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Investigation of the presence of *Mycoplasma* as an etiologic agent of inflammatory airway diseases in thoroughbred racehorses in İstanbul Province

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Abstract: *Mycoplasma equirhinis* and *Mycoplasma felis* are thought to be infectious etiologic agents of inflammatory airway disease (IAD), which, after musculoskeletal injuries, is the second most common cause of poor performance in thoroughbred racehorses. The aims of this study were to investigate for the first time the presence of *M. equirhinis*, *M. felis*, and other *Mycoplasma* spp. in the lower respiratory tract of racehorses in İstanbul Province in Turkey and to statistically evaluate the association between clinical signs of IAD and the presence of mycoplasmas. Tracheal wash (TW) samples were collected from 111 English and Arabian thoroughbred horses that showed clinical signs of IAD. TW samples were examined by culture and PCR methods. The detection rates of *Mycoplasma* spp., *M. felis*, and *M. equirhinis* were found to be 16.2%, 0%, and 18% by culture and 59.5%, 1.8%, and 6.3% by PCR in TW samples, respectively. According to statistical evaluations, a significant association was found for the presence of *M. felis* in English thoroughbred racehorses. However, no significant association was found between clinical signs of IAD and the presence of *M. equirhinis*, *M. felis*, and other *Mycoplasma* spp.

Key words: *Mycoplasma* spp., *Mycoplasma equirhinis*, *Mycoplasma felis*, inflammatory airway disease

1. Introduction

Besides musculoskeletal injuries, diseases of the airway in thoroughbred racehorses are the second most frequently encountered disorders (1), and inflammatory airway disease (IAD) is one of the most common of these diseases in racehorses (2,3). Poor performance, increased amounts of mucus in the trachea, coughing, and nasal discharge are all clinical signs associated with IAD (2). Horses of any age can be affected, but IAD is more commonly reported in younger horses (2). A variety of etiological agents may be involved in IAD, and their relative contribution in the development of the disease varies within different populations according to many factors such as the genetic makeup of the animal and the type of infectious agent but also environmental conditions, feeding, housing season, and preventive medicine practices. Beta-hemolytic streptococci, *Actinobacillus*/*Pasteurella* spp., *Klebsiella pneumoniae*, *Bordetella bronchiseptica*, and *Streptococcus pneumoniae* are isolated from IAD cases as bacterial pathogens (1,4,5). In addition to these agents, *Mycoplasma equirhinis* and *Mycoplasma felis* can also be isolated from the lower respiratory tract of horses and are determined to be the etiologic agents of IAD (1,3,4,6–8).

Mycoplasma species are small prokaryotes that do not synthesize peptidoglycan and therefore lack cell walls. Many *Mycoplasma* species are the causative agents of significant diseases such as pneumonia, pleuritis, arthritis, urogenital infections, and mastitis in humans, ruminants, pigs, poultry, and horses (9,10).

Previous studies in different populations have showed that the presence of *Mycoplasma* spp. was determined to be 9.5% (11), 3% (12), and 10% (13), while the presence of *M. equirhinis* was determined to be 12.3% (3), 10.2% (4), 14% (7), and 10.4% (14) in tracheal wash (TW) samples by culture. The presence of *M. felis* was determined as 1.4% (3), 1.4% (4), 8% (7), and 1.35% (14) by culture. Unfortunately, no previous studies could be found to detect these microorganisms by PCR in TW samples. *M. felis*, which was isolated from a female hypogammaglobulinemic patient with septic arthritis, also had zoonotic significance (15). *M. equirhinis* has no significance as a zoonotic infectious agent. There have been no previous studies showing the presence and prevalence of *Mycoplasma* in the respiratory tract and its association with signs of respiratory disease in any horses, including thoroughbreds and non-thoroughbreds, in Turkey. The

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objectives of the present study were to investigate for the first time the presence of *Mycoplasma* spp., *M. equirhinis*, and *M. felis* in TW samples in İstanbul Province and to evaluate the statistical association between clinical signs of IAD and the presence of these agents.

2. Materials and methods

2.1. Samples

A total of 111 TW samples were collected from 82 (73.88%) English and 29 (26.12%) Arabian thoroughbred horses. The samples were submitted to the İstanbul Race Horse Hospital along with complaints of poor performance and/or abnormal respiratory sounds at rest or while training. The same clinician evaluated the clinical signs of the horses, and the amount of tracheal mucus accumulation (classified as none, little, intermediate, or high), poor performance, coughing at rest or during exercise, and nasal discharge were recorded while collecting samples from the horses (2,5,16–18). The sex, age, and strain (English or Arabian thoroughbred) of the horses were also recorded. TW samples were collected during the endoscopic examination of the respiratory tract. A flushing catheter was passed through the biopsy channel of the endoscopy, and the carina was washed with 30 mL of sterile 0.9% saline solution. The content was then aspirated back to a sterile 60-mL sterile syringe (19). The specimens were immediately sent to the laboratory and stored at –20 °C prior to laboratory analysis.

2.2. Culture

The TW samples were subjected to *Mycoplasma* cultivation as previously described (18–20). The specimens were inoculated in a *Mycoplasma* broth base (Oxoid CM 0403) and a *Mycoplasma* agar base (Oxoid CM 0401, Thermo Scientific, Waltham, MA, USA) supplemented with *Mycoplasma* selective supplement-G (Oxoid SR0059C, Thermo Scientific), both of which were prepared according to the manufacturer's instructions. The TW samples were inoculated into the mediums by adding 1 mL of sample to the 9 mL of broth medium, and 1 drop (50 µL) of sample was streaked onto the surface of the agar medium. Both the broth and the agar mediums were inoculated at the same time. The plates and broth cultures were incubated at 37 °C in a highly moist atmosphere containing 5% CO_2. They were checked daily for growth by evaluating the turbidity of the broth culture and stereomicroscopic examination of the agar culture plates. Two blind passages were made from the broth cultures to eliminate contaminant bacterial and fungal agents and to isolate mycoplasmas. After two passages, the broth and agar cultures were inoculated for an additional 14 days. If no growth was seen at the end of the 14-day period, the sample was evaluated as negative by culture. If growth was seen, the suspected colonies were subcultured on a fresh broth medium. The subcultured broth medium and *Mycoplasma*-suspected broth cultures were stored at –20 °C until PCR assays were carried out for identification.

2.3. DNA extraction

DNA was extracted from the suspected broth cultures and from the TW samples according to the manufacturer's instructions using a commercial kit (Sigma GenElute Bacterial Genomic DNA Kit, Sigma-Aldrich Life Sciences, Darmstadt, Germany).

2.4. PCR

Three types of PCR were used in the study (Table 1). *Mycoplasma* spp. PCR was performed to detect *Mycoplasma* spp. in the TW samples and for genus-specific identification of the isolated colonies (21). The PCR reaction mix of 25 µL included 0.5 µL of each primer (10 µM), 0.5 µL of dNTP mix (10 mM), 2.5 µL of 10X PCR buffer, 1.5 µL of $MgCl_2$ (50 mM), 0.2 µL of Platinum Taq Polymerase (5 U/µL), and 5 µL of clinical sample. PCR conditions were as follows: initial denaturation at 94 °C for 5 min and 35 cycles of denaturation at 95 °C for 60 s, annealing at 55 °C for 60 s, and extension at 72 °C for 60 s. *M. felis* PCR was performed to detect *M. felis* in the TW samples and to identify the isolated colonies (22). The PCR reaction mix of 25 µL included 0.25 µL of each primer (10 µM), 0.5 µL of dNTP mix (10 mM), 2.5 µL of 10X PCR buffer, 0.75 µL of $MgCl_2$ (50 mM), 0.5 µL of Platinum Taq Polymerase (5 U/µL), and 5 µL of clinical sample. PCR conditions were as follows: initial denaturation at 94 °C for 5 min and 35 cycles of denaturation at 94 °C for 30 s, annealing at 51.6

Table 1. Target genes, amplicon sizes, and oligonucleotide sequences used during PCR analysis.

Agent	Target gene	Amplicon size	Oligonucleotide sequence	Reference
M. equirhinis	16s rRNA	211 bp	5'-CAC CGC CCG TCA CAC CA-3' 5'-GAT CTC TCA AAA CTG AAT ACG-3'	Robinson (14)
M. felis	16s rRNA	238 bp	5'-CAC CGC CCG TCA CAC CA-3' 5'-GGA CTA TTA TCA AAA GCA CAT AAC-3'	Chalker et al. (22)
Mycoplasma spp.	16S rRNA	280 bp	5'-GGG AGC AAA CAG GAT TAG ATA CCC T-3' 5'-TGC ACC ATC TGT CAC TCT GTT AAC CTC-3'	Van Kuppeveld et al. (21)

°C for 30 s, and extension at 72 °C for 20 s followed by a final extension at 72 °C for 5 min. *M. equirhinis* PCR was performed to detect *M. equirhinis* in the TW samples and to identify the isolated colonies (14). The PCR reaction mix of 50 µL included 0.5 µL of each primer (10 µM), 1 µL of dNTP mix (10 mM), 5 µL of 10X PCR buffer, 1.5 µL of $MgCl_2$ (50 mM), 0.5 µL of Platinum Taq Polymerase (5 U/µL), and 10 µL of clinical sample. PCR conditions were as follows: initial denaturation at 94 °C for 5 min and 35 cycles of denaturation at 94 °C for 30 s, annealing at 46 °C for 30 s, and extension at 72 °C for 20 s followed by a final extension at 72 °C for 5 min. All PCR products were analyzed using 1% agarose gel electrophoresis with ethidium bromide staining. The samples were accepted as positive if 280-bp, 238-bp, and 211-bp bands were seen in the agarose gel electrophoresis, respectively. Reference culture DNAs (NCTC 10160, NCTC 10160, and NCTC 10148, respectively) as the positive control and nuclease-free water as the negative control were used in every PCR cycle.

2.5. Statistical analysis

The statistical analysis of the association between clinical signs and the presence of *Mycoplasma* spp., *M. felis*, and *M. equirhinis* in the TW samples was evaluated using Pearson's chi-square (χ^2) test with SPSS (23).

3. Results

3.1. Culture

Mycoplasma-suspected colonies were isolated in 18 (16.2%) of the 111 TW samples (Figure 1). In 33 (29.7%) specimens, bacterial and fungal contaminants were detected in the first inoculation. All contaminants were eliminated using a 0.45-µm syringe filter during the first passage.

3.2. *Mycoplasma* spp.-specific PCR

Sixty-six (59.5%) of the 111 TW samples were positive by *Mycoplasma* spp.-specific PCR. All isolated colonies (n = 18) were also identified as *Mycoplasma* spp. by PCR (Figure 2).

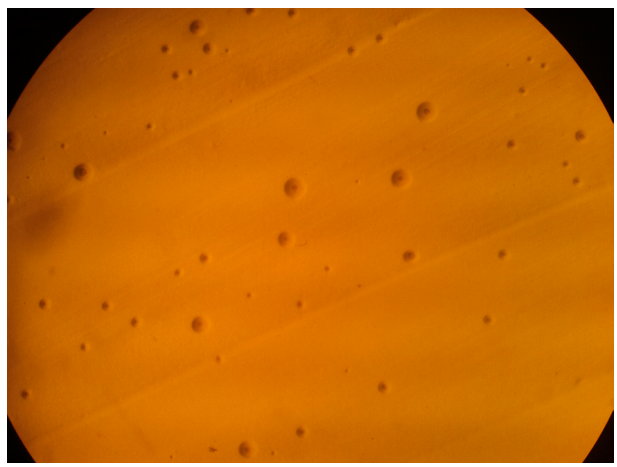

Figure 1. *M. equirhinis* colonies isolated from a tracheal wash sample.

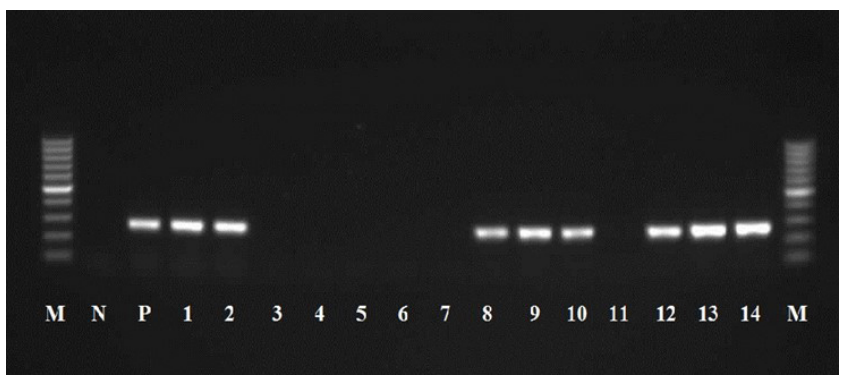

Figure 2. *Mycoplasma* spp. PCR findings in the cultures. M: Marker, 100–1000 bp, 500 ref. band; P: positive control; N: negative control; 1, 2, 8–10, 12–14: positive samples; 3–7, 11: negative samples.

3.3. *M. felis*-specific PCR

Two (1.8%) of the 111 TW samples were positive by *M. felis*-specific PCR (Figure 3). None of the isolated colonies were identified as *M. felis* by PCR.

3.4. *M. equirhinis*-specific PCR

Seven (6.3%) of the 111 TW samples were *M. equirhinis*-positive by PCR. All of the isolates (n = 18) that were identified as *Mycoplasma* spp. were also *M. equirhinis*-positive by PCR (Figure 4).

3.5. Statistical results

Statistical analysis showed that no associations were found between clinical signs of IAD (large amounts of mucus in the trachea, coughing, nasal discharge, and poor performance) and the presence of *Mycoplasma* spp., *M. felis*, and *M. equirhinis* (Table 2). There was also no association between age groups (comparing young horses of 2–4 years old and older horses) and the presence of *Mycoplasma*. There was no statistical association between the presence of *Mycoplasma* spp. or *M. equirhinis* and English or Arabian thoroughbred horses. However, a statistical association between the presence of *M. felis* and thoroughbred English horses was found ($P < 0.05$). In addition to these results, no statistical association was found between the presence of *Mycoplasma* and the sex of the horses.

4. Discussion

Until now, no studies have shown the status of *Mycoplasma* spp. in the respiratory tract of thoroughbred racehorses or other horse groups and horse strains in Turkey; however, there have been some studies conducted in other countries that showed the presence of *Mycoplasma* in the respiratory tract of horses. The presence of *Mycoplasma* spp. was found as 10% and 9.5%, respectively, by culture in the tracheal swabs of horses in retrospective studies (11,13). In Canada, the prevalence of *Mycoplasma* was found to be 3% in TW samples by culture (12). In the present study, the presence of *Mycoplasma* spp. in the respiratory tract of horses was found to be 16.2% by culture. *Mycoplasma* species are known to be more prevalent in countries that have hot climates with high humidity (9). This study revealed that *Mycoplasma* has a high isolation rate in Turkey compared with Canada and the United Kingdom. This high detection rate may be due to Turkey's hot and humid climate.

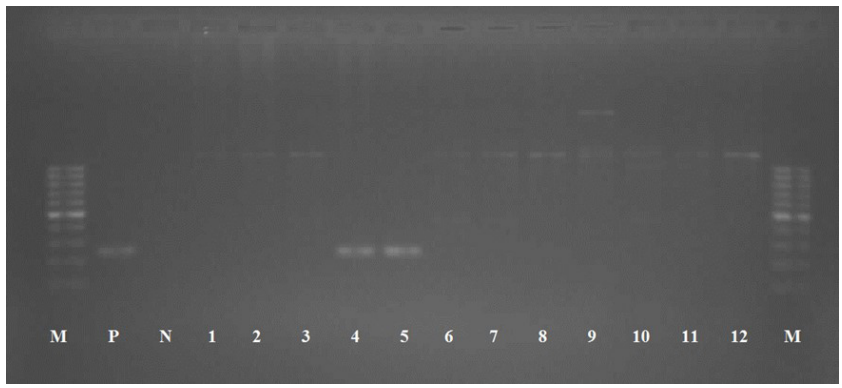

Figure 3. *M. felis* PCR findings in the tracheal washes. M: Marker, 100–1000 bp, 500 ref. band; P: positive control; N: negative control; 4, 5: positive samples; 1, 2, 3, 6, 7, 8, 9, 10, 11, 12: negative samples.

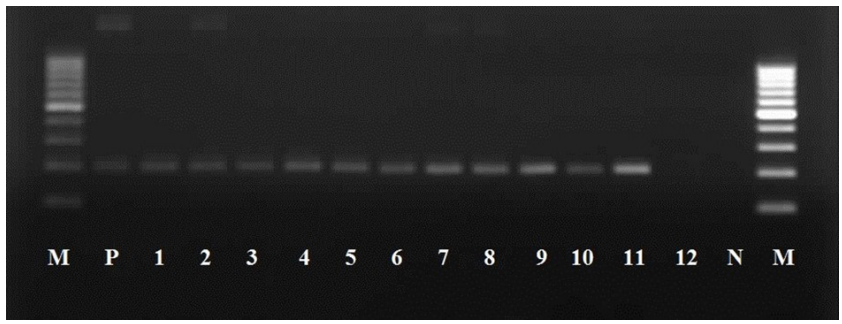

Figure 4. *M. equirhinis* PCR findings of the *Mycoplasma* spp.-confirmed isolates. M: Marker, 100–1000 bp, 500 ref. band; P: positive control; N: negative control; 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11: positive samples; 12: negative sample.

Table 2. Distribution of *Mycoplasma* detected in racehorses according to strain, age, sex, and clinical signs in Turkey.

		Detected microorganism			Total number of horses in the group
		Mycoplasma spp.	*M. felis*	*M. equirhinis*	
Strain information	English	53 (64.6%)	2 (2.4%)	15 (18.3%)	82
Age, years	Arabian	13 (44.8%)	0 (0%)	5 (17.2%)	29
	2	33 (60.0%)	2 (3.6%)	11 (20.0%)	55
	3	24 (66.6%)	0 (0%)	9 (25.0%)	36
	4	4 (40.0%)	0 (0%)	0 (0%)	10
	5	2 (50.0%)	0 (0%)	0 (0%)	4
	6	2 (50.0%)	0 (0%)	0 (0%)	4
	7	1 (50%)	0 (0%)	0 (0%)	2
Sex	Female	27 (57.4%)	0 (0%)	12 (25.5%)	47
	Male	39 (60.9%)	2 (3.1%)	8 (12.5%)	64
Amount of mucus in trachea	Little	23 (62.1%)	0 (0%)	3 (8.1%)	37
	Intermediate	26 (56.5%)	1 (2.1%)	10 (21.7%)	46
	High	17 (60.7%)	1 (3.5%)	7 (25.0%)	28
Nasal discharge		16 (48.4%)	1 (3.0%)	5 (15.1%)	33
Poor performance		46 (59.7%)	0 (0%)	13 (16.9%)	77
Coughing		37 (71.2%)	2 (3.9 %)	12 (23.0%)	52

No previous study has shown the presence of *M. equirhinis* and *M. felis*, which were both thought to be the etiologic agents of IAD in Turkey. Some studies have revealed the presence of *M. equirhinis* and *M. felis* in other countries. In the United Kingdom, the presence of *M. felis* was found to be 1.4%, 1.4%, 8%, and 1.35%, respectively, in different studies (3,4,7,14). The presence of *M. equirhinis* was found to be 12.3%, 10.2%, 14%, and 10.4% by the same researchers. All of the samples handled in those studies were carried out using culture. In our study, *M. felis* was not isolated from any samples; however, *M. equirhinis* was isolated from 16.2% (n = 18) of the samples. These results showed that *M. equirhinis* had a higher isolation rate than *M. felis*, which was not isolated from TW samples in Turkey. It was also concluded that *M. equirhinis* had higher isolation rates compared with other countries.

Classification of the presence of *M. equirhinis* and *M. felis* according to age groups revealed that *M. felis* was isolated in 1.9% of 2-year-old, 1.7% of 3-year-old, and 0.7% of 4-year-old horses. *M. equirhinis* was isolated in 14% of 2-year-old, 11.4% of 3-year-old, and 9.4% of 4-year-old horses and, overall, *M. felis* was isolated in 1.4% of horses of all age groups while *M. equirhinis* was detected in 12.3% by culture in the United Kingdom (5). In our study, *M. felis* was not isolated in any age group. *M. equirhinis* was isolated from 18% in all age groups, specifically in 20% of 2-year-old and 19.4% of 3-year-old horses. However, it was not isolated in horses older than 3 years. These results indicated that in all age groups the presence of *M. felis* was lower in Turkey. *M. equirhinis* was also found to be higher in 2- and 3-year-old horses and in all age groups.

Two *M. felis*-positive samples were obtained from English thoroughbred horses, and the presence of *M. felis* in English thoroughbred horses was found to be statistically significant ($P < 0.05$). The presence of *Mycoplasma* spp. and *M. equirhinis* was not associated with this horse strain. Unfortunately, no previous studies could be found indicating the presence of *Mycoplasma* species in different horse strains.

The culture method has been used in many retrospective studies (3–5,7,10,12,24). In our study, both culture and PCR methods were used in order to detect *M. felis* and *M. equirhinis*, which were thought to be associated with IAD in horses. *Mycoplasma* spp. were isolated from 18 (16.2%) culture samples while *Mycoplasma* spp. were detected in 66 (59.5%) PCR-positive samples taken directly from a TW.

The detection of *M. felis* and *M. equirhinis* from TW samples and the identification of isolates were carried out by *M. felis*- and *M. equirhinis*-specific PCR. In this study, we found 2 (1.8%) *M. felis*-positive samples by PCR but no positive samples by culture. Within the TW samples, all of the isolates (n = 18) were identified as *M. equirhinis* by PCR. Five (27.8%) of the 18 isolates identified as *M. equirhinis* were also positive by the PCR carried out directly from the TW samples. Of the 18 samples that were detected as *M. equirhinis* by culture, 13 (72.2%) were not found positive by PCR. Two (28.6%) of the 7 TW samples found to be positive by *M. equirhinis* PCR were not grown in culture. It was considered that the empirical use of antibiotics without any recommendation by veterinarians and high amounts of nontarget bacteria in the TWs may have resulted in 48 *Mycoplasma* spp., 2 *M. felis*, and 2 *M. equirhinis* PCR-positive but culture-negative samples. There were also 13 *M. equirhinis* culture-isolated samples that could not be detected by PCR. The inhibition of PCR might have been due to the large number of PCR inhibitors such as heme molecules, enzymes, proteins, and nonspecific DNA content in the TW samples.

Unfortunately, studies could not be found that investigated the presence of *Mycoplasma* by PCR in the TW samples of horses. For this reason, we were unable to compare our PCR results with any other previous studies.

Wood et al. (3) observed that there was a strong association between *M. equirhinis*, *S. zooepidemicus*, *S. pneumoniae*, and *Actinobacillus/Pasteurella* spp. and IAD. They also indicated that mixed etiology plays a key role in this association. The investigation of other etiologic agents of IAD in TW samples was not performed in this study. An investigation of the other agents in conjunction with *Mycoplasma* should be carried out to better understand the role of *Mycoplasma* species in IAD.

In conclusion, the detection rates of *Mycoplasma* spp., *M. felis*, and *M. equirhinis* were found to be 16.2%, 0%, and 18% by culture and 59.5%, 18%, and 6.3% by PCR, respectively. The presence of these agents in TW samples could not be associated with clinical signs of IAD from the statistical analysis. The presence of *M. felis* in the TW samples of English thoroughbred racehorses was found to be statistically significant ($P < 0.05$). More research, including cytological evaluation of TW samples and investigation of other bacteriological agents occurring with *Mycoplasma* species, is needed to corroborate the relationship between IAD and *Mycoplasma*.

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References

1. Chanter N. Bacterial Infections Including Mycoplasmas. Ithaca, NY, USA: International Veterinary Information Service; 2002.
2. Couëtil LL, Cardwell JM, Gerber V, Lavoie JP, Léguillette R, Richard EA. Inflammatory airway diseases of horses-revised consensus statement. J Vet Intern Med 2016; 30: 503-515.
3. Wood JLN, Newton JR, Chanter N, Mumford JA. Association between respiratory disease and bacterial and viral infections in British racehorses. J Clin Microbiol 2005; 43: 120-126.
4. Cardwell JM, Smith KC, Wood JLN, Newton JR. A longitudinal study of respiratory infections in British national hunt racehorses. Vet Rec 2013; 172: 637.
5. Wood JLN, Newton JR, Chanter N, Mumford JA. Inflammatory airway disease, nasal discharge and respiratory infections in young British racehorses. Equine Vet J 2005; 37: 236-242.
6. Hodgson JL, Hodgson DR. Inflammatory Airway Disease. Ithaca, NY, USA: International Veterinary Information Service; 2002.
7. Newton JR, Wood JLN, Chanter N. A case control study of factors and infections associated with apparent respiratory disease in UK thoroughbred racehorse. Prev Vet Medi 2003; 60: 107-132.
8. Wood JLN, Newton JR, Burrell MH, Dugdale D, Windsor HM, Windsor GD, Rosendal S, Townsend HGG. An outbreak of respiratory disease in horses associated with *Mycoplasma felis* infection. Vet Rec 1997; 140: 388-391.
9. Aydın N, İzgür M, Diker KS, Yardımcı H, Esendal Ö, Paracıklıoğlu J, Akan M. Veteriner Mikrobiyoloji (Bakteriyel Hastalıklar). Ankara, Turkey: İlke Emek Yayınları; 2006 (in Turkish).
10. Queen PJ, Carter ME, Markey BK, Carter GR. Clinical Veterinary Microbiology. 1st ed. Dublin, Ireland: Wolfe Publishing; 1994.
11. Allam NM, Lemcke RM. Mycoplasmas isolated from respiratory tract of horses. J Hyg-Camb 1975; 74: 385.
12. Clark C, Greenwood S, Boison JO, Trejo MC, Dowling PM. Bacterial isolates from equine infections in western Canada (1998-2003). Can Vet J 2008; 49: 153-160.
13. Poland P, Lemcke R. Mycoplasmas of the respiratory tract of horses and their significance in upper respiratory tract disease. In: Proceedings of the 4th International Conference on Equine Infectious Diseases. Princeton, NJ, USA: ICEID; 1978. pp. 437-446.
14. Robinson C. HBLB Funded Project Ref. 697. HBLB Veterinary Newsletter 2006; 10-1: 2.

15. Bonilla HF, Chenoweth CE, Tully JG, Blythe LK, Robertson JA, Ogenovski VM, Kaufmann CA. *Mycoplasma felis* septic arthritis in a patient with hypogammaglobulinemia. Clini Infect Dis 1997; 24: 222-225.

16. Carman S, Rosendal S, Huber L, Gyles C, McKee S, Willoughby A, Dubovi E, Thorsen J, Lein D. Infectious agents in acute respiratory disease in horses in Ontario. J Vet Diagn Invest 1997; 9: 17-23.

17. Dixon PM. Ancillary diagnostic techniques for the investigation of equine pulmonary disease. Equine Vet Educ 1997; 9: 72-80.

18. Laus F, Attili AR, Cerquetella M, Spaterna A, Tessei B, Cutteri V. Endoscopic findings, microbiological and cytological evaluation of tracheal aspirates in a population of Standardbred horses with poor performances. Vet Medi Praha 2009; 9: 444-450.

19. Christley RM, Hodgson DR, Rose RJ, Wood JLN, Reid SWJ, Whitear KG, Hodgson JL. A case-control study of respiratory disease in thoroughbred racehorses in Sydney, Australia. Equine Vet J 2001; 33: 256-264.

20. Moorthy ARS, Spradbrow PB. Isolation of mycoplasmas from the respiratory tract of horses in Australia. Vet Rec 1976; 98: 235-237.

21. Van Kuppeveld FJ, Johansson KE, Galama JM, Kissing J, Bolske G, van der Logt JT, Galama MD, Melchers WJ. Detection of mycoplasma contamination in cell cultures by a mycoplasma group-specific PCR. Appl Environ Microbiol 1994; 60: 149-152.

22. Chalker VJ, Owen WMA, Paterson CJI, Browlie J. Development of a polymerase chain reaction for the detection of *Mycoplasma felis* in domestic cats. Vet Microbiol 2004; 100: 77-82.

23. Akdamar K. SPSS ile Biyoistatistik. 3. Baskı. Eskişehir, Turkey: Kaan Kitapevi; 1999 (in Turkish).

24. Allam NM, Powell DG, Andrews BE, Lemcke RM. The isolation of *Mycoplasma* species from horses. Vet Rec 1973; 93: 402.