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Research Article

The Effects of High and Low Altitudes on Conjunctival Flora in Sport and Work Horses: A Field Study in the Northeast Anatolia Region of Turkey (Kars and Iğdır) [1]

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Abstract

The purpose of this study was to identify the conjunctival microflora in healthy horses living at various altitudes and used for both sports as well as work activities, and determine the predispositions they may cause in terms of ocular diseases. A total of 400 eyes of 200 horses (50 sports and 50 work horses living at high altitudes, and likewise, 50 sports and 50 work horses living at low altitudes) aged 6 year (min: 5, max: 13) of English and Arabian and native breed from both sexes were clinically and ophthalmoscopically examined, and swab samples were taken from the conjunctival recesses of horses with no eye problems. After microbiological examinations, bacteria was isolated in 125 (31.2%) of the 400 swab samples, while fungi was isolated in 194 (48.5%) of the samples. It was determined that the isolated bacteria were mostly Gram-positive (71.5%) with *Staphylococcus* spp. being the most frequently isolated and *Bacillus* spp. the second-most frequently isolated. In terms of fungi isolation, Yeast were the most isolated with a rate of 39.6%, *Penicillium* spp. were the second-most isolated with 22.6% and *Aspergillus* spp. were the third-most isolated with 17.5%. The most effective antibiotics on isolated bacteria were determined as cephalosporin, oxytetracycline, lincomycin/neomycin (L/N) and ceftiofur. It was concluded that the data collected from the study, taken into consideration as reference values, could help in the creation of a more effective treatment protocol in the case of an eye infection. It was also concluded that it is necessary to carefully evaluate the bacteria and fungi that are identified in horses used for these activities, both during check-ups and with regard to creating a treatment plan when an inflammatory eye problem occurs.

Keywords: *Horse, Conjunctival flora, High altitude, Low altitude, Antibiogram*

Yüksek ve Alçak Rakımın Spor ve Hizmet Atlarındaki Konjunktival Flora Üzerine Etkisi: Kuzeydoğu Anadolu'da (Kars ve Iğdır) Bir Saha Çalışması

Özet

Bu çalışmada gerek spor amaçlı gerekse hizmet faaliyetlerinde kullanılan ve farklı rakımlarda yaşayan sağlıklı atlara ait konjunktival mikrofloranın belirlenerek bunların oküler hastalıklar yönünden yaratabilecekleri predispozisyonların tesbit edilmesi amaçlanmıştır. Yaşları ortalama 6 olan İngiliz, Arap ve yerli ırklara mensup her iki cinsiyette toplam 200 ata (yüksek rakımlı bölgede yaşayan 50 spor ve 50 hizmet ile alçak rakımlı bölgede yaşayan 50 spor ve 50 hizmet atı olmak üzere) ait 400 gözün klinik ve oftalmoskopik muayeneyesi yapılarak herhangi bir göz problemi olmayan bu atlara ait konjunktival resesustan sürüntü örnekleri alındı. Mikrobiyolojik incelemeler sonucu 400 sürüntü örneğinin 125'inden (%31.2) bakteri, 194'ünden (%48.5) ise mantar izolasyonu gerçekleştirilmiştir. İzole edilen bakterilerin çoğunlukla Gram pozitif olduğu (%71.5) ve ilk sırada *Staphylococcus* spp, ikinci sırada ise *Bacillus* spp.'nin yer aldığı tespit edilmiştir. Mantar izolasyonu açısından en sık izole edilen %39.6 oranla Maya, ikinci sırada %22.6 ile *Penicillium* spp., ve üçüncü sırada %17.5 ile *Aspergillus* spp.'dir. İzole edilen bakteriler üzerine en etkili antibiyotikler sefalosporin, oksitetrasiklin, linkomisin/neomisin (L/N) ve seftiofur olarak belirlendi. Farklı amaçla kullanılan atların hem rutin sağlık kontrollerinde, hem de bakteri ve mantar nedenli göz yangılarında sağaltım planı oluşturulurken, çalışmamızla belirlenen konjunktival mikrofloranın dikkate alınması ve çalışmadan elde edilen verilerin referans değer olarak göz önünde bulundurulması, atlarda göz enfeksiyonu varlığında daha etkin bir sağaltım protokolü oluşturulmasına yardımcı olabilektir.

Anahtar sözcükler: *At, Konjunktival flora, Yüksek rakım, Alçak rakım, Antibiyogram*

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INTRODUCTION

Fungi and bacteria may be hosted in the ocular microflora of humans and animals. These microorganisms remain at a balanced state in the microflora depending on the immunity of the host. This resident flora also helps protect eye health by preventing potential pathogens from settling on the eye's surface layers. However, when the ocular defense mechanisms weaken, these members of the normal flora may become pathogenic and cause infection [1-3]. The bacterial and fungal flora of normal conjunctiva [4,5] has been studied in many animal species [6-10]. It has been reported in these studies that the animal's age, geographic changes experienced, environment and home range affect the conjunctival flora [3]. Upon literature review, no studies were found concerning the identification of the conjunctival flora of horses, including those used for javelin the traditional Turkish sport of *jeered* throwing on horseback. Studies report that conjunctivitis may develop mostly as a result of infection (viral, bacterial or fungal) and allergic reactions. In studies done on the conjunctival flora of healthy horses, what stands out is the abundance of Gram-positive bacteria. The most commonly isolated bacteria are staphylococcus and streptococcus [8]. The most commonly isolated fungi are *Aspergillus* [11], *Penicillium* and *Mucor*. In the occurrence of erosion or tissue damage caused by trauma or any other reason, the bacteria in the flora become active and cause infections [3].

The aim of this study was to identify the conjunctival microflora of horses used for work and sports living in two different areas with different altitudes, determine the predispositions they may cause in terms of ocular diseases, and set up tests to measure the resistance of conjunctival flora against microbial agents.

MATERIAL and METHODS

Study Area

The study was carried out in the Northeast Anatolia Region in the provinces of Kars and Iğdır/Turkey, which have completely different geographical and climatic characteristics even though they border each other.

Horses in this region are commonly used for both work and sports purposes. Both provinces have a significant potential for horses, and it is thought that there are around 30.000 horses at present.

1. High Altitude (Kars Region): The province of Kars has long, harsh winters and cool, rainy summers. It is located in the Northeast Anatolia Region at 40°26'N and 43°05'E. The region largely consists of plateaus with an average elevation of 1.747 meters [12].

2. Low Altitude (Iğdır Region): The province of Iğdır, a broad plain located at 39°55'N and 43°51'E with an average elevation of 858 meters, has a continental climate. It has short, warm winters and hot, dry summers [13].

Animals and Study Groups

The study material consisted of a total of 200 horses used for sports and work purposes, living in the two regions described above.

The animals in both study groups were examined in the regions in which they lived/worked, and cases with no eye problems were included in the study.

Sports Horses: This term usually describes riding horses and horses used in *jereed* games, including strong native breeds as well as purebreds (Arabian and English).

Work Horses: This term describes horses used for carrying cargo (kaşka [14]) and passengers (carriages and sleighs), as well as horses used for agricultural labor, and mostly consisting of native breeds.

The study took into consideration the animals' purpose of use and the elevation at which they lived, and was conducted in two main groups (Group I and Group II) and their corresponding subgroups (Group I-A, Group I-B, Group II-A and Group II-B). The study groups and their characteristics are shown in *Table 1*.

All samples were taken in the month of September.

Swaps Taken from Conjunctival Sac

Swab samples were taken from the conjunctival sac of the horses deemed healthy upon clinical and ophthalmological examinations. A sterile swab moistened with sterile saline was the applied to the anterior surface of the third eyelid, and rooled along the lower inferior conjunctival fornix, avoiding contact with eyelid margins or vibrissae. Swab samples were brought to Microbiology Laboratory of Veterinary Faculty, Kafkas University.

Microbiological Examinations

Each sample taken was planted on blood agar, MacConkey agar and Saboraud agar. The blood agar and MacConkey agar cultures were incubated at 37°C for 24-

Table 1. *Groups of the study*
Tablo 1. *Çalışma grupları*

Groups	Subgroups	The Number of Eyes Taken Swaps
Group I (n = 100) Kars region (High altitude)	Group I-A Sport horses (n = 50)	100
	Group I-B Work horses (n = 50)	100
Group II (n = 100) Iğdır region (Low altitude)	Group II-A Sport horses (n = 50)	100
	Group II-B Work horses (n = 50)	100

48 h, and the bacteria that multiplied were identified with Gram staining, morphology and biochemical tests [15]. The cultured Saboraud agars were incubated for 2 weeks at 20°C, and the fungi that multiplied were identified according to their species, taking into consideration their macroscopic and microscopic features [16].

Antibiotic Susceptibility Test

The Kirby-Bauer disk diffusion method was used in determining the sensitivity of the isolated bacteria to antibiotics [17], and cloxacillin, lincomycin/neomycin, oxitetracycline, cephalosporin, penicillin G and ceftiofur disks were utilized.

RESULTS

The average age of all horses used in the study was 6 years (min: 5, max: 13). Group I-A included 10 English, 15 Arabian and 25 native breeds, while all the horses in Group I-B were native breeds (n=50). Group II-A included 5 English, 20 Arabian and 25 native breeds, whereas all the horses in Group II-B were native breeds.

Despite the fact that the Kars and Iğdır regions have completely different geographical and climatic characteristics, it was observed that the horses bred in these regions had very similar purposes of use and types of shelter.

No abnormalities were detected during the clinical and ophthalmological examinations of any of the animals included in the study. In addition, no complications arose during the process of taking conjunctival swabs.

Bacteria were isolated in 125 (31.2%) of the 400 swab samples examined, while fungi were isolated in 194 (48.5%) of the samples. It was determined that the isolated bacteria were mostly Gram-positive (71.5%) with *Staphylococcus* spp. being the most isolated and *Bacillus* spp. the second-most isolated. In terms of fungi isolation, *Yeast* were the most frequently isolated with a rate of 39.6%, *Penicillium* spp. were the second-most isolated with 22.6% and *Aspergillus* spp. were the third-most isolated with 17.5%.

In terms of isolation results, a total of 32 bacterial species were isolated in the group of high elevation sports horses: 18 *Staphylococcus* spp., 6 *Bacillus* spp., 5 *Acinetobacter* spp. and 3 *Escherichia coli*. Thirty-two fungi were isolated in the fungal examination. Of these, 19 were identified as yeasts and 14 as moulds (8 *Aspergillus* spp., 5 *Penicillium* spp. and 1 *Mucor* spp.). It was observed that *Staphylococcus* spp. were the most isolated species. *Aspergillus* spp. were determined to be the most common type of mould. Microbiological test results of the groups are shown comparatively in *Table 2*.

The sensitivity rates of the isolated bacteria to antibiotics, measured using the Kirby-Bauer disk diffusion method, are shown in *Table 3*.

DISCUSSION

Activities such as games of *jereed* in particular, as well as flat racing and pacing horse races are done in the Eastern Anatolian Region, and horses are bred in the region by riding centers specifically for this purpose [18-20]. Sports horses raised in the Kars region for these purposes are becoming more and more common and gaining prominence by the day. For this reason our study aimed to comparatively evaluate the effects of the horses purpose of use as well as the different geographic elevations at which they lived on the normal conjunctival flora.

Studies have been done on the normal flora of the conjunctiva in horses. Of these, in the study carried out by Araghi-Sooreh et al.[1], it is stated that Gram-positive bacteria were detected at the rate of 59.51%, while *Bacillus*

Table 2. *Distribution of bacteria and fungi species and the number isolated from the samples according to the groups*
Tablo 2. *Örneklerden izole edilen bakteri ve mantar cins ve sayısı ile gruplara göre dağılımı*

Isolated Bacteria and Fungi	Group I High Altitude (Kars Province)		Group II Low Altitude (Iğdır Province)	
	Sports Horse	Work Horse	Sports Horse	Work Horse
Staphylococcus spp.	18	14	14	15
Bacillus spp.	6	5	8	6
Acinetobacter spp.	5	3	5	3
Eschericia coli	3	4	5	3
Aspergillus spp.	8	14	5	7
Penicillium spp.	5	10	16	13
Mucor spp.	1	-	8	7
Yeast	-	9	36	31
Alternaria spp.	-	-	5	3
Rhizopus spp.	-	-	3	3

Table 3. *Isolated bacteria and sensitivity rates (%) to antibiotics*
Tablo 3. *İzole edilen bakteriler ve antibiyotiklere olan duyarlılık oranları (%)*

Isolates	Antibiotic Sensitivity Rates of the Isolates (%)						
	Number of Isolates	Cloxacillin	Lincomycin/Neomycin	Oxytetracyclin	Cephalosporin	Penicillin G	Ceftiofur
Staphylococcus spp.	61	44	73	70	85	70	63
Bacillus spp.	28	28	53	82	71	46	53
Eschericia coli	20	60	85	85	85	80	80
Acinetobacter spp.	16	56	62	56	68	81	87

spp. were the most frequently identified species at 27.68%. Fungi was isolated at the rate of 96.85%, while the most common species were *Aspergillus* spp. at 48.03%. In another study done by Khosravi et al.[8], *Aspergillus spp*. were isolated as the most common fungus with a rate of 19.9%. In a study done in England [2], Gram-positive bacteria were detected at a rate of 52%, while the isolated species of fungi were detected to be 13%.

No comparative studies have been done on the Kars and Iğdır region in terms of altitude and the way in which the horses were raised. For this reason, our study is thought to contribute to the recording of the normal conjunctival flora of the horses in the region. This study, just like the rest of the literature [1-3,6], came across mostly Gram-positive bacteria in terms of the isolated bacteria group. The species of bacteria and fungi taken from the high elevation group for isolation have similar rates of isolation. These rates are in accordance with the literature [6-10]. For this reason, no difference was noticed in the microbial flora of the conjunctival recesses in terms of the horses purpose of use and their upbringing. On the other hand, the results obtained from horses at low elevations in especially hot and humid areas revealed that the species and isolation rates of the bacteria and fungi in the conjunctival sac samples of both the sports horse group and the work horse group were quite similar. Yet when the Iğdır region (low elevation) and Kars region (high elevation) are compared, significant differences in fungi species [4,5] and isolation rates become obvious when compared with the literature [1,2]. While *Aspergillus* spp.[11] were the most frequently isolated fungi in the first group, in the other group the ranking was Yeast followed by *Penicillium* spp. In addition, fungi species such as *Mucor* spp., *Alternaria* spp. and *Rhizopus* spp. were found in the second group at certain rates. This difference between the groups in the rates detected may linked to the different altitudes at which the horses live, as well as differences in climate conditions such as heat, humidity and rainfall.

While an antibiogram test done in England [2] showed gentamicin and chloramphenicol to be responsive, the antibiogram results obtained in this study revealed varying rates. For this reason, agents in eye infections occurring in horses must be isolated. In cases where this is not possible, depending on the agent, cephalosporin, oxytetracycline, lincomycin/neomycin (L/N) and ceftiofur could be considered options for empirical treatment.

In the spring and summer months, the animals in the Kars region were generally grazed in the area they were already in, while most of the animals in the Iğdır region, which has a very hot climate during those months, were sent to surrounding plateaus and brought back in the fall season. Taking this situation into consideration, in order to make a standard assessment, samples were taken from both regions in the month of September, as it is the end of summer and beginning of the fall season.

Based on the data obtained in this study, we are of the opinion that it is necessary to carefully evaluate the bacteria and fungi that are identified in horses used for these activities, both during check-ups and with regard to creating a treatment plan when an inflammatory eye problem occurs. Furthermore, it should not be forgotten that the normal flora of the eye may be affected by various factors [2], that therefore the microbiology of the eye must be constantly monitored and that knowing the normal flora of the eye will ease the assessment of potential eye infections.

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