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Risk factors of Gram-negative bacteria infection isolated from equine ophthalmic lesions

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ABSTRACT

In this study, we conducted an epidemiological investigation of Gram-negative bacteria associated with ocular lesions in equines. A total of 100 animals (86 horses and 14 donkeys) were investigated in this case-control study. Clinical and microbiological investigations were performed for each patient. Twenty-eight animals (25 horses and three donkeys) were found to have ophthalmic infections. Regarding clinical signs, horses and donkeys with ophthalmic infection had conjunctival hyperemia (n=10, 35.7%), continuous lacrimation (n=11, 39.3%), blepharospasm (n=1, 3.6%), eyelid edema in 5/28 (17.9%), mucopurulent ocular discharge (n=9, 32.1%), corneal edema (n=1, 3.6%), and corneal opacity (n=5, 17.9%). There was a significant association between *Pseudomonas* species and age. The highest percentage was recorded in horses aged < 5 years (n = 36, 57.1%). There was a significant association between season and the spread of ocular infection with *E. coli*, where 10 (76.9%) horses were infected in spring compared to three (23.1%) horses in the winter season. There was a significant association between the occurrence of mixed infections in the eyes and each season and the level of hygiene. The highest percentage of infection was recorded in winter (19, 61.3%) than in spring (12, 38.7%). However, for the level of hygiene, the highest percentage (87.1%) was recorded in horses with poor hygiene compared to those with good hygiene (12.9%). The risk factors associated with both *E. coli* and *Pseudomonas* spp. and ophthalmic infections in horses revealed a multifaceted relationship between microbial pathogens and environmental influences.

Introduction

Ocular disorders and their associated complications significantly impact both the quality of life of horses and their overall value, particularly regarding athletic performance (Flores *et al*., 2020) . Beyond the pain that accompanies these conditions, such disorders can lead to blindness and effectively disqualify horses from engaging in performance-related activities (Paschalis-Trela and Cywinska, 2017).

The ocular surfaces of horses, donkeys, and mules are characteristically populated by a diverse array of microorganisms referred to as normal microflora (Foti *et al*., 2013; Tamarzadeh and Araghi-Sooreh, 2014). The typical ocular microflora is believed to contribute to a protective function against pathogenic microorganisms through several mechanisms, including restricting nutrient availability, synthesizing antimicrobial compounds, and occupying ecological niches within the corneal and conjunctival epithelia (Carter *et al*., 1995). In pathological states, typical microbial flora can transition to opportunistic pathogens (Petersen-Jones, 1997; Kodikara *et al*., 1999).

Gram-positive bacteria such as *Staphylococcus*, *Streptococcus*, *Corynebacterium*, and *Bacillus* species comprise part of the normal conjunctival microflora (Hernández Vidal *et al*., 2010; Foti *et al*., 2013; Tamarzadeh and Araghi-Sooreh, 2014). In a comprehensive analysis of the microbiological profiles of normal equine eyes, gram-negative bacteria, including *Escherichia coli*, *Pseudomonas* species, *Moraxella*, *Acinetobacter*, and *Neisseria*, were infrequently isolated during culture procedures. This observation suggests a limited prevalence of these bacterial genera in the ocular environment of healthy horses (Andrew *et al*., 2003; Johns *et al*., 2011).

Some factors that might increase the chances of a horse getting infectious keratitis include where they are kept, if they are using topical steroids or antibiotics, and even factors such as the weather, temperature, and humidity during different seasons. (Andrew *et al*., 1998; Gaarder *et al*., 1998). In investigations involving humans, pathogenic microorganisms typically lead to ocular issues owing to their inherent virulence. This is further exacerbated by diminished host resistance, which can be attributed to various factors, including hygiene practices, living conditions, nutritional status, genetic predispositions, physiological conditions, the occurrence of fever, and age-related vulnerabilities (Alfonso and Miller, 1990; Prescott *et al*., 2002).

Bacterial keratitis has been documented in nearly all animal species including horses. It can be linked to various bacterial genera, such as *Staphylococcus*, *Streptococcus*, and *Bacillus*, as well as to the normal microflora of the ocular surface, which may acquire pathogenic capabilities. Additionally, it may involve pathogenic bacteria that colonize the cornea, notably *Pseudomonas* and *Escherichia* species (Sauer *et al*., 2003; Keller and Hendrix, 2005). *Leptospira* spp. have also been isolated from cases of recurrent equine uveitis (Himebaugh and Gilger, 2022; Wollanke *et al*., 2022).

A variety of veterinary ophthalmic formulations combining antimicrobials and corticosteroids are available; however, their use is generally discouraged, particularly for combinations of these agents. Such combinations are contraindicated in cases of infectious keratitis. Additionally, ocular conditions that require corticosteroids for inflammation management do not typically require the concurrent administration of antimicrobials (Dowling and Grahn, 1998).

While gram-positive bacteria are the dominant constituents of the typical conjunctival microflora found in horses across the globe, instances of isolation have revealed a more frequent occurrence of gram-negative bacteria and fungi in cases of equine ulcers (Brooks *et al*., 2000). *Pseudomonas*, *Staphylococcus*, and *Streptococcus* spp. are commonly identified in horses with bacterial keratitis and concurrent infections (Andrew *et al*., 1998; Reed *et al*., 2013). Information about the role of Gram-negative bacteria in ophthalmic affections and their associated risk factors is

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scarce. Therefore, the aim of this study was to explore the infection rate and risk factors associated with *E. coli* and *Pseudomonas* spp. in Horses with ocular lesions.

Materials and methods

Horses

In total, 100 horses were randomly selected for this study. Of these, 86 horses (61 clinically healthy and 25 with ophthalmic lesions) aged 6 months to 22 years [median (range)] [11.5 (0.5-22)] and weighed 100 – 500 kg [Median (Range)] [330 (100-500)]. In addition, 14 donkeys (Equus asinus) (11 clinically healthy and 3 with ophthalmic lesions) aged 4 months to 13 years [median (range)] [6.8 (0.3 - 13)] and weighed 80–350 kg [median (range)] [220 (80–350)]. The horses selected lived in a semi-open house. All horses were in the open yard of the farm on 8 AM until 2 PM and then entered the closed pens. All horses were fed a concentrated diet and barseem in winter and a concentrated diet and hay during the summer season. At the time of the visit, a questionnaire consisting of objective questions was administered to managers to obtain data regarding age, breed, sex, housing, season, hygiene, bedding, any stress factor related to the animal, and antimicrobial drugs that were used in the treatment of previous ophthalmic infections. This study was conducted between October 2018 and May 2019. This study was approved by the Animal Welfare and Ethics Committee of the Mansoura University, Mansoura, Egypt.

Clinical examination

Data concerning the case history, clinical findings, and medical records of each horse and donkey were also recorded. Ophthalmic history was obtained through a series of questions directed at the owners and farm personnel regarding observed clinical signs. A detailed clinical examination of the animals, including eye examination, was performed, and the clinical findings were recorded.

The ophthalmic examination procedures included general inspection of the eyes and detailed ocular examination. General inspection of the eyes included observation of animal vision and its response to visual stimuli, close inspection to reveal normal or abnormal eye movement, signs of ocular pain (i.e., blepharospasm, photophobia, epiphora), and the presence of ocular discharge or opacities. Detailed examinations included palpation of the boundaries of the orbit, abnormalities of the eyelids (movement, position, and thickness), and inspection of the anterior aspect of the nictitating membrane (third eyelid). The conjunctiva, cornea, eyeball movement, and deep structures of the eyes were thoroughly examined according to standard procedures (Hakanson and Merideth, 1987). The menace response was assessed from several different angles bilaterally in each animal by rapid movement of a hand within the horse's visual field, and care was taken to avoid creating wind currents or contact with eye lashes, which could lead to false-positive reactions (Radostits *et al.*, 2007).

Sample collection

A swab from the ocular conjunctiva was collected from the inferior conjunctival fornix of both eyes without touching the eyelashes or eyelids using a sterile swab from each animal under investigation for microbiological examination. All samples were collected before the animals received any type of local or systemic antibiotic or anesthetic (Ferreira *et al.*, 2017). The collected swabs were placed directly in sterile test tubes containing tryptone soya broth (Oxoid, Oxoid Ltd, 3rd Floor, 1 Ashley Road, Altrincham, Cheshire, England WA14 2DT) as enrichment medium and sent directly to the laboratory for further bacteriological examination.

Isolation and identification of bacterial species

Two loops were generated for each colony. The first loop was spread out on a *Pseudomonas* agar base (from Conda, Madrid) with some C.F.C. supplement thrown in, then we incubated it at 25°C for 48 h to specifically isolate *Pseudomonas* species. *Pseudomonas* isolates were confirmed using an API 20NE (BioMérieux, Marcy-l'Étoile, France). The second loop was placed on MacConkey's agar (Oxoid) and maintained at 37°C for another 24 h to detect *E. coli*. All these isolates were found to be *E. coli* and *Pseudomonas* species, which were identified by looking at their colony shapes and performing biochemical tests. All pale colonies were then moved to *Pseudomonas* agar base for isolation of these *Pseudomonas* species, and we performed a double-check motility test. In addition, we biochemically verified all *E. coli* isolates using the API 20E system and PCR.

DNA extraction and PCR amplifications

According to standard techniques using specific primer sequences for *E. coli* (16S rRNA) (Wang *et al.*, 2002), The complete DNA extraction and PCR amplifications of *E. coli* were carried out (Table 1).

Table 1. Primer sequences for *E. coli* (16S rRNA).

Gene	Sequence	Tm	Amplicon size	Reference
E. coli	F CCCCCTGGACGAAGACTGAC RACCGCTGGCAACAAAGGAT	58 °C	401 bp	Wang *et al*. (2002)

Antibiotic susceptibility testing

Antibiotic sensitivity was tested for all *Pseudomonas* spp. isolated using the Kirby-Bauer disc diffusion method (Ferreira *et al.*, 2017). The criteria for the choice of antimicrobial tests were based on the active principles most frequently used in ophthalmic routine for horses. Topical gentamicin and chloramphenicol are antibiotics used for the preventive treatment of corneal ulcers in horses (Johns *et al.*, 2011; Sauer *et al.*, 2003; Wada *et al.*, 2010).

Statistical analysis

SPSS for Windows (version 21.0) was used to analyze the data. The numerical data are shown as the median with a range, and for categorical data, we simply provided the numbers along with percentages. To check if there were any links between different risk factors, such as age, sex, breed, housing, season, hygiene, ground type, stress, and the isolation percentages of certain bacterial species from eye swabs, we ran a chi-squared test. For each variable, we noted the P-value, Odds ratio (OR), and 95% confidence interval (CI 95%). We considered anything with a p-value less than 0.05 as significant in all our analyses.

Results

In total, 100 animals were investigated (86 horses and 14 donkeys). Ophthalmic infections were confirmed in all 28 animals (25 horses and three donkeys). Regarding clinical signs, horses and donkeys with ophthalmic infection had conjunctival hyperemia (n=10, 35.7%), continuous lacrimation (n=11, 39.3%), blepharospasm (n=1, 3.6%), eyelid edema in 5/28 (17.9%), mucopurulent ocular discharge (n=9, 32.1%), corneal edema (n=1, 3.6%), and corneal opacity (n=5, 17.9%) (Table 2). There were no systemic signs in any animal, and the heart rate (29–37), respiratory rate (9 – 12), and rectal temperature (37 – 38°C) were within the normal ranges. Moreover, no behavioral abnormalities or signs of pain were observed in any animal.

Regarding the characteristics of *Pseudomonas* species isolated from the eyes of the studied horses, *Pseudomonas* species appeared light creamy to yellow on the surface of the *Pseudomonas* agar base. *E. coli* appeared as pink colonies on MacConkey agar because of lactose fermentation. *E. coli* by PCR, a band appeared at the expected target size of 401 bp (Figure 1).

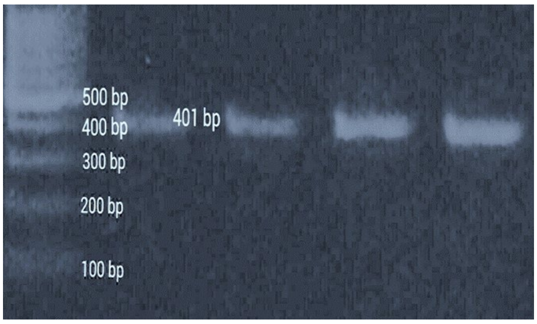

Figure 1. PCR for Detection of *E. coli* isolated from eyes of equines with ophthalmic infection, the band appeared at expected target size 401 bp.

Table 2. Frequency distribution of ocular signs in equine with ophthalmic disorders.

Clinical signs	Number of animals (percent %)
Continuous lacrimation	
Present	11 (39.3%)
Absent	17 (60.7%)
Conjunctival hyperemia	
Present	10 (35.7%)
Absent	18 (64.3%)
Mucopurulent discharge	
Present	9 (32.1%)
Absent	19 (67.9%)
Eyelid edema	
Present	5 (17.9%)
Absent	23 (82.1%)
Corneal opacity	
Present	5 (17.9%)
Absent	23 (82.1%)
Corneal edema	
Present	1 (3.6%)
Absent	27 (96.4%)
Blepharospasm	
Present	1 (3.6%)
Absent	27 (96.4%)

E. coli was mainly isolated from diseased horses (n = 13, 46.4%) and was not isolated from healthy horses. In addition, there was a significant increase in the number of mixed isolates isolated from diseased horses 17 (60.7%) compared to healthy ones 11 (39.3%) (Table 3).

Table 3. Frequency of isolated *Pseudomonas* spp. and *E. coli* from healthy and diseased equine eyes.

	Healthy (n = 72)	Diseased (n = 28)	Odds ratio 0.74	P-value 0.35	95 % CI 0.296 – 1.875
Pseudomonas species					
Positive	44 (61.1%)	19 (67.9%)			
Negative	28(38.9%)	9(32.1%)			
E. coli					
Positive	0 (0%)	13(46.4%)	-	0	0.109 – 0.273
Negative	72 (100%)	15(53.6%)			
Mixed					
Positive	14(19.4%)	17(60.7%)	0.16	0	0.060 – 0.407
Negative	58(80.6%)	11(39.3%)			

There was a significant association between *Pseudomonas* species and age. The highest percentage was recorded in horses < 5 years of age 36(57.1%) (Table 4).

Table 4. Risk factors of *Pseudomonas* species isolated from healthy and diseased equine eyes.

	Pseudomonas species		Odds ratio	P-value	95 % CI
	Negative	Positive			
Age					
<5	12(32.4%)	36(57.1%)	0.36	0.01	0.154 – 0.842
5-10	25(67.6%)	27(42.9%)			
Breed					
Arabian	4(10.8%)	1(1.6%)	7.52	0.06	0.807 – 70.004
Others	33(89.2%)	62(98.4%)			
Sex					
Male	12(32.4%)	12(19%)	2.04	0.10	0.803 – 5.182
Female	25(67.6%)	51(81%)			
Housing					
Indoor	14(37.8%)	15(23.8%)	1.95	0.10	0.806 – 4.704
Mixed	23(62.2%)	48(76.2%)			
Season					
Winter	32(86.5%)	51(81%)	1.51	0.34	0.485 – 4.676
Spring	5(13.5%)	12(19%)			
Hygiene					
Good	12(32.4%)	13(20.6%)	1.85	0.14	0.736 – 4.632
Bad	25(67.6%)	50(79.4%)			
Ground type					
Mud	11(29.7%)	9(14.3%)	2.54	0.06	0.936 – 6.883
Concrete	26(70.3%)	54(85.7%)			
Stress					
Individual	6(16.2%)	5(7.9%)	2.25	0.17	0.634 – 7.950
Over-crowding	31(83.8%)	58(92.1%)			

There was a significant association between season and the spread of ocular infection with *E. coli*, where 10 (76.9%) horses were infected in spring compared to three (23.1%) horses in the winter season (Table 5).

There was a significant association between the occurrence of mixed infections in the eyes and each season and the level of hygiene. The highest percentage of infection was recorded in winter (19, 61.3%) than in spring (12, 38.7%). However, regarding the level of hygiene, the highest percentage (87.1%) was recorded in horses with poor hygiene compared to those with good hygienic measures 4 (12.9%) (Table 6).

Pseudomonas isolates were sensitive to ampicillin (47.83%), tetracycline (39.13%), cephalosporin (47.83%), streptomycin (86.96%), ciprofloxacin (95.66%), chloramphenicol (91.3%), sulfamethoxazole (86.96%), and gentamycin (100%) (Table 7). All *E. coli* isolates were sensitive to ampicillin (53.85%), tetracycline (92.31%), cephalosporin (84.62%), streptomycin (76.92%), ciprofloxacin (92.31%), chloramphenicol (100%), sulfamethoxazole (92.31%), and gentamycin (100%) (Table 8).

Discussion

Horse eyes are some of the largest in the animal kingdom, giving them a bigger retinal surface and a better image magnification—50% more than humans. The shape of their eyeballs is more oval than that of primates, with the long part pointing straight out, in line with what they are looking at (Timney and Macuda, 2001). Thus, they have relatively

Table 5. Risk factors of *E. coli* isolated from healthy and diseased equine eyes.

	E. coli		Odds ratio	P-value	95 % CI
	Negative	Positive			
Age					
<5	39(44.8%)	9(69.2%)	0.36	0.09	0.103 – 1.262
5-10	48(55.2%)	4(30.8%)			
Breed					
Arabian	84(96.6%)	11(84.6%)	0.20	0.13	0.029 – 1.309
Others	3(3.4%)	2(15.4%)			
Sex					
Male	21(24.1%)	3(23.1%)	1.06	0.62	0.267 – 4.218
Female	66(75.9%)	10(76.9%)			
Housing					
Indoor	25(28.7%)	4(30.8%)	0.91	0.56	0.256 – 3.218
Mixed	62(71.3%)	9(69.2%)			
Season					
Winter	80(92%)	3(23.1%)	38.10	0	8.468 – 171.382
Spring	7(8%)	10(76.9%)			
Hygiene					
Good	23(26.4%)	2(15.4%)	1.98	0.32	0.407 – 9.598
Bad	64(73.6%)	11(84.6%)			
Ground type					
Mud	17(19.5%)	3(23.1%)	0.81	0.51	0.201 – 3.266
Concrete	70(80.5%)	10(76.9%)			
Stress					
Individual	8(9.2%)	3(23.1%)	0.34	0.15	0.077 – 1.484
Over-crowding	79(90.8%)	10(76.9%)			

Table 6. Risk factors of Mixed isolates isolated from healthy and diseased equine eyes.

	Mixed		Odds ratio	P-value	95 % CI
	Negative	Positive			
Age					
<5	36(52.2%)	12(38.7%)	1.73	0.15	0.728 – 4.096
5-10	33(47.8%)	19(61.3%)			
Breed					
Arabian	66(95.7%)	29(93.5%)	0.66	0.50	0.104 – 4.157
Others	3(4.3%)	2(6.5%)			
Sex					
Male	20(29%)	4(12.9%)	2.76	0.07	0.854 – 8.893
Female	49(71%)	27(87.1%)			
Housing					
Indoor	23(33.3%)	6(19.4%)	2.08	0.12	0.750 – 5.789
Mixed	46(66.7%)	25(80.6%)			
Season					
Winter	64(92.8%)	19(61.3%)	8.08	0	2.529 – 25.844
Spring	5(7.2%)	12(38.7%)			
Hygiene					
Good	21(30.4%)	4(12.9%)	2.95	0.05	0.918 – 9.502
Bad	48(69.6%)	27(87.1%)			
Ground type					
Mud	17(24.6%)	3(9.7%)	3.05	0.07	0.823 – 11.315
Concrete	52(75.4%)	28(90.3%)			
Stress					
Individual	7(10.1%)	4(12.9%)	0.76	0.46	0.206 – 2.822
Over-crowding	62(89.9%)	27(87.1%)			

Table 7. Antimicrobial susceptibility pattern for *Pseudomonas* species isolated from eyes of equine with ophthalmic infection.

Antimicrobial agent	Antimicrobial sensitivity for *Pseudomonas species*		
	Resistant	Intermediate	Sensitive
Ampicillin (10 µg)	3 (13.04%)	9 (39.13%)	11 (47.83%)
Tetracycline (30 µg)	0 (0%)	14 (60.87%)	9 (39.13%)
Cephalosporin (10 µg)	2 (8.7%)	10 (43.47%)	11 (47.83%)
Streptomycin (25 µg)	2 (8.7%)	1 (4.34%)	20 (86.96%)
Ciprofloxacin (5 µg)	1 (4.34%)	0 (0%)	22 (95.66%)
Chloramphenicol (30 µg)	2 (8.7%)	0 (0%)	21 (91.3%)
Sulfamethoxazole/trimethoprim (25 µg)	3 (13.04%)	0 (0%)	20 (86.96%)
Gentamicin (10 µg)	0 (0%)	0 (0%)	23 (100%)

Table 8. Antimicrobial susceptibility pattern for *E. coli* isolated from eyes of equine with ophthalmic infection.

Antimicrobial agent	Antimicrobial sensitivity for *E. coli*		
	Resistant	Intermediate	Sensitive
Ampicillin (10 µg)	2 (15.38%)	4 (30.77%)	7 (53.85%)
Tetracycline (30 µg)	1 (7.69%)	0 (0%)	12 (92.31%)
Cephalosporin (10 µg)	2 (15.38%)	0 (0%)	11 (84.62%)
Streptomycin (25 µg)	3 (23.08%)	0 (0%)	10 (76.92%)
Ciprofloxacin (5 µg)	1 (7.69%)	0 (0%)	12 (92.31%)
Chloramphenicol (30 µg)	0 (0%)	0 (0%)	13 (100%)
Sulfamethoxazole/trimethoprim (25 µg)	1 (7.69%)	0 (0%)	12 (92.31%)
Gentamicin (10 µg)	0 (0%)	0 (0%)	13 (100%)

large and prominent eyes that are susceptible to damage by straw or dirt from the environment, resulting in more frequent ocular infections than in other domestic animals (Ihrke *et al*., 1988). In the current study, clinical signs of conjunctivitis included mucopurulent ocular discharge, corneal opacity of the eye, and continuous lacrimation. In addition, signs of ocular pain, including epiphora, photophobia, and periocular hyperesthesia, were observed. It has been stated that when bacterial organisms are the primary infectious agents or, as in cases of secondary bacterial infections, mucopurulent material is the characteristic ocular discharge (Davidson, 1991). It occurs frequently in large animals and manifests as rudeness of the eyes with copious amounts of mucopurulent ocular exudate. The clinical signs recorded in horses with ocular infections were similar to those recorded previously (Sanchez *et al*., 2001; Edwards *et al*., 2015).

Changes associated with the microbiota, such as alterations in microbial biodiversity or dysregulation of host-microbe relationships, may increase the risk of disease (Gemensky-Metzler *et al*., 2005). Various potentially pathogenic bacterial species, such as *Staphylococcus*, *Pseudomonas*, and members of the Enterobacteriaceae family, including *E. coli*, along with fungal organisms, such as *Aspergillus* and *Penicillium*, are widely present in equine habitats. This prevalence in dust and plant materials contributes to an increased vulnerability to corneal infections in horses compared with other animal species (Andrew *et al*., 2003; Brooks *et al*., 2000; Cooper *et al*., 2001).

In this study, we found that 61.1% of the eye samples from healthy horses contained *Pseudomonas* species, which is quite high compared to what we have seen before. In Poland, only 2.3% of healthy Silesian foals and adult horses have these bacteria (Zak *et al*., 2018). In horses, there is a large difference in how often *Pseudomonas* species are found in the conjunctiva of the eye (Foti *et al*., 2013; Johns *et al*., 2011; Tamarzadeh and Araghi-Sooreh, 2014; Vidal *et al*., 2010). Differences in ambient temperature and management practices may explain these discrepancies (Johns *et al*., 2011; Pisani *et al*., 1997).

Pseudomonas species attach to the corneal epithelium using fimbriae, penetrate the epithelium, and migrate trans-cellular to reach the stroma (Lee *et al*., 2003; Sauer *et al*., 2003). *Pseudomonas* species release exotoxins, endotoxins, and proteinases that destroy corneal tissue. Furthermore, proteinases activated in the cornea and tear film by these microorganisms cause collagenolysis of the corneal stroma with concomitant corneal ulceration (Sauer *et al*., 2003). *Pseudomonas* species are metabolically versatile bacteria that can survive in soil, water, plants, animals, and human-associated habitats (Khan *et al*., 2007; Kidd *et al*., 2012; Pirnay *et al*., 2005). Therefore, the isolation percentage from the diseased eyes of the examined horses increased and reached 67.9%, as the resident microorganisms may become potentially pathogenic following increased virulence (Cogen *et al*., 2008; Willcox, 2013).

E.coli was not isolated from the eyes of healthy horses, indicating that it is a pathogenic microorganism, which is in agreement with previous reports previously (Moore *et al*., 1988). However, it was isolated in 24% of healthy horses following experimental application of topical antimicrobial or antimicrobial-corticosteroid ophthalmic preparations (Gemensky-Metzler *et al*., 2005). Pathogenic bacteria are ubiquitous in the environment; however, the low isolation rate of these organisms from healthy ocular surfaces suggests that normal flora may suppress the colonization of such pathogens (Tamarzadeh and Araghi-Sooreh, 2014). *E. coli* is naturally found in the intestinal tracts of many farm animals that shed these germs in their feces, contaminating the animals' skin, wool, and the areas and roams where they live. However, they still appear healthy and clean. Animals can appear healthy and clean but can spread *E. coli* to humans or other animals. Moreover, it may be disseminated through water and soil, where it can attach to itself and colonize plants (Feary and Hassel, 2006).

Regarding the response of *Pseudomonas* species to antibiotics, veterinary eye care literature shows many differences in resistance to aminoglycosides. Interestingly, the *Pseudomonas* samples did not show much resistance to aminoglycosides, and they were completely susceptible to gentamicin (100%), which is consistent with the findings of previous studies have found (Sweeney and Irby, 1996; Sauer *et al*., 2003). The use of topical antimicrobial drugs causes a shift in the total population of normal ocular bacterial flora (Whitley and Moore, 1984; Moore *et al*., 1995). In our study, just one isolate showed resistance to ciprofloxacin (95.66%). This is mainly because ciprofloxacin is not commonly used to treat ulcerative keratitis or conjunctivitis in animals (Keller and Hendrix, 2005).

Similar to previous UK surveys in 1982 (Williams *et al*., 1984), 1993 (Livermore and Chen, 1999), and 1999 (Henwood *et al*., 2001), the present investigation found that resistance was infrequent in *Pseudomonas* species. Similar studies conducted in Europe showed little variation in the rates of resistance observed in *Pseudomonas aeruginosa* (Fluit *et al*., 2000).

E. coli isolates exhibited the highest levels of resistance to several antibiotics, with a prevalence of 23.08%. In addition, resistance to ampicillin was noted at 15.38%, whereas tetracycline and sulfametoxazole each demonstrated a resistance rate of 7.69%. These findings may correlate with those of a study performed in southern Finland (Suojala *et al*., 2011). The writers mentioned that the most common resistance was to ampicillin (18.6%), streptomycin (16.4%), tetracycline (15.7%), and sulfamethoxazole (13.6%). In the current study, it appears that *E. coli* strains had the highest resistance rates against certain antibiotics, such as ampicillin, streptomycin, sulfonamide, and tetracycline, which have been used in horses for quite some time. However, gentamicin was not detected in southern Finland, as previously reported (Suojala *et al*., 2011).

Conclusion

The risk factors associated with both *E. coli* and *Pseudomonas* spp. and ophthalmic infections in horses revealed a multifaceted relationship between microbial pathogens and environmental influences.

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Conflict of interest

The authors have no conflict of interest to declare.

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