were isolated from a single abscess. For example, *C. renale* was isolated with *S. aureus*, *Pseud.aeruginosa* with *C. pseudotuberculosis*, and *Enter. aerogenes* with *A. pyogenes*. The histopathological sections of the lung abscesses revealed central necrotic areas, acute interstitial pneumonia, chronic interstitial pneumonia, bronchopneumonia, oedema and emphysema. Most the lungs abscesses contained *S. aureus*, *Strept. pyogenes*, *C. pseudotuberculosis*, *A. pyogenes* and *Pseud. aeruginosa*. In addition, *K. pneumoniae* subsp.*pneumoniae* and *S. aureus* were isolated from acute interstitial pneumonia and oedema while *Strept. pyogenes*, *Past. multocida* and *Pseud. aeruginosa* were isolated from chronic interstitial pneumonia and bronchopneumonia. This study showed a high incidence of pulmonary abscesses and we recommend working to avoid this infection in order to ensure access to safe meat for human consumption.

Key words: Bacterial, Histopathological, Pulmonary abscesses, Dromedary camels, South Darfur State.

Introduction

Camel is an economically important animal in the Sudan, where it is used for production of meat, milk, hides and in transportation. These attributes have stimulated our interest to study the disease agents which affect the health and productivity of camels. Abscesses located in the visceral organs represent the most common problem in the camel meat industry as they lead to wasting animals slaughtered for human consumption, a public health hazard to abattoir workers, meat inspectors as well as consumers (Bakhsh Alhendi, 2000). Abscesses of the visceral organs are only detected after the slaughter of animals, because even hundreds of small abscesses or several large abscesses rarely cause clinical manifestation (Nagaraja and Chengappa, 1998). Abscesses of the visceral organs in camels are among the most prominent emerging problems due to considerable losses in production and varying mortality rates (Bekele, 1999). The pulmonary abscesses of the camel develop as single or multiple lesions that affect the upper and lower parts of the lung, causing cough and emaciation. Suppurative pulmonary abscesses can be caused by septicaemic emboli of the lung capillaries, fungal infections, parasitic infections, aspiration pneumonia, traumatic reticulopericaditis and bacterial pneumonia (Jones *et al.*, 1997). Several studies have focused on bacterial pathogens as possible causative agents of abscesses in camels (Songer *et al*., 1988; Radwan *et al*., 1989; Afzal *et al*., 1996 and Tejedor *et al*., 2008). *Staphylococcus* spp., *Streptococcus* spp., *Corynebacterium pseudotuberculosis*, *Arcanobacterium pyogenes*, *Pseudomonas aeruginosa, Klebsiella pneumoniae* subs. p*neumoniae* and *Rhodococcus equi* were found to be the most frequent agents involved in pulmonary abscesses (Al-Tarazi, 2001; Azmi, 2008; Younan *et al*., 2005; Abubakar *et al*., 2010; Kinne *et al*., 2011and Shiferaw *et al*., 2012). Like arise, lung may constitute an important source of abscesses dissemination to other vital organs such as the liver resulting in great economical losses due to condemnation at meat inspection. Accordingly, this study was designed to isolate and identify the bacteria associated with pulmonary abscesses and to study correlate the histopathological findings in affected camels slaughtered at Nyala slaughterhouse, South Darfur State.

Materials and methods

Study area and target host

A total of 712 camels were presented for slaughter at Nyala slaughterhouse during the period from March 2009 to May 2010. Post-mortem examinations were carried out using routine visual, palpation and incision methods. Ninety five camels showed abscess lesions in their lungs. Samples were collected from all affected lungs and each one was divided into two portions.

The first part was placed into sterile plastic bag on ice and transported to the Bacteriology Department at Nyala Veterinary Research Laboratory for examination within two hours of collection. The second portion was fixed in 10 % buffered neutral formalin and transported to the Department of Pathology at Veterinary Research Institute (VRI) to be processed, paraffin embedded and sectioned at 5µm thickness. These sections were stained with Haematoxylin and Eosin (H&E) (Bancroft and Stevens, 1990).

Bacterial isolation and phenotypic identification

The surface of the affected lungs was touched by a heated spatula. An incision was made with a sterile scalpel blade; impression smears were made and stained with Gram stain. Pus from the inner part of the lesion was removed using a sterile loop and inoculated into blood agar with 7% defibrinated sheep blood and MacConkey agar plates. The plates were incubated both aerobically and anaerobically (Gas-pack anaerobic system, Cat. no. 70304) at 37 ºC for 24 h. If no growth was observed after 24 h, the plates were incubated for additional 48 -72 h. Bacterial colonies were characterized morphologically (e.g., colour, size, and edge). If the culture had showed a variety of bacterial colonies, a portion of a single colony was picked and subculturing was performed to obtain pure culture (Carter, 1984). Identification of bacteria isolated to the species level was performed using biochemical analysis (Don *et al*., 2005; Barrow and Feltham 1993). In addition, analytical prolife index kits were also used to identify *Enterobacteriaceae* spp. (KB003: Hi25), *Staphylococcus* spp. (KB004: HiStaph) and *Streptococcus* spp. (KB005: HiStrep) (Quinn *et al*., 1994).

Results

Out of 712 camels presented for slaughter at Nyala slaughterhouse during the period from March 2009 to May 2010. Ninety five camels showed abscess lesions in their upper and lower parts of the lungs; the lesions were encapsulated by a relatively thick layer of fibrous tissue and contained odourless thin, creamy or watery pus; yellowish white, white, grey or yellow in colour and sometimes contained blood. The overall prevalence rate was 13.3%. Twenty five (26.3%) abscess samples failed to yield bacterial growth, while bacterial growth was observed in seventy (73.7%) of the samples. sixty (85.7%) showed pure growth, while ten (14.3%) showed mixed growth.

The recovered bacterial species are shown in Table 1. Furthermore, some isolates were identified to the species level as shown in Table 2. In 10 samples, more than one species were isolated from a single abscess. For example, *C. renale* was isolated

with *S. aureus*, *Pseud.aeruginosa* with *C. pseudotuberculosis*, and *Enter. aerogenes* with *A. pyogenes*.

Table 1: Bacterial isolates from lung abscesses of dromedary camels slaughtered at Nyala slaughterhouse, South Darfur State during 2009-2010 (n = 95).

Bacterial isolates	Frequency	Percentage (%)
Staphylococcus spp.	27	32.9
Streptococcus spp.	18	21.9
Corynebacterium spp.	10	12.2
Pseudomonas spp.	5	6.0
Arcanobacterium pyogenes	4	4.9
Proteus spp.	4	4.9
Micrococcus spp.	3	3.8
Pasteurella ***multocida***	3	3.8
Enterococcus spp.	2	2.4
Klebsiella pneumoniae subsp.*pneumoniae*	2	2.4
Enterobacter ***aerogenes***	2	2.4
Bacillus cereus	1	1.2
Rhodococcus equi	1	1.2
Total	82	100

(n = Total of samples)

Table 2: Bacterial species isolated from lung abscesses of dromedary camels slaughtered at Nyala slaughterhouse, South Darfur State during 2009-2010 (n = 95).

Bacterial species	Frequency	Percentage (%)
Staphylococcus aureus	16	19.5
S. *lentus*	3	3.7
S. *chromogens*	4	4.9
S. *epidermidis*	4	4.9
Streptococcus pyogenes	12	14.8
Streptococcus equi subsp. *zooepidemicus*	2	2.4
Strept. uberis	4	4.9
Corynebacterium pseudotuberculosis	8	9.8
C. ***diphtheria****e*	1	1.2
C. ***renale***	1	1.2
Pseudomonas ***aeruginosa***	3	3.7
Ps. ***vesicularis***	2	2.4
Arcanobacterium pyogenes	4	4.9
Proteus ***vulgaris***	2	2.4
P. ***mirabilis***	2	2.4
Micrococcus ***luteus***	2	2.4
M. ***Kristinae***	1	1.2
Pasteurella ***multocida***	3	3.7
Klebsiella pneumoniae subsp.pneumoniae	2	2.4
Enterococcus ***durans***	1	1.2
Enterococcus ***faceium***	1	1.2
Enterobacter ***aerogenes***	2	2.4
Bacillus cereus	1	1.2
Rhodococcus equi	1	1.2
Total	82	100

(n = Total of samples)

Most of the histopathological sections of the lung abscesses revealed central necrotic areas and inflammatory cells mainly macrophages, lymphocytes, plasma cells and neutrophils that were surrounded by fibrous tissue (Figure 1). Acute interstitial pneumonia, chronic interstitial pneumonia, bronchopneumonia, oedema and emphysema were also seen in some sections, they were characterized by thickening of alveolar septa, infiltration of inflammatory cells and surrounded proliferation with fibrous tissue and enlarged alveolar spaces (Figures 2, 3, 4, 5, 6).

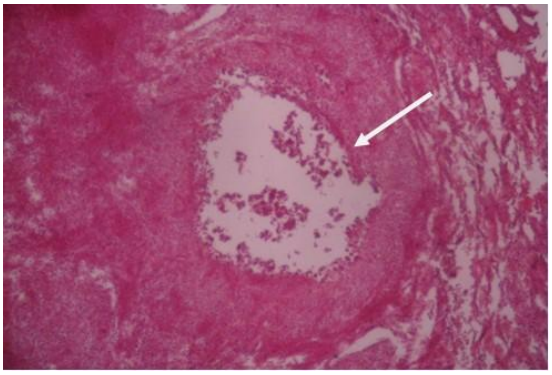

Fig. (1): Lung abscess showing central necrotic area and inflammatory cells surrounded by fibrous capsule. H&E stain, x100 magnification

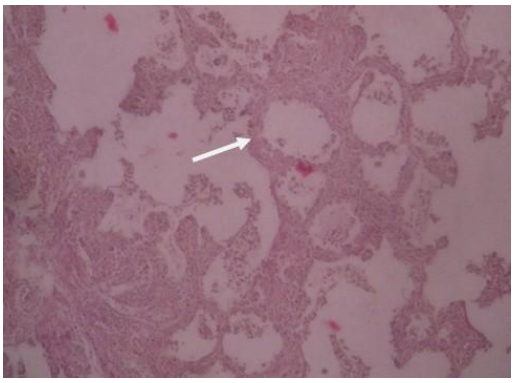

Fig.(2): Acute interstitial pneumonia showing thickening of alveolar septa and infiltration of inflammatory cells (lymphocytes). H&E stain, x100 magnification

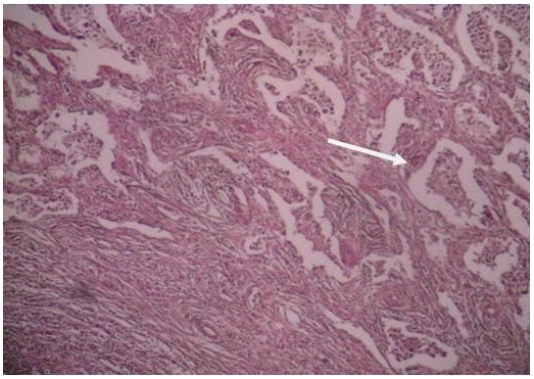

Fig. (3): Chronic interstitial pneumonia showing thickening of alveolar septa with proliferation of fibrous tissue and inflammatory cells. H&E stain, x100

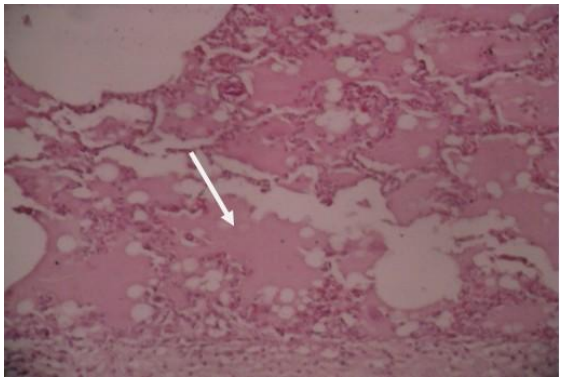

Fig. (4): Bronchopneumonia showing infiltration of inflammatory cells around the bronchiole and in the alveoli. H&E stain, x100 magnification

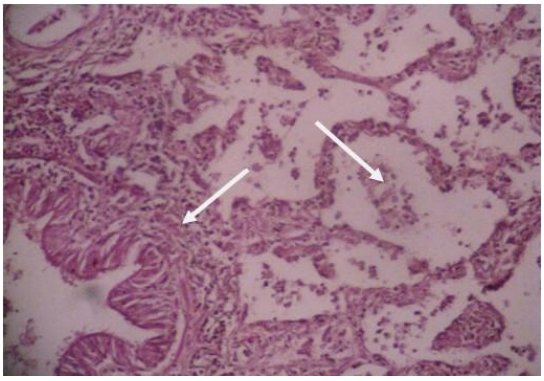

Fig. (5): Oedema in alveoli and interstitial area. H&E stain, x100 magnification **.**

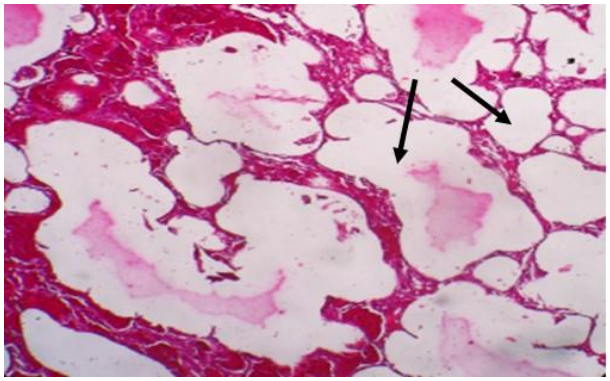

Fig. (6): Lung emphysema (enlarged alveolar spaces). H&E stain, x100 magnification.

Discussion

This study investigated the bacteria associated with abscesses in the lungs of camels slaughtered in Nyala. The prevalence rate was 13.3 %; this is similar to the findings of Al-Tarazi, (2001) who reported 10.2 % lungs with gross lesions in camels slaughtered in Jordan.

Staph. aureus, *Streptococcus* spp., *C. pseudotuberculosis*, *A. pyogenes* were isolated from the lungs abscesses. These findings were in consistent with previous reports showing that *Staphylococcus* spp., *Streptococcus* spp. and *Corynebacterium* spp. are

bacterial agents commonly isolated from abscesses in the lungs of camels (Al-Tarazi, 2001; Azmi, 2008; Abubakar *et al*., 2010 and Kinne *et al*., 2011). The isolation of *A. pyogenes* in this study showed its involvement with these abscesses. Based on available literature, *A. pyogenes* has not been previously reported as a pathogen in the abscesses of camels in the Sudan. Abubkar *et al*. (2010) isolated *A. pyogenes* from dromedary camels having pulmonary abscesses. This pathogen is often isolated from the abscesses of lungs in ruminants, pigs and sometimes humans (Hommez *et al*., 1991).

The recovery of *R. equi* in this study supports the findings of Kinne *et al*. (2011) who isolated *R. equi* from camel lungs that were diffusely consolidated with large caseous areas. The organism was also associated with diffuse necrotizing lymphadenitis in the bronchial and mediastinal lymph nodes of a 2-year-old male llama (Hong and Donahue, 1995).

The isolation of *C. pseudotuberculosis* serotype I and serotype II in this study, strongly supports the findings of Tejedor *et al*. (2008), who reported that dromedary camels can be susceptible to serotype II and that preventive measures should be considered when camels are in contact with horses or cattle affected by pseudotuberculosis.

Other bacterial isolates in this study, such as *Pseud. aeruginosa*, *M. luteus*, *E. durans*, *B. cereus*, *Prot. vulgaris*, *Enter. aerogenes*, *Past. multocida* and *K. pneumoniae* subsp.*pneumoniae* were previously reported as having association with pneumonia and mastitis in the dromedary camel (Barbour *et al*., 1985; Bekele, 1999 and Younan *et al*., 2005).

The histopathological sections of the affected lungs revealed pulmonary necrotic areas, acute interstitial pneumonia, chronic interstitial pneumonia and bronchopneumonia. These results are in agreement with similar findings related to pulmonary lesions of the dromedary camel (Bekele, 2008; Al-Tarazi, 2001; Kane *et al*., 2005; Shiferaw *et al*., 2012). Al-Tarazi, (2001) reported that chronic pneumonia that affected older camels was mainly caused by *M. haemolytica* and *Pseudomonas aeruginosa*, whereas interstitial pneumonia that affected young camels was caused by *Klebsiella* spp. and *E. coli*.

The evidence of emphysema in this study supported the results of Lopez, (2001) who reported that emphysema in animals is always a secondary lesion. The oedema as found in this study is generally reported in association with pulmonary lesions at the early stage and is an integral part of inflammatory response as indicated by Lopez (2001).

Conclusion

This study showed a high incidence of prevalence rate (13.3), it appears that although camels generally feed away from contaminated soils, they are still infected with several microorganisms due to environmental changes and husbandry practices herds mixing, sharing of water and pastures, and migration with other animal species. These microorganisms include bacteria which lead to abscesses formation in the lungs; some of these were *S. aureus*, *Strept. pyogenes*, *C. pseudotuberculosis*, *A. pyogenes* and *Pseud. Aeruginosa*, and Actinomyces pyogenes and Rhodococcus . Furthermore, more than one species may be isolated from a single abscess e.g. *C. renale* was isolated with *S. aureus*, *Pseud.aeruginosa* with *C. pseudotuberculosis*, and *Enter. aerogenes* with *A. pyogenes*. On the other hand, some species were including *Strept. pyogenes*, *Past. multocida* and *Pseud. aeruginosa* were isolated from areas of chronic interstitial pneumonia and bronchopneumonia, while *K. pneumoniae* subsp.*pneumoniae* and *S. aureus* were isolated from sites of acute interstitial pneumonia and oedema. Twenty five abscess samples failed to yield bacteria when cultured, which suggested that either bacteria were not viable, due to treatment with antibiotics just before slaughters or that the abscesses were caused by other causative agents. Finally, we recommend working to avoid this infection in order to ensure access to safe meat for human consumption and further studies are necessary to verify the impact of isolated bacteria on human heath.

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