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Full Length Research Paper

Bacteriological studies on pulmonary lesions of camel (*Camelus dromedarius*) slaughtered at Addis Ababa abattoir, Ethiopia

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This study was carried out with the aim of identifying bacterial species involved in lung lesions of camels slaughtered between October 2009 and April 2010 at Addis Ababa abattoir enterprise, Ethiopia. All camels were originated from Borana and Kereyu pastoral areas. A total of 387 lungs were inspected during the study period, of which 300 (77.5%) possessed gross pulmonary lesions. Of which 72 lungs with lesions were processed for bacteriology and bacterial growth was observed from 50 of the pneumonic lung samples. A total of 54 bacterial species were isolated and identified. These included coagulase negative staphylococci (21.1%), *Streptococcus* species (19.3%), *Escherichia coli* (17.5%), *Francisella tularensis* (5.3%), *Flavobacterium* species (5.3%), *Rhodococcus equi* (5.3%), *Bordetella bronchoseptica* (3.5%), *Aeromonas hydrophila* (3.5%), *Neisseria* species (3.5%), *Streptococcus agalactia* (1.8%), *Staphylococcus aureus* (1.8%), *Pasteurella trehalosi (*1.8%), *Pasteurella anatipestifer (*1.8%), *Pseudomonas aeruginosa* (1.8%), *Micrococcus* species (1.8%) and *Mycobacterium* species (5.3%). These pathogens could induce respiratory diseases under stressful conditions or predispose camels to other opportunistic infections.

Key words: Bacteria, camel, pulmonary lesion, Ethiopia.

INTRODUCTION

Various lower respiratory tract diseases have been reported in camels, but the definitive etiology of most respiratory diseases is not determined. A variety of viral, fungal, bacterial and parasitic microorganisms have been associated with outbreaks of respiratory disease among camels. Viruses encountered in respiratory infections in camels are parainfluenza virus 3 (PIV3), influenza viruses A and B, adenovirus, respiratory syncytial virus (RSV), and infectious bovine rhinotracheitis (IBR) (Dioli and Stimmelmary, 1992; Intisar et al., 2010).

The camel is a comparatively hardy animal and is less susceptible to many of the diseases that affect other livestock species in the same areas (Schwartz and Dioli, 1992; Dirie and Abdurhaman, 2003). However, it is apparent that little is known about the diseases from which it suffers. The dromedary of Ethiopia has suffered from different diseases like trypanosomosis, camel pox, mange, respiratory disease complex, hemorrhagic septicemia, cephalopsis, pustular dermatitis, dermato-mycosis, gastrointestinal parasites and acute plant poisoning for the past so many years (Tefera, 2004). In Ethiopia few studies were conducted on the extent of respiratory problems of camels compared to other livestock species (Bekele, 2008). Respiratory diseases have been a major threat to the camel population of Ethiopia with the whole camel population affected during the camel respiratory disease outbreak of 1995. The causative agent has not yet been conclusively identified; however, some reports indicate the involvement of peste des petits ruminants (PPR)-like virus, *Streptococous equi*, *Pasteurella* and *Mycoplasma* species (Bekele,

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1999; Roger et al., 2001).

Respiratory disease outbreak in camels characterized by sudden death has occurred recently in the Afar and Oromia regions during 2005/2006 (Wernery et al., 2006). In 2007 a similar disease was reported from the Somali and Oromia regions of Ethiopia. Investigations of these outbreaks by a number of Veterinary Institutions and Laboratories in Ethiopia have failed to isolate the exact etiological agent of the disease. Yet there is a need to identify the causes of respiratory diseases in camels in order to design better control strategies. The present study was conducted with the objectives of isolating and identifying the types of bacterial species involved in pulmonary lesions of camel and to determine their association with types of pneumonia.

MATERIALS AND METHODS

Study area

The study was conducted from October 2009 to April 2010 at Addis Ababa abattoir enterprise. All camels slaughtered were originated from the Borana and Kereyu areas of Ethiopia. Borana is located at approximately 600 km South of Addis Ababa at an altitude of 500 to 2500 m above sea level. The climate of Borana is semi arid. Kereyu is located at about 250 km East of Addis Ababa at an altitude of 930 m above sea level. The prevailing climate in Kereyu area is arid (NMSA, 1999).

Study animals

The study was conducted on 387 camels slaughtered at Addis Ababa abattoir enterprise. Camels were transported from their origin to the abattoir by trucks and kept at lairage for 3 to 4 days. All of them appeared healthy during antemortem inspection.

Data collection and sampling procedure

Before slaughter, general physical examination was conducted on each camel in the lairage and postmortem examination was made by visual examination and thorough palpation of the airways, lungs and the associated bronchial lymph nodes for the presence of any lesion.

Immediately after slaughter, lung tissue of size greater than 10 x 10 cm and when possible the whole lung showing pneumonic lesion was collected using sterile forceps and scissors or scalpel blade and wrapped separately using sterile polyethylene bags. Samples were then transported to the laboratory using ice box. Sampling was done following the procedures given by Carter (1984), Quinn et al. (1999) and the bench protocol of National Veterinary Institute bacteriology laboratory, Debre-Zeit, Ethiopia. The surface of tissue samples was burned by hot scalpel blade and inoculum samples were taken from the inner part of the tissue using sterile pasteur pipette. Inoculum samples were inoculated onto appropriate primary media (blood agar or other media enriched with 10% horse serum). The cultures were incubated at 37°C for 24 to 48 h depending on the growth of bacteria. Bacteria colonies were characterized by their color, size, edge, etc. Single colony was taken and smear was prepared for Gram's reaction. If the culture had showed different bacterial colonies, sub-culturing was performed to obtain a single colony. Finally, identification of bacteria to the species level was performed using different biochemical tests.

Data analysis

Data were analyzed using SPSS software version 15.0 and descriptive statistics like percentage and frequency distribution were used to determine the proportion of bacterial isolates. Chi-square test statistic with 5% absolute precision was used to test the significance of the association between bacterial isolates and different pulmonary lesions.

RESULTS

In total, 387 lungs were examined, of which 300 (77.5%) possessed one or more types of lesion. The remaining 87 lungs (22.5%) had no evidence of gross lesion. From the 300 lungs containing lesions, 72 were suitable for bacteriological isolation and were processed for aerobic bacterial isolation. The remaining 228 lungs were affected with conditions like hydatidosis, emphysema, atelectasis, aspiration of blood, pneumoconiosis, pulmonary edema and congestion and hence, bacterial isolation was not attempted. Only 69.4% (n = 50) of lung tissue samples yielded bacteria colonies and identified either to the genus or species level. The remaining 30.6% (n = 22) of the lung lesion samples showed no bacterial growth. Among these 53.7% (n = 29) were gram-positive and 46.3% (n = 25) were gram-negative. In addition, acid-fast positive bacteria (*Mycobacterium* species) were identified on the basis of Ziehl-Neelson staining of 3 lung tissue samples suspected of tuberculosis. The frequency of isolation of the identified bacteria from camel lung tissue samples is presented in Table 1.

Association of bacterial isolation rate and types of pneumonia

The isolation rate of bacterial species and their involvement in different types of pneumonia are shown in Tables 2 and 3. Statistically, there was significant difference ($p < 0.05$) in the isolation rate of bacteria from the different types of pulmonary lesion. This difference is mainly attributed to absence of bacteria from acute interstitial pneumonia (AIP).

DISCUSSION

This study assessed the types of aerobic bacteria involved in different types of pulmonary lesions encountered in camel lungs slaughtered at Addis Ababa abattoir enterprise. The isolation rate of bacteria experienced in this study was lower than the studies of Al-Doughaym et al. (1999), Al-Tarazi (2001), Zubair et al. (2004), Kane et al. (2005) and Tigani et al. (2006). In these studies, bacterial species were isolated from all

Table 1. Aerobic bacterial species isolated from pneumonic camel lungs.

Bacterial isolate	Frequency	Percentage (%)
Coagulase negative staphylococci (CNS)	12	21.1
Streptococcus species	11	19.3
Escherichia coli	10	17.5
Francisella tularensis	3	5.3
Flavobacterium species	3	5.3
Rhodococcus equi	3	5.3
Bordetella bronchoseptica	2	3.5
Aeromonas hydrophila	2	3.5
Neisseria species	2	3.5
Streptococcus agalactia	1	1.8
Staphylococcus auerus	1	1.8
Pasteurella trehalosi	1	1.8
Pasteurella anatipestifer	1	1.8
Pseudomonas aeruginosa	1	1.8
Micrococcus species	1	1.8
Mycobacterium species*	3	5.3
Total	57	100

* Based on Ziehl Neelson staining.

Table 2. Types of pneumonia and isolation rate of bacteria.

Types of pneumonia	Total number of lungs	Bacteria	
		Present (%)	Absent (%)
Acute suppurative bronchopneumonia (ABP)	32	32 (100)	0 (0)
Chronic suppurative bronchopneumonia (CBP)	16	9 (56.25)	7 (43.75)
Fibrinous bronchopneumonia (FBP)	2	2 (100)	0 (0)
Acute interstitial pneumonia (AIP)	6	0 (0)	6 (100)
Chronic interstitial pneumonia (CIP)	16	7 (43.75)	9 (56.25)
Granulomatous pneumonia (GP)	3	3 (100)	0 (0)
Total	75	53	22

pneumonic lung samples of camel. In addition, Azizollah et al. (2009) also isolated various bacterial species from lung lesions of apparently healthy camels. This variation in the isolation rate of bacteria could be due to variation in sample size, sample collection and processing, types of pneumonia or it could also be due to variation in the cause of pneumonia among geographical areas. The pathogenesis of pneumonia is complex and multifactorial. In the present study, failure of bacteriological isolation in 22 (30.6%) pneumonic lung tissue samples might be due to involvement of other organisms commonly involved in respiratory problems such as *Mycoplasma*, viruses, parasite, fungi or other anaerobic bacteria.

A total of 54 bacteria species were isolated and identified from 50 pneumonic lung samples and these are discussed as follows:

a). Coagulase negative *staphylococci* (CNS) were the commonest bacteria isolated at a rate of 21.1%. This is a little lower than Tigani et al. (2006) who recovered CNS at a rate of 27.9% from pneumonic lungs lesions of camels but considerably 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. They also occur as environmental contaminants. Staphylococcus infections are opportunistic and associated with trauma, immunosuppression, intercurrent infections and other stress factors (Quinn et al., 2002). Isolation of staphylococci from the lungs of camel in this study may be attributed to the stress of transportation and confinement. The camels are exposed to dusty conditions for prolonged periods (3 to 4 days) in the lairage without sufficient feed and water.

b). *Streptococcus* species were recovered at a rate of 19.3% which is considerably higher than that of Al-Tarazi

Table 3. Association of bacterial species isolated and types of pneumonia.

Bacterial isolate	Type of pneumonia					
	ABP	CBP	FBP	AIP	CIP	GP
Coagulase Negative Staphylococci (CNS)	7	4	-	-	1	-
Streptococcus species	7	-	-	-	4	-
E. coli	6	2	-	-	2	-
Francisella tularensis	1	-	2	-	-	-
Flavobacteria species	2	1	-	-	-	-
Rhodecoccus equi	2	-	-	-	1	-
Bordetella bronchoseptica	2	-	-	-	-	-
Aeromonas hydrophila	2	-	-	-	-	-
Neisseria species	2	-	-	-	-	-
Streptococcus agalactia	1	-	-	-	-	-
Staphylococcus aureus	-	1	-	-	-	-
Pasteurella trehalosi	1	-	-	-	-	-
Pasteurella anatipestifer	1	-	-	-	-	-
Pseudomonas aeruginosa	-	1	-	-	-	-
Micrococcus species	-	1	-	-	-	-
Mycobacterium species*	-	-	-	-	-	3

* Based on Zihel Neelson staining.

(2001) and Zubair et al. (2004) who recovered at a rate of 5.33 and 7%, respectively. However, this finding is in agreement with Tigani et al. (2006) who recovered at a rate of 13.9%. *Streptococci* species live as commensals in the mucus membrane of the upper respiratory, alimentary and lower genital tract. Many infections of *Streptococci* are probably endogenous and stress related.

c). *Escherichia coli* was isolated at a rate of 17.5% in this study. This is lower than the results of Al-Tarazi (2001) who recovered at a rate of 26.7% but much higher than 6.2 and 3% recovery rate reported by Al-Doughaym et al. (1999) and Zubair et al. (2004), 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).

d). *Francisella tularensis* was isolated at rate of 5.3%. It has been found in an array of warm- and cold-blooded hosts (mammals, insects, arthropods, fresh water protozoans) indicating remarkable bacterial adaptability (Petersen et al., 2009). There are no previous reports of isolation of this bacterium from camels and other species of animals in Ethiopia. *F. tularensis* is highly invasive and after infection bacteraemia develops with localization and granuloma formation in parenchymatous organs and lymph nodes (Hirsh and Zee, 1999; Quinn et al., 1999).

e). *Rhodococcus equi* was isolated at a rate of 5.3%. There are no previous reports of *R. equi* isolation from camels suffering respiratory disease. It inhabits soil and also the intestinal tracts of animals. It is generally acquired by inhalation of dust contaminated with *R. equi* (Quinn et al., 2002).

f). *Flavobacterium* species were isolated at a rate of 5.3%. These are widespread in the environment, soil, water and are acquired by the inhalation of contaminated dust (Quinn et al., 1999). There are no previous reports of *Flavobacterium* species from camels with respiratory disease.

g). *Bordetella bronchoseptica* was recovered at a rate of 3.5%. *B. bronchoseptica* infects a wide range of animals including man. *Bordetella* species are commensals on the mucous membranes of the upper respiratory tract (Quinn et al., 2002). Therefore, it is likely that the isolation of these bacteria is due to the camels suffering from predisposing factors such as stress of transportation, shortage of feed and water and concurrent infections.

h). *Aeromonas hydrophila* was recovered from 3.5% of pneumonic lung tissue samples. It is opportunistic pathogen of fish, reptile and rarely mammals (Quinn et al., 2002). There are no previous reports of isolation of this organism from lung lesions of the camel and the camels might acquire it from contaminated drink water.

i). *Streptococcus agalactiae* was recovered from 1.8% pneumonic lung tissues. There are no reports about its role in the respiratory disease of animals. However, it is the leading cause of neonatal pneumonia, sepsis, and meningitis leading to significant morbidity and mortality in human (Baker and Edward, 2001).

j). *Pseudomanas aeruginosa* was isolated from 1.8% of pneumonic lung samples. This result agrees with the study of Tigani et al. (2006) who isolated the bacteria at a rate of 1.07% but lower than Al-Tazari (2001) and Zubair et al. (2004) who recovered at a rate of 12 and 5%, respectively. *P. aeruginosa* is isolated from cases of pneumonia in all large animal species (Radostits et al., 2007).

k). *Pasteurella trehalosi* and *Pasteurella anatipestifer* were isolated each at a rate of 1.8% from pneumonic lung tissue samples. *Pasteurella* species are commensals on the mucosa of the upper respiratory tract of animals (Quinn et al., 2002). *Pasturella* species are involved as a primary or secondary agent in pneumonia of cattle, sheep, goats, donkeys and horses following stressful conditions (Quinn et al., 2002; Roadostits et al., 2007).

l). *Staphylococcus aureus* was recovered at a rate of 1.8% from pulmonary lesions. This is lower than the results of Al-Doughaym et al. (1999) and Al-Tarazi (2001) where *S. aureus* was isolated at rates of 10.6 and 24.8%, respectively. *S. aureus* occurs both as commensal on skin and mucous membranes and as environmental contaminant. Infection of *S. aureus* can be either endogenous or exogenous in origin (Quinn et al., 2002).

m). *Micrococcus* species were isolated from 1.8% of pneumonic lungs. There are no previous reports of this organism being isolated from diseased camel lungs. *Micrococcus* species are commensals of the respiratory tract. Despite the fact that *Micrococcus* species are considered non-pathogenic (Quinn et al., 1999), its isolation from pneumonic lung samples of animals by various workers (Al-Tarazi, 2001; Kane et al., 2005; Tigani et al., 2006) indicated that it could have a role in the development of respiratory infections.

n). *Neisseria* species were recovered from 1.8% of lung samples. Several researchers (Quinn et al., 2002; Kane et al., 2005; Tigani et al. 2006) have suggested that this organism has a role as an opportunistic pathogen in respiratory tract infection and have isolated from pneumonic lung samples of camel.

o). *Mycobacterium* species was identified from three lung samples with granuloma on the basis of Ziehl-Neelson staining only and culture was not attempted. This result is in agreement with the study of Zubair et al. (2004) who isolated *Mycobacterium* species from 2% of camel lungs. Many species of *Mycobaterium* are pathogenic and the principal cause of tuberculosis in human and animals (Dungworth, 1993; Lopez, 2001; Quinn et al., 2002; Radostits et al., 2007).

Association of bacterial species isolated with types of pneumonia

In agreement with this study, Dungworth (1993), Lopez (2001) and Radostits et al. (2007) determined that, the most common bacterial pathogens causing acute suppurative bronchopneumonia in cattle, sheep, goats, donkeys and horses domestic animals were *Pasteurella* species*, Bordetella bronchoseptica, Streptococcus* species*, E. coli,* several species of *Mycoplasma, Rhodococcus equi* and *Staphylococcus* species in attempts made to isolate and characterize aerobic bacteria.

Al-Tarazi (2001) identified *Pasteurella haemolytica, P. aeruginosa, E. coli* and *Klebsiella* species from chronic suppurative bronchopneumonia in camels. The absence of bacterial growth in 53.25% of lung samples showing chronic suppurative bronchopneumonia could be either due to the involvement of pathogens other than aerobic bacteria or due to removal of pathogens by inflammatory process during the acute stage of the disease (Lopez, 2001; Radostits et al., 2007).

Fibrinous bronchopneumonia occurred in two lung samples with both yielding *F. tularensis*. According to Dungworth (1993) and Lopez (2001), pathogens causing fibrinous bronchopneumonia in domestic animals include *Pasteurella* species*, Actinobacillus pleuropneumoniae, Mycoplasma mycoides* species and *Haemophilus* species. However, the role of *F. tularensis, Flavobacterium* species*, A. hydrophila and Micrococcus* species in pneumonia and respiratory tract infection in camels has not previously been studied or documented. The role of these bacteria in respiratory infection of camels requires further study.

Most interstitial pneumonias in animals are infectious and caused by viruses, bacteria or parasites. In addition, pneumoconiosis can also cause interstitial pneumonia (Dungworth, 1993). The inability to culture bacteria from some of the lung samples presenting interstitial pneumonia, plus the absence of parasites in histological sections and the presence of parafollicular hyperplasia in their associated bronchial lymph nodes, leads to the conclusion that a virus may have been the causative agent in such cases.

In this study 54 bacterial species were isolated from pneumonic lung samples of camel. Failure to isolate bacteria from 22 lung samples may be due to the involvement of other pathogens such as anaerobic bacteria, virus, *Mycoplasma* and fungi. All camels included in the study were apparently healthy at the time of slaughter whilst possessing one or more type of lesions in their lung. The lesions encountered in this study may act as predisposing factors for respiratory disease outbreaks in camels influenced by stress factors such as environmental change, extremes of climatic conditions, transportation and shortage of feeds and/or water. Therefore, controlling respiratory diseases of the camel's should give due attention in alleviating stress during different managemental practices including transportation, lairaging, feeding, watering, etc. and on those measures that has to be taken during stressful conditions.

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This study was carried out with the aim of identifying types of gross and histopathological lesions in lungs of camels slaughtered between October 2009 and April 2010 at Addis Ababa abattoir enterprise, Ethiopia. All camels were originated from Borana and Kereyu areas. A total of 387 slaughtered camel lungs were inspected during the study period. Of which, one or more gross lesions were encountered on 300 lungs. Lesions were further subjected for detail gross and histopathological examinations. The occurrence of pulmonary lesions was 77.5%. 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.Â Bacteriological studies on pulmonary lesions of camel (Camelus dromedarius) slaughtered at Addis Ababa abattoir, Ethiopia. African Journal of Microbiology Research, 5: 522-527. 19. Bekele, S.T., 2008.