The Aerobic Bacterial flora of the Respiratory passageways of healthy goats in Dire Dawa Abattoir, Eastern Ethiopia

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SUMMARY

The aerobic bacterial flora of the respiratory passageways were investigated using 50 healthy goats slaughtered at Dire Dawa abattoir, Eastern Ethiopia. Samples were collected aseptically from the nasal cavity, tonsil, trachea and the lungs for bacteriological examination. Standard microbiological techniques were used for isolation and identification of bacterial species. From a total of 200 specimens collected for bacteriological examination from nasal cavity, tonsil, trachea, and the lung (50 each), 154 (77%) contained bacteria. Bacterial species identified were coagulase negative Staphylococcus (22.8%), Mannheimia (Pasteurella) haemolytica (18.2%), Staphylococcus aureus (17.2%), Pasteurella multocida (11.9%), Corynebacterium pseudotuberculosis (8.8%), Bacillus species (7.4%), Actinomycyes pyogenes (6.7%), E. coli (6.0%), and Micrococcus species (1.0%). Six of the 9 isolates namely, S. aureus, Coagulase Negative Streptococcus, M. (P.) haemolytica, P. multocida, A. pyogenes, and Bacillus species were isolated from all the anatomical sites examined. On the other hand, C. pseudotuberculosis, E. coli, and Micrococcus species were isolated only from the nasal cavity and tonsils. In a nutshell, Gram-positive bacteria were dominant in this environment, followed by Mannheimia and Pasteurella species.

Keywords : Abattoir - Aerobic bacteria - Dire Dawa - Healthy goats - Pneumonia - Respiratory tract.

RÉSUMÉ

La flore bactérienne aérobie des voies respiratoires des chèvres saines dans l’abattoir de Dire Dawa, Est de l’Ethiopie. Par T. MEGRA, T. SISAY et B. ASSEGED.

La flore bactérienne aérobie des voies respiratoires en utilisant 50 chèvres saines abattues à l’abattoir de Dire Dawa, Ethiopie a été étudiée. Des échantillons de la cavité nasale, des amygdales, de la trachée et des poumons ont été recueillis de manière aseptique pour examen bactériologique.

Sur un total de 200 échantillons prélevés (50 échantillons de chaque site anatomique), 154 (77%) contenaient des bactéries. Les espèces bactériennes identifiées étaient des Staphylococcus coagulase négative (22.8%), Mannheimia (Pasteurella) haemolytica (18.2%), Staphylococcus aureus (17.2%), Pasteurella multocida (11.9%), Corynebacterium pseudotuberculosis (8.8%), Bacillus spp. (7.4%), Actinomycoses pyogenes (6.7%), Escherichia coli (6.0%) et Micrococcus spp. (1.0%). Six espèces bactériennes (S. aureus, Staphylococcus coagulase negative, M. (P.) haemolytica, P. multocida, A. pyogenes, et Bacillus spp.) ont été retrouvées dans tous les sites anatomiques examinés, alors que C. pseudotuberculosis, Escherichia coli et Micrococcus spp. ont seulement été isolées de la cavité nasale et des amygdales. Il ressort donc que les bactéries Gram positives étaient dominantes dans cet environnement suivies par les espèces de Mannheimia et de Pasteurella.

Mots-clés : Abattoir - Bactérie aérobie - Chèvre - Dire Dawa - Pneumonie - Voies respiratoires.

Introduction

Owing to their remarkable adaptability to adverse environments, goats assume important position in Ethiopian livestock economy. In combination with sheep, they supply more than 30% of all domestic meat consumption, and generate income from exports of live animal, meat and skin [3]. Moreover, due to less opportunity for alternative land uses, livestock (mainly goats) production is the only economic activity supporting the livelihood of the communities in the arid areas of the country [15]. Hence increase in goat production is needed both to maintain food self-sufficiency and to increase export earnings.

In spite of their large number (above 23.5 m) and enormous contribution to the national economy [17], goat production is not well developed due to inadequate nutrition, poor management and prevailing diseases [11]. Of all the diseases of goats, those affecting the respiratory tract: PPR, CCPP and pasteurellosis impose a substantial loss through high morbidity and mortality [16, 35, 37].

Infectious pneumopathies are commonly attributed to Mannheimia (Pasteurella) haemolytica, which causes the most severe damage to the lung. In addition, bacterial agents such as Mycobacterium, Mycoplasma, Haemophilus, Fusobacterium and Actinomyces inflict damage on the pulmonary tissues of most domestic animals [6, 21, 24, 29]. Nonetheless, it is becoming increasingly difficult to make an etiological diagnosis because, although a single agent may be a primary invader [13], when the local resistance of respiratory mucosa is lowered, bacteria growing in the nose and throat extend downwards; usually producing multiple bacterial infections [22]. Besides, most of the infectious agents that cause respiratory disease are ubiquitous in nature and are normal inhabitants of the nasopharynx [29].

Most studies conducted on the bacterial flora of the respiratory tract of domestic animals in Ethiopia mainly focus on pneumatic lungs of sheep, cattle and camel [1, 7, 18, 19, 23, 35], and there are no published reports regarding the microflora of apparently normal respiratory passageways. Moreover, studies have not been extended to include goats, one of the most important livestock species in the arid areas of the country. This study was designed to isolate and characterize bacteria from different anatomical sites of the respiratory passageways of apparently healthy goats.
Material and Methods

STUDY AREA

The study was conducted in Dire Dawa, 518 km east of Addis Ababa, at an altitude of 950 meters above sea level. The mean annual rainfall and the mean day-night air temperature ranges are respectively 550-850 mm and 14-30°C [28].

STUDY ANIMALS AND STUDY PROTOCOL

The study was carried out from November 2003 through April 2004 on 50 goats (40 males and 10 females) randomly selected from animals brought to Dire Dawa abattoir for slaughter. The animals were raised in a communal pasture under traditional management practices. Although records were not available concerning the age and previous health status, all the goats brought to the abattoir were adults and, were found to be apparently normal at the ante-mortem examination.

SAMPLE COLLECTION

The nasal samples were collected, during ante-mortem inspection, by inserting sterile cotton-tipped applicator sticks into the nasal passageways after proper cleaning and disinfection of the external nares. After flaying, the trachea of each goat was grasped with tissue forceps and opened by sterile scalpel blade to take sample by inserting sterile cotton-tipped swab into the tracheal tube. The mucosae were thoroughly rubbed by rolling the swabs to attain effective contact. The swabs were put in separate sterile test tubes, labeled and kept in a cool box, and transported to the nearby Dire Dawa Veterinary Laboratory for further processing.

Before collecting tonsils and lung samples, the external surfaces were disinfected with 70% alcohol to minimize surface contamination. Using sterile scissors and tissue forceps, pieces of the lung and the corresponding tonsil were collected separately into sterile screw-capped universal bottles and similarly transported in a cool box for further processing.

BACTERIOLOGICAL EXAMINATION

The swabs were removed from the bottles and streaked over the plates containing blood agar-base supplemented with 7% sheep blood. Whereas, the surfaces of lung and tonsil samples were seared with a hot spatula, incised and printed on a blood agar and streaked with a wire loop. The streaking was further spread with inoculating loop to aid colony isolation. The plates were labeled and incubated aerobically at 37°C for 24-48 h [13]. Mycoplasma culture was not done due to lack of facilities, and anaerobic incubation was not included because the predominant anaerobic organism, Fusobacterium necrophorum, is often part of the normal flora of the mouth, intestine and genital tract of many herbivores and omnivores and is not of particular importance in the respiratory tract of normal sheep and goats [8, 10].

After taking note of cultural growth characteristics, positive cultures were subjected to Gram’s staining to study staining properties and cellular morphology under 100X objective of light microscope. Mixed colonies and Gram-negative bacteria were sub-cultured on both blood and McConkey agars and incubated aerobically for further 24 h. Pure cultures of single colony type, from both blood and McConkey agars, were transferred onto nutrient agar-slants for a series of biochemical tests including, catalase, oxidase, and fermentative/oxidative tests for final identification, following standard procedures [13, 27].

DATA ANALYSIS

Descriptive statistics was used to summarize the data generated from the study. The relative abundance of each species/genera was expressed as a percentage in comparison to the total number of isolates. Similarly, the relative and absolute infection rates at each anatomical site were expressed as percentages.

Result

Of the total 200 samples, comprising 50 each of the specimens from the nasal cavity, trachea, tonsils and lungs, 154 (77%) contained bacteria. In general, 285 bacteria were recovered from 154 infected specimens, accounting for 1.85 isolates per infected sample. The Gram-positive cocci were dominant among the isolates: the frequently isolated species being Coagulase Negative Staphylococcus (CNS) (22.8%) and S. aureus (17.2%). The second dominant bacterium was Pasteurellae species including Mannheimia (Pasteurella) haemolytica (18.2%) and P. multocida (11.9%). Other species were isolated at relatively lower rates. The bacterial isolates and their absolute/relative abundance are presented on Table I and Fig. 1.

On the other hand, 6 of the 9 isolates: S. aureus, CNS, Mannheimia (P) haemolytica, P. multocida, A. pyogenes and Bacillus species were isolated from all the anatomical sites. Micrococcus species, C. pseudotuberculosis and E. coli were isolated only from the nasal cavity and tonsils (Table I).

Discussion

This study has shown that a variety of bacterial flora colonizes the respiratory passageways of apparently healthy goats. Several workers [4, 23, 25, 39] reported similar bacteria from pneumatic caprine lungs, while others [5, 9, 30, 33] isolated them from pneumatic lungs of sheep. In addition, SHEMSEDIN [32] and SHIGIDI [34] isolated similar species from pneumatic lungs of camels. The invariable isolation of these organisms from the pneumatic lungs of various animal species might indicate their role in different respiratory syndromes.

The more pathogenic species, Mannheimia (P.) hemolytica, was isolated in higher proportion from the nasal cavity (50%), followed by tonsil (34%), then lung (14%) and trachea (6%), as opposed to earlier report of 35.5% [4] and 47.5% [23] lung infection rates. The lower isolation rate in

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this study might have come from the type of the lung samples, which are apparently healthy tissues [12]. According to BAKER [8], stress factors with or without viral infections interact to suppress the muco-ciliary clearance mechanism, which allow the proliferation of commensal bacteria in the respiratory tract. The isolation of this organism from nasal cavity and tonsil at a higher rate compared to trachea and lung (Table I) indicates that the organism lives as a commensal in the upper respiratory tract, but invades the lung under conditions of stress [14]. Similarly, \( P. \) multocida mainly infected the nasal cavity and tonsils followed by lung and trachea (Table I). Different workers [4, 23, 34] also isolated \( P. \) multocida from respiratory tracts of sheep and goats although recovery rate from lung was much lower. In agreement with previous reports [7, 29, 35] both in terms of infection intensity and pathogenicity, \( M. (P.) \) haemolytica assumes greater prominence in caprine pasteurellosis in this environment.

CNS was isolated from 68%, 34%, 16% and 12% of the nasal, tonsilar, tracheal and lung samples respectively (Fig. 1). SHEMSEDIN [32] and SHIGIDI [34] previously isolated CNS from pneumonic camel's lung, whereas AJUWAPE and AREGBESOLA [2] failed to isolate the agent from nasal cavity of normal rabbit. CNS is inordinately inhaled in pharyngeal and lung abscesses, and suppurus of the remaining respiratory tract when the defense mechanism of the host is compromised [31].

\( S. \) aureus was isolated from all sites, with the lowest level in the lung (Table I). Although this finding is consistent with reports from the ovine lung [9, 23], much higher rates have been reported from pneumonic caprine lungs [23, 39]. Conversely, ALMEIDA et al. [4] reported an infection rate as low as 3% from caprine lungs. According to ROBINS et al. [31], \( S. \) aureus resides in the upper respiratory tract and involves in disease processes only when stress conditions prevail. In line with this, AJUWAPE and AREGBESOLA [2] isolated \( S. \) aureus invariably from the nasal swab of normal rabbits, compared to 17.6% isolation rate in our study. This variation could be attributed to a feeding habit, as rabbits are coprophagous animals while goats are mainly browsers [20] but the extensive management under which the goats were kept might have also contributed to the comparatively lower nasal involvement.

The relatively higher prevalence of \( A. \) pyogenes in tonsil (18%) and nasal cavity (14%) indicates that this organism normally inhabits the upper respiratory tract and extends downward following environmental stress [36, 38]. Previous studies have similarly isolated \( A. \) pyogenes from nasal cavity [32, 34] and pneumonic lungs [4, 9, 23].

Although \( \text{Micrococcus} \) species were isolated from nasal cavities of camels [32], normal rabbits [2], and nasal cavity and tonsils of goats in the present study, they are assumed to be non pathogenic [13]. Their ubiquity (compared to other non-pathogenic species) is primarily due to contamination from skin [13]. Similarly, \( C. \) pseudotuberculosis was isolated only from nasal cavity and tonsils (Table I). Previous workers [32, 34] similarly isolated the organism only from the nasal cavity and tonsil of camels although others [4, 23] additionally isolated from trachea and lungs of the diseased goats. It has been established that \( C. \) pseudotuberculosis is inhaled into respiratory tract from its normal habitats: skin and mucus membranes [13]. Other non-pathogenic organism, \( \text{Bacillus} \) species, was isolated mainly from the nasal cavity followed by tonsils (Table I). This was at variance with previous works that isolated \( \text{Bacillus} \) species only from camel’s lung [23, 32, 34], and goat’s trachea and lung [23] under disease conditions. On the other hand, AJUWAPE and AREGBESOLA [2] could not isolate the agent from the nasal swab of normal rabbits, implying that the organism might preferentially colonize diseased organs.

About 22% of the nasal samples and 12% of the tonsils contained \( E. \) coli, although the trachea and lung samples...
were free (Table I). This finding was in agreement with previous study in sheep and goats [23] and normal rabbits [2]. However, OKOLO [25] isolated the agent from condemned goat’s lung. Although E. coli is considered to be transient in the respiratory tract when inhaled with dust particles and do not play a pathogenic role [14], its isolation from clinically healthy goats in the absence of clinical enteritis is noteworthy. PELCZAR et al. [26] suggested that E. coli, which is usually harmless in their normal habitat, could cause pulmonary and urgenital tract infections.

The present study has indicated that several bacterial species inhabit the respiratory passages of apparently normal goats. Considering the extremes of weather and other poor managerial conditions, which subject the animals to a considerable stress under this environment, the pathogenic role of these apparently commensal organisms could be enormous. We therefore recommend a detailed study to look into the economic impact of these organisms as well as other anaerobic bacteria and mycoplasmas.

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