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Bacterial Examinations in the Nasal Cavity of Apparently Healthy and Unhealthy Holstein Cattle

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Abstract: The aim of this study was to examine the bacterial flora of nasal cavity in apparently healthy and unhealthy Holstein cattle and determine the antimicrobial susceptibilities of *Pasteurella multocida* and *Mannheimia haemolytica* strains isolated from samples. Nasal swab samples were collected from 100 Holstein cattle, 70 of these were apparently healthy and 30 were unhealthy with the following respiratory signs: nasal discharge, coughing and dyspnoea. A total of 220 bacterial isolates were detected on Columbia blood agar plates, 102 (46.4%) of these were recovered from healthy and 118 (53.6%) from unhealthy Holstein cattle. The most frequently isolated species from the nasal cavity of the healthy animals sampled were *Staphylococcus epidermidis* (32.9%) and *S. aureus* (24.3%), while the most prevalent species in unhealthy cattle were *Pseudomonas aeruginosa* (40.0%), *P. multocida* (40.0%) and *M. haemolytica* (100.0%). According to antibiotic susceptibility test results, enrofloxacin (95.0%) was the most effective antibiotic on *P. multocida* isolates, while 95.0% of *M. haemolytica* isolates were susceptible to gentamicin. Among the *P. multocida* isolates the highest resistance was found against to kanamycin (70.0%), while erythromycin resistance were detected as 90.0% for *M. haemolytica* isolates.

Key words: Nasal cavity, cattle, *P. multocida*, *M. haemolytica*, antibiotic susceptibility test

INTRODUCTION

It is considered that the bacterial flora of upper respiratory system includes both resident and transitory microflora. When resident microflora is damaged, the first balance tends to be restored spontaneously. However, some opportunistic pathogens may cause diseases if they pass the normal defenses of the host. Transitory microflora, which is originated from environment, remains for only short periods within the host. Generally, it is not easy to detect primer etiological agent in the respiratory system diseases because of the mutual effects of many bacterial agents. This state usually concludes with multiple bacterial infections in the respiratory system (Quinn *et al*., 2002). Although, some bacterial agents such as *Mannheimia haemolytica* are found in the nasopharynx and tonsils of apparently healthy animals (Rowe *et al*., 2001), they can be isolated as the main microorganism responsible for respiratory system diseases (Quinn *et al*., 2002).

Many researchers usually have been studied on unhealthy domestic and wild animals have clinical signs ssuch as pneumonic lungs, nasal discharge and cough (Welsh *et al*., 2004; Katsuda *et al*., 2008). Also, the studies regarding to nasal and tonsillar bacterial flora have been reported in apparently healthy animals (Queen *et al*., 1994; Megra *et al*., 2006). However, studies investigating the bacterial flora of upper respiratory system in healthy and unhealthy animals are limited (Barbour *et al*., 1997; Jaramillo-Arango *et al*., 2007). DeRosa *et al*. (2000) reported that a nasal swab culture can be predictive of the bacterial pathogen within the lung when the isolates are from an acutely ill animal and can be used to determine antibiotic susceptibility.

The aim of this study was to examine the bacterial flora of nasal cavity in apparently healthy and unhealthy Holstein cattle and determine the antimicrobial susceptibilities of *P. multocida* and *M. haemolytica* strains isolated from samples.

MATERIALS AND METHODS

Collection of samples: A total of 100 Holstein cattle aged 5 months through 3 years breeding in Afyonkarahisar province of Turkey were examined. Seventy of these were apparently healthy and 30 were unhealthy with the following respiratory signs: nasal discharge, coughing and dyspnoea. Sterile cotton swabs were used

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in sampling the nasal cavities of animals. The swabs were introduced directly into nasal cavity and rubbed smoothly against the mucosa in a circular motion. Two swabs collected from each animal were put into Stuart transport medium (Oxoid Ltd., Hampshire, England) and transported to the laboratory in a cool box on ice.

Microbiological analysis: A total of 200 nasal swab samples belong to 100 Holstein cattle were cultured in Columbia blood agar (Oxoid Ltd., Hampshire, England), containing 7% of sheep blood. The plates were incubated under aerobic conditions for 24-48 h at 37°C. After the incubation each different colony was examined macroscopically (colony morphology, hemolysis, pigment producing) and microscopically (Gram staining). Then, each different colony was subcultured in blood agar media (Oxoid Ltd., Hampshire, England), containing 7% of sheep blood and tryptone soya broth (Oxoid Ltd., Hampshire, England) for further characterizations. Identification of microorganisms was done using conventional methods according to standard manuals (Quinn *et al*., 1999; Holth *et al*., 2000).

Antimicrobial susceptibility test: Antimicrobial susceptibility profiles of *P. multocida* and *M. haemolytica* isolates were determined using the Kirby-Bauer disk diffusion method on Mueller-Hinton Agar (Oxoid Ltd., Hampshire, England) supplemented with 7% sheep blood according to the National Committee of Clinical Laboratory Standards (NCCLS). Pure colonies from the blood agar medium, incubated at 37°C for 18 h, suspended in 2 mL sterile saline to a density approximately equal to McFarland opacity standard No. 0.5. A dry cotton wool swab was placed into the suspension and excess liquid was expressed against the inside of the tube. The bacterial suspension was then inoculated onto Mueller-Hinton agar with the swab in such a way that the whole surface of the agar was covered. The plates were incubated at 37°C for 24 h. The results were recorded by measuring the inhibition zone diameter according to the interpretive standards of NCCLS. The following 12 antimicrobial agents were assayed: ampicillin (10 μg), chloramphenicol (30 μg), enrofloxacin (5 μg), erythromycin (15 μg), gentamicin (10 μg), kanamycin (30 μg), oxacillin (1 μg), oxytetracycline (30 μg), penicillin G (10U), streptomycin (10 μg), tetracycline (30 μg), trimethoprim/sulphamethoxazole (25 μg).

Statistical analysis: Pearson and Fisher's exact Chi-square (χ^2) tests were used to determine the relationship between the bacteria isolated from samples and the health condition of cattle.

RESULTS AND DISCUSSION

Bacterial identification findings: A total of 220 bacterial isolates were detected on Columbia blood agar plates, 102 (46.4%) of these were recovered from healthy and 118 (53.6%) from unhealthy Holstein cattle. Sixty-seven (65.7%) of 102 isolates observed from healthy cattle were Gram positive and 35 (34.3%) were Gram negative. Fifty-one (43.2%) isolates isolated from cattle with clinical signs were detected as Gram positive and 67 (56.8%) as Gram negative. Twelve different bacterial genera including *Staphylococcus*, *Streptococcus*, *Micrococcus*, *Corynebacterium*, *Arcanobacterium*, *Bacillus*, *Escherichia*, *Moraxella*, *Neisseria*, *Pseudomonas*, *Pasteurella* and *Mannheimia* were isolated. The most frequently isolated species from the nasal cavity of the healthy animals sampled were *Staphylococcus epidermidis* (32.9%) and *S. aureus* (24.3%), while the most prevalent species in unhealthy cattle were *Pseudomonas aeruginosa* (40.0%), *P. multocida* (40.0%) and *M. haemolytica* (100.0%). The other species isolated and identified from healthy and unhealthy cattle were shown in Table 1. There was statistically significant difference ($p<0.005$) between apparently healthy and unhealthy cattle in terms of the species including *Streptococcus* sp., *Arcanobacterium pyogenes*, *Moraxella bovis*, *P. aureginosa*, *P. multocida* and *M. haemolytica* isolated from animals (Table 1).

Antimicrobial susceptibility test: Twenty *P. multocida* and 40 *M. haemolytica* isolates were assayed for antimicrobial susceptibilities using standard disk diffusion tests and interpreted via NCCLS guidelines. Enrofloxacin was found to be the most effective antibiotic as 95.0% of *P. multocida* isolates were susceptible to this drug, followed by chloramphenicol 90.0%, streptomycin 90.0%, oxytetracycline 85.0%, ampicillin 85.0%, gentamicin 80.0%, oxacillin 80.0% and tetracycline 80.0%, while rest of the antibiotics were less effective (Table 2).

Trimethoprim/sulphamethoxazole 75.0% was moderately effective on *P. multocida* isolates. Among the *P. multocida* isolates, the highest resistance was seen against to kanamycin 70.0%. Ninety-five percent of *M. haemolytica* isolates were susceptible to gentamicin. Similar to *P. multocida* isolates, *M. haemolytica* isolates were susceptible to enrofloxacin, chloramphenicol and tetracycline, 90.0, 87.5 and 85.0%, respectively. Erythromycin resistance were detected as 90.0% for *M. haemolytica* isolates (Table 2).

Numerous microorganisms, including *Pasteurella*, *Arcanobacterium*, *Neisseria*, *Moraxella*, *Staphylococcus* and *Streptococcus* sp. are commonly isolated from nasal

Table 1: Bacteria isolated from nasal cavities of apparently healthy and unhealthy cattle and their percentage

	Healthy Holstein cattle (n = 70)		Unhealthy Holstein cattle (n = 30)		
Bacterial species	Frequency (n)	Proportion (%)	Frequency (n)	Proportion (%)	p-value
Gram positive bacteria					
Staphylococcus epidermidis	23	32.9	10	33.33	0.963
S. intermedius	6	8.6	5	16.70	0.298
S. aureus	17	24.3	8	26.70	0.801
Streptococcus sp.	5	7.1	9	30.00	0.005
Micrococcus luteus	5	7.1	1	3.30	0.665
Corynebacterium bovis	1	1.4	2	6.70	0.213
Arcanobacterium pyogenes	4	5.7	11	36.70	0.000
Bacillus sp.	6	8.6	5	16.70	0.298
Gram negative bacteria					
Escherichia coli	6	8.6	3	10.00	1.000
Moraxella bovis	4	5.7	8	26.70	0.006
Neisseria sp.	5	7.1	2	6.70	1.000
Pseudomonas aeruginosa	2	2.9	12	40.00	0.000
Pasteurella multocida	8	11.4	12	40.00	0.001
Mannheimia haemolytica	10	14.3	30	100.00	0.000

Table 2: Antibiotic susceptibilities of *P. multocida* and *M. haemolytica* strains isolated from Holstein cattle

	P. multocida (n = 20)			*M. haemolytica* (n = 40)		
Antimicrobial agent	S	I	R	S	I	R
Ampicillin (10 μg)	15 (75.0)	2 (10.0)	3 (15.0)	29 (72.5)	1 (2.5)	10 (25.0)
Chloramphenicol (30 μg)	17 (85.0)	1 (05.0)	2 (10.0)	34 (85.0)	1 (2.5)	5 (12.5)
Enrofloxacin (5 μg)	19 (95.0)	-	1 (05.0)	36 (90.0)	-	4 (10.0)
Erythromycin (15 μg)	8 (40.0)	-	12 (60.0)	5 (12.5)	2 (5.0)	33 (82.5)
Gentamicin (10 μg)	16 (80.0)	-	4 (20.0)	38 (95.0)	-	2 (05.0)
Kanamycin (30 μg)	2 (10.0)	4 (20.0)	14 (70.0)	31 (77.5)	-	9 (22.5)
Oxacillin (1 μg)	14 (70.0)	2 (10.0)	4 (20.0)	9 (22.5)	4 (10.0)	27 (67.5)
Oxytetracyline (30 μg)	16 (80.0)	1 (05.0)	3 (15.0)	10 (25.0)	1 (2.5)	29 (72.5)
Penicillin G (10 U)	4 (20.0)	4 (20.0)	12 (60.0)	28 (70.0)	2 (5.0)	10 (25.0)
Streptomycin (10 μg)	17 (85.0)	1 (05.0)	2 (10.0)	8 (20.0)	2 (5.0)	30 (75.0)
Tetracycline (30 μg)	12 (60.0)	4 (20.0)	4 (20.0)	34 (85.0)	-	6 (15.0)
Trimethoprim/sulphamethoxazole (25 μg)	15 (75.0)	-	5 (25.0)	31 (77.5)	-	9 (22.5)

S: Sensitive; I: Intermediately sensitive; R: Resistant

mucosa and the nasopharynx of ruminants. Most of these microorganisms are considered to have a predisposition for these sites and capable of colonizing the upper respiratory system (Quinn *et al*., 2002).

In the present study, of the 220 bacterial isolates, 102 were recovered from healthy and 118 isolates from unhealthy Holstein cattle. Gram positive bacteria determined as dominant among the isolates obtained from apparently healthy animal, while Gram negative bacteria were commonly isolated from unhealthy cattle. Most of the Gram positive bacteria isolated from healthy cattle are common commensals on the mucous membranes of upper respiratory tract of healthy animals. Generally, it is accepted that Gram negative bacteria are commonly associated with systemic infections in human and animals (Quinn *et al*., 2002). Therefore, it was considered that discrepancy in the isolation rates of Gram positive and Gram negative bacteria may be associated with health condition of examined cattle.

Staphylococcus epidermidis 32.9% and *S. aureus* 24.3%, which are common commensals on the mucous membranes of upper respiratory tract of healthy animals, were the most frequently isolated species from the nasal cavity of the apparently healthy cattle in this study. Also, *P. multocida* 11.4% and *M. haemolytica* 14.3%, which are opportunistic and primary pathogens as well as hosts of the upper respiratory system of domestic and wild animals were recovered in higher proportion from healthy cattle as opposed to isolation rate of other bacteria. Similarly, Megra *et al.* (2006) reported the isolation of *P. multocida* and *M. haemolytica* from nasal cavity of healthy goats at a higher rate compared to trachea and lung indicates that the microorganisms live as a commensal in the upper respiratory system, but invades the lung under condition of stress.

P. multocida and *M. haemolytica* cause severe infections such as hemorrhagic septicemia or bovine pneumonic pasteurellosis as well as secondary infections in ruminants. They are the most pathogenic bacterial agents commonly isolated from bovine respiratory system diseases. Although, *Pseudomonas aeruginosa* is usually accepted as opportunistic pathogen, it can cause pneumonia in cattle, also (Quinn *et al*., 2002). In the present study, the most prevalent species

were *P. aeruginosa* (40.0%), *P. multocida* (40.0%) and *M. haemolytica* (100.0%) in apparently unhealthy cattle. These results were consistent with other reports from the cattle with clinical signs of respiratory system diseases (Barbour *et al*., 1997; DeRosa *et al*., 2000; Welsh *et al*., 2004).

According to antibiotic sensitivity test results, enrofloxacin 95.0% was the most effective antibiotic on *P. multocida* isolates, followed by chloramphenicol 90.0%, streptomycin 90.0%, oxytetracycline 85.0%, ampicillin 85.0%, gentamicin 80.0%, oxacillin 80.0% and tetracycline 80.0%. Similar efficacy of enrofloxacin and chloramphenicol was reported by Kumar *et al.* (2009) in an *in vitro* study conducted on isolates of ruminant origin. In another study, enrofloxacin, chloramphenicol, streptomycin, oxytetracycline, ampicillin, gentamicin were found quite effective against to bovine and ovine *P. multocida* isolates (Shayegh *et al*., 2009). In the present study, the highest resistance belong to *P. multocida* isolates was found against to kanamycin (70.0%). Similarly the finding, Kumar *et al.* (2009) were reported kanamycin was effective only against 43.0% of isolates.

Gentamicin 95.0% was found as the most effective antibiotic on *M. haemolytica* isolates. In a study made by Post *et al.* (1991), 90.0% of *M. haemolytica* isolates recovered from cattle with bovine respiratory disease complex were markedly found as susceptible to gentamicin. We found that enrofloxacin, chloramphenicol and tetracycline were the other effective antibiotics on *M. haemolytica* isolates. These findings were consistent with the results of Kaspar (2006) belong to 102 *M. haemolytica* strains isolated from cattle with acute respiratory system disease. In this study, 90.0% of *M. haemolytica* isolates were resistant to erythromycin. Fales *et al.* (1982) reported that only 9.0% *M. haemolytica* strains isolated from 1976-1980 were resistant to erythromycin and recommended that erythromycin be used as the first-choice antibiotic for treatment of respiratory system diseases of cattle. Conversely, Watts *et al.* (1994) reported resistance to erythromycin was frequently detected among cattle strains of *M. haemolytica*. The level of resistance to erythromycin observed in the present study indicates that erythromycin probably has limited usefulness in the treatment of respiratory system problems related to cattle *M. haemolytica* isolates.

CONCLUSION

This study shown that the various of bacterial flora colonized the nasal cavities a part of the upper respiratory tract of apparently healthy Holstein cattle as well as unhealthy cattle. Although, the species isolated from healthy animal have not a role as primer pathogen for important infections, some of species isolated such as *P. multocida* and *M. haemolytica* may be the main reason of respiratory system problems under poorly conditions. Nasal sampling can provide the prediction of lower respiratory system problems in the cattle with clinical signs of respiratory disease. In the present study, antibiotic susceptibility profiles of *P. multocida* and *M. haemolytica* isolates were determined, also. Sampling with nasal swab and this data can help for choosing of proper antibiotic in the treatment of cattle with clinical signs of respiratory diseases.

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