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# Isolation of Aerobic and Anaerobic Bacteria from Pneumonic One Humped Camels (*Cameleus dromedarius*) in Iran

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**Respiratory tract infections, especially pneumonia, are common disease in camel. The aim of this study was bacterial isolation and identification of aerobic and anaerobic bacterial agent from camels in central part of Iran. This study was conducted to 150 slaughtered pneumonic camels in the Najaf-abbad abattoir in Iran. Samples were collected aseptically from the nasal cavity, trachea, and the lungs for bacteriological examination. Standard microbiological and biochemical techniques were used for isolation and identification of bacterial genera. Out of 450 samples, 59.65% of these isolates were Gram-positive, include 55.71% aerobic and 3.94% anaerobic bacteria, and while 40.35% were Gram-negative bacteria include 35.78% aerobic and 4.48% anaerobic bacteria. The aerobic Gram-positive bacteria were *Staphylococcus* spp. (27.85%), *Bacillus* spp. (10.57%), *Corynebacterium* spp. (9.18%), *Streptococcus* spp. (8.11%) and the anaerobic Gram-positive bacteria were *Clostridium* spp. (3.84%) and *Lactobacillus* spp. (0.1%). The aerobic Gram-negative bacteria were *Neisseria* spp. (12.38), *Pasteurella multocida* (5.87%), *Escherichia coli* (5.37%), *Pseudomonas aeruginosa* (3.82%), *Proteus* spp. (3.73%), *Klebsiella* spp. (2.77%) and *pasteurella haemolytica* (1.92%). Anaerobic Gram-negative include *Fusobacterium necrophorum* (2.03%) and *Bacteroides* spp. (0.1%). On the basis of our study, more prevalent aerobic and anaerobic bacteria in pneumonia camels were *Staphylococcus* spp. and *Clostridium* spp. respectively.**

**Key word:** Bacteria, *Cameleus dromedarius*, Pneumonia, Iran.

Camel is one of the important factor's in the life of a number of shepherd ethnic groups in Iran. Camel has the ability to survive in very difficult conditions, so it is possible for it to be used in marginal and desert ecosystems. Nowadays it is known that camel's in one of the most important sources of milk and meat and these days both shepherds and urban population consume these productions. (Abbas *et al.,* 1992; Azizollah *et al.,* 2009).

Respiratory tract infections, especially pneumonia, are common disease in various species of domestic animals like camel. Cause of infections has been incriminated as the most important reason of pneumonia in mammals (Schwartz and Dioli, 1992; Al-Tarazi and Elsheikh, 2006). The possible contribution of anaerobic bacteria to pneumonic process in one humped camels (*cameleus*

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*dromedaries*) has not been studied as well as aerobic bacteria, although lung infections due to anaerobic bacteria are common and important in human (Mustafa, 1992). There are suggesting the occasional involvement of anaerobic bacteria in pneumonias (Herthelius *et al.,* 1989; Prescott, 1979).

Pneumonia is manifested clinically by an increase in the respiratory rate, changes in the depth and character of respiration, coughing, abnormal breath sounds on lung auscultation and, in most bacterial pneumonias, evidence of toxemia. The economic losses due to pneumonia in camels are represented by loss of weight, losses due to condemnations during meat inspection and mortality rate (Al-Tarazi and Elsheikh, 2006).

To best our knowledge, only one study was reported aerobic bacterial population of the respiratory passageways of healthy camels from Iran (Azizollah et al., 2009) and most of research on camels were about parasitic infections (Oryan *et al.,* 2008; Nourani and Rohani, 2009). Therefore, the aim of this study was investigation of presence and identity of aerobic and anaerobic bacteria from pneumonic camel lungs in slaughter house in central part of Iran by standard bacteriological methods.

## MATERIALS AND METHODS

**Study area**

The study was carried out in Najaf-abbad abattoir in (Esfahan province) central part of Iran and occupies a total land area of 8955 km2. Najaf-abbad is located between latitudes 32° and 38°_N and longitude 35° and 51°_E. The altitude ranges from 1754 meters above sea level, and the average temperature ranges from 9°C to 14°C. The annual rain fall in the study area is estimated to be 163 mm. season in this zone is categorized into hot and dry (June - August), warm and wet (March – April) and cool and dry (September - February).

**Sampling and data collection**

This study was formed on 150 pneumonic camels (2 to 7 years old) of both sexes from November 2010 to April 2011. Samples were collected aseptically from the nasal cavity, trachea, and the lungs for bacteriological examination. Pneumonic lungs were chosen base on postmortem examinations were carried out using the routine visual, palpate and incise method (Taiwo, 2005).

**Tissue samples for bacteriological examination**

Nasal samples were collected, during post-mortem inspection, by inserting sterile cotton-tipped applicator sticks into the nasal passageways after proper cleaning and disinfection of the external nares. After slaughter the trachea of each camel was opened using a sterile scalpel blade to take a sample by inserting sterile cotton tipped swab into the midway of tracheal tube. The swabs were put in separate sterile test tubes containing Stuart transport media labeled and kept in a cool box. A sharp incision was made in the lesion area of pneumonic lungs by a sterile blade and the lung specimens were collected in separate plastic bags. All of the samples were transported to the microbiologic laboratory of Islamic Azad University-Shahrekord Branch on the day of sampling for further processing.

**Isolation and identification of bacteria**

For aerobic culture a full loop was taken from the lung specimens and inoculated on 5% sheep blood agar (Merck 1108860500) and MacConkey agar (Merck 1054650500). The nasal and tracheal sample inoculated as the same method also. For anaerobic culture of lung specimens blood agar plate was used. The inoculated plates were placed in anaerobic jars after preparation immediately. The anaerobic environment was established in standard anaerobic jars using a commercial anaerocult A (Merck 1.13829.0001) according to Merck Company protocol. The using a gas mixture consisting of kieselguhr, iron powder, citron acid and sodium carbonate to fill jars which had chemical bind oxygen quickly and completely, creating an oxygen free (anaerobic) milieu and a $CO_2$ atmosphere.

All of the inoculated plates were incubated at 37 °C for 24-48 hrs. After incubation, colonies were identified on the basis of Gram stained morphology, cultural characteristics, motility, and the production of catalase, oxidase, oxidative, fermentative and other biochemical properties.

## RESULTS

A total 450 specimens collected from the nasal cavity, trachea and lung (150 from each site). From this 450 specimens, 858 aerobic and 39 anaerobic bacteria were isolated and identified

(Table 1) and 59.65% of these isolated were Gram-positive include 55.71% aerobic and 3.94% anaerobic bacteria; while 40.35% of them were Gram-negative bacteria include 35.78% aerobic and 4.48% anaerobic bacteria.

The isolated aerobic Gram-positive bacteria were, *Staphylococcus* spp. (27.85%), *Bacillus* spp. (10.57%), *Corynebacterium* spp. (9.18%), *Streptococcus* spp. (8.11%) and the anaerobic Gram-positive bacteria were *Clostridium* spp. (3.84%) and *Lactobacillus* spp. (0.1%). The aerobic Gram-negative bacteria were *Neisseria spp.* (12.38), *Pasteurella multocida* (5.87%), *Escherichia coli* (5.37%), *Pseudomonas aeruginosa* (3.82%), *Proteus* spp. (3.73%), *Klebsiella* spp. (2.77%) and *pasteurella haemolytica* (1.92%). Anaerobic Gram-negative bacteria include *Fusobacterium necrophorum* (2.03%) and *Bacteroides* spp. (0.1%).

The majority of the isolated bacteria, colonized in all the anatomical sites of investigation except of *Bacillus* spp. which was absent in the lung, *proteus* spp. which was absent in the nasal tract.

Also from anaerobic yield of the 150 lungs, 99 facultative bacteria were isolated (Table 2). They belonged to 9 different genera. The predominant isolated species were *Staphylococcus* spp., *Corynebacterium* spp., *Streptococcus* spp., *Pseudomonas* spp. From anaerobic yield of bacteria in 150 lungs, 84 milieu contained facultative bacteria, and 66 had no growth.

**Table 1.** Prevalence of bacteria from nasal tract, trachea and Lung in pneumonic the one humped camels in Esfahan, Iran

| Types of bacteria | Nasal tract | Trachea | Lung | Total (%) |
|---|---|---|---|---|
| *Staphylococcus* spp. | 107 | 89 | 65 | 261(27.85%) |
| *Corynebacterium* spp. | 38 | 35 | 13 | 86 (9.18%) |
| *Streptococcus* spp. | 32 | 26 | 18 | 76 (8.11%) |
| *Bacillus* spp. | 54 | 45 | - | 99 (10.57%) |
| *Neisseria* spp. | 49 | 40 | 27 | 116 (12.38%) |
| *Klebsiella* spp. | 12 | 7 | 7 | 26 (2.77%) |
| *Pasteurella multocida* | 13 | 27 | 15 | 55 (5.87%) |
| *Escherichia coli* | 24 | 19 | 7 | 50 (5.37%) |
| *Pseudomonas aeruginosa* | 16 | 11 | 9 | 36 (3.82%) |
| *Pasteurella haemolytica* | 8 | 3 | 7 | 18 (1.92%) |
| *Proteus* spp. | - | 10 | 25 | 35 (3.73%) |
| *Bacteroides* spp. | - | - | 23 | 23(2.45%) |
| *Fusobacterium necrophorum* | - | - | 19 | 19 (2.02%) |
| *Clostridium* spp. | - | - | 36 | 36 (3.84%) |
| *Lactobacillus* spp. | - | - | 1 | 1 (0.1%) |
| Total | 353 (37.68%) | 312 (33.30%) | 271 (28.92%) | 937 (100%) |

In this study bacteriological findings revealed that *Staphylococcus* spp. were the most prevalent organism (27.85%). This organism is found to be incriminated in different pneumonic lesions severely and it often isolates from cultures while it is mixed with other bacteria. Nonbacterial pathogens (viruses, mycoplasma and parasite) were not included in the examinations.

**Table 2.** Facultative bacteria recovered from camel pneumonias in the Presence or Absence of Anaerobic Bacteria

| Facultative bacteria | Number of isolation |
|---|---|
| *Staphylococcus* spp. | 33 |
| *Corynebacterium* spp. | 27 |
| *Streptococcus* spp. | 10 |
| *Klebsiella* spp. | 6 |
| *Pseudomonas* spp. | 11 |
| *Pasteurella haemolytica* | 3 |
| *Pasteurella multocida* | 5 |
| *Escherichia coli* | 3 |
| *Salmonella* spp. | 1 |

## DISCUSSION

According to our knowledge, this is the first study reported the anaerobic bacteria that cause pneumonia in camels in Iran.

In our study *staphylococcus* spp. was the most commonly isolated bacteria in the cultures (27.85 %). This agrees with the results of other studies conducted in Sudan, where the rate of infection with *Staphylococcus* spp. in lungs was 20.4% (Al-Doughaym *et al.,* 1999). In Nigeria, *Arcanobacterium pyogenes*, *Mannheimia haemolytica* and *Pasteurella multocida* were recovered from pulmonary lesions; however *Staphylococcus aureus* and other *Staphylococcus* spp were the most commonly isolated (Abubakar *et al.,* 2010). Our findings were in agreement with previous reports that *Staphylococcus* spp., *Corynebacterium* spp. and *Streptococcus* spp. were microorganisms commonly isolated from pneumonic lesions in camels (Kebede and Gelaye, 2010; Zubair *et al.,* 2004). *Neisseria* spp. was as the second most important pathogens involved pneumonic lungs which are mainly pyogenic lesions and this agreed with the results obtained by Mustafa (Mustafa, 1992). Bacteriological study conducted in Jordan, shown that the *E. coli* was the most frequent bacterial which is isolates from pneumonic camels (Al-Tarazi, 2001). In Iraq, another study suggested that 56% of 150 camel lungs were pneumonic and *Pasteurella multocida*, *Mannheimia haemolytica*, *E. coli*, *Pseudomonas aeruginosa* and *Actinomyces pyogenes* were isolated (Al-Ani, 1990).

In Ethiopia, parainfulenza-3 was found as a primary causative agent as 70.5% in outbreak and *Manhaemiya hemolytica* was involved as secondary bacterial complications (Al-Tarazi, 2001) *Pasteurella multocida* and *Mannheimia haemolytica* are commensals, residing in the nasopharyngeal microflora and are capable of causing infection when the body defense mechanisms are impaired (Zamri-Saad *et al.,* 2006). *Clostridium* spp., *Lactobacillus* spp., *Fusobacterium necrophorum, Bacteroides* spp was isolated from pneumonic lungs for the first time in this study.

In our study, *Staphylococcus* spp. and *Neisseria* spp. were the most prominent extraction out of total bacteria in the cultures, while *Pasteurella haemolytica* and *Klebsiella* spp. were less prominent aerobic bacteria and might be considered as secondary invaders.

Several factors such as the herd, climb, environment, kind of usage, nutrition and immunological status of the animal can predispose respiratory disease. The suppression of the normal bacteria frequently allows development of potential pathogens, leading to disease (Hirsh et al., 1979). The bacteria isolated during this study are comparable to those reported from the respiratory tract of apparently healthy camel dromedaries in Najaf-abbad abattoir (Azizollah et al., 2009).

Therefore, it is recommended to develop a vaccine containing the responsible pathogens in order to protect this unique animal species from such kind of unpredictable disastrous diseases. It suggests that the respiratory tract may play as a reservoir for many microorganisms. They are capable of to invading the different parts of respiratory tract and cause pathologic lesions under suitable predisposing conditions.

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