

Investigation of healthy horse eyes microbiota and evaluation of its antimicrobial susceptibility

Bahar Nayeri Fasaee¹, Hesameddin Akbarein², Sepideh Asadi², and Maryam shahbazi²

¹Affiliation not available

²University of Tehran

September 12, 2023

Abstract

Background: The conjunctiva of the eye is a mucosal surface that is colonized by various bacteria. Following surgeries, trauma, or other underlying conditions the normal flora of these bacteria may cause some eye infections such as conjunctival, corneal infections, or endophthalmitis. **Objectives:** This study aimed to investigate the bacterial population and antibiotic resistance of the conjunctiva in healthy horses. **Study design:** Random sampling of ocular samples from 20 horse during the winter (2019). **Methods:** Swab samples were collected from the eyes of 20 healthy horses of different breeds, ages, and sexes in Tehran province, Iran. The swabs were cultured on blood agar and MacConkey agar and incubated at 37°C for 24-48 hours. The isolated bacteria were characterized using cellular morphology, gram-staining, and biochemical tests. Sensitivity and resistance to 10 different antibiotics were measured using an antibiogram test. **Results:** Results showed that the bacterial population consisted of various species, with *Bacillus cereus* being the most frequent. There were no significant differences in the frequency of isolated bacteria between sexes and age groups. There were no significant differences in the sensitivity of bacterial flora of the eyes to Colistin, ceftiofur, florfenicol, amoxicillin, and ampicillin between neither different age groups nor different sexes of the horses ($P>0.05$). However, sensitivity to enrofloxacin and ciprofloxacin was significantly correlated with the age groups of the horses ($P>0.05$). Significant difference in sensitivity to trimethoprim-sulfamethoxazole between various sexes of horses was observed. **Main limitations:** The non-uniformity of the follow-up examination reliance owners or caregiver of the horse for follow-up information and Lack of adequate financial resources to study more horses. **Conclusions:** The study suggests that the frequency of bacterial flora in horses' eyes is influenced by housing and management conditions rather than age and sex. **Keywords:** Microbial flora, Conjunctiva, Eyes, Horse, Mucosal surface.

Investigation of healthy horse eyes microbiota and evaluation of its antimicrobial susceptibility

Bahar Nayeri Fasaee¹, Hesameddin Akbarein², Sepideh Asadi¹, Maryam shahbazi¹

¹Department of Microbiology and Immunology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran.

²Department of Food Hygiene and Quality Control, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran.

***Corresponding author:** Bahar Nayeri Fasaee, Department of Microbiology and Immunology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran. Tel: +989122270229, Tel: +982161117050, Fax: +982166933222, E-mail: Nayerib@ut.ac.ir

Abstract

Background : The conjunctiva of the eye is a mucosal surface that is colonized by various bacteria. Following surgeries, trauma, or other underlying conditions the normal flora of these bacteria may cause some eye infections such as conjunctival, corneal infections, or endophthalmitis.

Objectives : This study aimed to investigate the bacterial population and antibiotic resistance of the conjunctiva in healthy horses.

Study design : Random sampling of ocular samples from 20 horse during the winter (2019).

Methods : Swab samples were collected from the eyes of 20 healthy horses of different breeds, ages, and sexes in Tehran province, Iran. The swabs were cultured on blood agar and MacConkey agar and incubated at 37°C for 24-48 hours. The isolated bacteria were characterized using cellular morphology, gram-staining, and biochemical tests. Sensitivity and resistance to 10 different antibiotics were measured using an antibiogram test.

Results : Results showed that the bacterial population consisted of various species, with *Bacillus cereus* being the most frequent. There were no significant differences in the frequency of isolated bacteria between sexes and age groups. There were no significant differences in the sensitivity of bacterial flora of the eyes to Colistin, ceftiofur, florfenicol, amoxicillin, and ampicillin between neither different age groups nor different sexes of the horses ($P > 0.05$). However, sensitivity to enrofloxacin and ciprofloxacin was significantly correlated with the age groups of the horses ($P > 0.05$). Significant difference in sensitivity to trimethoprim-sulfamethoxazole between various sexes of horses was observed.

Main limitations : The non-uniformity of the follow-up examination reliance owners or caregiver of the horse for follow-up information and Lack of adequate financial resources to study more horses.

Conclusions : The study suggests that the frequency of bacterial flora in horses' eyes is influenced by housing and management conditions rather than age and sex.

Keywords: Microbial flora, Conjunctiva, Eyes, Horse, Mucosal surface.

Introduction

Eye surfaces are continually exposed to the environment, providing a habitat for different bacterial and fungal microorganisms. This microbiota contributes to the ocular defense mechanism by producing antibacterial substances that prevent colonization by pathogenic microorganisms (Chiang and Chern, 2022, LiYi and Wei, 2020). In physiological conditions, the surface of the eye is protected by several innate and adaptive immune systems, such as mucin and epithelium barrier functions, tear film that enables flushing of the ocular surface, antimicrobial tear components (lysozyme, beta-lysin, lactoferrin, blood cells, IgA), antigen-presenting cells and special antigen-recognition receptors called Toll-like receptors (Bolaños-Jiménez et al., 2015, Zak et al., 2018, SkopińskiKrawczyk and Ambroziak, 2013, Santibáñez et al., 2022). Eye infections are prevalent in horses, despite the protective mechanisms of the ocular surface. These infections are often caused by members of the conjunctival commensal microbiota that act as opportunistic pathogens after damage to the integrity of the ocular surface or surrounding areas (Santibáñez et al., 2022). The injured equine cornea may be more susceptible to infection due to the ubiquitous nature of bacteria and fungi in the outdoor or barn environment and the poor efficacy of host immune responses to prevent infection (Plummer, 2017). In horses, gram-positive bacteria are the predominant normal conjunctival flora, whereas gram-negative bacteria are isolated with greater frequency in eyes with extraocular disease (Zak et al., 2018). The most common gram-positive bacteria isolated from normal equine eyes are *Staphylococcus*, *Streptococcus*, *Corynebacterium*, and *Bacillus* spp. On the other hand, gram-negative bacteria such as *Escherichia coli*, *Pseudomonas*, *Moraxella*, *Acinetobacter*, and *Neisseria* spp are less frequently isolated from normal equine eyes. Fungal organisms such as *Cladosporium*, *Alternaria*, *Fusarium*, *Aspergillus*, and *Penicillium* spp have also been isolated from normal equine eyes (Hampson et al., 2019, Awosile et al., 2018). Several studies have shown the type of bacterial flora in the conjunctival sac may vary depending on the geographical location, the climate zone, age, and breed of the horses. Identifying the physiological commensal flora in the conjunctival sac is crucial for diagnosing inflammatory ocular disorders and selecting the most effective antibacterial treatment before obtaining antibiogram results (Johns et al., 2011). Risk factors for equine infectious keratitis include seasonal influences like weather, temperature, humidity as well as housing conditions, and use of topical corticosteroids and antimicrobials (MustikkaGrönthal and Pietilä, 2020, Tahoun et al., 2020). Bacterial resistance to certain

antimicrobials was found to be higher in isolates obtained after antimicrobial treatment compared to those obtained before treatment (Hashemi et al., 2020, Hashemizadeh et al., 2022). This study aimed to investigate the population of bacterial flora in the eyes of healthy adult horses in Iran and to assess their antibiotic resistance to prevent the emergence of further antibiotic resistance in horses. This study was also performed to identify any sex and age-related differences in bacterial populations and antibiotic resistance, which could be useful for developing targeted antibiotic therapies in the future.

Materials and methods

Animals

This study was conducted on 20 healthy horses with different breeds including Koninklijk Warmbloed Paardensamboek Nederland (KWPN), crossbreed, thoroughbred, and Friesian from different breeding centers located in Tehran province. Horses under study had neither clinical signs nor a history of eye disorders. No evidence of ocular inflammation or infection was observed during ophthalmic examinations conducted by a veterinarian. Examinations consisted of menace test and assessment of both eyeballs' location, size of both eyes, motility of both eyes, and the existence of any ocular discharge. No history of antimicrobials or antifungal drugs administration in 2 weeks before specimen collection was also confirmed. Horses were divided into three different age intervals including 1-5 years old, 5-10 years old, and older than 10 years old. Characteristics of animals are shown in Table 1.

Specimen collection

In the winter of 2019, eye specimens were collected from horses without local anesthesia. A sterile cotton swab moistened with saline solution was gently run along the surface of the conjunctiva adjacent to the medial canthus of both eyes. Special care was taken to avoid contamination of the swab by unwanted contact with nostrils, eyelids, and eyelashes. Individual swabs were used for each eye, and the swabs were placed in separate tubes containing Amies transfer medium. The tubes were stored at 4°C in refrigerator until they were transported to the laboratory for culture.

Bacterial culture

To assess the growth of aerobic bacteria, swab samples were cultured on blood agar as well as MacConkey agar, and also cultured in broth agar. The cultures were incubated at 37°C for 24-48 hours to allow sufficient time for bacterial growth. Bacterial colonies were isolated from 17 out of 20 horses. The grown bacterial colonies were separately cultured again in the aforementioned mediums. A total of 101 bacteria were isolated from the samples. The bacterial genera were identified based on cellular morphology, gram-staining, and biochemical tests, including the oxidation/fermentation (OF) test, catalase, oxidase, gelatinase, urease, and potassium hydroxide tests. In cases where necessary, the species of the bacteria were also identified using the same methods (Wang et al., 2008).

Antibiotic sensitivity test

After the identification of bacteria, cultured medium plates were used for antibiogram tests. Antibiotic discs of 10 effective antibiotics for horses highly utilized in similar studies, including Colistin, ceftiofur, florfenicol, amoxicillin, ampicillin, enrofloxacin, ciprofloxacin, trimethoprim-sulfamethoxazole, neomycin, and gentamycin were placed on the media using forceps. Plates' incubation was done at 37°C for 24-48 hours. Zones of growth inhibition were measured and recorded as sensitive or intermediate (Tahoun et al., 2022, Baran et al., 2015).

Statistical analysis

Statistical analysis was performed using SPSS version 26. Qualitative data was analyzed by chi-squared and Fisher's exact tests. For quantitative data, one-way ANOVA test was done. $P \leq 0.05$ was considered statistically significant.

Results

The frequency of bacteria isolated from ocular swabs and the correlation between the frequency of the isolated bacteria and either age or sex of the studied horses along with the correlation between antibiotic sensitivity and resistance of bacteria and sex of horses as well as age groups were statistically evaluated.

Isolated bacteria

The relative frequency percentage of the isolated bacteria from studied horses' eyes is indicated in Table 2 and Figure 1. The most frequently isolated bacterial was *Bacillus cereus* (24.75%) followed by *Micrococcus luteus* (11.88%), *Bacillus licheniformis* (10.89%), *Pasteurella multocida* (10.89%), *Bacillus mutans* (8.91%) *Staphylococcus* spp. (6.93%), *Staphylococcus epidermidis* (4.95%), alpha-hemolytic *streptococcus* species (4.95%), *Trueperella pyogenes* (3.96%), *Corynebacterium* spp. (3.96%), *Bacillus subtilis* (2.97%), *Corynebacterium pseudotuberculosis* (1.98%), *Listeria monocytogenes* (0.99%), *Actinomyces* spp. (0.99%), *Corynebacterium bovis* (0.99%) and *Mannheimia haemolytica* (0.99%). There were no significant differences between *Bacillus cereus*, *Bacillus licheniformis*, *Micrococcus luteus*, *Pasteurella multocida*, *Bacillus mutans*, *Staphylococcus* spp., *Bacillus subtilis*, alpha-hemolytic *streptococcus* species, *Trueperella pyogenes*, *Staphylococcus epidermidis*, and *Corynebacterium pseudotuberculosis* isolated from eyes of 17 horses using chi-squared and Fisher exact tests ($p > 0.05$). Statistical analysis of the frequency of *Listeria monocytogenes*, *Actinomyces* spp., *Mannheimia haemolytica*, and *Corynebacterium bovis* was not possible due to isolating each of them from one individual horse.

Antibiogram test

No significant correlation between neither different age groups nor different sexes of the horses and sensitivity of the eyes' bacterial flora to Colistin, ceftiofur, florfenicol, amoxicillin, and ampicillin was indicated using Chi-squared and Fisher exact statistical tests ($P > 0.05$) (Tables 3,4). However, unlike trimethoprim-sulfamethoxazole, significant differences in sensitivity to enrofloxacin, as well as ciprofloxacin between age groups of 1-5 and 5-10 along with 1-5 and older than 10, were indicated ($p < 0.05$) (Table 5). Among all utilized antibiotics, only differences in sensitivity to trimethoprim-sulfamethoxazole were significantly correlated with the sex of horses in which neutered horses and intact mares were significantly different ($p < 0.05$) while the difference between mare and stallion was insignificant (Table 6). As shown in Tables 5 and 6 since all isolated bacteria were sensitive to gentamicin and neomycin, statistical analysis was not possible.

Correlation between frequency of isolated bacteria and age

There were no significant differences in the frequency of *Bacillus cereus*, *Bacillus licheniformis*, *Micrococcus luteus*, *Pasteurella multocida*, *Corynebacterium* spp., *Bacillus mutans*, *Staphylococcus* spp., *Staphylococcus epidermidis*, alpha-hemolytic *streptococcus* species, *Bacillus subtilis*, *Trueperella pyogenes* and *Corynebacterium pseudotuberculosis* between different age groups using one-way ANOVA statistical test ($p > 0.05$).

Correlation between frequency of isolated bacteria and sex

Significant differences in the frequency of isolated bacteria between various sexes of the horses were not indicated ($p > 0.05$). Obviously, due to the isolation of each of *Listeria monocytogenes*, *Actinomyces* spp., *Mannheimia haemolytica*, and *Corynebacterium bovis* from only one individual horse, statistical analysis is impossible.

Discussion

The Conjunctiva of the eye is a mucosal surface which is colonized by different bacteria. Following surgeries, trauma, or other underlying conditions the normal flora of the conjunctiva may cause some kinds of infections in the eye including conjunctivitis, keratitis, or endophthalmitis (Sthapit and Tuladhar, 2014). *Staphylococcus*, *Streptococcus*, *Corynebacterium*, and *Bacillus* spp. are the most common gram-positive bacteria isolated from normal equine eyes (Hampson et al., 2019). Conjunctiva and cornea have a very strong unique immune system against infections which consists of tear, phagocytosis, mechanical barriers, and bacteriostatic functions of lysozyme (Sousa et al., 2011). Any agents including stress, dysfunctions of

the immune system, and systemic as well as traumatic diseases that cause an imbalance of the mentioned immune system can put the eye at risk of probable infections with internal and external origins(Rosa et al., 2003). The primary objective of this study was to determine the frequency of bacterial isolates from the eyes of healthy horses and to investigate any potential correlation between this frequency and the age or sex of the horses. Additionally, the study aimed to assess the sensitivity of the bacterial isolates. This study found that *Bacillus cereus* was the most frequently isolated bacteria from the conjunctiva of healthy horses, followed by *Micrococcus luteus* , *Bacillus licheniformis* , *Pasteurella multocida* , *Bacillus mutans* ,*Staphylococcus* spp., and others. Gram-positive bacteria were the most commonly found. In a study conducted by Zak et al, the majority of the isolated bacteria consisted of gram-positive bacteria besides the lack of significant effect of neither age nor sex on the frequency of the isolated bacteria resulted the same as the current study(Zak et al., 2018). Several previous studies have indicated that most frequent bacteria species are gram-positive(Johns et al., 2011, Gemensky-Metzler et al., 2005). Despite the similarities of the mentioned studies to the current one, the major isolated species were different from each other. As an assumption, this difference may be resulted by various climate and housing conditions, including the ingredient of the feed. However, in a study conducted by Johns et al, the effects of geographic location and type of housing (either housing in the field or stable) on bacteria isolation were not significant(Johns et al., 2011). It was also resulted from this study that sex and age have no significant effect on the frequency of bacteria isolation which is similar to the results of the current study (Johns et al., 2011). One of the reasons for the high frequency of bacterial flora in horse eyes is the mechanical transmission of microorganisms by insects contacting eyes which can be considered an important factor in determining the type of bacterial population of the conjunctiva. In a study conducted by Butler et al, isolated *Staphylococcus sciuri* from horse eyes was isolated from *Musca domestica* fly as well. This result proves the correctness of the aforementioned claim(Butler et al., 2010). Health condition is another reason for the variety in the bacterial flora of eyes as indicated in the current study that sensitivity to trimethoprim-sulfamethoxazole was significantly higher in neutered horses compared with intact mares which can be due to the better health conditions in housing neutered horses resulting in the lower bacterial population of neutered horses (total of 22 isolated bacteria) compared with intact ones (total of 26 isolated bacteria). Therefore, the lower the frequency of the isolated bacteria, the lower the amount of sensitivity to antibiotics. Araghi-Sooreh et al. (2013) in 2013 indicated 100% sensitivity of isolated bacteria to ciprofloxacin and florfenicol as well as 100% sensitivity of gram-negative bacteria to gentamycin. In the current study, 100% of isolated bacteria were sensitive to gentamycin and neomycin. In another study in Brazil, the bacterial flora of 100 healthy horses with no clinical disorders was assessed. Like the current study, the majority of isolated bacteria were gram-positive (65.5%). Sensitivity to antibiotics was also examined and 94%, 85%, 81%, and 68% of isolated bacteria were sensitive to ciprofloxacin, gentamycin, chloramphenicol, and tobramycin, respectively(Ferreira et al., 2017). In a study conducted by Hampson et al, bacteria and fungi of the normal flora of eyes in 95 horses were evaluated and the most frequent isolated bacteria were detected as gram-positive. Furthermore, most of the isolated bacteria were sensitive to neomycin and florfenicol(Hampson et al., 2019). In a study in Iran, Tamarzadeh et al studied bacterial flora of the eyes in 50 healthy mules with 5-12 years old of age using swab sample collection from conjunctiva as well as the sensitivity of the isolated bacteria to different 12 antibiotics. 85% of the isolated bacteria were gram-positive and the bacteria were mostly sensitive to florfenicol (90.82%) followed by flumequine (70.64%) and gentamycin (67.88%) (Tamarzadeh and Araghi-Sooreh, 2014).

In conclusion, this study aimed to evaluate the frequency of the bacterial flora in the conjunctiva of horses with different sex and age groups as well as their sensitivity to various antibiotics in order to prevent any further bacterial resistance to antibiotics by administration of antibiotics according to sex and age groups of the horses in future. Results of the current study indicated that the frequency of bacterial flora mainly depends on housing and management conditions while it was not significantly correlated with age and sex. Among 10 examined antibiotics, only sensitivity to ciprofloxacin and enrofloxacin was correlated with age which was only significantly different between age groups of 1-5 and 5-10 as well as 1-5 years old and older than 10. Sensitivity to ciprofloxacin and enrofloxacin was increased by age increment as no resistance to antibiotics was shown in horses older than 10 years of old which may be related to more administration of these drugs in young ages, and consequently altering bacterial population which results in resistance of

bacteria to the antibiotics in younger horses. In addition to previous results, the sensitivity of the isolated bacteria to trimethoprim-sulfamethoxazole in intact mares was significantly more than in neutered horses which may be due to the lower number of total isolated bacteria from neutered horses' eyes (total number of 22) compared with intact mares (total number of 26) probably because of better care and health conditions in neutered horses. It should be beard in mind that this result may be also related to the lower number of studied neutered horses (table 1).

Availability of data and materials

The data are available from the corresponding author upon request.

Consent for publication

There is no limit to the publication.

Competing interests

The authors declare that they have no competing interests

Funding

Funding for this study was provided by University of Tehran, Tehran, Iran.

Acknowledgements

The authors of the current study would like to express their deep thanks to the staff of University of Tehran.

References

- AWOSILE, B. B., HEIDER, L. C., SAAB, M. E. & MCCLURE, J. 2018. Antimicrobial resistance in bacteria isolated from horses from the Atlantic Provinces, Canada (1994 to 2013). *The Canadian Veterinary Journal*, 59, 951.
- BARAN, V., OZAYDIN, İ., GENC, O., OTLU, S., YAYLA, S., KILIC, E., ÇELEBİ, Ö., AYDIN, U. & CELIK, E. 2015. 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). *Kafkas Üniversitesi Veteriner Fakültesi Dergisi*, 21.
- BOLAÑOS-JIMÉNEZ, R., NAVAS, A., LÓPEZ-LIZÁRRAGA, E. P., DE RIBOT, F. M., PEÑA, A., GRAUE-HERNÁNDEZ, E. O. & GARFIAS, Y. 2015. Ocular surface as barrier of innate immunity. *The open ophthalmology journal*, 9, 49.
- BUTLER, J. F., GARCIA-MARUNIAK, A., MEEK, F. & MARUNIAK, J. E. 2010. Wild Florida house flies (*Musca domestica*) as carriers of pathogenic bacteria. *Florida Entomologist*, 93, 218-223.
- CHIANG, M.-C. & CHERN, E. 2022. Ocular surface microbiota: Ophthalmic infectious disease and probiotics. *Frontiers in Microbiology*, 13, 952473.
- FERREIRA, A. R. D. A., SANTANA, A. F., ALMEIDA, A. C. D. V. R. D., SOUSA, R. F., PERECMANIS, S. & GALERA, P. D. 2017. Bacterial culture and antibiotic sensitivity from the ocular conjunctiva of horses. *Ciência Rural*, 47.
- GEMENSKY-METZLER, A. J., WILKIE, D. A., KOWALSKI, J. J., SCHMALL, L. M., WILLIS, A. M. & YAMAGATA, M. 2005. Changes in bacterial and fungal ocular flora of clinically normal horses following experimental application of topical antimicrobial or antimicrobial-corticosteroid ophthalmic preparations. *American journal of veterinary research*, 66, 800-811.
- HAMPSON, E. C., GIBSON, J. S., BAROT, M., SHAPTER, F. M. & GREER, R. M. 2019. Identification of bacteria and fungi sampled from the conjunctival surface of normal horses in South-East Queensland, Australia. *Veterinary ophthalmology*, 22, 265-275.

- HASHEMI, B., AFKHAMI, H., KHALEDI, M., KIANI, M., BIALVAEI, A. Z., FATHI, J., SARLEY, H., DIVSALAR, S. & AHANJAN, M. 2020. Frequency of Metallo beta Lactamase genes, bla IMP1, INT 1 in *Acinetobacter baumannii* isolated from burn patients North of Iran. *Gene Reports*,21, 100800.
- HASHEMIZADEH, Z., HATAM, G., FATHI, J., AMINAZADEH, F., HOSSEINI-NAVE, H., HADADI, M., SHAKIB, N. H., KHOLDI, S. & BAZARGANI, A. 2022. The spread of insertion sequences element and transposons in carbapenem resistant *Acinetobacter baumannii* in a hospital setting in Southwestern Iran. *Infection & Chemotherapy*, 54, 275.
- JOHNS, I. C., BAXTER, K., BOOLER, H., HICKS, C. & MENZIES-GOW, N. 2011. Conjunctival bacterial and fungal flora in healthy horses in the UK. *Veterinary ophthalmology*, 14, 195-199.
- LI, J. J., YI, S. & WEI, L. 2020. Ocular microbiota and intraocular inflammation. *Frontiers in Immunology*, 11, 609765.
- MUSTIKKA, M. P., GRONTHAL, T. S. & PIETILA, E. M. 2020. Equine infectious keratitis in Finland: Associated microbial isolates and susceptibility profiles. *Veterinary ophthalmology*, 23,148-159.
- PLUMMER, C. E. 2017. Corneal response to injury and infection in the horse. *Veterinary Clinics: Equine Practice*, 33, 439-463.
- ROSA, M., CARDOZO, L. M., DA SILVA PEREIRA, J., BROOKS, D. E., MARTINS, A. L. B., FLORIDO, P. S. S. & STUSSI, J. S. P. 2003. Fungal flora of normal eyes of healthy horses from the State of Rio de Janeiro, Brazil. *Veterinary ophthalmology*, 6, 51-55.
- SANTIBANEZ, R., LARA, F., BARROS, T. M., MARDONES, E., CUADRA, F. & THOMSON, P. 2022. Ocular microbiome in a group of clinically healthy horses. *Animals*, 12, 943.
- SKOPIŃSKI, P., KRAWCZYK, P. & AMBROZIAK, A. M. 2013. Immunology of the ocular surface and contact lens wear: theoretical fundamentals. *Central European Journal of Immunology*, 38, 254-259.
- SOUSA, M. E. D., ARAÚJO, M. A. D. S., MOTA, R. A., PORTO, W. J. N., SOUZA, A. K. P., SANTOS, J. L. D. & SILVA, P. P. D. 2011. Fungal microbiota from ocular conjunctiva of clinically healthy horses belonging to the Military Police Cavalry of Alagoas. *Brazilian Journal of Microbiology*, 42, 1151-1155.
- STHAPIT, P. R. & TULADHAR, N. R. 2014. Conjunctival flora of normal human eye. *JSM Ophthalmol*, 2, 1021.
- TAHOUN, A., ELNAFARAWY, H. K., EL-SHARKAWY, H., RIZK, A. M., ALORABI, M., EL-SHEHAWI, A. M., YOUSSEF, M. A., IBRAHIM, H. M. & EL-KHODERY, S. 2022. The prevalence and molecular biology of staphylococcus aureus isolated from healthy and diseased equine eyes in Egypt. *Antibiotics*, 11, 221.
- TAHOUN, A., ELNAFARAWY, H. K., ELMAHALLAWY, E. K., ABDELHADY, A., RIZK, A. M., EL-SHARKAWY, H., YOUSSEF, M. A., EL-KHODERY, S. & IBRAHIM, H. M. 2020. Epidemiological and molecular investigation of ocular fungal infection in equine from Egypt. *Veterinary Sciences*, 7,130.
- TAMARZADEH, A. & ARAGHI-SOOREH, A. 2014. Bacterial flora of the conjunctiva in healthy mules (*Equus mulus*). *Revue de Médecine Vétérinaire*, 165, 334-337.
- WANG, L., PAN, Q., ZHANG, L., XUE, Q., CUI, J. & QI, C. 2008. Investigation of bacterial microorganisms in the conjunctival sac of clinically normal dogs and dogs with ulcerative keratitis in Beijing, China. *Veterinary ophthalmology*, 11, 145-149.
- ZAK, A., SIWINSKA, N., SLOWIKOWSKA, M., BOROWICZ, H., PLONECZKA-JANECZKO, K., CHORBINSKI, P. & NIEDZWIEDZ, A. 2018. Conjunctival aerobic bacterial flora in healthy Silesian foals and adult horses in Poland. *BMC veterinary research*, 14, 1-6.

Table 1. Characteristics of animals.

Table 2. Isolated bacteria and percent of their relative frequency ($p > 0.05$).

Table 3. Correlation between some antibiotics and age.

Table 4. Correlation between some antibiotics and sex

Table 5. Correlation between some antibiotics and age.

Table 6. Correlation between some antibiotics and sex.

Figure 1. Relative frequency percentage of isolated bacteria from studied horses

Hosted file

Figures.docx available at <https://authorea.com/users/663833/articles/665860-investigation-of-healthy-horse-eyes-microbiota-and-evaluation-of-its-antimicrobial-susceptibility>

Hosted file

Tables.docx available at <https://authorea.com/users/663833/articles/665860-investigation-of-healthy-horse-eyes-microbiota-and-evaluation-of-its-antimicrobial-susceptibility>