

## Investigation of biotype, genotype and virulence associated genes in *Pasteurella multocida* capsular type A strains from the respiratory tract of cattle and their relationship with disease cases

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**Abstract:** *Pasteurella (P) multocida*, which causes respiratory disease in cattle, can also be found commensally in the upper respiratory tract. Therefore, it is epidemiologically important to determine the possible difference between pathogenic and commensal strains for the diagnosis of the disease. In this study, phenotypic and genotypic characteristics and virulence associated genes of *P. multocida* capsular type A strains isolated from the respiratory tract of sick and healthy cattle were investigated. Also, the relationship between the disease status of animals and the characteristics of the isolates were evaluated. Total of 51 *P. multocida* capsular type A strains were biotyped and genotyped by using commercial bacteria identification kits and PCR-fingerprint, respectively. Four major biotypes generated from xylose and sucrose test results were detected in 39 (76.47%) isolates. Eleven different biotypes were detected in the remaining 12 isolates (23.53%), which were separated from the 4 major biotypes by mannitol, indole, sorbitol, nitrate, VP, lactose, arabinose, salicin, and rhamnose test. Four genotypes were detected in *P. multocida* isolates and most of them (90.2%) had genotype I profile. Virulence associated genes, such as *hgbA*, *exbB*, *exbD*, *tpfA*, *ptfA*, *hsf-2*, *tadD*, *nanB*, *nanH*, *pmHAS*, *sodA*, *sodC*, and *oma87* were detected in all isolates whereas *hgbB*, *toxA*, and *hsf-1* genes were not found by PCR. *PfhA* gene were found in 49 isolates (96.07%) but, *ompH* gene could be detected only in 10 (18.86%) isolates. The findings of the study indicated that *P. multocida* capsular type A strains generated as xylose positive/sucrose negative (biotype II) and genotype II may be associated with respiratory diseases of cattle. However, *ompH* gene was observed to be associated with sucrose negative isolates, but not with disease cases. As a result, it was concluded that xylose positive/sucrose negative biotype and genotype II determined in *P. multocida* capsular type A strains isolated from respiratory diseases of cattle, might be important epidemiological criteria in the differentiation of commensal and pathogenic isolates.

**Key words:** Cattle, genotype, *Pasteurella multocida*, phenotype, virulence genes

### 1. Introduction

*Pasteurella (P) multocida* is a primary or secondary pathogen in poultry, cattle, pig, and rabbits in the cases of cholera, atrophic rhinitis, hemorrhagic septicaemia, respiratory disease and rhinitis. *P. multocida* strains are divided into 5 serogroups: A, B, D, E, and F according to capsule antigens and type A is considered one of the primary agents of bovine respiratory diseases [1,2]. Additionally, it has been reported that the agent can be transmitted to humans by cat and dog bites [3]. *P. multocida* is found in the natural flora of the upper respiratory tracts of cattle and spreads to lower respiratory system in animals in which the immune system is suppressed. The agent is transmitted by direct contact with the infected animals or by aerosol [4]. However, it has not already been fully elucidated whether the pathogenic strains that cause infection differ from the commensal strains [5–9].

*P. multocida* isolates can be identified by conventional bacteriological methods, but the differences in biochemical properties lead to the emerge of various biotypes [4,10,11]. However, there are few studies in which *P. multocida* biotypes are associated with pathogenic strains that cause disease. Barbour et al. [12] found that the relationship between the trehalose fermentation of the bovine isolates and the health status of the animals was significant, while Autio et al. [6] emphasized the need to investigate the relationship of indole-negative isolates with disease cases.

Pulsed-field gel electrophoresis, multilocus sequence analysis, ribotyping, restriction endonuclease analysis, random-amplified polymorphic DNA analysis, PCR-fingerprint analysis are the most commonly used methods for genotyping of *P. multocida* isolates. RAPD and PCR fingerprint analysis are quick and easy to apply and are more advantageous than other techniques [2,13]. However, limited number of studies were reported on biochemical

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properties of *P. multocida* genotypes determined by PCR-fingerprint method [14,15].

To date, 23 virulence genes including capsule (*capA*, *B*, *D*, *E*, and *F*), adherence and colonization factors (*ptfA*, *fimA*, *hsf-1*, *hsf-2*, *pfhA*, and *tadD*), factors associated with iron binding (*exbB*, *exbD*, *tonB*, *hgbA*, *hgbB*, *Fur*, and *tbpA*), hyaluronidase (*pmHAS*) and sialidase (*nanB* and *nanH*), superoxide dismutase (*sodA* and *sodC*), dermonecrotin (*toxA*), outer membrane and porin proteins (*ompA*, *ompH*, *oma87*, and *plpB*) have been identified which play different roles in the pathogenesis of *P. multocida* infections [7,16]. There are several studies investigating the association of virulence genes with infections caused by *P. multocida* at bovine respiratory tract [7–9]. However, no study was reported to determine an association between the virulence genes and biotype or genotype distribution of *P. multocida* strains isolated from lower or upper respiratory tract of cattle.

In this study, the distribution of biotype, genotype, and virulence associated genes were investigated in *P. multocida* capsular type A strains isolated from respiratory tract of sick or healthy cattle. The relation between the strain characteristics and health status of animals were analysed and the epidemiological significance of the findings was evaluated.

## 2. Material and methods

### 2.1. Bacterial strains

In this study, a total of 51 *P. multocida* capsular type A strains isolated from clinically healthy and sick cattle were used. The isolates were collected between 2016–2017 in Van/ Turkey and identified by PCR using species specific gene [17]. Determination of capsular type of the strains were performed according to previously reported by Townsend et al. [18]. Thirty-one of the isolates were obtained from nasopharyngeal swaps of clinically healthy animals and 13 of them were from nasopharyngeal swap samples of sick animals that had pneumonia symptom. Also, 7 of them were from trachea-bronchial swaps of slaughtered cattle that had pneumonic lung.

### 2.2. Reference strains

In this study, *P. multocida* subsp. *multocida* ATCC 43137 capsular type A and *P. multocida* ATCC 12948 capsular type D were used as reference strains.

### 2.3. Biotyping

Microgen GN-ID A + B commercial bacteria identification kit was used for typing of *P. multocida* capsular type A isolates according to their biochemical properties. The kit strip contains standardised biochemical substrates (lysine, ornithine, H<sub>2</sub>S, glucose, xylose, O-nitrophenyl-beta-D-galactopyranoside, indole, urease, Voges-Proskauer, citrate, tryptophan deaminase, nitrate, gelatine, malonate, inositol,

sorbitol, rhamnose, sucrose, lactose, arabinose, adonitol, raffinose, salicin, and arginine) which have been selected for identification of the family *Enterobacteriaceae* and oxidase positive and Gram negative bacilli. Manufacturer recommendations were taken into consideration in the application and evaluation of the tests.

### 2.4. Genotyping

The isolates were genotyped by PCR-fingerprint analysis using the M13 core primer (5'-GAG GGT GGC GGT TCT-3') [15]. Genomic DNA was obtained by boiling method [7]. PCR mix was consisted of 11 mL mastermix (Abm 2X PCR Taq Plus MasterMix, G014), 1 mL genomic DNA, 1 mL (10 µM) M13 core primer, and 12 µL PCR nuclease free water. The amplification process was applied as a total of 35 cycles including denaturation at 94 °C for 40 s, extension at 51 °C for 40 s, elongation at 72 °C for 40 s and 1 cycle final elongation at 72 °C for 6 min. The obtained amplicons were electrophoresed in a 1.5% agarose gel at 60 V for 2 h and examined in a gel imaging system (Spektroline, GL-500).

### 2.5. Detection of virulence associated genes

The oligonucleotide sequences and references used for the detection of virulence associated genes by PCR are shown in Table 1. PCR mix consisted of 10 µL master mix, 1 µL genomic DNA, 1 µL (10 µM) of each primer specific for the genes and 12 µL PCR water. The PCR protocol was applied according to previous report [7,19]. The amplicons were electrophoresed in a 1.5% agarose gel at 80 V for 1.5 h and visualized in a gel imaging system (Spektroline, GL-500).

### 2.6. Statistical analysis

The relationship between the biotypes and genotypes of *P. multocida* capsular type A, virulence genes, health status of animals as well as isolation region were analysed by using Z ratio (2-sample proportion test) and chi-square test (Minitab, Demo ver 16-access to: 10.01.2018).

## 3. Results

### 3.1. Biotyping

All of the isolates were positive for glucose and negative for lysine decarboxylase, ornithine decarboxylase, H<sub>2</sub>S, O-nitrophenyl-beta-D-galactopyranoside (ONPG), urease, citrate, tryptophan deaminase (TDA), gelatine, malonate, inositol, adonitol, raffinose, and arginine tests. Some isolates were found to be variable in mannitol, xylose, nitrate, indole, Voges-Proskauer (VP), sorbitol, rhamnose, sucrose, lactose, arabinose, and salicin fermentation tests. Fifteen different biotypes were determined based on the biochemical properties of *P. multocida* isolates.

The differences between the isolates were mostly related to xylose and sucrose test results while mannitol, indole, sorbitol, nitrate, VP, lactose, arabinose, salicin and rhamnose test results were limited with a few isolates. Thus, 4 major biotypes (I, II, III, IV) generated

**Table 1.** Oligonucleotide sequences used for the detection of virulence related genes by PCR.

Gene	Oligonucleotide sequences (5' - 3')	Amplicon size (bp)	References
<b>Iron binding genes</b>			
<i>tbpA</i>	F: TTG GTT GGA AAC GGT AAA GC R: TAA CGT GTA CGG AAA AGC CC	728	[7]
<i>hgbA</i>	F: TGG CGG ATA GTC ATC AAG R: CCA AAG AAC CAC TAC CCA	420	[7]
<i>hgbB</i>	F: ACC GCG TTG GAA TTA TGA TTG R: CAT TGA GTA CGG CTT GAC AT	789	[7]
<i>exbD</i>	F: CGT TCT GAT TAC AGC CTC TT R: AAC GAA ATC TTG GAA ACT GG	247	[19]
<i>exbB</i>	F: TTG GCT TGT GAT TGA ACG C R: TGC AGG AAT GGC GAC TAA A	283	[19]
<b>Adhesins</b>			
<i>pfhA</i>	F: AGC TGA TCA AGT GGT GAA C R: TGG TAC ATT GGT GAA TGC TG	276	[7]
<i>ptfA</i>	F: TGT GGA ATT CAG CAT TTT AGT GTG TC R: TCA TGA ATT CTT ATG CGC AAA ATC CTG CTG G	488	[19]
<i>hsf-1</i>	F: TTG AGT CGG CTG TAG AGT TCG R: ACT CTT TAG CAG TGG GGA CAA CCT C	654	[19]
<i>hsf-2</i>	F: ACC GCA ACC ATG CTC TTA C R: TGA CTG ACA TCG GCG GTA C	433	[19]
<i>tadD</i>	F: TCT ACC CAT TCT CAG CAA GGC R: ATC ATT TCG GGC ATT CAC C	416	[19]
<b>Sialidase</b>			
<i>nanB</i>	F: GTCCTA TAA AGT GAC GCC GA R: ACA GCA AAG GAA GAC TGT CC	585	[7]
<i>nanH</i>	F: GAA TAT TTG GGC GGC AAC A R: TTC TCG CCC TGT CAT CAC T	361	[7]
<b>Hyaluronidase</b>			
<i>pmHAS</i>	F: TCA ATG TTT GCG ATA GTC CGT TAG R: TGG CGA ATG ATC GGT GAT AGA	430	[19]
<b>Superoxide dismutase</b>			
<i>sodA</i>	F: TAC CAG AAT TAG GCT ATG C R: GAA ACG GGT TGC TGC CGC T	365	[19]
<i>sodC</i>	F: AGT TAG TAG CGG GGT TGG CA R: TGG TGC TGG GTG ATC ATC ATG	236	[19]
<b>Toxin</b>			
<i>toxA</i>	F: CTT AGA TGA GCG ACA AGG TT R: GGA ATG CCA CAC CTC TAT A	866	[7]
<b>Outer membrane porin and protectins</b>			
<i>oma87</i>	F: ATG AAA AAA CTT TTA ATT GCG AGC R: TGA CTT GCG CAG TTG CAT AAC	949	[7]
<i>ompH</i>	F: CGC GTA TGA AGG TTT AGG T R: TTT AGA TTG TGC GTA GTC AAC	438	[19]

based on the positive or negative test results of xylose and sucrose were detected in 39 (76.47%) isolates. These major biotypes were also positive in mannitol, indole and sorbitol tests, but negative in nitrate, VP, lactose, arabinose, salicin, and rhamnose tests. Nine of the other 12 (23.53%) isolates forming 11 other biotypes were observed to be separated by negative reaction detected only in indole (V) or mannitol (VI, X) or positive reaction detected only in salicine (IX), nitrate (XII), rhamnose (XIV), or arabinose (XV), from the major biotypes. The other 3 isolates were separated from the major biotypes, by positive reaction detected in more than one test such as nitrate, VP, lactose, arabinose and salicin (VII) or arabinose and salicine

(VIII) or by negative reaction such as indol and sorbitol (XI). Thus, according to the xylose and sucrose test results, 24 of the isolates were xylose-sucrose positive (biotype I), 11 were xylose positive-sucrose negative (biotype II), 6 were xylose negative-sucrose positive (biotype III), 10 were xylose-sucrose negative (biotype IV). *P. multocida* subsp. *multocida* ATCC 43137 and *P. multocida* ATCC 12948 reference strains were determined to have a biotype I profile (Table 2).

### 3.2. Genotyping

Four different PCR-fingerprint profiles (genotypes) were determined in the examined isolates. Forty-six (90.2%) of the isolates had genotype I fingerprint profile while

**Table 2.** Distribution of *Pasteurella multocida* capsule Type A isolates\* from diseased and healthy cattle based on their biochemical activity, virulence associated genes (*ompH*) and genotypes\*\*.

Parameter	<i>P. multocida</i> capsule Type A biotypes														
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV	XV
Biochemical tests															
Xylose	+	+	-	-	+	-	+	+	+	+	+	-	-	+	+
Sucrose	+	-	+	-	+	+	+	+	+	+	+	-	-	-	+
Mannitol	+	+	+	+	+	-	+	+	+	+	-	+	+	+	+
Indole	+	+	+	+	-	+	+	+	+	+	-	+	-	+	+
Sorbitol	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+
Nitrate	-	-	-	-	-	-	+	-	-	-	-	+	-	-	-
VP	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
Lactose	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
Arabinose	-	-	-	-	-	-	+	+	-	-	-	-	-	-	+
Salicine	-	-	-	-	-	-	+	+	+	-	-	-	-	-	-
Rhamnose	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-
	Major biotypes					The remaining biotypes									
Number of isolates (51)	16+2***	10	5	8	2	1	1	1	1	1	1	1	1	1	1
Diseased (20)	<b>5</b>	<b>7</b>	<b>2</b>	<b>2</b>	<b>1</b>	0	0	0	0	0	0	0	<b>1</b>	<b>1</b>	<b>1</b>
Healthy (31)	11	3	3	6	1	1	1	1	1	1	1	1	0	0	0
Virulence gene															
<i>OmpH</i> (10)	2	<i>1;3</i>	0	4	0	0	0	0	0	0	0	0	0	0	0
Genotypes															
I (46)	<i>11;4</i>	<i>3;4</i>	<i>3;1</i>	<i>6;2</i>	<i>1;1<sup>a</sup></i>	<i>1</i>	<i>1</i>	<i>1</i>	<i>1</i>	<i>1</i>	<i>1</i>	<i>1</i>	<b>1</b>	<b>1</b>	<b>1</b>
II (3)		<b>3</b>													
III (1)	<b>1<sup>b</sup></b>														
IV (1)			<b>1</b>												

\* + = positive reaction; - = negative reaction

Number in brackets indicate the total numbers of isolates.

Number in bold indicate the number of isolates from diseased cattle.

Number in italic indicate the number of isolates from healthy cattle.

\*\*; different isolates from tracheal<sup>a</sup> and nasal<sup>b</sup> samples of the same diseased cattle.

\*\*\*; *P. multocida* subsp. *multocida* ATCC 43137 and *P. multocida* ATCC 12948.

genotype II, genotype III, and genotype IV fingerprint profile were detected only in other 3 (5.88 %), 1 (1.96%), and 1 (1.96%) isolates, respectively. Fingerprint band profile of approximately 1500, 1000, and 300 bp observed in genotype I isolates were determined in all genotypes whereas the isolates evaluated as genotype II, genotype III, and IV were detected to have additional bands of approximately 1800 bp, 450 bp, and 750 bp, respectively. *P. multocida* subsp. *multocida* ATCC 43137 strain and *P. multocida* ATCC 12948 strain had the band profile observed in genotype I whereas, an additional band of 900 bp was found in *P. multocida* ATCC 12948 strain (Figure).

### 3.3. Detection of virulence associated genes

*HgbA*, *exxB*, *exxD*, *ptfA*, *hsf-2*, *nanB*, *nanH*, *sodA*, *sodC*, and *oma87* genes were detected in all *P. multocida* Type A isolates and in both reference strains while *tbpA* gene was determined only in *P. multocida* Type A isolates. *PfhA*, *tadD*, and *pmHAS* genes were detected in both *P. multocida* capsular type A reference strain and the isolates. But, these genes could not be determined in *P. multocida* capsular type D reference strain. *HgbB*, *hsf-1*, and *toxA* genes determined in *P. multocida* capsular type D reference strain were not detected in both *P. multocida* capsular type A reference strain and isolates examined in this study. *OmpH* gene detected in both reference strains could be determined only in 10 (19.60%) *P. multocida* Type A isolates examined in this study (Table 3).

### 3.4. Relationship among biotypes, genotypes, and virulence associated genes of *Pasteurella multocida* and health status of cattle

*P. multocida* capsular type A strains generated as xylose positive/sucrose negative (biotype II) were found to be associated with respiratory diseases in cattle ( $P < 0.05$ ). However, the virulence associated *ompH* gene was observed to be associated with sucrose negative isolates ( $P < 0.01$ ), but not with disease cases ( $P > 0.05$ ). A similar association could not be found in xylose negative or positive *P. multocida* capsular type A isolates. When genotype II compared to genotype I, genotype II isolates were found higher rate in disease cases ( $P < 0.05$ ). Also, it was determined that there was a significant association among genotype II and *ompH* gene positivity ( $P < 0.05$ ). Because of the limited number, isolates which had genotype III and genotype IV profiles were not statistically evaluated (Table 4).

### 4. Discussion

Although *P. multocida* is widespread in a large number of animal species, there are a limited number of epidemiological studies on respiratory diseases caused by these bacteria in cattle. The presence of the bacterium in the upper respiratory tract is not sufficient for the diagnosis of the disease and currently it is discussed whether *P. multocida* is a primary factor or an opportunistic pathogen of the respiratory diseases of cattle [4,5].



**Figure.** Band profile obtained from *Pasteurella multocida* capsular Type A isolates by PCR-fingerprint using M13 core primer. M: 100 bp DNA ladder (Thermo Scientific, SM0321); 1: *Pasteurella multocida* subsp. *multocida* ATCC 43137, 2: *Pasteurella multocida* ATCC 12948, 3: Negative control, 4-7: Genotype I isolates, 8-10: Genotype II isolates, 11: Genotype III isolate, 12: Genotype IV isolate.

**Table 3.** Presence of virulence genes in *Pasteurella multocida* reference strains and type A isolates.

Genes	Reference strains		Number of isolates (%)
	Tip A*	Tip D**	
<i>Hgb A</i>	+	+	51 (100)
<i>Hgb B</i>	-	+	0 (100)
<i>Exb D</i>	+	+	51 (100)
<i>Exb B</i>	+	+	51 (100)
<i>Tbp A</i>	-	-	51 (100)
<i>Tox A</i>	-	+	0 (100)
<i>Pfh A</i>	+	-	49 (96.2)
<i>Ptf A</i>	+	+	51 (100)
<i>Hsf-1</i>	-	+	0 (100)
<i>Hsf-2</i>	+	+	51 (100)
<i>Tad D</i>	+	-	51 (100)
<i>PmHAS</i>	+	-	51 (100)
<i>Nan B</i>	+	+	51 (100)
<i>Nan H</i>	+	+	51 (100)
<i>Sod A</i>	+	+	51 (100)
<i>Sod C</i>	+	+	51 (100)
<i>OmpH</i>	+	+	10 (19.6)
<i>Oma 87</i>	+	+	51 (100)

+, positive isolates -; negative isolates

\*: *P. multocida* subsp. *multocida* ATCC 43137 \*\*: *P. multocida* ATCC 12948.

In recent years, few studies have been published to determine the possible difference between the pathogenic *P. multocida* strains associated with the disease cases and the commensal isolates [6–9]. In these studies, the relationship between different capsule type, biotype, genotype or virulence associated genes detected in *P. multocida* isolates and disease cases was emphasized. In this study, the association of disease cases with the virulence associated genes, phenotypes and genotypes of *P. multocida* capsule type A strains obtained from lower or upper respiratory tract of cattle were investigated.

In several studies investigating the biochemical properties of *P. multocida* isolates from healthy and/or sick animals, it was reported that indole test results might vary [9,12,20–22]. In a study on the aetiology of respiratory diseases in calves, 75% of the isolates were reported to be indole negative and it was emphasized that the relationship of the indole negative *P. multocida* isolates with the disease cases in calves should be clarified [6]. In this study, indole negative isolates were found to be low (7.84%) and this

**Table 4.** Association between *Pasteurella multocida* capsule Type A isolates from diseased and healthy cattle and their biochemical activity, virulence associated genes (*ompH*) and genotypes.

Parameter	<i>Pasteurella multocida</i> Type A major biotypes			
	I	II	III	IV
Biochemical tests				
Xylose	+	+	-	-
Sucrose	+	-	+	-
Number of isolates (51)	24	11	6	10
Diseased (20)	<b>7<sup>a</sup></b>	<b>8<sup>a</sup></b>	<b>2</b>	<b>3</b>
Healthy (31)	17	3	4	7
Virulence gene				
<i>OmpH</i> (10)	<b>2<sup>b,c</sup></b>	<b>1;3<sup>c</sup></b>	0	<b>4<sup>c</sup></b>
Genotypes				
I (46)	<b>17;6<sup>d</sup></b>	<b>3;5<sup>d</sup></b>	<b>4;1<sup>d</sup></b>	<b>7;3<sup>d</sup></b>
II (3)		<b>3<sup>d</sup></b>		
III (1)	<b>1</b>			
IV (1)			<b>1</b>	

+ = positive reaction; - = negative reaction.

Number in brackets indicate the total numbers of isolates.

Number in bold indicate the number of isolates from diseased cattle.

Number in italic indicate the number of isolates from healthy cattle.

<sup>a</sup>; Significance of association (Z-ratio) between biotype II isolates and disease status of animals (P < 0.05).

<sup>b</sup>; Significance of association (X<sup>2</sup>) between the sucrose negative isolates and *ompH* positive isolates (P < 0.01).

<sup>c</sup>; Significance of association (Z-ratio) between the *ompH* positive isolates and disease status of animals (P > 0.05).

<sup>d</sup>; Significance of association (X<sup>2</sup>) between genotype II isolates and disease status of animals (P < 0.05).

phenotypic feature was not found to be related to health status of animals.

Another study reported that *P. multocida* isolates were sucrose positive, but xylose test results were to be variable [21]. Similarly, Sellyei et al. [14] and Verma et al. [9] reported that different results can be obtained in xylose and sucrose fermentation of *P. multocida* isolates, but in each study it was not found any association of xylose and/or sucrose test results with the health status of cattle. In contrast, in this study, it was determined that 41.17% of *P. multocida* Type A isolates were sucrose negative and sucrose negative isolates were also found to be related with the disease cases.

PCR-fingerprint method was frequently used in genotyping of *P. multocida* isolates from rabbits, poultry and pigs [23–28]. However, there are few studies on bovine isolates [14,15,29].

Gerardo et al. [15] found that PCR-fingerprint profiles of *P. multocida* subsp. *septica* and *P. multocida* subsp. *multocida* strains were associated with  $\alpha$ -glycosidase activity, however, Selley et al. [14] reported that PCR-fingerprint profiles of *P. multocida* capsule type A and D isolates from pneumonic bovine lungs were associated with trehalose fermentation and  $\alpha$ -glycosidase activity. In another study, 13 different PCR-fingerprint profiles were determined in 41 *P. multocida* isolates from diseased calves, but PCR-fingerprint profiles were not reported to be related to any phenotypic feature or disease cases [29]. In this study, 4 genotypes were found in *P. multocida* isolates and genotype II isolates were found to be associated with disease cases. Similarly, xylose positive/sucrose negative (biotype II) isolates were also found to be associated with disease cases. Additionally, it was determined that 23 (50%), 8 (17.4%), 5 (10.86%), and 10 (21.73%) of 46 genotype I isolates were biotype I, biotype II, biotype III, and biotype IV, respectively. Genotype II profile was detected in only 3 isolates which were biotype II and related to disease cases. However, no other study was found in which reported that xylose positive/sucrose negative *P. multocida* isolates were associated with the genotype profile.

To date, many studies have been conducted to investigate the presence of virulence associated genes in *P. multocida* isolates [7–9,16,30–32]. However, there are a limited number of studies evaluating the relationship between virulence genes and the health status of animals [7–9].

Ewers et al. [7] reported that *pfhA*, *hgbB*, and *tpbA* genes detected in *P. multocida* isolates from cattle and the *toxA* gene from pigs were related to disease cases. Similarly, Katsuda et al. [8] also stated that these genes could be used to differentiate pathogen and commensal *P. multocida* strains in cattle. In another study, it was reported that *ptfA* gene was higher in *P. multocida* isolates obtained from infected cattle than in isolates from healthy ones and this

gene could be used as epidemiological marker in disease cases [9]. Unlike other studies, in this study, *tbpA*, *ptfA*, and *pfhA* genes were detected in all or in the majority of *P. multocida* capsular type A isolates, whereas *hgbB* and *toxA* genes were not determined. However, *ompH* gene detected in 19.60% of the isolates was found to be related to xylose positive/sucrose negative (biotype II) *P. multocida* capsular type A strains, but *ompH* gene alone was not associated with disease cases.

However, in the other studies [16,19,30–32] it has been observed that virulence associated genes reported in *P. multocida* isolates were detected in different ratios. The variability observed in the presence of virulence associated genes might be due to the use of *P. multocida* isolates with different capsule types obtained from different hosts.

As a result, it was concluded that xylose positivity/sucrose negativity (biotype II) and genotype II detected in *P. multocida* capsular type A isolates might be epidemiological criteria that can be used for the identification of commensal and pathogenic *P. multocida* capsular type A isolates. However, considering the reports of other researchers, the findings from this study showed that it is necessary to investigate the phenotypic and genotypic properties of *P. multocida* strains by using different capsule types which would be obtained from different host species in future studies.

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#### Conflict of Interest

The authors declare that there is no conflict of interest.

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