

Isolation and Identification of Aerobic Bacterial Flora from the Upper Respiratory Tract of Donkeys in Central Ethiopia

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ABSTRACT

This study was conducted from December 2006 to June 2007 in order to isolate aerobic bacterial micro flora residing in the upper respiratory tract (URT) of working donkeys. Eighty apparently healthy donkeys (APHD) and 20 donkeys with respiratory tract disease (DRTD) were used for the study. Swab samples were collected from the nasopharynx. Isolation and identification of the bacteria were conducted under aerobic condition. Each of the samples collected yielded at least one bacteria species. A total of 189 bacteria species/genus were recovered from both groups of donkeys. The majority (159/189, 84.1%) of the isolates were gram-positive and the rest (30/189, 15.9%) were gram-negative. Bacterial isolates identified in order of magnitude were *Streptococcus* species (28.1%), *Corynebacterium* species (15.4%), *S. aureus* (13.2%), CNS species (9.5%), *Bacillus* species (9.0%), *K. pneumoniae* (5.8%), *E. coli* (4.2%), *Micrococcus* species (4.2%), *Rhodococcus* species (2.7%), *Proteus vulgaris* (2.1%), *A. pyogenes* (2.1%), *Pasteurella caballi* (1.6%), *Actinomyces* species (1.1%), *Pseudomonas* species (0.5%), and *Pasteurella hemolytica*

(0.5%). This result indicates the predominance of gram-positive bacteria in both cases ($P < 0.01$). Bacteria were recovered at a higher rate in DRTD (2.4) than in APHD (1.8). The quality of bacteria isolated in both cases was comparable, except *Pseudomonas* spps, *Pasteurella hemolytica*, and *Actinobacillus* spps, which were recovered only from APHD.

INTRODUCTION

Forty-four million donkeys are known to exist in the world, of which 40 million (96.35%) are in developing countries³². Ethiopia has approximately 5.2 million donkeys²⁰, which represents more than 55% of the national equine species, and constitutes 11.4% of the 34.4% of the entire African donkey population. This figure not only puts Ethiopia first in Africa, but also second in the world next to China. According to the recent regional classification of Ethiopia, 97% of the donkeys are found in three regions: 44% in Oromiya, 34% in Amhara, and 19% in Tigray regional states¹⁰.

Despite its prominent role in the rural and agricultural system of the country, the welfare of donkeys has been subjected to poor management, lack of health care, lack of knowledge, and negative attitudes from the community. Despite these factors,

donkeys have continued to serve as draught animals (packing, carting, threshing, farm cultivation, riding), and for milk and meat production. Donkeys account for 50% of the animal energy in the Ethiopia ¹¹.

Donkeys in Ethiopia are subject to a variety of health disorders, including multi-parasitism, sores, respiratory problems, hoof problems, ophthalmic problems, colic, various infectious diseases such as strangles and tetanus, and African horse sickness ^{12,21,1}.

Respiratory problems are the most severe health issue observed in working donkeys ⁸. Respiratory disease results from a complex interaction of parasitic, bacterial, and viral factors, as well as environmental conditions. The lungs are continuously exposed to air that contains dust, bacteria, fungi, viruses, and various noxious agents. The donkey's defenses against these potentially harmful materials are controlled by many protective mechanisms ¹⁴.

Respiration is a cellular activity, dependent on the respiratory tract to take place.

Table 1: Bacterial species isolated from nasopharynx of both apparently healthy and donkeys with respiratory diseases

Types of bacteria	Number of isolates	% of isolates
Gram positives	159	(84.1%)
Bacillus spp.	17	9.0%
Streptococcus spp.	53	28.1%
Coagulase negative staphylococcus Spp.	18	9.5%
Staphylococcus aureus	25	13.2%
Micrococcus spp.	8	4.2%
Corynebacterium spp.	29	15.4%
Actinomyces pyogenes	4	2.1%
Rhodococcus equi	5	2.7%
Gram negatives	30	15.9%
E.coli	8	4.2%
Pasteurella caballi	3	1.6%
Pasteurella haemolytica	1	0.5%
Actinobacillus spp	2	1.1%
Pseudomonas spp.	1	0.5%
Klebsiella pneumoniae	11	5.8%
Proteus vulgaris	4	2.1%
	189	100.00%

Equines are obligatory nasal breathers. The movement of air through the respiratory tract is achieved by the creation of pressure gradients during inspiration and expiration. Therefore, problems of the upper respiratory tract (URT) are more critical in these species than other domestic animals. The primary functions of the nasal cavity and paranasal sinuses of the URT are to conduct air flow, warm and humidify air, and remove particulate debris from inspired air prior to exposure to the lower respiratory tract (LRT) ². Consequently, the upper respiratory tract is subject to secondary bacterial infections, including mucopurulent nasal discharge, depression, persistent fever, and abnormal lung sounds. The most common opportunistic bacterial isolates are *S. equi var zooepidermicus*, *Actinobacillus equuli*, *Bordetella bronchiseptica*, *E. coli*, *Pasteurella spp.*, *Pseudomonas aurogenosa*, and *S. equi var* ²⁶. There has been no formal research conducted in Ethiopia concerning the bacterial flora in the upper respiratory tract of donkeys.

The present study was conducted to investigate bacterial isolates from the URT of donkeys, both with and without reparatory diseases. Therefore, the specific objectives of the study were:

- Isolation of various aerobic bacterial agents from the upper respiratory tract of apparently health donkeys.
- Isolation of various aerobic bacterial agents from the upper respiratory tract of donkeys with reparatory tract disease.

- Comparison of the nature of isolates recovered from both groups.

MATERIALS and METHODS
Study Area

The study was conducted in Debre Zeit, located at 9o N latitude and 40o E longitude at an altitude of 1850m a.s.l in the central highlands of Ethiopia. It has an annual rainfall of 866mm, of which 84% falls in the long rainy season (June-September). The dry season extends from October to February. The mean annual minimum and maximum temperatures are 8.9oC and 26oC respectively, with mean relative humidity of 58.9%¹⁸. Samples were processed at the Donkey Health and Welfare project Laboratory, Faculty of Veterinary Medicine, Addis Ababa University.

Study Animals

Donkeys arriving at the Donkey Health and Welfare Project (DHWP) stationary clinic were the target population. The present study was conducted on 80 apparently health donkeys (APHD) and 20 donkeys with upper respiratory tract diseases (DRTD) brought to the stationary clinic from the nearby villages. The group of animals investigated was working donkeys of mixed age, sex, and types.

Apparently healthy donkeys (APHD): Donkeys with no obvious signs of diseases. They had no sores or of history of respiratory diseases, vital signs in normal range, body condition score 2 and above in a 1-5 scale (reference), and were brought to the

Table 2: Bacterial special isolated from nasopharyngeal swab of apparently healthy donkeys

Types of bacteria	Number of isolates	% of isolates
Gram positives	122	85.9%
Bacillus spp.	14	9.0%
Streptococcus spp.	36	28.1%
Coagulase negative staphylococcus Spp.	13	9.5%
Staphylococcus aureus	18	13.2%
Micrococcus spp.	6	4.2%
Corynebacterium spp.	27	15.4%
Actinomyces pyogenes	4	2.1%
Rhodococcus equi	4	2.7%
Gram negatives	20	14.1%
E.coli	6	4.2%
Pasteurella caballi	2	1.4%
Pasteurella haemolytica	1	0.7%
Actinobacillus spp	2	2.1%
Pseudomonas spp.	1	0.7%
Klebsiella pneumoniae	5	2.1%
Proteus vulgaris	3	3.5%
	142	100.00%

clinic for regular de-worming or castration.

Donkeys with respiratory tract diseases (DRTD): Donkeys with obvious signs of respiratory diseases. They had nasal discharge, with or without fever, dyspnoea, dysphagia, history of respiratory diseases, and abnormal lung sounds.

Study Protocol

Among donkeys admitted to the Donkey Health and Welfare Stationary Clinic during the period December 2005-June 2006, 80 APHD and 20 DRTR were randomly selected and subjected to a thorough physical and clinical examination. History (previous exposure to respiratory diseases, antimicrobial treatment, any concurrent diseases, duration of the current illness) and symptoms, including loss of appetite, fever cough, nasal discharge, enlargement of head and neck lymph nodes, quality and rate of respiration, were noted.

Sample Collection and Processing

Before sampling, sterile swabs and media were made ready for use. The nares and an-

Table 3: bacterial species isolated from nasopharyngeal swab of donkeys with respiratory diseases (relative recovery rate of bacterial isolates/total isolates)

Types of bacteria	Number of isolates	% of isolates
Gram positives	37	78.8%
Bacillus spp.	3	6.4%
Streptococcus spp.	17	36.2%
Coagulase negative staphylococcus Spp.	5	10.6%
Staphylococcus aureus	7	14.9%
Micrococcus spp.	2	4.3%
Corynebacterium spp.	2	4.3%
Actinomyces pyogenes	0	0%
Rhodococcus equi	1	2.1%
Gram negatives		
E.coli	2	4.3%
Pasteurella caballi	1	2.1%
K. pneumoniae	6	12.7%
Proteus vulgaris	1	2.7%
	47	100.00%

terior part of the nasal mucosa was cleaned and disinfected with 70% alcohol. Then, a 20-25 cm sterile nasal swab was directed via the ventral nasal meatus into the nasopharynx and samples were collected. As soon as possible, samples were inoculated into Brain Heart Infusion agar (BHI) and incubated. Then, the growth was streaked on 7% sheep blood agar. Representative colonies from culture positive plates were further streaked on blood agar, then subcultures on blood agar and MacConkey agar. At least two cultures were made from each specimen. All cultures were incubated aerobically at 37°C for 24-48hrs. Colony morphology, color, and status of hemolysis were recorded. Pure colonies were transferred to slant nutrient agar for further tests. These culture isolates were subjected to Gram stain, a potassium hydroxide (KOH) test, and tests for primary identification and secondary biochemical tests adopting standard recommended procedures^{7,3,23}.

Data Analysis

Descriptive statistics (mean and percentage) were used to summarize the generated data. SAS was used as a statistical package to ob-

serve the association between isolates, nature, and proportion of bacterial population between APHD and donkeys with DRTD²⁷.

RESULTS

Descriptive Analysis of Bacterial Isolates

All of the 100 samples (80 nasopharyngeal swabs from APHD and 20 nasopharyngeal swabs from donkeys with DRTD) collected for aerobic bacterial isolation yielded bacteria. Except for a few Bacillus spp.,

Corynebacterium spp. and S. aureus, which were isolated as pure cultures in samples taken from the nasopharynx, the majority of the isolates were mixed. A total of 189 isolates were obtained from the 100 culture positive specimens, of which 159 (84.1%) were gram-positive and 30 (15.9%) were gram-negative.

The isolation rates of bacteria from the nasopharynx of both APHD and donkeys with DRTD are shown in Tables 1, 2, and 3. Overall, isolated bacteria include: *Streptococcus spp.* (28.1%), *Corynebacterium spp.* (15.4%), *S. aureus* (13.2%), *CNS spp.* (9.5%), *Bacillus spp.* (9.0%), *K. pneumoniae* (5.8%), *E.coli* (4.2%), *Micrococcus spp.* (4.2%), *Rhodococcus spp.* (2.7%), *Proteus vulgaris* (2.1%), *A. pyogenes* (2.1%), *Pasteurella caballi* (1.6%), *Actinomyces spp.* (1.1%), *Pseudomonas spp.* (0.5%) and *Pasteurella haemolytica* (0.5%).

Major proportions of gram-positive isolates were recovered from both apparently healthy and sick donkeys, except *pseudomonas spp.*, *Pasterella haemolytica*, and *Actinobacillus spp.*, which were not isolated from sick donkeys.

DISCUSSION

In the present study, detailed investigation was carried out to isolate and assess the types of bacteria inhabiting the upper respiratory tract of apparently healthy donkeys, and those with upper respiratory tract diseases brought to DHWP open-air clinic. Moreover, the quality and quantity of bacterial isolates from both apparently healthy donkeys and donkeys with respiratory diseases were compared.

Out of 189 bacteria isolated, 159 (84.1%) were gram-positive and 30 (15.9%) were gram-negative. Of 142 bacteria isolated from apparently healthy donkeys, 122 (85.9%) were gram-positive and 20 (14.1%) were gram-negative. From 47 bacterial isolates from donkeys with respiratory diseases, 37 were gram-positive and 10 were gram-negative. There was no statistically significant variation in the quality of bacterial isolates from APHD and DRTD. This might be attributed to the disparity in sample size compared (80 APHD versus 20 DRTD). However, the recovery rate in DRTD (2.4%) was greater than the rate for both APHD and DRTD cases ($P < 0.01$). This is comparable to previous findings¹⁵. The quality of bacterial isolates in both cases was similar, except *pseudomonas spp.*, *pasterella hamolytica* and *Actinobacillus spp.*, which were recovered from APHD only.

Streptococcus spp., the predominant bacteria recovered in this study, was isolated from the nasopharynx at a rate of 28.1% (36 from APHD and 17 DRTD). Comparable proportions of bacteria were recorded from a tracheal wash of horses with pneumonia^{17,35,34,39}. It has been established that streptococci are widely distributed among animals. Bacteria may be potentially pathogenic, or non-pathogenic on the upper respiratory tract of equine species^{6,23}. Isolation of *Streptococcus spp.* from donkeys with respiratory disease indicates the role of these bacteria as a primary opportunistic pathogen, following viral infections or stressful conditions.

Corynebacterium species were en-

countered as a second dominant bacterium among those recovered from APHD. They were isolated at a rate of 19% from apparently healthy donkeys, and 4.3% from donkeys with respiratory tract diseases.

These species were also recovered as common isolates from URT infections in foals⁴. *Corynebacteria* are pyogenic bacteria located on mucous membranes and skins of animals. They are known to cause a variety of suppurative conditions²³.

Staphylococcus aureus was isolated at a rate of 12.7% from apparently healthy donkeys, and 14.9%, second dominant, from donkeys with respiratory problems. A related study indicated that *staphylococcus aureus* was isolated from horses with pneumonia at a rate of 1.7%. This is a lower rate compared to the present study³⁴. *S. aureus* has been located from the respiratory tracts of healthy and pneumonic cases of domestic animals in many other cases as well^{28, 38, 37}. *S. aureus* is the main inhabitant of the mucous membranes in the URT of animals. It can be involved as an opportunistic bacterium, following the pathologic role of stress conditions such as viral infections, and other cause of infection in immuno-suppressed hosts.^{25, 23}

Coagulase negative staphylococcus (CNS) was one of the commonly recovered bacterial isolates in the study. It was isolated at a rate of 9.2% from apparently healthy donkeys, and 10.6% from donkeys with respiratory problems. The isolation rate of these bacteria is similar in both cases. *CNS* was also isolated as dominant species of bacteria in URT infections of foals⁴. *CNS* species are involved in pharyngeal abscess, lung abscess, and suppuration of other parts of the respiratory tract in cases where the defense mechanisms of the lung are compromised.^{25, 13, 23}

Bacillus species were isolated at a rate of 9.9% from apparently healthy donkeys, and 6.4% from donkeys with respiratory problems. Several researchers have isolated *Bacillus spp.* at different rates of recovery from different species of animals.^{30,33,29, 38,16}

Most of the *Bacillus spp.* are saprophytes that are widely distributed in air, soil, and water.²³ The presence of *Bacillus species* usually reflects contamination during either acquisition or handling of specimens. They are usually ignored when isolated from clinical materials, except *Bacillus anthracis*^{7,33}.

Klebsiella pneumoniae was the dominant isolate among gram-negatives, with a recovery rate of 2.1% from apparently healthy donkeys, and 12.7% from donkeys with respiratory problems. It was recovered more frequently from DRTD (30%) than APHD (6%). Sweeny et al. (1991) isolated *Klebsiella pneumoniae* at a rate of 13.9% from horses with pneumonia. The bacterium was also recovered dominantly from the respiratory tracts of sheep inhabiting the same study area.¹⁶ Since *Klebsiella pneumoniae* inhabits the intestinal tracts of animals, fecal contamination of the environment accounts for the wide distribution of the organism, and contributes to the occurrence of opportunistic infection²³.

Escherichia coli was isolated as a second dominant bacterium among gram-negatives, with the isolation rate of 4.2% from apparently healthy donkeys, and 4.3% from donkeys with respiratory problems. *E. coli* was also isolated in the nasal cavities of other animal species.^{9, 19, 28 37}

Actinobacillus spp. was recovered rarely (1.4%) from apparently healthy donkeys. In this study, none of these species were isolated from donkeys with respiratory problems. *Actinobacilli* are commonly found on the mucous membranes of domestic animals.²³ These bacteria were also isolated at a rate of 2.56% from goats.³⁷

Actinomyces pyogenes was isolated only from apparently healthy donkeys at a relatively low rate, 2.8%. The bacterium is commonly found on the mucous membranes of the nasopharynx of domestic animals, and causes disseminated purulent infections when the animal is stressed.^{31, 23}

Rhodococcus equi was isolated at a rate of 2.8% from apparently healthy donkeys, and 2.1% from donkeys with respiratory

problems. The bacterium is an opportunistic pathogen and common soil inhabitant²³. Severe *equine pneumonia*, usually found with abscess formation in the lung tissue, is most often associated with *Rhodococcus equi infection*^{36, 4}. *R. equi* is one of the most common isolates from equine respiratory tracts^{33, 15, 24}.

Micrococcus spp. were isolated from eight apparently healthy donkeys (4.2%), and in 4.3% of donkeys with respiratory disease. *Micrococci* are a non-pathogenic species of upper respiratory bacteria found in domestic animals²³.

Pseudomonas spp., *Pasteurella haemolytica*, and *pasteurella caballi* were recovered at a rate of 0.5%, 0.5%, and 1.6% of the total isolates, respectively. Studies by Cabbassai et al. indicate that *Pseudomonas spp.* are typically included in the transient flora of the nasal mucosa of horse because 17-*pseudomonas spp.* strains were isolated from the nasal mucosa of 40 horses during their study⁵.

CONCLUSION and RECOMMENDATIONS

In the present study, an attempt was made to isolate and identify the major bacterial flora from the nasopharynx of donkeys. The results showed a variety of bacteria that may be resident and/or transient. The majority of the bacteria are gram-positive in both APHD and DRTD. Analysis of bacteria from both apparently healthy donkeys and donkeys with respiratory problems suggests that the bacteria that reside in the URT have a chance to invade the lower respiratory tract and cause opportunistic infections.

The respiratory tract is invariably exposed to the surrounding environment. Most of the bacteria isolated in this study are naturally found in the soil, water, and feces of animals. Hence, from these results we can conclude that the microbial build-up in the respiratory tract from the immediate environment can cause opportunistic infections in donkeys.

Due to limited resources, the present study did not include the antibiogram sen-

sitivity patterns of the isolates identified to species level, isolation of other microorganisms, including anaerobic bacteria, viruses, and fungal species expected to reside in the respiratory tract of donkeys.

Furthermore, the results of the present study were compared with work done in horses and other domestic animals. Based on all of the study results, the following recommendations are made:

- Further thorough investigation and identification of bacterial flora involved directly or indirectly in the respiratory diseases of donkeys should be carried out.
- The study sample should include not only nasopharyngeal, but also the whole URT.
- The pathogenic role of each species of bacteria isolated from the respiratory tract (nasopharynx) has to be further investigated.
- Antibigram sensitivity should be conducted for each species and varieties of bacterial isolates identified.
- The involvement of both gram-positive and gram-negative bacteria in the respiratory problems of donkeys should prompt clinicians to consider the use of antimicrobials when handling cases of respiratory problems.
- Since the causative agents of respiratory diseases are so complex, further investigation is imperative to institute effective control measures.

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