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Frequency and antibiotic susceptibility of *Pasteurella multocida* and *Mannheimia haemolytica* isolates from nasal cavities of cattle

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Abstract: The aim of the present study was to determine the frequency of *Pasteurella multocida* and *Mannheimia haemolytica* from nasal cavities of cattle, and to find antibiotic susceptibility profiles of the isolates. Bilateral nasal swab samples were collected from 47 clinically healthy Holstein cattle, with no history of antimicrobial treatment prior to sampling. Respectively, 5 and 27 isolates were identified as *M. haemolytica* and *P. multocida*. Seventeen samples from cattle of 1 year or younger were found to have *P. multocida*. On the other hand, from 29 samples from cattle of 2 years or older, 10 *P. multocida* and 5 *M. haemolytica* strains were isolated. Each isolate was tested for susceptibility against florfenicol, sulfamethoxazole/trimethoprim, enrofloxacin, ampicillin, and erythromycin. All *P. multocida* and *M. haemolytica* isolates were found susceptible against florfenicol, and 27 *P. multocida* and 5 *M. haemolytica* isolates showed susceptibility against enrofloxacin, sulfamethoxazole/trimethoprim, erythromycin, and ampicillin at the rates of 85.1% and 80% ; 92.6% and 80% ; 44.5% and 60% ; 100% and 80% , respectively.

Key words: *Pasteurella multocida, Mannheimia haemolytica,* cattle, antibiotic susceptibility

Sığırların burun boşluklarından izole edilen *Pasteurella multocida* ve *Mannheimia haemolytica* izolatlarının in vitro antibiyotik duyarlılık sonuçları

Özet: Bu çalışmanın amacı sığırların burun boşluklarında *Pasteurella multocida* ve *Mannheimia haemolytica* prevalansını ve izolatların antibiyotik duyarlılık sonucu profillerini belirlemektir. Klinik olarak sağlıklı ve örneklemeden önce herhangi bir antibiyotik tedavisi geçmişi bulunmayan 47 adet Holstein ırkı sığırdan bilateral burun svab örnekleri alındı. Beş izolat *M. haemolytica,* 27 izolat *P. multocida* olarak identifiye edildi. Bir yaşında ve daha genç olan sığırlardan izole edilen 17 örnek *P. multocida* olarak bulundu. Buna karşın, 2 ve daha üzeri yaşlı sığırlardan alınan 29 örneğin 10 adedi *P. multocida,* 5 adedi *M. haemolytica* olarak izole edildi. Her izolatın florfenicol, sulphamethoxazole/trimethoprim, enrofloxacin, ampicillin ve erythromycin'e karşı duyarlılıkları test edildi. *Pasteurella multocida* ve *Mannheimia haemolytica* izolatlarının tümü florfenicol'e karşı duyarlı bulundu, 27 *P. multocida* ve 5 *M. haemolytica* izolatı enrofloxacin, sulphamethoxazole/trimethoprim, erythromycin and ampicillin'e karşı sırasıyla % 85,1 ve % 80 ; % 92,6 ve % 80; % 44,5 ve % 60; % 100 ve 80 % oranlarında duyarlılık gösterdi.

Anahtar sözcükler: *Pasteurella multocida, Mannheimia haemolytica,* sığır, antibiyotik duyarlılığı

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Pasteurella and *Mannheimia* are known to act as opportunistic pathogens in the bovine respiratory disease (BRD), and both belong to the family Pasteurellaceae (1). Antimicrobial agents are currently used for the treatment of BRD (2) and antibiotic susceptibility tests are important, because resistance to antibiotics is frequent in *Pasteurella multocida* and *Mannheimia haemoytica*, although it is less common in other pasteurellae (3). The aim of the present study was to determine the frequency of *P. multocida* and *M. haemolytica* from nasal cavities of clinically healthy cattle, and to determine antibiotic susceptibility profiles of the isolates.

This study was performed at the Uludağ University cattle farm, Bursa, Turkey. The calves and the cattle are housed separately from each other. None of the cattle and calves had signs or history of clinical respiratory problems and received antibiotherapy prior to sampling. A total of 47 clinically healthy Holstein breed cattle, including 18 calves (3-12 months old) and 29 cattle (2 years old), were examined. External ports of the nostrils were disinfected using alcohol (90%) and bilateral nasal swabs were taken and each swab was put into a tube containing 3 mL sterile 0.9% NaCl solution and vortexed in order to release whole collected material from swabs to the aqueous phase. The materials in tubes were transferred to sterile Eppendorf tubes, and centrifuged at 3000 ×g for 10 min. Sediments were used as inocula. Sediments were mixed with 30 µL sterile 0.9% NaCl and 20 µL mixture was inoculated onto 5% ovine blood agar plates (Oxoid) and the plates were incubated in 5.2% CO_2 for 24 h at 37 °C. A single colony was subcultured and tested by Gram staining, and catalase and oxidase features. Each isolate, which is gram negative, coccobacilli shape, catalase (3% H_2O_2) positive, cytochrome oxidase positive and having the typical colony morphology of Pasteurellaceae, was taken into consideration for identification procedure. Morphological features of the isolates were re-evaluated. Differentiation between *P. multocida* and *M. hemolytica* was determined according to ß-hemolysis on blood agar (Blood agar base No:2, Oxoid, containing 5% ovine blood), growth on Mac Conkey agar (Oxoid), catalase (3% H_2O_2), oxidase (Merck), and ornithine decarboxylation [in Mueller decarboxylase broth medium (Difco)]. Motility was examined in SIM medium (Oxoid); urease activity was tested in Urea Medium (Urea agar base, Oxoid); indole production was examined by adding Kovac's reagent to a 48-h culture of the isolates in Brain Heart Infusion broth; production of acid from: L(+) arabinose, D(+) mannose, trehalose, lactose, glucose, sucrose, maltose, and mannitol were examined on 48-h Brain Hearth Infusion broth culture with brom-thymole reagent. After inoculation, the media were incubated aerobically at 37 °C. The readings were taken after 24 h for all tests. A second reading was taken after 48 h only for the sugar fermentations, which were negative after the first reading results were interpreted in accordance with the recommendations of Quinn et al. (1) and MacFaddin (4). Each isolate was tested for susceptibility against florfenicol, sulfamethoxazole/trimethoprim, enrofloxacin, ampicillin, and erythromycin. Antimicrobial susceptibilities were performed by modified Kirby-Bauer methods on Muller-Hinton agar (Oxoid) with 5% ovine blood added. Procedures and susceptibility interpretation were standardized according to the published criteria by the National Committee for Clinical Laboratory Standards as outlined by Watts et al. (5). The zone diameters in the intermediate category were considered to be nonsusceptible.

Forty-three suspected colonies on 5% sheep blood agar plates were examined for their biochemical characteristics and 5 and 27 isolates were identified as *M. haemolytica* and *P. multocida*, respectively. Seventeen samples from cattle of 1 year or younger were found to have *P. multocida* strains. On the other hand, 10 *P. multocida* and 5 *M. haemolytica* strains were isolated from 29 samples of cattle of 2 years or older (Table 1). Thirty-two isolates were subjected to antibiotyping (Table 2).

One of the interesting findings of our study was high frequency of *P. multocida* isolation rate (94%) and no isolation of *M. haemolytica* (0%) in 1 year and younger clinically healthy calves which is in contrast with other reports where it was found in 46.6% and 31.6% of 60 (6), 0% and 35 % of 40 (7), and 57.4% and 3.2% of 61 (2) nasal swab samples, respectively. Differences with the above-mentioned studies maybe due to our clinical samples and because our samples were consisted of calves from the same herd. Another

Table 1. Distrubition and percentages of *Mannheimia haemolytica* and *Pasteurella multocida* isolates on the basis of age.

Age	*M. haemolytica* (%)	*P.multocida* (%)	Total Samples
Less than one year of age	no isolation (0%)	17 (94.4%)	18
More than two year of age	5 (17.2%)	10 (34.4%)	29
Total	5 (10.6%)	27 (57.4%)	47

Table 2. Number of isolates tested and sensitivity percentages

Antibiotic	*M. haemolytica*				*P. multocida*			
	N	% S	% Int	% R	N	% S	% Int	% R
Florfenicol	5	100	0	0	27	100	0	0
Enrofloxacin	5	80	20	0	27	85.1	11.2	3.7
Trimethoprim/Sulphamethoxazole	5	80	20	0	27	92.6	3.7	3.7
Erythromycin	5	60	40	0	27	44.5	55.5	0
Ampicillin	5	80	20	0	27	100	0	0

N: Number of isolates, S: Sensitive, Int: Intermediate, R: Resistant

reason for the difference is that a subclinical *P. multocida* infection may have been in progress in our herd.

It is important to monitor the antimicrobial susceptibility of *Pasteurella* species to determine resistance development. Increases in resistance against antibiotics in both *P. multocida* and *M. haemolytica* isolates have been reported in recent years (2,8,9). In our study, according to the antimicrobial susceptibility test results, florfenicol was found to be the most effective antibiotic for both *P. multocida* and *M. haemolytica*. However, *P. multocida* and *M. haemolytica* isolates showed minimal susceptibility against erythromycin at the rates of 54.5% and 60%, respectively. Post et al. (10) found that 90% of the *P. multocida* isolates were moderately susceptible to ampicillin and erythromycin while *M. haemolyica* isolates were resistant to ampicillin. In our study susceptibility rates to ampicillin of *P. multocida* and *M. haemolytica* were found as 100% and 80%, respectively, in contrast to previous studies (8-10). However, susceptibility to enrofloxacin and sulfamethoxazole/trimethoprim were over 80%, which is very close to the rates reported previously (8,11,12). Antibiotic susceptibility profiles of *P. multocida* and *M. haemolytica* help veterinarians to choose appropriate antibiotic against BRD; however, antibiotic susceptibility studies should be renewed periodically because of possible resistance development among the BRD pathogens.

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