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Typing of *Mannheimia haemolytica* Isolated From Respiratory Tract and Investigation of Virulence Genes

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Mannheimia hamolytica, Typing, Virulence genes

Abstract: This study aimed to determine the biochemical properties and distribution of important virulence genes of *Mannheimia haemolytica* isolates from the respiratory tracts of diseased and healthy cattles and sheeps. Forty-eight *Mannheimia haemolytica* isolates from naso-pharyngeal and trachea-bronchial swaps were identified by Real Time-PCR. According to the differences in arginine and sorbitol tests, four different biochemical profiles were determined in the isolates examined. Three virulence gene profiles were detected in the isolates examined by Real-Time PCR. 37.5%, 33.3%, and 12.5% of the isolates examined were identified as profiles I, III and II, respectively. At the same time, it was determined that biochemical profile II was associated with disease cases and this was related to arginine negativity. In addition, it was determined that isolates with virulence gene profile I were associated only with the biochemical profile I and that this was due to arginine negativity. In contrast, the relationship between arginine-positive isolates and virulence gene profile III was found to be significant. As a result; arginine negativity and *gcp*, *gs60*, *tbp*B, *lkt*C, *adh* positive, *nma*A negative isolates may be the epidemiological criteria that can be used to differentiate commensal and pathogen *Mannheimia haemolytica* isolates and new studies on the subject should be done.

Solunum Yollarından İzole Edilen *Mannheimia haemolytica* İzolatlarının Tiplendirilmesi ve Virulens Genlerinin Araştırılması

Anahtar Kelimeler
Mannheimia hamolytica, Tiplendirme, Virülens genler

Öz: Bu çalışmada, hasta/sağlıklı sığır ve koyunların solunum yollarından izole edilen *Mannheimia haemolytica* izolatlarının biyokimyasal özellikleri, önemli virülens genlerinin dağılımı tespit edilmesi amaçlandı. Nazo-farengiyal ve trake-bronşiyal svap örneklerinden izole edilen ve Real-Time PCR ile identifiye edilen 48 (%87.3) adet *Mannheimia haemolytica* izolatı kullanıldı. İncelenen izolatlarda, hastalık ve virülens genleri ile ilişkili biyokimyasal özelliklerin arginin ve sorbitol testlerindeki farklılıklara göre 4 farklı biyokimyasal profil belirlendi. Real Time-PCR yöntemiyle virülens genleri incelenen izolatlarda, %37.5'i profil I, %33.3'ü profil III ve %12.5'i profil II olmak üzere 3 farklı virülens gen profili tespit edildi. Bununla birlikte biyokimyasal profil II'nin hastalık olguları ile ilişkili ve bunun arginin negatiflik ile ilişkili olduğu belirlendi. Ayrıca virülens gen profil I özelliğine sahip izolatların sadece biyokimyasal profil I ile ilişkili olduğu ve bunun arginin negatiflikten kaynaklandığı belirlenirken, arginin pozitif izolatlar ile virülens gen profil III arasındaki ilişkinin önemli olduğu gözlendi. Sonuç olarak; arginin negatiflik ile *gcp*, *gs60*, *tbp*B, *lkt*C, *adh* pozitif, *nma*A negatif izolatların kommensal ve patojen *Mannheimia haemolytica* izolatlarının ayrımında kullanılabilecek epidemiyolojik kriter olabileceği ve konu ile ilgili yeni çalışmaların yapılması gerektiği düşünülmektedir.

1. INTRODUCTION

The importance of animal husbandry in terms of both economy and health has brought animal health to the agenda [1, 2]. According to the Global Animal Medicine Association [3], one out of every five farm animals dies around the world every year due to completely preventable diseases and approximately 20 billion

dollars are lost [4, 5, 6]. Researchers have reported that pneumonic pasteurellosis is responsible for 50% of small ruminant deaths and 30% of cattle deaths [7, 8]. The effect of the disease on the global economy has been recognized all over the world, and it has been reported that it causes an annual economic loss of approximately 1 billion dollars in some countries [9, 6]. In bacterial investigations performed in pneumonia cases in cattle and sheep, *Mannheimia haemolytica (M. haemolytica), Pasteurella multocida (P. multocida), Mycoplasma* spp., *Histophilus somni*, *Trueperella pyogenes*, *Streptococcus* spp. and *Staphylococcus* spp. are the primary and secondary factors in the formation of the disease. In addition, viruses and parasites are other infectious agents that cause pneumonia [10, 11, 12, 13, 14]. Pneumonia caused by *M. haemolytica* is characterized by acute cranio-ventral fibrinous pneumonia and fibrinopurulent pleura pneumonia [15]. Although *M. haemolytica* is found as a primary and secondary bacterial agent in respiratory tract infections due to its commensal presence in the respiratory tract flora of various animal species, there are not enough epidemiological studies especially in Turkey. Whether there is a difference between commensal and pathogenic *M. haemolytica* and the mechanism by which the strains in the flora become pathogenic in disease cases are still the subjects of interest to researchers. [16, 17, 18]. *M. haemolytica* is included in Pasteurellaceae family, *Mannheimia* genus [19, 20, 21, 22, 23, 24]. Twelve different serotypes were detected in the serotyping performed according to the capsular surface antigens of the agent [25, 26]. *M. haemolytica* is a Gram-negative, non-motile, bipolar stainable, coccobacilli-shaped encapsulated bacterium [24, 27). It has many virulence factors that allow *M. haemolytica* to evade host defense and colonize and play an important role in the pathogenesis of the disease. Adhesins, capsular polysaccharide, outer membrane proteins, lipopolysaccharide, neurominidase, leukotoxin, lipoprotein, sialoglycoprotease, serotype-specific protein and transferrin binding proteins detected in studies among these factors have been reported by many researchers [28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39]. Outside of conventional bacteriological methods for the identification of *M. haemolytica* isolates, PCR methods using 12S rRNA and 16S rRNA gene regions and specific primers for different genes related to the virulence of the agent have also been frequently used [39, 40, 41, 42, 43, 44, 45, 46, 47].

This study investigated the biochemical properties and virulence-related genes of *M. haemolytica* isolates obtained from the respiratory tract of healthy and diseased cattle and sheep. It also evaluated possible relationships between the isolates' phenotypic and genotypic characteristics and the animals' disease status.

2. MATERIAL AND METHOD

In this study, 650 swab samples taken from the nasopharyngeal (NF) and tracheobronchial (TB) regions of 157 (157 NF and 138 TB) cattle and 182 sheep (186 NF and 173 TB) slaughtered, and diagnosed with pneumonia in veterinary clinics in Van province between 2018-2019 were examined (Table 3).

Forty-eight *M. haemolytica* strains examined in the study were isolated and identified from the swab samples. *M. haemolytica* reference strain were supplied by Prof. Dr. Arzu Fındık from Ondokuz Mayıs University Faculty of Veterinary Medicine Department of Microbiology.

In the study, swab samples were placed in Stuart transport medium and delivered to the laboratory of the Microbiology Department of the Faculty of Veterinary Medicine of Van Yüzüncü Yıl University in a short time [48].

The study was approved by the Animal Ethics Committee of Van Yuzuncu Yil University (approval date and no: 06/03/2018, YUHADYEK-17310).

2.1. Isolation and Identification of *M. haemolytica* Isolates

Swab samples taken from cattle and sheep were cultured on 7% blood agar for bacteriological culture and incubated at 37°C for 24-72 hours under aerobic conditions. Mucoid and haemolytic, 1-2 mm in diameter, greyish, transparent, smooth, round bacterial colonies were selected and stored at -20°C until identification [23, 49, 50]

In the preliminary identification of the isolates examined, Gram-negative small bacilli, oxidase and catalase positive bacterial isolates that formed acid (yellow colour) at the bottom of TSI agar, could and/or could not grow on MacConkey agar and were immobile on SIM medium were accepted as suspected *M. haemolytica* in preliminary identification [51]. BD™ Pheonix automated bacterial identification system (Becton Dickinson, USA) was used for identification and biochemical characteristics of suspected isolates, BD Pheonix identification panel (BD Phoenix™ NMIC-308, USA) containing 45 different biochemical tests was used [52].

2.2. Identification by Real Time-PCR

DNA isolation: According to the recommended protocol, the genomic DNA of suspected *M. haemolytica* isolates was obtained using a commercial bacterial DNA isolation kit (Cat≠ GF-BA-100, Vivantis, Malaysia).

Real Time-PCR: Identification of *M. haemolytica* at species level was performed according to the optimised protocol of Hawari et al. [53] with minor modifications. In Real Time-PCR, commercial SYBR Green qPCR Master Mix (Amplicon (2X), 5000830, Denmark) was used according to the recommended protocol. To prepare the PCR mixture, 12 µl of Master Mix, 2 µl of genomic DNA and 1 µl of each primer (10 mM) (Table 1) were added and the mixture was completed to 25 µl with PCR grade water. The PCR mixture was pre-denaturated in a thermal cycler (Qiagen, Corbet Rotor Gene, 6000, USA) at 94°C for 5 minutes. Then, in the amplification process

including 35 cycles; denaturation at 94°C for 1 min; annealing at 56°C for 45 s; elongation at 72°C for 1 min and final elongation at 72°C for 5 min protocol was applied and the dynamic sigmoidal curves obtained in the software of the Real Time-PCR device were evaluated. For the confirmation of PCR products obtained at the amplification stage, electrophoresis (Owl Easycast, B1-BP, USA) was performed in 1% agarose gel (1xTAE solution) for one hour and then visualised in the imaging system (ImageSCI, GL 5000, USA). The detected amplicons were compared with a 100 bp DNA ladder (VC 100 bp plus, Vivantis, Malaysia) and samples with a 325 bp band were considered positive for *M. haemolytica*. Reference *M. haemolytica* strain was used as positive control, and DNA/RNA free PCR grade water was used as negative control.

Table 1. *M. haemolytica* specific oligonucleotide sequence [53].

Gene		Primer Sequence (5'-3')	Amplicon (bp)
PHSSA	**F**	TTCACATCTTCATCCTC	325
	R	TTTTCATCCTCTTCGTC	

2. Detection of Virulence Related Genes of *M. hemolytica*

In the study, O-sialoglycoprotease (*gcp*), outer membrane lipoprotein (*gs*60), transferrin binding protein (*tbp*B), leukotoxin (*lkt*C), UDP-N-acetyl-D-glucosamine-2-epimerase (which is associated with virulence in *M. haemolytica* isolates) *nma*A) and adhesion (*adh*) genes were investigated by Real Time-PCR. The oligonucleotide sequences and binding temperatures used for each virulence-related gene are given in Table 2 [54].

Table 2. Oligonucleotide sequences and binding temperatures of virulence related genes [54].

Gen		Primer Sequence (5'-3')	Annealing (°C)/Cycles
gcp	**F**	CGCCCCTTTTGGTTTTCTAA	54/35
	R	GTAAATGCCCTTCCATATGG	
*gs*60	**F**	GCACATTATATTCTATTGAG	50/40
	R	AGGCATACTCTAACTTTTGC	
*tbp*B	**F**	CTACTTGCTGCTTGTTCCTC	56/35
	R	AGAACCGCTTACTGTACGTC	
*lkt*C	**F**	GGAAACATTACTTGGCTATGG	54/40
	R	TGTTGCCAGCTCTTCTTGATA	
*nma*A	**F**	CTGTAGAAGCCGGAACAGTA	56/35
	R	CATCGCCATAAGGGTTGTGA	
adh	**F**	CTGCAAGTAAGGCAACATTG	54/35
	R	GAATCCGCACCAATAGCAAT	

Commercial SYBR Green qPCR Master Mix (Ampliqon, 5000830, Denmark) was used for Real Time PCR for Real-Time PCR for virulence-related genes. After optimization of the PCR mix, 2 µl of genomic DNA and 1 µl of each primer (10 mM) (Table 2) were added into 10 µl of Master Mix, and the mixture was completed to 20 µl with PCR grade water. The mixture was incubated for 5 min at 95 °C for preliminary denaturation in a Thermal cycler (Qiagen, Corbet Rotor Gene, 6000, USA). Denaturation at 94°C for 30 s, binding for 60 s at the temperatures specified for each gene in Table 2, elongation at 72°C for 60 s, and finally final elongation at 72°C for 8 min were applied. The protocol was applied as 35 amplification cycles for adh, *gcp*, *nma*A and *tbp*B genes and 40 cycles for *gs*60 and *lkt*C genes. Sigmoidal curves obtained dynamically in the software of the Real Time-PCR device were evaluated as positive.

2.3. Statistical Analysis

The SPSS (2013) package program was used to determine the degree of affinity of *M. haemolytica* isolates according to their biochemical properties by dendogram analysis. The Z ratio test (Two-Sample proportion test) and Chi-square test methods were used by using the Minitab (Demo version 15-Access 15.11.2019) statistical program to determine the correlation between the biochemical properties of isolates and genes related to disease and virulence [55].

3. RESULTS

3.1. Identification with Real-Time PCR

After preliminary identification for 650 swabs samples, 48 isolates were identified as *M. haemolytica* by Real Time-PCR using primers specific to the *M. haemolytica* gene (Figure 1, Figure 2).

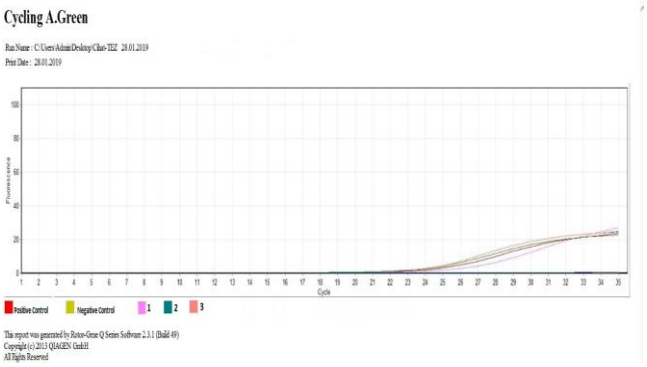

Figure 1. Positive amplification results from *M. haemolytica* isolates analyzed by Real Time-PCR.

Of the 48 isolates identified as *M. haemolytica* in the study, 44 (91.6%) were obtained from sheep and 4 (8.4%) from cattle (Table 3).

Table 3. Distribution of *M. haemolytica* isolates according to animal species and health/disease conditions.

Animal Species	Material Source	Number of Animals	Number of Isolates		Total Number of Isolates	Isolation Rate (%)
			NF	TB		
Cattle	Slaughterhouse/Healthy	133	–	–		
Cattle	Slaughterhouse/Diseased	5	–	1	4	2.54
Cattle	Field/Diseased	19	3	–		
Sheep	Slaughterhouse/ Healthy	133	19	–		
Sheep	Slaughterhouse/Diseased	40*	11	10	44	24.17
Sheep	Field/Diseased	9	4	–		
Total		**339**	**37**	**11**	**48**	**26.71**

*: Six different *M. haemolytica* were isolated from the NF (Naso-Pharyngial) and TB (Trache-Bronchial) regions of three patient sheep.
–: *M. haemolytica* was not isolated.

3.2. Distribution of Biochemical Properties

Biochemical characteristics of 48 isolates identified as *M. haemolytica* by Real Time-PCR were determined by the BD Pheonix™ automated bacterial identification system using a test panel containing 45 biochemical tests. According to the differences in arginine and sorbitol tests, four different biochemical profiles were determined in the isolates examined (Table 4).

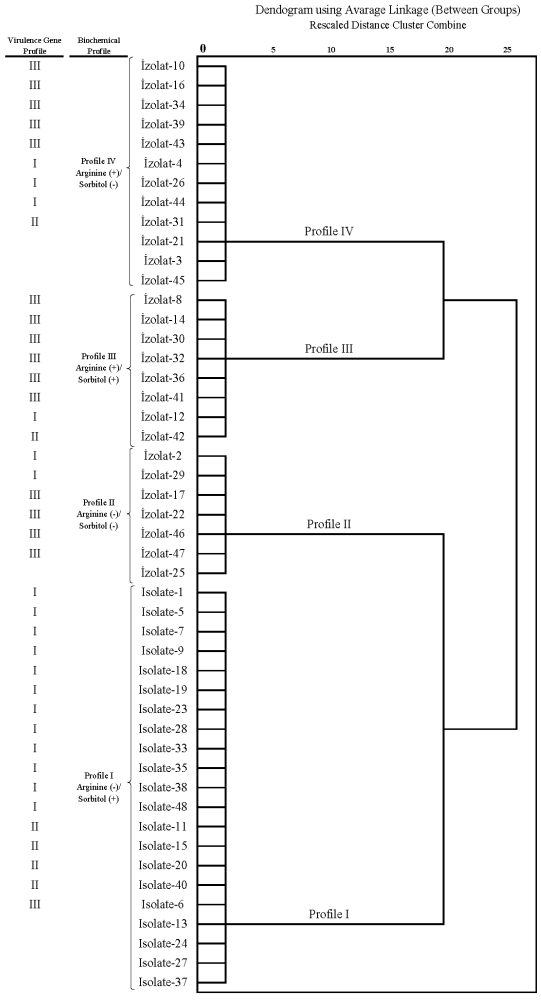

Figure 2. Dendrogram analysis of biochemical profiles determined according to arginine and sorbitol test results and virulence-related gene profiles, and the distribution of antimicrobial resistance genes in *M. haemolytica* isolates.

3.3. Identifying virulence associated genes

Of the isolates analysed by Real Time-PCR, 95.8% were positive for *gcp*, 87.5% for *gs*60, 58.3% for *tbp*B, 93.8% for *Ikt*C, 16.7% for *nma*A and 91.7% for *adh* genes (Table 4).

3.4. Determining Gene Profiles Associated with Virulence

It was determined that different gene profiles were found according to the distribution of virulence-related genes in the isolates. Isolates with three or more virulence-associated genes were considered as a separate profile, while isolates with less than three virulence-associated genes were not considered as virulence gene profiles. Accordingly, three different gene profiles were detected in the isolates (Table 4).

3.5. Distribution of Virulence-Related Gene Profiles in the Main Biochemical Profiles Determined in *M. haemolytica* Isolates

Biochemical profile I, which was determined in 17 (42.5%) of the *M. haemolytica* isolates, was determined in 12 (66.66%), 4 (66.66%) and 1 (6.25%) of virulence-related gene profiles I, II, and III respectively. Biochemical profile II, which was determined in 6 (15%) of the isolates, was detected in 2 (11.11%) of 18 virulence gene profile I and 4 (25%) of 16 virulence gene profile III. Biochemical profile III, which was determined in 8 (20%) of the *M. haemolytica* isolates, was determined in 1 (5.55%), 1 (16.66%) and 6 (37.5%) of virulence-related gene profiles I, II, and III respectively. Biochemical profile IV, which was determined in 9 (22.5%) of the isolates, was detected in 3 (16.66%) of 18 virulence gene profile I, 1 (16.66%) of 6 virulence gene profile II and 5 (31.25%) of 16 virulence gene profile III (Table 4). Isolates belonging to profiles IV, V, VI, VII, VIII and IX are presented in Table 4, but they were not evaluated because of their poor correlation with biochemical profiles.

Table 4. Distribution of virulence-related gene profiles detected in *M. haemolytica* isolates in major biochemical profiles.

Virulence-Related Gene Profile								Biochemical Profile (%)			
								I	II	III	IV
Profile	***gcp***	***gs60***	***tbpB***	***lktC***	***nmaA***	***adh***	**n**	**Arg – Sor +**	**Arg – Sor –**	**Arg+ Sor+**	**Arg+ Sor –**
I	+	+	+	+	-	+	**18**	12 (66.66)	2 (11.11)	1 (5.55)	3 (16.66)
II	+	+	+	+	+	+	**6**	4 (66.66)	-	1 (16.66)	1 (16.66)
III	+	+	-	+	-	+	**16**	1 (6.25)	4 (25)	6 (37.5)	5 (31.25)
IV	+	-	+	+	-	+	**2**	1	1	-	-
V	+	-	-	-	+	-	**1**	-	-	-	1
VI	+	-	-	-	-	-	**1**	-	-	-	1
VII	-	-	-	-	-	-	**1**	1	-	-	-
VIII	-	+	-	+	-	+	**2**	1	-	-	-
IX	+	+	+	+	-	-	**1**	1	-	-	1
Total							**48**	**21**	**7**	**8**	**12**

Arg: Arginine, **Sor:** Sorbitol, **Profiles IV, V, VI, VII, VIII, IX:** Isolates that were not evaluated as virulence gene profile.

3.6. Relation of biochemical profiles to virulence-related gene profile and animal health status

In the evaluation made according to the statistical analysis of the obtained data; the isolates with biochemical profile I were associated only with virulence gene profile I ($p<0.01$), while biochemical profile II was associated with isolates obtained from disease cases ($p<0.01$) (Table 5), and this was determined that it was caused by only arginine negativity ($p<0.05$) (Table 5).

On the other hand, it was determined that isolates with virulence gene III profile were not associated with biochemical profile I ($p>0.05$), but the relationship between arginine positive isolates and virulence gene profile III was found as significant ($p<0.01$) (Table 5).

Also, it was determined that sorbitol positive or negative isolates were not associated with disease cases ($p>0.05$). In addition, no correlation was found between sorbitol positive or negative isolates and virulence gene profiles (Table 5). In contrast virulence gene profile II was not associated with biochemical profiles and disease cases ($p>0.05$).

Table 5. The relationship of arginine positive/negative *M. haemolytica* isolates with health situations and virulence factors.

	Arginine(+) n=20	**Arginine (-) n=28**	**Sorbitol (+) n=29**	**Sorbitol (-) n=19**
Health situation				
Diseased (%)	9 (45)	20 (71)[a]	17 (59)	12 (63)
Healthy (%)	11(55)	8 (29)	12 (41)	7 (37)
Virulence gene profile II				
Pozitive (%)	4 (25)	14 (50)[b]	13 (45)	5 (26)
Negative (%)	16 (75)	14 (50)	16 (55)	14 (74)
Virulence gene profile V				
Pozitive (%)	11 (55)[c]	5 (18)	7 (24)	9 (47)
Negative (%)	9 (45)	23 (82)	22 (76)	10 (53)

a; The relationship between arginine negative isolates and disease cases ($p<0.05$), **b;** The relationship between arginine negative isolates and virulence gene profile I ($p<0.05$), **c;** The relationship between arginine positive isolates and virulence gene profile III ($p<0.01$).

4. DISCUSSION AND CONCLUSION

This study investigated the biochemical properties and virulence-related genes of *M. haemolytica* isolates obtained from the respiratory tract of healthy/diseased cattle and sheep. In addition, possible relationships between the isolates' phenotypic and genotypic characteristics and the animals' disease status were evaluated.

A study conducted in Antakya reported that *M. haemolytica* was not isolated from 122 bovine lung samples [56]. In a similar study conducted in Kars and its surroundings, it was reported that *M. haemolytica* was isolated from 61 (26.4%) of the lung samples taken from a total of 231 cattle and sheep examined [57]. Önat et al. [50] reported that *M. haemolytica* was isolated from 5 (10.6%) of bilateral nasal swab samples taken from 47 cattle. Noyes et al. (58) *M. haemolytica* was isolated from 1596 (29%) of the naso-pharyngeal swab samples taken from 5498 cattle with respiratory tract infection symptoms. Castillo et al. [59] reported that they isolated *M. haemolytica* from 70 (79.5%) of the nasal swab samples taken from 88 cattle with respiratory tract infection. Demissie et al. [60] reported that *M. haemolytica* was isolated from 57 (34.1%) of 167 sheep lung samples with macroscopic pneumonia lesions. In studies conducted in some other regions (Aydın and Urfa) [61, 62], Kırkan et al. (61) reported that they isolated *M. haemolytica* from 22 (11%) of 200 sheep lung samples with pneumonia lesions, and Tel and Keskin [62] reported that they isolated *M. haemolytica* from 30 (12.5%) of 240 sheep lung samples.

In this study, four (2.54%) of the *M. haemolytica* isolates were obtained from cattle, while fourty four were isolated from diseased and healthy sheep (Table 3). While the *M. haemolytica* isolation rate obtained from cattle was determined to be higher than the rates reported by Ülker et al. [56], it was higher than the isolation rates reported by Gürbüz [57], Önat et al. [50], Noyes et al. [58] and Castillo et al. [59]. was found to be low. However, the isolation rate obtained from sheep was found to be higher than the isolation rates reported by

Gürbüz [57] and Demissie et al. (2014) and Kırkan and Kaya [61], Tel and Keskin [62]. It was thought that the differences observed in the studies were due to the differences in cultivation methods, climate and barn conditions from region to region.

Biochemical properties of *M. haemolytica* isolates show great variability. Bisgaard [63] reported that indole, MR, VP, arabinose, and esculin test results were negative in *M. haemolytica* strains isolated from the tracheal cavity of cattle, while lactose fermentation showed variability. Daphal et al. [64] also reported that indole, MR-VP and citrate test results of 7 *M. haemolytica* isolates obtained from small ruminants were negative. Angen et al. [65] reported that indole, sorbitol and MacConkey agar growth test results of 246 trehalose negative *M. haemolytica* isolates isolated from cattle and sheep showed variability. In the study in which ten different biogroups were determined among the isolates, it was reported that growth on MacConkey agar was variable in 5 biogroups and isolates in the other five biogroups did not grow on MacConkey agar. In a study conducted in Baghdad [45], it was reported that all 5 *M. haemolytica* isolates, 3 of which were identified from lung tissue and 2 of which were identified from the nasal cavity, were positive for rhamnose, indole, nitrate and negative for gelatinase, urea, inositol, xylose, mannitol, arabinose, maltose with commercial identification kit. Taunde et al. [66] found that 16 (15%) of 40 *M. haemolytica* isolates obtained from goats with severe respiratory infection symptoms were β-haemolytic, 34 (85%) of them were non-haemolytic, all of the isolates were esculin positive, ONPG negative and did not grow on MacConkey agar (atypical strain). The researchers reported that *M. haemolytica* case isolates may have atypical characteristics under unfavourable environmental conditions. Gürbüz [57] reported that all *M. haemolytica* isolates (61 isolates) obtained from cattle and sheep were indole negative, and reactions to arabinose, xylose, salicin, and trehalose were variable. In a study conducted by İlhan and Keleş [67] in Van region, it was reported that 57 (86.3%) of 66 (11.3%) *M. haemolytica* isolates isolated from lung samples of sheep slaughtered in slaughterhouse were identified as biotype A and 9 (13.6%) as biotype T according to arabinose, xylose and trehalose fermentation test results. Another study [68] determined that all 16 *M. haemolytica* isolates obtained from sheep were trehalose negative, arabinose and xylose positive, and 13 (81.3%) were lactose positive. According to the results obtained, the researchers reported that all of the isolates were defined as biotype A (xylose and arabinose positive). The present study determined that all 48 *M. haemolytica* isolates were β haemolytic on blood agar but did not grow on MacConkey agar and were indole negative. However, among the biochemical tests in the commercial kits used to identify the isolates, it was determined that the isolates had four different biochemical profiles according to arginine-arginine and sorbitol fermentation properties. It was observed that biochemical profile II was associated with the isolates obtained from disease cases and this relationship was observed only in arginine negative isolates. It is thought that biochemical characteristics may differ among *M. haemolytica* case isolates and this may be caused by unfavourable environmental conditions, host differences and genetic transmission among Gram negative bacteria.

In a study on the detection of the leukotoxin (*lkt*A) gene in 248 *M. haemolytica* and *Biberstenia trehalosi* isolates obtained from different animal groups [69], the leukotoxin gene was detected in 108 of the isolates. The study, it was reported that leukotoxin was the major virulence factor in the formation of pneumonic pasteurellosis, and other virulence factors played a role in inducing factors in the colonization of the agent and disease formation. In a similar study conducted in our country [70], the *lkt*A gene was detected in all 22 *M. haemolytica* and 2 *Biberstenia trehalosi* isolates isolated from sheep with pneumonic lesions in their lungs, and it was reported that the leukotoxin gene is the most important virulence factor in cases of pneumonic pasteurellosis. On the other hand, it was reported that 7 (13%) of 54 *M. haemolytica* isolates obtained from lung samples taken from calves with respiratory tract infection in Siberia and Kazakhstan did not contain the leukotoxin gene [27]. In an experimental study conducted on *M. haemolytica* isolates obtained from 29 calves infected with *M. haemolytica* [32], it was reported that lkt, *gs*60, adh and *gcp* genes were expressed in the lung tissue in all isolates. In contrast, tbp and nma genes were not detected. The study reported that the most important virulence factor in *M. haemolytica* isolates was leukotoxin, and the failure to detect nma and tbp genes may be related to the infection stage of the disease. El Dokmak et al. [71] conducted another study on the virulence-related genes of 7 *M. haemolytica* isolates isolated from cattle and sheep, it was reported that *gcp* and *gs*60 genes were detected in all of the isolates. In another study in which 16 genes related to virulence were examined in 121 *M. haemolytica* isolates obtained from sheep [72], *lkt*A, *tbp*A, *tbp*B and *ton*B genes were detected in all isolates, while *adh* (97.5%), *fha*C (%). 94.2), *gcp* (79.3%), *hf* (79%), *irp* (59.5%), *lps*A (65%), *nan*H (99.2%), *pil*A (95.8%), *plp*D (95.8%), *pom*A (97.6%), *sod*A (91.7%) and *sod*C (19%) genes have been reported to be present in different proportions. In the study, it was reported that only the irp gene was found to be associated with the disease in isolates obtained from sheep with pneumonia, and it was concluded that the distribution of virulence-related genes was homogeneous between *M. haemolytica* isolates isolated from diseased and healthy animals.

In this study, gcp genes were detected in 46 (95.8%), *gs*60 in 42 (87.5%), *tbp*B in 28 (58.3%), lktC in 45 (93.8%), *nma*A in 8 (16.7%) and *adh* in 44 (91.7%) of 48 *M. haemolytica* isolates obtained from the respiratory tract of cattle and sheep. The number of leukotoxin gene positive isolates detected in the study was higher than reported by Fisher et al. [69] and Nefedchenko et al. [27] and lower than reported by Kırkan [70], Lo et al. [32] and Garcia-Alvarez et al. [72]. It was concluded that the detection of leucotoxin genes at different rates may be due to the presence of more than one gene region encoding leucotoxin, and the presence of genes may also

vary according to regional conditions and animal species. The number of positive isolates in the direction of *nma*, iron binding protein *tbp*, *adh* gene was higher than reported by Lo et al. [32] and Garcia-Alvarez et al. [72]. The number of *gcp* and *gs*60 gene positive isolates was lower than that found by Lo et al. [32] and higher than that found by El Dokmak et al. [71]. In the present study, it was thought that the high rate (95.8% and 87.5%) of *gcp* and *gs*60 genes in the field isolates of *M. haemolytica* may differ from strain to strain in terms of the need for synthesis against immune response in adverse environmental conditions and in vivo environments.

In this study, it was concluded that the heterogeneity in *tbp*B genes was high in *M. haemolyitca* isolates and since it could be detected in isolates obtained from disease cases, it was thought that it could be a criterion that could be used to distinguish between pathogenic and commensal *M. haemolytica* isolates. *nma*A gene was not detected in the majority of isolates (91.7%), and it is thought that there may be other gene regions encoding the capsule gene. However, different gene profiles were determined according to the distribution of virulence-related genes in the isolates. While all virulence-related genes were found in all isolates with profile II, nmaA was not detected in isolates with profile I, and *nma*A and *tbp*B genes were not detected in isolates with profile III. Virulence gene profile I (37.5%), III (33.3%) and II (12.5%) were detected in 83.3% of the isolates. Isolates with virulence gene profile I were associated with arginine-arginine negative isolates, and isolates with virulence gene profile III were associated with arginine-arginine positive isolates. In general, virulence genes in *M. haemolytica* isolates have heterogeneity and one of the most important reasons for this is that the agent causes mixed infection with different bacterial species. Therefore, it was concluded that new genes encoding some virulence factors may be transferred via extrachromosomal genetic materials such as plasmids and transposons, and the other reason may be due to the control of virulence factors by more than one gene.

It was concluded that arginine negativity and virulence gene I profile (*gcp*, *gs*60, *tbp*B, *lkt*C and *adh* positive; *nma*A negative) may be epidemiological criteria that can be used to distinguish between commensal and pathogenic *M. haemolytica* isolates and further studies should be conducted on the subject.

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REFERENCES

[1] Et ve Süt Kurumu. Et ve Süt Kurumu sektör değerlendirme raporu. Ankara. 2016.
[2] TÜİK Hayvancılık İstatistikleri (İnternet). 2019. [Erişim tarihi: 29.03.2020]. Erişim adresi: https://biruni.tuik.gov.tr/medas/?kn=101&locale=tr.
[3] Global Animal Healt Association, Animal breeding [İnternet]. 2019, [Erişim tarihi 17 Mayıs 2019]. Erişim_adresi: https://healthforanimals.org/zoonoses.html.
[4] Bell S. Respiratory disease in sheep: 2. Treatment and control. In Practice. 2008; 30: 278-283.
[5] Ermilio EM, Smith MC. Treatment of emergency conditions in sheep and goats. Vet Clin Food Anim. 2011; 27: 33-45.
[6] Statham J. Respiratory disease in cattle a practical approach. Livestock. 2018; 23 (5): 206-13.
[7] Kumar A, Tikoo SK, Malik P, Kumar AT. Respiratory diseases of small ruminants. Hindawi Publishing Corporation Veterinary Medicine International. 2014; 373642: 2.
[8] Şentürk B. Türkiye'de salgın hayvan hastalık sorunu ve yeni model önerileri. Harran Üniv Vet Fak Derg. 2015; 4 (1), 27-9.
[9] Rahal A, Ahmad AH, Prakash A, Mandil R, Kumar RT. Environmental attributes to respiratory diseases of small ruminants. Vet Med INT. 2014; 853627: 10.
[10] Öztürk D, Çorlu M. Pnömonili Koyun akciğerlerinden izole edilen bakteriler ve antibiyotik duyarlılıkları. Vet. Bil. Derg. 2006; 22 (1-2): 59-63
[11] Yüzbaşıgil AF (2010). Kuzu pnömonilerinde patolojik ve bakteriyolojik incelemeler ile *Parainfluenza 3* (PI 3) virüsünün etiyolojideki rolü. Selçuk Üniversitesi Sağlık Bilimleri Enstitüsü, Doktora Tezi.
[12] Rosadio R, Cirilo E, Manchego A, Rivera H. *Respiratory syncytial* and *parainfluenza type 3* viruses coexisting with *Pasteurella multocida* and *Mannheimia hemolytica* in acute pneumonias of neonatal alpacas. Small Ruminant Res. 2011; 97: 110–6.
[13] Arslan HH, Özcan U. Current approach to bovine respiratory disease. Dairy and Vet Sci J. 2018; 5 (2): 1-3
[14] Jesse FFA, Mubin HNA, Hambali IU, Lila MAM, Chung ELT, Abba Y, et al. Review on clinical management involving respiratory diseases in ruminants. Adv Anim. Vet. Sci. 2019; 7(4): 321-5.
[15] Confer AW. Update on bacterial pathogenesis in BRD. Anim Health Res Rev. 2009 Dec;10(2):145-8. doi: 10.1017/S1466252309990193. PMID: 20003651.
[16] Tomassini L, Gonzales B, Weiser GC, Sischo W. An ecologic study comparing distribution of *Pasteurella trehalosi* and *Mannheimia haemolytica* between Sierra Nevada bighorn sheep, White Mountain Bighorn Sheep, and Domestic sheep. J Wildl Dis. 2009; 45 (4): 930-40.
[17] Hossain MM, Islam MS, Kamal AHM, Rahman AKMA, Cho HS. Dairy cattle mortality in an

organized herd in Bangladesh. VeterinaryWorld. 2014; 7 (5): 331-6.
[18] Caswell JL, Williams KJ. Respiratory system. Maxie M.G. (Ed.) Jubb, Kennedy and Palmer's Pathology of Domestic. 5. baskı. Edinburgh: Elsevier; 2016.
[19] Angen O, Ahrens P, Kuhnert P, Christensen H, Mutters R. Proposal of *Histophilus somni* gen. nov., sp. nov. for the three species *incertae sedis Haemophilus somnus*, *Haemophilus agni* and *Histophilus ovis*. Int J Syst Evol Microbiol. 2003; 53: 1449-56.
[20] Christensen H, Bisgaard M. Revised definition of *Actinobacillus* sensu stricto isolated from animals. A review with special emphasis on diagnosis. Vet. Microbiol. 2004; 99: 13-30.
[21] Blackall PJ, Bojesen AM, Christensen H, Bisgaard M. Reclassification of *Pasteurella trehalosi* as *Bibersteinia trehalosi* gen. nov., comb. nov. Int J Syst Evol Microbiol. 2007; 57: 666−74.
[22] Yavrucu E. *Mannheimia haemolytica*'nın sığır epitel hücreleri üzerindeki apoptotik etkisi [Yüksek Lisans tezi]. Ankara: Ankara Üniversitesi; 2008.
[23] Quinn PJ (Editor), Markey BK, Leonard FC, Fitzpatrick ES, Fanning S, Hartigan PJ. Veterinary Microbiology and Microbial Disease, UK: John Wiley and Sons Ltd; 27: 300-308. 2011.
[24] Mutters R, Angen Q, Bisgaard M. Bergey's manual of systematics of archaea and bacteri, Online Bergey's Manual Trust. (e-book). Published by John Wiley and Sons. in association with Bergey's
[25] Manual Trust. 2015. Doi: 10.1002/9781118960608.gbm01200.
[26] Angen Ø, Mutters R, Caugant DA, Olsen JE, Bısgaard M. Taxonomic relationships of the [*Pasteurella*] *haemolytica* complex as evaluated by DNA-DNA hybridizations and 16S rRNA sequencing with proposal of *Mannheimia haemolytica* gen. nov., comb. nov., *Mannheimia granulomatis* comb. nov., *Mannheimia glucosida* sp. nov., *Mannheimia ruminalis* sp. nov. and *Mannheimia varigena* sp. nov. Int J Syst Evol Microbiol 1999; 49: 67-86.
[27] Omaleki L, Browning FG, Allen JL, Barber SR. Molecular epidemiology of *Mannheimia haemolytica* and *Mannheimia glucosida* associated with ovine mastitis. JVDI. 2012; 24(4): 730-4.
[28] Nefedchenko AV, Glotova TI, Glotov AG. Isolation and antimicrobial resistance of *Mannheimia haemolytica* on dairy farms in Siberia. Bulg J *Vet* Med. 2019; 22 (4): 428-38.
[29] Highlander SK. Molecular genetic analysis of virulence in *Mannheimia (Pasteurella) haemolytica*, Frontiers in Bioscience. 2001; 6: 1128-50.
[30] Lee RWH, Pool AN, Ziauddin A, Lo RYC, Shewen PE, Strommer JN. Edible vaccine development: stability of *Mannheimia haemolytica* A1 leukotoxin 50 during post-harvest processing and storage of field-grown transgenic white clover. Kluwer Academic Publshers Molecular Breeding. 2003; 11: 259-66.
[31] Zecchinon L, Fett T, Desmecht D. How *Mannheimia haemolytica* defeats host defence through a kiss of death mechanism. Vet Res. 2005; 36: 133-56.
[32] De la Mora A, Trigo F, Jaramillo L, Garfies Y, Solórzano C, Agundis C et al. The N-acetyl-D-glucosamine specific adhesin from *Mannheimia haemolytica* activates bovine neutrophils oxidative burst. Vet Immunol Immunopathol. 2006; 113 (1-2): 148-56.
[33] Lo RYC, Sathiamoorthy S, Shewen PE. Analysis of *in vivo* expressed genes in *Mannheimia haemolytica* A1. FEMS Microbiol Lett. 2006; 265: 18-25.
[34] Lee RWH, Cornelisse M, Ziauddin A, Slack PJ, Hodgins DC, Strommer JN, et al. Expression of a modified *Mannheimia haemolytica gs60* outer membrane lipoprotein in transgenic alfalfa for the development of an edible vaccine against Bovine Pneumonic Pasteurellosis. J Biotechnol. 2008; 135: 224-31.
[35] Mohamed RA, Abdelsalam EB. A review on Pneumonic Pasteurellosis (Respiratory Mannheimiosis) with emphasis on pathogenesis, virulence mechanisms and predisposing factors. Bulg J Vet Med. 2008; 11 (3): 139-60.
[36] Rice JA, Carrasco-Medina L, Hodgins DC, Shewen PE. *Mannheimia haemolytica* and bovine respiratory disease. Anim Health Res Rev. 2008; 8 (2): 117-28.
[37] Hounsome JDA. Comparative outer membrane proteomic analyses of bovine and ovine isolates of *Mannheimia haemolytica* and *Mannheimia glucosida* [PhD thesis]. UK; University of Glasgow. 2012.
[38] Puchalski A, Chmiel RU, Dec M, Wernicki A. An electrophoretic characterization of ıron-transporting proteins in *Mannheimia haemolytica* A1. Pol J Vet. 2013; 3: 527-32.
[39] Legesse A, Abayneh T, Mamo G, Gelaye E, Tesfaw L, Yami M, et al. Molecular characterization of *Mannheimia haemolytica* Isolates associated with Pneumonic cases of sheep in selected Areas of Central Ethiopia. BMC Microbiol. 2018; 18: 205.
[40] Singh F, Sonawane GG, Meena RK. Molecular detection of virulent *Mannheimia haemolytica* and *Pasteurella multocida* in lung tissues of pneumonic sheep from semiarid tropics, Rajasthan, India. Turk J Vet Anim Sci. 2018; 42: 556-1.
[41] Özbey G, Muz A. Pnömonili Koyun ve Keçilerin Akciğerlerinden Aerobik Bakteri izolasyonları ve İzole *Pasteurella multocida* ve *Mannheimia haemolytica*'nın Polimeraz Zincir Reaksiyonu ile Saptanması. Turk J Vet Anim Sci. 2004; 28: 209-16.
[42] Gioia J, Qin X, Jiang H, Clinkenbeard K, L0 R, Liu Y, et al. The genome sequence of *Mannheimia haemolytica* A1: Insights into virulence, natural competence, and *Pasteurellaceae* phylogeny. J. Bacteriol. 2006; 7257-66.
[43] Alexander TW, Cook SR, Yanke LJ, Booker CW, Morley PS, Read RR, et al. A multiplex polymerase

chain reaction assay for the identification of *Mannheimia haemolytica*, *Mannheimia glucosida* and *Mannheimia ruminalis*. Vet Microbiol. 2008; 130 (1-2): 166-75.

[44] Kumar J, Shivendra D, Kumar R. Rapid detection of *Mannheimia haemolytica* in lung tissues of sheep and from bacterial culture. Veterinary World. 2015; 8 (9): 1073-7.

[45] Shanthalingam S, Goldy A, Bavananthasivam J, Subramaniam R, Batra SA, Kugadas A, et al. PCR assay detects *Mannheımıa haemolytıca* in culture negatıve pneumonıc lung tıssues of Bıghorn Sheep (Ovıs Canadensıs) from outbreaks in the Western Usa, 2009–2010. J Wildl Dis. 2014; 50 (1): 1-10.

[46] Ahmed WA, Mohammed RJ, Khalaf IA. Molecular and phenotypical characterization of *Mannheimia haemolytica* ısolated from goats in Baghdad Province. AIM. 2017; 7: 304-14.

[47] Dağ S, Gürbüz A, Özen H, Büyük F, Çelebi Ö, Karaman M, et al. Immunohistochemical and molecular detection of *Mannheimia* spp. and *Pasteurella* spp. in sheep with pneumonia in Kars Province – Turkey. Kafkas Univ Vet Fak Derg. 2018; 24 (2): 281-8.

[48] Rawat N, Gilhare VR, Kushwaha KK, Hattimare DD, Khan FF, Shende RK et al. Isolation and molecular characterization of *Mannheimia haemolytica* and *Pasteurella multocida* associated with pneumonia of goats in Chhattisgarh. Vet World. 2019; 12 (2): 331-6.

[49] Koneman EW (Editor), Allen SD, Dowell VR, Janda WM, Sommers HM, Winn WC. Color Atlas and Text Book of Diagnostic Microbiology. Third Edition. Lippincott Comp., Philadelphia, USA. 1988.

[50] Carter GR (1984). *Pasteurella*, *Yersinia* and *Francisella*, chapter 11, in "Diagnostic Procedures in Veterinary Bacteriology and Mycology" Fourth Edition, 111-121, Charles C Thomas, U.S.A.

[51] Önat K, Kahya S, Çarlı T. Frequency and antibiotic susceptibility of *Pasteurella multocida* and *Mannheimia haemolytica* isolates from nasal cavities of Cattle. Turk J Vet Anim Sci. 2010; 34 (1): 91-4.

[52] Angen Ø, Ahrens P, Bisgaard M. Phenotypic and genotypic characterization of *Mannheimia (Pasteurella) haemolytica*-like strains isolated from diseased animals in Denmark. Vet Mic. 2002; 84: 103-14.

[53] Laishevtcev AI, ОСОБЕННОСТИ БИОХИМИЧЕСКОЙ ИДЕНТИФИКАЦИИ И ДИФФЕРЕНЦИАЦИИ БАКТЕРИЙ ВИДА *Mannheimıa haemolytica* (Features of biochemical identification and differentiation of *Mannheimıa haemolytica*). RJOAS. 2016; 6 (54): 70-7.

[54] Hawari AD, Hassawi DS, Sweiss M. Isolation and identification of *Mannheimia haemolytica* and *Pasteurella multocida* in sheep and goats using biochemical tests and Random Amplified Polymorphic DNA (RAPD) Analysis. J Biol Sci. 2008; 8(7): 1251-4.

[55] Klima CL, Alexander TW, Hendrick S, McAllister AT. Characterization of *Mannheimia haemolytica* isolated from feedlot cattle that were healthy or treated for bovine respiratory disease. Can J Vet Res.2014; 78 (1): 38-45.

[56] Gülaydın Ö, Gürtürk K. İdentification of *Pasteurella multocida* strains isolated from respiratory tract of healthy and diseased cattle and determination of capsular types by PCR in Van Region. Van Vet J. 2018; 29 (3): 143-6.

[57] Ülker H, Kucuk D, Cantekin Z, Solmaz H (2012). Hatay yöresinde kesimhanede kesilen sığır akciğerlerinden *Pasteurella multocida* ve *Mannheimia haemolytica* izolasyonu ve antibiyotiklere duyarlılığı. AVKAE Derg, 2, 2, 10-14.

[58] Gürbüz A. Sığır ve koyunlara ait pnömonili akciğerlerden *Pasteurella haemolytica*'nın izolasyonu, identifikasyonu, biyotiplendirilmesi ve antibiyotiklere olan duyarlılıklarının belirlenmesi. [Doktora Tezi]. Kars: Kafkas Üniversitesi. 2003.

[59] Noyes NR, Benedict KM, Gow SP, Booker CW, Hannon SJ, McAllister TA, Morley PS (2015). *Mannheimia haemolytica* in feedlot cattle: prevalence of recovery and associations with antimicrobial use, resistance, and health outcomes. Journal of Veterinary Internal Medicine 29, 705–713.

[60] Castillo JLR, Valencia GL, Monge-Navara FJ, Medina-Basulto GE, Hori-Oshima S, Cueto-Gonzalez SA, ve ark. Detection and economic impact related to bovine respiratory disease, shrink, and traveling distance in feedlot cattle in Northwest Mexico. Turk J Vet Anim Sci. 2017; 41: 294-301.

[61] Demissie T, Dawo F, Sisay T. Biochemical and antigenic characterization of *Mannheimia*, *pasteurella* and *Mycoplasma* species from naturally infected pneumonic sheep and goats, Bishoftu, Ethiopia. AJBAS. 2014; 6 (6): 198-204.

[62] Kırkan Ş, Kaya O. Serotyping of *Mannheimia haemolytica* Strains Isolated from Pneumonic Lungs of Sheep in the Aydın Region of Turkey. Turk J Vet Anim Sci. 2005; 29: 491-4.

[63] Tel OY, Keskin O. Koyun akciğerlerinden *Pasteurella multocida* ve *Mannheimia haemolytica* izolasyonu ve antibiyotiklere duyarlılığı. YYÜ Vet Fak Derg. 2010; 21 (1): 31.

[64] Bisgaard M. Incidence of *Pasteurella haemolytica* in the respiratory tract of Apparently healthy chickens and chickens with infectıous bronchitis. Characterisation of 213 Strains. Avian Pathology. 1977; 6: 285-292.

[65] Daphal SH, Mhasa PP, Pawade MM, Shelke PP, Sangle JD, Kolhe RP. Emergence of virulent *Pasteurella multocida* and *Mannheimia hemolytica* in sheep and goats of Western Maharashtra, India. Int J Curr Microbiol App Sci. 2018; 7 (9): 1990-1998.

[66] Angen Q, Aalbjek B, Falsen E, Olsen JE, Bisgaard M. Relationships among strains classified with the ruminant *Pasteurella haemolytica*-complex using quantitative evaluation of phenotypic Data. Zbl. Bakt. 1997; 285: 459-79.

[67] Taunde PA, Argenta FF, Bianchi RM, De Cecco BS, Vielmo A, Lopes BC, et al. *Mannheimia*

haemolytica pleuropneumonia in goats associated with shipping stress. Ciencia Rural. 2019; 49(1): 1-6.

[68] İlhan Z, Keleş İ. Biotyping and serotyping of *Mannheimia (Pasteurella) haemolytica* isolated from lung samples of slaughtered sheep in the Van Region. Turk J Vet Anim Sci. 2007; 31 (2): 137-41

[69] Şahin İ. Samsun ili ve çevresinde koyun pnömonilerinden izole edilen *Mannheimia haemolytica* türlerinin fenotipik karakterizasyonu ve antibiyotik duyarlılıklarının belirlenmesi. Samsun, Ondokuz Mayıs Üniversitesi. 2017.

[70] Fisher MA, Weiser GC, Hunter DL, Ward ACS. Use of a Polymerase Chain Reaction method to detect the leucotoxine gene *lktA* in biogrup and biovariant isolates of *Pasteurella haemolytica* and *Pasteurella trehalosi*. Am. J. Vet. Res. 1999; 11: 1402-6.

[71] Kırkan Ş. Aydın yöresinde koyunların solunum sisteminde infeksiyon nedeni *Mannheimia (Pasteurella) haemolytica*'nın biyotip ve Serotip tayini, elektroforez ve PCR ile tanısı. [Doktora Tezi]. Aydın: Adnan Menderes Üniversitesi. 2003.

[72] El Dokmak MM, Khalil SA, Ebied SKHM. Genetic diversity of *Mannheimia haemolytica* strains. AJVS. 2015; 47: 166-74.

[73] Garcia-Alvarez A, Garayzabal JF, Chaves F, Pinto C, Cid D. Ovine *Mannheimia haemolytica* Isolates from Lungs with and without Pneumonic Lesions Belong to Similar Genotypes. Vet Microbiol. 2018; 219: 80-6.