Shahid Bahonar University of Kerman

Veterinary and Comparative Biomedical Research

Original Research

Doi:10.22103/Vcbr.2024.23172.1004

Detection of Staphylococcus and *Escherichia coli* in the ocular microbiota of Persian cats

Y. Noorzadeh [1], G. Aftab [2], M. Razaghi Manesh [1], T. Ahmadi [2]*

[1]*Department of Clinical Sciences, Faculty of Veterinary Medicine, Shoushtar branch, Islamic Azad University, Shoushtar, Iran*

[2]*Department of Clinical Sciences, Faculty of Veterinary Medicine, Science and Research branch, Islamic Azad University, Tehran, Iran*

***Correspondence:**

Author email:
Aftab_ghazal@yahoo.com

Article history:

Received: 02 March 2024
Revised: 28 March 2024
Accepted: 29 April 2024
Published: 03 May 2024

Keywords:

Bacterial flora
Fungal flora
Ophthalmic flora
Persian cat

Abstract This study aimed to characterize the microbial and fungal flora in the eyes of 100 Persian cats and investigate their potential role in ocular health and disease in this breed. The composition and prevalence of microorganisms were determined using laboratory techniques such as culture and fungal isolation. The results revealed a diverse range of bacterial and fungal species present in the conjunctiva of Persian cats. Gram-positive bacteria, including *Staphylococcus spp., Streptococcus spp., and Corynebacterium spp.*, were commonly identified as commensals, suggesting a healthy ocular microbiome. However, certain bacterial species associated with ocular disease in other feline breeds were also found, such as *Pseudomonas spp*. The presence of pathogenic bacteria, including *Staphylococcus aureus* and *Escherichia coli*, highlights the potential role of specific species in the development and progression of ocular conditions in Persian cats. Fungal species, including *Aspergillus spp., Candida spp.,* and *Malassezia spp*., were also detected, emphasizing the need to consider fungal etiologies in the diagnosis and management of ocular diseases in this breed. This knowledge will contribute to improved veterinary ophthalmology practices and aid in the diagnosis and treatment of ocular conditions in Persian cats and other feline breeds.

Introduction

The ocular surface is a dynamic ecosystem that harbors a diverse array of microorganisms, including bacteria and fungi [1]. Understanding the composition and dynamics of the ocular microbiota is crucial for effective diagnosis and management of ocular diseases [2]. Persian cats, known for their distinctive ocular anatomy and predisposition to ophthalmic conditions, present a unique population to investigate the microbial and fungal flora within their ocular environment [3].

The ocular microbiota plays a critical role in maintaining ocular health by contributing to the production of antimicrobial peptides, inhibiting pathogenic colonization, and modulating immune responses. Alterations in the composition or balance of the ocular microbial community can lead to dysbiosis, resulting in ocular surface disorders such as conjunctivitis, keratitis, and corneal ulcers. Furthermore, certain microorganisms may act as opportunistic pathogens, causing severe and potentially sight-threatening ophthalmic conditions [4].

While previous studies have investigated the ocular microbiota in humans and various animal species, including dogs, rabbits, horses, and also DSH cats, limited information is available regarding the ophthalmic microbial and fungal flora in Persian cats [5-7]. Given their predisposition to ophthalmic diseases, it is imperative to gain insights into the microbial composition of their ocular surface, which may be distinct from other feline breeds [8].

This study aimed to characterize the ophthalmic microbial and fungal flora in 100 Persian cats, investigating the prevalence, diversity, and potential pathogenicity of these microorganisms. By elucidating the ocular microbial community in this breed, this research can contribute to the development of targeted therapeutic strategies and enhance our understanding of the etiology and progression of ophthalmic diseases in Persian cats.

Materials and Methods

Sample collection

Microbiologic culture samples were collected from both eyes in a random sequence, using gentle physical restraint. The collection took place 30 seconds after applying a single drop of topical anesthetic (Minims®, 0.4% oxybuprocaine hydrochloride, Bausch & Lomb UK Ltd, Surrey, England). To collect the samples, a sterile swab applicator was rolled over the surface of the cornea and the ventral conjunctival fornix, being careful not to touch the surrounding skin or hair. Cultures were promptly started right after the samples were collected.

This study involved the participation of 100 healthy Persian cats (200 eyes). The cats met specific inclusion criteria, which included being physically and ophthalmologically normal, having normal blood count and biochemistry profile, testing negative for feline herpes virus-1 through polymerase chain reaction (PCR), living indoors with constant access to food, and being the only animal in the household. Cats younger than 12 months or older than 36 months, cats with systemic or ocular diseases, and cats with obstructions in their nasolacrimal duct were excluded from the study.

.

Bacterial culture

Each swab was streaked on agar plates, including selective and non-selective plates such as blood agar, Mac-Conkey agar, and Chocolate agar. The plates were incubated at 37 °C for 24-48 hours, in both aerobic and anaerobic conditions as needed. After the incubation period, the plates were inspected for the presence of bacterial growth. Colonies with distinct characteristics were chosen for further analysis, which involved Gram staining and standard biochemical tests to identify the bacteria.

Fungal culture

Swabs were introduced onto Sabouraud Dextrose Agar (SDA) plates supplemented with chloramphenicol to prevent bacterial growth. The SDA plates were incubated at 25°C for a maximum of 7 days. Daily observations were made to check for the development of fungal growth on the plates. Fungal colonies were transferred to fresh SDA plates to obtain pure cultures.

The fungal isolates were identified by examining their colony morphology, using microscopic methods, and conducting standard biochemical tests like lactophenol cotton blue staining.

Statistical analysis

The prevalence and diversity of bacterial and fungal isolates were recorded. The identified bacterial species and fungal genera were tabulated. Descriptive statistics were used to summarize the data, including frequencies and percentages. Descriptive statistics were used to analyze the data, using SPSS 20.0 (SPSS Inc., Chicago, IL, USA).

Results

All animals were aged between 1 and 2 years, with 50 being female and 50 being male. None of the cats were neutered or castrated. No

statistically significant differences were found between gender and bacterial or fungal growth.

The study on the ophthalmic flora of Persian cats revealed a diverse range of microorganisms in their eyes. Both bacteria and fungi were identified within the ocular microbiota of these cats.

Among the bacteria, the most prevalent species were *Staphylococcus felis*, *Staphylococcus epidermidis*, and *Staphylococcus pseudintermedius*. These Staphylococcus species accounted for the highest number of isolates, indicating their common presence in the ophthalmic flora of Persian cats. Other gram-positive bacteria, including *Staphylococcus aureus*, *Staphylococcus hemolyticus, Staphylococcus hominis, Staphylococcus cohnii, Streptococcus agalactea, Streptococcus alpha-hemolyticus, Bacillus firmus,* and *Bacillus subtilis*, were also identified, albeit in smaller quantities (Figure 1).

In terms of gram-negative bacteria, Escherichia coli was the most prominent species. Other gram-negative bacteria identified included *Moraxella osloensis, Neisseria, Pasteurella, Acinetobacter johnsonii, Pseudomonas spp., Chlamydophila felis,* and *Mycoplasma* (Figure 2).

Moving on to the fungal species, our study identified *Aspergillus niger, Aspergillus brasiliensis, Chaetomium globosum, Fusarium, Candida,* and *Pichia guilliermondii* in the ophthalmic flora of Persian cats. These findings highlight the presence of various fungi in the ocular microbiota of these cats (Figure 3).

Discussion

The analysis of the microbial and fungal flora in the eyes of 100 Persian cats offers important insights into the types, prevalence, and potential harm caused by microorganisms related to ocular health and diseases in this breed. This study enhances our understanding of the ocular microbiome in Persian cats and can help in developing specific treatment strategies for managing eye conditions in this breed.

According to Athanasiou et al. (2018), common laboratory techniques for evaluating conjunctival samples include examining cytological preparations under a microscope,

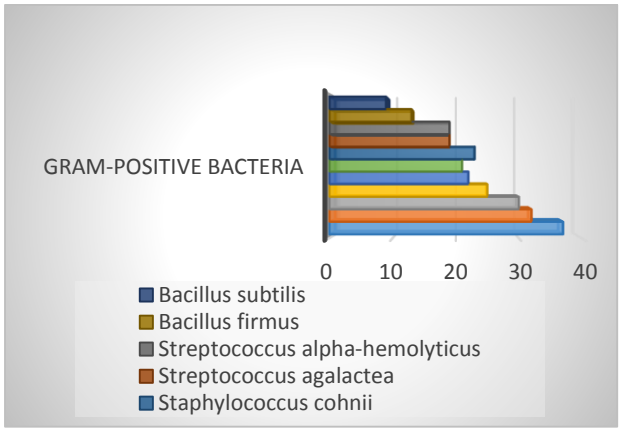

Fig 1. Gram-positive bacterial distribution in 200 feline eyes

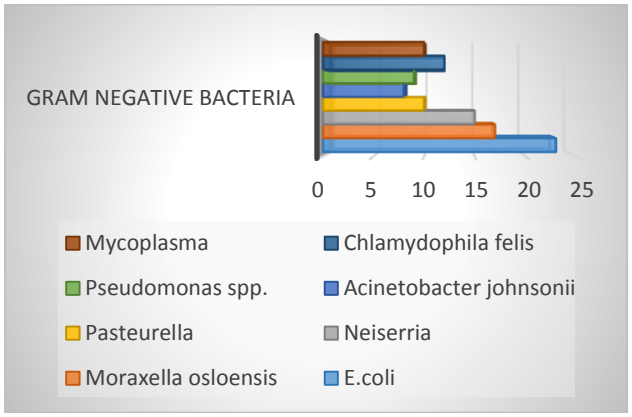

Fig 2. Gram-negative bacterial distribution in 200 feline eyes

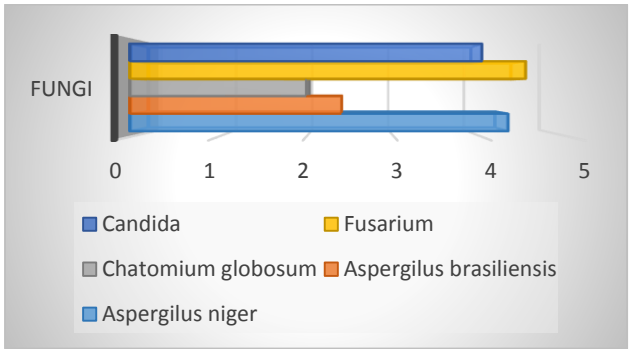

Fig 3. Fungal distribution in 200 feline eyes

conducting culture and susceptibility testing, isolating live viruses, using polymerase chain

reaction (PCR), performing direct immunofluorescent antigen tests, and conducting histopathological examination for snip biopsies. In the current study, the culture method was used [9].

Aftab et al. (2019) discovered that gram-positive bacteria were dominant in the ophthalmic flora of Persian cats throughout all seasons, although gram-negative bacteria were more prevalent during spring and summer. Since the current study took place in fall 2022, the season may have had less influence on the conjunctival microflora. In the current study, *Escherichia coli* was the most frequently identified gram-negative bacteria, consistent with previous studies on Persian cats. Aftab et al. (2019) reported a fall percentage of 10% for *Staphylococcus epidermidis*, whereas the current study detected it in nearly 30% of cases. The larger sample size of 200 eyes, compared to the 30 eyes from 15 Persian cats in the mentioned study, may have contributed to this difference [3].

The results of this study revealed a diverse range of bacterial and fungal species in the conjunctiva of Persian cats. Bacterial culture identified common ocular commensals such as *Staphylococcus spp., Streptococcus spp.,* and *Corynebacterium spp*., which aligns with previous studies in different feline, canine, exotic breeds, and human populations [10, 11]. The presence of these commensal bacteria suggests a healthy ocular microbiome, as they help maintain the balance of the ocular surface and prevent the colonization of pathogenic microorganisms[12].

Interestingly, certain bacterial species associated with ocular diseases in other feline breeds were also found in Persian cats. For example, *Pseudomonas spp*., an opportunistic pathogen linked to keratitis and corneal ulcers, was isolated in some cats [13]. This finding emphasizes the potential role of specific bacterial species in the development and progression of eye conditions in Persian cats. Further investigation is needed to understand the factors that contribute to the susceptibility of Persian cats to certain ocular pathogens.

Among the identified bacteria, several are known potential pathogens, including Staphylococcus aureus, a common cause of various infections in humans and animals, including ocular infections [14]. Other potential pathogens include *Escherichia coli, Moraxella osloensis, Neisseria, Pasteurella, Acinetobacter johnsonii, Pseudomonas spp., Chlamydophila felis,* and *Mycoplasma*. In a study by Arteaga et al., *Staphylococcus epidermidis* was the most frequently isolated bacteria, followed by β-hemolytic *Streptococcus spp*., *Corynebacterium spp., Staphylococcus aureus,* and *Escherichia coli* as the most common gram-negative bacteria in Persian cats during fall[3, 15].

Regarding fungal culture, various genera were identified, including *Aspergillus spp., Candida spp.,* and *Malassezia spp*. These findings are consistent with previous studies on feline and human ocular mycology. While some fungal species are considered commensals, others can cause opportunistic infections, especially in immunocompromised individuals or those with underlying ocular surface abnormalities. The identification of pathogenic fungal species, such as *Aspergillus spp*., highlights the importance of considering fungal causes in the diagnosis and management of ocular diseases in Persian cats. A study by Büttner et al. (2019) on the ophthalmic flora of 120 cats also found that gram-positive bacteria, particularly Staphylococcus species, were the most common conjunctival flora [16]. Fungal species were detected at a lower rate of 3%, indicating that the specific ocular structure of Persian cats may increase the likelihood of fungal growth. In the study by Arteaga et al. (2021), *Aspergillus spp*. was the most prevalent fungus identified, followed by Alternaria spp. and Cladosporidium spp. in Persian cats [15].

The findings of this study lay the groundwork for future research on the role of the ocular microbiota in feline ocular health and disease. Advanced molecular techniques, such as next-generation sequencing, could further enhance our understanding of the microbial composition and dynamics on the ocular surface of Persian cats. Additionally, investigating the interplay between the ocular microbiota and the host immune response in Persian cats may shed light on the mechanisms underlying the development and progression of ocular diseases

in this breed. Future studies could also benefit from incorporating molecular techniques to obtain a more comprehensive understanding of the ocular microbial and fungal flora in Persian cats.

Conclusion

In conclusion, the characterization of the microbial and fungal flora in the eyes of 100 Persian cats provides valuable insights into the composition and potential harm caused by microorganisms associated with ocular health and diseases in this breed. The identification of specific bacterial and fungal species linked to ocular diseases emphasizes the need for targeted therapeutic interventions in managing eye conditions in Persian cats. Further research on the ocular microbiota's role in feline ocular health and disease will contribute to advancements in veterinary ophthalmology, leading to improved diagnosis and management of ocular conditions in Persian cats and potentially other feline breeds.

Acknowledgements:

Not applicable

Conflict of interest

There is no conflict of interest.

Ethical approval

The study was conducted in accordance with ethical guidelines and regulations for animal research. The cats' welfare and comfort were prioritized throughout the study. Appropriate measures were taken to minimize stress and discomfort during sample collection. The study received approval from the Iran Society for Prevention of Cruelty to Animals, in accordance with the ethical guidelines for laboratory animal studies in Iran. Additionally, the study was conducted in compliance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

References

1. Baim AD, Movahedan A, Farooq AV, Skondra D. (2019) The microbiome and ophthalmic disease. Experimental Biology and Medicine, 244(6):419-29.
2. Aftab G, Arfaee F, Asghari A, Zahraei Salehi T. (2023) The evaluation of normal ocular parameters in two breeds of hedgehogs. Veterinary Medicine and Science, 9(2):738-43.
3. Aftab G, mehdi Rajaei S, Pot SA, Faghihi H. (2019) Seasonal effects on the corneoconjunctival microflora in a population of Persian cats in Iran. Topics in companion animal medicine, 34:30-2.
4. Petrillo F, Petrillo A, Marrapodi M, Capristo C, Gicchino MF, Montaldo P, et al. (2022) Characterization and comparison of ocular surface microbiome in newborns. Microorganisms, 10(7):1390.
5. Cooper S, McLellan G, Rycroft A. (2021) Conjunctival flora observed in 70 healthy domestic rabbits (Oryctolagus cuniculus). Veterinary Record, 149(8):232-5.
6. Samuelson D, Brooks D. Small animal ophthalmology: Self-assessment color review: CRC Press; 2011.
7. Meekins JM, Eshar D, Rankin AJ. (2015) Tear production, intraocular pressure, and conjunctival bacterial flora in a group of captive black-tailed prairie dogs (C ynomys ludovicianus). Veterinary Ophthalmology., 18:132-6.
8. Mirshahi A, Shafigh S, Azizzadeh M. (2014) Ultrasonographic biometry of the normal eye of the Persian cat. Australian veterinary journal, 92(7):246-9.
9. Athanasiou L, Psemmas D, Papaioannou N. (2018) Conjunctival cytology assessment in dogs and cats. Sampling, diagnostic techniques and findings. Journal of the Hellenic Veterinary Medical Society, 69(1):701-10.
10. Aslan O, Teberlk K, Yucel M, Gur N, Karakoc A. (2008) Effect of topical netilmicin on the reduction of bacterial flora on the human conjunctiva. European journal of ophthalmology, 18(4):512-6.
11. Whitley RD. (2000) Canine and feline primary ocular bacterial infections. Veterinary Clinics of North America: Small Animal Practice, 30(5):1151-67.
12. Gerding PA, Kakoma I. (1990) Microbiology of the canine and feline eye. Veterinary Clinics of

North America: Small Animal Practice, 20(3):615-25.
13. Elmenshawy YM, Ali KM, Samir A. (2021) Current Evidence of Coryneform Bacteria on The Ocular Surface of Immunocompromised Cats. Journal of Applied Veterinary Sciences, 6(3):86-93.
14. Teweldemedhin M, Gebreyesus H, Atsbaha AH, Asgedom SW, Saravanan M. (2017) Bacterial profile of ocular infections: a systematic review. BMC ophthalmology, 17(1):1-9.
15. Arteaga K, Aftab G, Rajaei SM, Faghihi H, Crasta M. (2021) Comparison of conjunctival microbiota of clinically normal Persian cats with and without nasolacrimal duct obstruction. Veterinary Ophthalmology, 24(5):455-9.
16. Büttner JN, Schneider M, Csokai J, Müller E, Eule JC. (2019) Microbiota of the conjunctival sac of 120 healthy cats. Veterinary ophthalmology, 22(3):328-36.

How to cite this article:
Noorzadeh, Y., Aftab, G., Razaghi Manesh, M., Ahmadi, T. Detection of Staphylococcus and Escherichia coli in the ocular microbiota of Persian cats. Veterinary and Comparative Biomedical Research, 2024, 1(1): 33 – 38. http://doi.org/10.22103/Vcbr.2024.23172.1004