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Microbiota of ocular and peri-ocular areas in Blanford fox (*Vulpes cana*) and Pallas cat (*Felis manul*)

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Abstract The ocular microbiota of healthy and diseased wild mammals remains relatively understudied, particularly within the Canidae and Felidae families. This study aimed to investigate the microbial flora present on the ocular and peri-ocular regions of Blanford fox (*Vulpes cana*) and Pallas cat (*Felis manul*), two wild species commonly found in zoos. A total of five Pallas cats and five Blanford foxes were included in the study, undergoing anesthesia for routine physical examinations. Ophthalmic examinations were conducted, followed by bacterial and fungal culture sampling from the ocular surface and peri-ocular skin. The results revealed a diverse range of bacterial and fungal species, with *Staphylococcus epidermidis* being the most commonly isolated bacteria. Additionally, *Aspergillus spp.* and *Penicillium spp*. were found in the peri-ocular skin of Pallas cats. No Gram-negative bacteria or fungal organisms were isolated from the ocular surface. This study provides valuable insights into the normal commensal flora of these wild mammals, serving as a reference for future comparisons with diseased states and enhancing our understanding of ocular microbiota in these species.

Introduction

A diverse range of bacteria and fungi can be found as commensal or transient organisms on the ocular surface of healthy animals [1, 2]. These commensal organisms play a crucial role in maintaining a balanced environment and preventing the colonization of pathogenic organisms on the ocular surface [3, 4]. However, there are few investigations available on the ocular microbiota of healthy and diseased wild mammals. Previous studies have primarily focused on the conjunctival microbiota of various wild species under managed care, such as *Capra ibex, Odocoileus hemionus, Mazama gouazoubira, Alces alces,* and *Rhinoceros unicornis*. However, there is a lack of research specifically examining the ocular microbiota of wild members of the Canidae and Felidae families [5, 6].

Understanding the normal microorganisms present in the conjunctiva is essential for identifying potential pathogenic bacteria and fungi that may contribute to ocular

diseases in these animals. Blanford fox (*Vulpes cana*) and Pallas cat (*Felis manul*) are wild species belonging to the Canidae and Felidae families, respectively, and are commonly found in zoos under managed care. However, there is limited published research on the ocular tissue of these animals, with only a few studies focusing on topics such as normal intraocular pressure in the healthy Pallas cat (*Felis manul*) and intrascapular cataract extraction in a captive *Felis manul*. To fill this knowledge gap, this study aims to determine the microbiota present on the ocular and peri-ocular regions of Blanford fox (*Vulpes cana*) and *Felis manul*, providing valuable insights into the normal commensal flora and serving as a reference for future comparisons with diseased states in these species.

Materials and Methods

This study was conducted following the guidelines set forth by the Association for Research in Vision and Ophthalmology for the Use of Animals in Ophthalmic and Vision Research. A total of five Pallas cats (*Felis manul*) (10 eyes) and five Blanford foxes (*Vulpes cana*) (10 eyes) undergoing anesthesia for routine physical examinations were included in the study. All animals were kept under managed care in Tehran, Iran.

The Blanford fox (*Vulpes cana*) was anesthetized using an intramuscular injection of medetomidine (0.1 mg/kg) and ketamine (6 mg/kg). The Pallas cat (*Felis manul*) was anesthetized with an intramuscular injection of medetomidine (0.05 mg/kg) and ketamine (5 mg/kg). Oxygen supplementation (1-2 L/min) was provided to each animal via a face mask, and a heating pad was used to maintain their body temperature. A comprehensive ophthalmic examination was performed on all animals, including Schirmer tear test I, fluorescein staining, Jones test, slit lamp biomicroscopy, and direct and indirect ophthalmoscopy. These examinations were carried out using specific equipment and materials.

For bacterial and fungal cultures, samples were obtained from the ocular surface using sterile swabs. Two swabs were used, one for bacterial culture and one for fungal culture, and they were obtained from the cornea and lower conjunctival fornix. Care was taken to avoid contact with the peri-ocular skin and eyelashes to prevent contamination. Bacterial samples were also obtained from the peri-ocular skin using sterile swabs, while plucked peri-ocular hair was used for fungal culture in both species.

No local anesthesia was used during sampling, and it was performed before fluorescein staining of the cornea. The swabs were immediately transferred in phosphate-buffered saline for transport to the laboratory. Each ocular sample (per swab) was cultured on two Mac Conkey agar plates, two blood agar plates, and two Sabouraud dextrose agar (SDA) plates. One Mac Conkey agar plate and one blood agar plate were placed in the aerobic condition at 37 ºC for 48 hours, while the remaining plates were incubated at 37 ºC with 5 % CO2. Cultures were examined after 24 hours, and further isolated subcultures were incubated as described above. Standard biochemical tests were performed for the identification of the isolated organisms. Bacterial growth quantification was not performed in this study [7]. Sabouraud dextrose agar (SDA) was incubated at 30-35 ºC for 3-5 days, and then subcultured in SDA and incubated at 30-35 ºC for 24-48 hours.

Polymerase chain reaction (PCR) was performed using the QIAamp DNA Minikit (QIAGEN, Hilden, Germany) to extract bacterial DNA from each ocular surface swab, following the instructions provided by the manufacturer. This PCR method was used to identify *Mycoplasma spp.* and *Chlamydia spp.* Descriptive statistics were performed to analyze the data obtained from these analyses [7].

Results

In the study, 10 bacterial and 10 fungal cultures were obtained from the ocular and peri-ocular surfaces of Blanford fox (*Vulpes cana*) and Pallas cat (*Felis manul*). The results showed that bacterial cultures from the ocular surface were positive for bacterial growth in all the eyes of the Blanford fox (*Vulpes cana*) (100%) and 8 out of 10 eyes of the Pallas cat (*Felis manul*) (81%). The

most commonly isolated bacteria from the ocular surface of Blanford fox (*Vulpes cana*) were *Staphylococcus epidermidis* (48%) and *Staphylococcus aureus* (31%). *Staphylococcus epidermidis* was also the most commonly isolated bacteria from the peri-ocular skin of Blanford fox (*Vulpes cana*) (100%). In Pallas cat (*Felis manul*), *Staphylococcus epidermidis* was the most commonly isolated bacteria from the ocular surface (62%) and peri-ocular skin (38%). *Aspergillus spp.* and *Penicillium spp*. were isolated from the peri-ocular skin of Pallas cat (*Felis manul*). No Gram-negative bacteria or fungal organisms were isolated from the ocular surface of the 20 eyes examined, and there were no notable differences observed in bacterial cultures within animals from the same species. The study provides insights into the microbial flora of the ocular and peri-ocular surfaces of Blanford fox (*Vulpes cana*) and Pallas cat (*Felis manul*), highlighting the prevalence of specific bacterial and fungal species in these animals.

Discussion

In the ocular surface of healthy animals, a diverse array of bacteria and fungi coexist as commensal or transient organisms. These microorganisms include *Staphylococcus spp., Corynebacterium spp., Streptococcus spp., Bacillus spp., Pseudomonas spp., Chlamydophila felis, Mycoplasma spp., Penicillium spp.,* and *Cladosporium spp*. In domestic dogs, the predominant ocular microbial and fungal flora consists of *Staphylococcus spp., Corynebacterium spp., Penicillium spp.,* and *Cladosporium spp. Similarly*, in the Blanford fox (*Vulpes cana*) and Pallas cat (*Felis manul*), the dominant ocular microbiota is also composed of Gram-positive bacteria [8-10].

In this study, *Staphylococcus epidermidis* was the most commonly isolated organism from Pallas cat (*Felis manul*), similar to domestic cats [11]. However, the Pallas cat (*Felis manul*) also had cultures of *Micrococcus spp*., which is not commonly found in domestic felids. Chlamydophila felis, commonly seen in domestic felids, was not found in the Pallas cat (*Felis manul*) in this study. In domestic species, the intact Corne-conjunctival epithelium and normal anatomical features, such as blinking and lacrimal flow, help prevent the colonization of Gram-negative bacteria and fungi [8, 11-13]. This may explain the absence of Gram-negative bacteria and fungal organisms isolated from the ocular surface of the Blanford fox (*Vulpes cana*) and Pallas cat (*Felis manul*) in this study.

Table 1. Ocular and peri-ocular microbial composition in Blanford fox and Pallas cat

Microorganism	**Host**			
	Blanford fox (*Vulpes cana*)		**Pallas cat (*Felis manul*)**	
	Ocular (%)	**Peri-ocular (%)**	**Ocular (%)**	**Peri-ocular (%)**
S. epidermidis	48	100	62	38
S. aureus	31	23	11	1
***Aspergillus* spp.**	11	23	0	1
***Penicillium* spp.**	21	10	0	0

Previous reports have shown that fluorescein stain can inhibit bacterial growth and alter PCR test results, which is why fluorescein staining was done after microbiological sampling [14]. The normal microbial and fungal flora of ocular surfaces can vary depending on species, age, geographical location, season, environment, and climate [7, 15-19]. Evaluating ocular microbiota in wildlife species is challenging due to the limited number of animals kept in captivity in each location and the need for sedation during microbial sampling [7, 16, 20]. Further multicenter studies in different geographical locations are needed to determine if there are any differences

in ocular microbiota among different parts of the world [9, 16, 20].

Conclusion

In conclusion, this study found similar Gram-positive bacterial flora on the ocular surface and peri-ocular skin of both Blanford fox (*Vulpes cana*) and Pallas cat (*Felis manul*), with no Gram-negative bacteria present. The samples collected from the peri-ocular skin were positive for fungi in both animal species, while no fungi were isolated from the ocular surface in these animals. The small sample size and the absence of colony count in the microbiological assessment of the ocular surface are limitations of this study.

Acknowledgements:

Not applicable

Conflict of interest

There is no conflict of interest.

Ethical approval

The study was conducted in compliance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

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