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Isolation and identification of aerobic bacterial species from upper respiratory tract of cart horses in Central Ethiopia

Tesfaye Debelu[1], Nigatu Akililu[2], Tesfaye Sisay[1] and Fanta Desissa[2*]

[1]College of Veterinary Medicine and Agriculture, Addis Ababa University, P. O. Box 34, Bishoftu, Ethiopia.
[2]College of Veterinary Medicine and Agriculture, Addis Ababa University, SPANA Project, P. O. Box 34, Bishoftu, Ethiopia.

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A cross sectional study to isolate and identify aerobic bacterial species from upper respiratory tract of horses was conducted from October, 2009 to April, 2010. Forty eight apparently healthy horses (APHH) and 56 horses with respiratory tract diseases (HRTD) brought to Society for protection of animals abroad (SPANA) clinics in Central Ethiopia were randomly selected. Swab samples were collected aseptically from nasopharynx of the horses. Isolation and identification of the bacteria was carried out following the recommended standard procedures. A total of 270 bacteria were recovered from the sample taken from both groups. Of the total isolates, 65.9% were Gram positives and the remaining 34.1% were Gram negatives. Bacterial species isolated in order of dominance include: *Bacillus* species, *Streptococcus* species, *Staphylococcus* species, *Escherichia coli*, *Pasteurella* species, *Micrococcus* species, *Bordetella* species, *Pseudomonas* species, *Actinobacillus* species and *Rhodococcus equi*. *Actinobacillus* species and *Rhodococcus equi* were only isolated from APHH. Despite the percentage variation of the isolates, there was no statistically significant ($p>0.05$) variation among the isolates with respect to altitude and health status of horses except significant variation of *Staphylococcus* species ($p\leq0.05$) between low and high altitudes. In conclusion, the study showed that wide variety of aerobic bacterial species inhabiting the upper respiratory tract of horses with similar distribution of the bacteria in APHH and HRTD suggesting bacteria which reside in the URT of healthy animals might cause opportunistic infections. It is recommended that clinicians should consider the dominance of Gram positives primarily as a cause of upper respiratory tract diseases in horses.

Key words: Bacterial species, horses, respiratory tract, Central Ethiopia, SPANA.

INTRODUCTION

Respiration is a cellular activity and respiratory tract is the organ that permits respiration to take place. The movement of air through the respiratory tract is achieved by the creation of pressure gradients during inspiration and expiration.

*Corresponding author. E-mail: fantadesissa@yahoo.com.

The primary functions of the nasal cavity and paranasal sinuses of the upper respiratory tract (URT) are to conduit air flow, warm, humidify air and remove particulate debris from inspired air prior to exposure to the lower respiratory tract. Equines are obligatory nasal breathers. Due to this fact, problems of the upper respiratory tract are more critical in these species than other domestic animals (Art et al., 2002). Respiratory disease is multifactorial resulting from a complex interaction of parasitic, bacterial, viral, management factor and environmental conditions. The lungs are continuously exposed to air that contains dust, bacteria, fungi, viruses and various noxious agents. Body defense against these potentially harmful materials is controlled by a complex of protective mechanisms (Jerome, 1993).

Most respiratory diseases in horses are originated from lesions located in the respiratory airways and lung (Quinn et al., 2002; Sellon and Long, 2007). The major causes of these respiratory diseases are microbial organisms. Foreign objects inhaled during feeding and oral drenching of horses with traditional medicines are predisposing factors for respiratory diseases (Quinn et al., 2002). The upper airway of healthy horses contains many bacterial flora including *Streptoccus equi subsp.zooepidemicus, Pasteruella spp., E coli, Actinomyces spp.,* and *Streptococcus spp.* Typically, horses with infectious lower airway diseases are infected with one of these bacteria, consistent with the concept that the contamination of the lower respiratory tract originates from the upper airways (Sellon and Long, 2007). In Ethiopia, despite the crucial role of cart horses in the livelihood of the owners, cart horse drivers and the economy of the country as a whole, the impact of respiratory diseases on their health and welfare and the potential microbes involved in the diseases have not been well addressed. Moreover, respiratory disease is one of the major health problems in society for protection of animals abroad (SPANA) Ethiopia project intervention areas being treated empirically (SPANA, 2008). Therefore, the objective of the study was to isolate and identify aerobic bacterial species from the upper respiratory tract of apparently health horses and horses with respiratory tract diseases in three selected SPANA project intervention towns of Central Ethiopia.

MATERIALS AND METHODS

Study area

The study was conducted in three towns of Central Ethiopia namely, Debre Zeit, Debre Brihan and Adama from October, 2009 to June, 2010. Debre-Zeit and Adama are lowland areas whereas Debre Brihan represents highland altitude. Debre Zeit is 45 km South East of Addis Ababa, located 9^0N and 4^0E at an altitude of 1850m above sea level. The rainfall is bimodal. It receives an annual rainfall of 1151.6 mm with a mean minimum and maximum temperature of 8.5^0C and 30.7^0C, respectively and a mean relative humidity of 61.3%. The cart horse population in the town is 1170 and the number of carts is 585. Adama town is located in Eastern Shoa zone of Oromia regional state 100 km South east of Addis Ababa at $8^0$32'N and 39^0 and 17`E. It has an altitude of 1622 m in the Great Rift Valley and receives an annual rainfall ranging from 400mm to 800mm and a temperature of 13.9^OC to 37^OC. There are 1580 cart horses and 790 carts in the town. Debre Berhan is located in Amhara regional state, in Central highlands of Ethiopia, 130 km North East of Addis Ababa at 9^0 36^0 N and 39^0 38'E with an altitude of 2780m above sea level. The average annual rainfall is 950 mm and the average minimum and maximum temperature is 17.6^0c to 22.5^0c in August and June, respectively. The mean relative humidity is 68.2%. There are 7114 cart horses and 357 carts in the town (NAMSA, 2003).

Study population

Cart horses that were presented to SPANA Ethiopia clinics at Debre Zeit, Debre Berhan and Adama towns during the study period were the target population. The study population was forty eight apparently health horses (APHH) and fifty six horses with respiratory tract diseases (HRTD) brought to the clinics. Apparently healthy horses (APHH) were horses with no obvious signs of diseases: no sores, no history of respiratory diseases, normal range of vital signs, body condition score 2 and above in a 1 to 5 scale brought to the clinic for regular deworming. Horses with respiratory tract diseases (HRTD) were horses with obvious signs of respiratory diseases: nasal discharges, with or without fever, dyspnoea, anorexia, respiratory distress, history of respiratory diseases and abnormal lung sounds.

Study design

A cross sectional study design was employed and the study population was selected using simple random sampling technique. All the study animals were subjected to a thorough physical and clinical examination prior to sampling.

Sample collection

Each study animal was individually identified, restrained by its owner and kept fixed. The nares and the external parts of the nose were cleaned using 70% ethyl alcohol. After disinfection, sterile cotton tipped swab in a sterile test tube having a length of 20 to 25cm was inserted into the nasopharynx, rotated and rubbed back and forth against the nasopharyngeal wall gently and carefully to take a nasopharyngeal swab sample. The swab was replaced back into the sterile test tube to which a transport medium, 5ml of tryptone soya broth, was added and tightly closed with a stopper. After labeling, the test tube containing the swab sample was kept in an ice box, shipped to a Microbiology laboratory and incubated aerobically overnight until culturing takes place on the subsequent day.

Isolation and Identification

The broth cultured samples, which were incubated overnight aerobically were thoroughly agitated and mixed. A loopful of broth culture was taken and streaked over an identified Petri dish plates containing blood agar base supplemented with 7% sheep blood. At least, two cultures were made from each specimen. The remaining samples in the test tubes were put as a sample pool source inside a refrigerator at $+4^0$C until the end of the investigation process. From culture positive plates representative of colonies were further streaked on blood agar, MacConkey agar and tryptic soya agar. Pure colonies again further transferred into slant nutrient agar for

further tests. The isolates were identified based on their growth characteristics, primary identification and secondary biochemical tests. All cultures were incubated aerobically at 37^0c for 24 to 48hrs (Quinn et al., 2002).

Data analyses

SPSS Version 16 as a statistical package was used to see variation, nature and proportion of isolates between APHH and HRTD. Descriptive statistics (percentage) was used to summaries the generated data. P value less than 0.5 was used to see the significance level.

RESULTS

All the samples collected for aerobic bacterial isolation yielded at least one isolate. A total of 270 bacterial isolates belonging to 10 genera were recovered from the entire samples with an average recovery rate of 2.6 bacteria per sample. Gram positives were recovered more often than Gram negative bacteria. Among the isolates, 178 (65.9%) were Gram positives and 92 (34.1%) were Gram negatives bacteria. The overall isolated bacterial species isolated in order of dominance include: *Bacillus* species (20%), *Streptococcus* species (17%), S*taphylococcus* species (16.7%), *E.Coli* (12.6%), *Pasteurella* species (11.5%), *Micrococcus* species (11.1%), *Bordetella* species (4.8%), *Pseudomona* species (3%), *Actinobacillus* species (2.2%) and *Rhodococcus equi* (1.1%) (Table1, 2 and 3). Almost all the isolates were recovered from both groups of horses except *Rhodococcus equi* and Actinobacillus species which were only isolated from APHH. The overall recovery rate of bacterial isolates in APHH was 52.2% (39% from highland and 61% from lowland horses) and 47.8% (32.6% from highland and 67.4% from lowland horses) in HRTD.

DISCUSSION

The present study has shown the presence of a wide variety of bacterial species in the upper respiratory tract of APHH and HRTD brought to SPANA Clinics at Adama, Debre Zeit and Debre Berhan towns of Central Ethiopia. Despite variation in the percentage of bacterial isolates, there was no statistically significant ($p> 0.05$) variation among the isolates except *Staphylococcus* species with respect to altitude and health status of the animals. *Bacillus* species, the predominant bacteria recovered in this study, were isolated from the upper respiratory tract of cart horses at a rate of 20%. Several researchers have isolated *Bacillus* species at different rate of recovery from different species of animals (Shemsedin, 2002, Tesfaye, 2004, Yimer, 2007, Desissa et al., 2009). Most Bacillus species are saprophytes and they are widely distributed in air, soil and water (Quinn et al., 2002). The presence of *Bacillus* organisms in nasopharyngeal cavity might reflect and associated with deep grazing behavior horses which exposed them to contaminate the cavity with the saprophytic bacilli in soil. *Streptococcus* species were encountered as second dominant bacteria. It was isolated at the rate of 17% equally from both APHH and HRTD. Streptococci are widely distributed in nature and commensal on the upper respiratory tract of equine species which way be potentially pathogenic (Carter and Chengappa, 1991, Quinn et al., 2002). As a matter of the fact, isolation of streptococcus species from horses with respiratory syndromes indicates the role of these bacteria as a primary opportunistic pathogen in respiratory tract infections following any stressful conditions. *Staphylococcus* species were isolated at a rate of 16.7% (17% from APHH, 16% from HRTD, 77%). Statistically significant variation in number of *Staphylococcus* species ($p<0.05$) between lowland and high altitudes, with high isolation rate from lowland horses was observed. The variation might attributable to horses in the lowlands are under continuous environmental stresses such as high environmental temperature during daytime that profoundly amplifies the impact of work stress which eventually leads to immunosupression. A related study indicated that *Staphylococcus auerus* was isolated from horses with cases of pneumonia (Sweeny et al., 1998). Coagulase negative *staphylococci* (CNS) were isolated as the dominant species of bacteria in upper respiratory infections of foals (Boguta et al., 2004). Coagulase positive *staphylococci* can be involved as opportunistic bacteria following pathogenic role of stress conditions such as viral infections and other causes of infection in immunosuppressed hosts (Quinn et al., 2002). *Escherichia coli* was the leading isolate among Gram negatives with an isolation rate of 12.6% (17% from both APHH and HRTD). It has been suggested that *E. coli*, which is usually harmless in their natural habitat, could cause disease when they gain access to other sites or tissue leading to pulmonary and urogenital infections (Pelczar et al., 1986).

Pasteurella species were the second dominant among the population of Gram negative bacteria isolated at a rate of 11.5% (12% from APHH, 14% from HRTD). Similar study conducted on sheep showed the isolation rate of pasteurella as high as 68.6% from tonsil tissue (Tesfaye, 2004) and at the rate of 8.7% from the lung of camels (Shemsedin, 2002). *Mannhemia hemolytica*, a normal flora of the upper respiratory tract, may play a secondary role after the primary initiating agent suppressed the host defense mechanism and favors the multiplication of *pasteucella* species leading to bronchopneumonia (Aiello and May,1998). *Micrococci* species were isolated at a rate of 11.1%. *Micrococci* are non-pathogenic species of upper respiratory tract of domestic animals (Quinn et al., 2002). Hence, the isolation of the bacteria from HRTD does not necessarily indicate their role in upper respiratory tract *diseases. Bordetella* species were isolated at a rate of4.8% (5% from APHH, 3% from HRTD.

Table 1. Bacterial species isolated from apparently healthy cart horses, Central Ethiopia.

Altitude	Isolated Species	Number	Percentage
Highland	*Streptococcus* species	10	41
	Micrococcus species	10	66
	Staphylococcus species	7	29
	Pasteurella species	6	50
	E. coli	7	41
	Bacillus species	7	25
	Actinobacillus species	2	33
	Bordetella species	4	50
	Pseudomonas species	2	40
		55	**39**
Lowland	*Streptococcus* species	14	59
	Micrococcus specie	5	34
	Staphylococcus species	17	73
	Pasteurella species	6	50
	E. coli	10	59
	Bacillus species	20	75
	Actinobacillus species	4	67
	Bordetella species	4	50
	Pseudomonas species	3	60
	Rhodococcus equi	3	100
		86	61
Total		**141**	**100**

Table 2. Bacterial species isolated from cart horses with respiratory diseases, Central Ethiopia.

Altitude	Isolated species	Number	Percentage
Highland	*Streptococcus* species	6	27
	Micrococcus specie	5	33
	Staphylococcus species	2	9
	Pasteurella species	6	31
	E. coli	7	41
	Bacillus species	12	44
	Bordetella species	3	60
	Pseudomonas species	1	33
		42	**32.6**
Lowland	*Streptococcus* species	16	73
	Micrococcus specie	10	67
	Staphylococcus species	19	91
	Pasteurella species	13	69
	E. coli	10	59
	Bacillus species	15	56
	Bordetella species	2	40
	Pseudomonas species	2	67
		87	**67.4**
Total		**129**	**100**

The bacteria are commensal in the upper respiratory tract of animals being frequently attributed to broncho-pneumonia in guinea pigs and other rodents, in swine and lower primates (Graves,1970) Hence, the isolation of the bacteria from HRTD might indicate its role in the upper respiratory tract diseases of the horses. *Actinobacillus*

Table 3. Overall bacterial species isolated from both apparently healthy and cart horses with respiratory diseases, Central Ethiopia.

	Isolated species	Number	Percentage
Gram positive	*Streptococcus* species	46	17
	Micrococcus species	30	11.1
	Staphylococcus species	45	16.7
	Bacillus species	54	20
	Rhodococcus equi	3	1.1
		178	**65.9**
Gram negative	*Pasteurella* species	31	11.5
	E. coli	34	12.6
	Actinobacillus species	6	2.2
	Bordetella species	13	4.8
	Pseudomonas species	8	3
		92	**34.1**
Total		**270**	**100**

species were recovered only from APHH at a rate of 2.2%. The finding was relatively in agreement with the findings of Teklesillasie (2005) and Desissa et al., (2009) who reported 1.4% and 2.56% of the bacteria from the upper respiratory tract of goats and donkeys, respectively. *Pseudomonas* species were the third least isolate as compared to the isolates of this study and isolated at a rate of 3% (3% from apparently health cart horses, 2% from cart horses with respiratory syndrome). Studies done by Cabbasi *et al.,* (1975) indicated as *Pseudomonas* species are transient flora of the nasal mucosa of horses. *Rhodococcus equi* was encountered as the least dominant among the isolates. It was isolated only from APHH in the lowland areas at a rate of 1.1% of the total isolates. The bacterium is an opportunistic pathogen and common soil inhabitant (Quinn et al., 2002). In agreement to this finding *Rhodococcus equi* was found as one of the common isolates of equine respiratory tract (Racklyeft and Love, 2000).

Conclusion

In conclusion, the study showed that wide variety of aerobic bacterial species inhabiting the upper respiratory tract of horses with similar distribution of the bacteria both in APHH and HRTD with the exception of *Actinobacillus species* and *Rhodococcus equi* which were only isolated from APHH. Isolation of most bacteria from both groups of horses might suggest that the bacteria which reside in the URT of healthy animals have a chance to cause opportunistic infections following various stress factors. Gram positives were more often recovered than Gram negatives. The authors recommended that clinician should consider the dominance of Gram positives primarily as a cause of upper respiratory tract diseases in selection of antibiotics when dealing with horses suffering from respiratory cases. Moreover, further comprehensive study ought to be conducted to ascertain the specific pathogenic role of anaerobic bacteria, fungal species and viruses and antibiogram of all pathogens involved in the respiratory tract diseases of horses which would help clinicians to select appropriate drugs for the management of the clinical cases.

Conflict of Interest

The author(s) have not declared any conflict of interests

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