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ORIGINAL ARTICLE/ARTICLE ORIGINAL

Conjunctival and cutaneous fungal flora in clinically normal dogs in Southern France



La flore fongique conjonctivale et cutanée chez des chiens sains vivant dans le sud de la France

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Summary The objectives of the present study were to describe the fungal flora of the conjunctiva in clinically normal dogs in southern France and to compare the fungal flora from the conjunctiva and that from adjacent skin areas (medial canthus and nose). Fifty dogs aged from 5 months to 13 years were investigated for conjunctival fungal flora. Specimens were obtained from both conjunctivae and the skin of the medial canthus and from the surface of the nose using sterile cotton-wool swabs and a piece of sterile carpet, respectively. Identification of fungal colonies was achieved recording the genus level on the basis of macro- and microscopic features. Fourteen fungal genera (most frequently *Alternaria*, *Cladosporium*, *Penicillium* and *Aspergillus*) were isolated from the conjunctival fornix of 7 dogs (14%). There was no significant effect of sex or age on frequency of fungal isolation. Eleven dogs (22%) had a positive mycological culture from at least one eye. Fungi found in the conjunctival fornix of dogs might represent transient seeding from the environment, as suspected in other animal species. The presence of conjunctival fungal organisms was correlated to the presence of fungi on the skin.

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Résumé Les objectifs de cette étude étaient d'identifier la flore fongique conjonctivale de chiens sains vivant dans le sud de la France et de la comparer à la flore cutanée des zones proches de l'œil : canthus médial et chanfrein. Cinquante chiens âgés de 5 mois à 13 ans ont été examinés. Les échantillons ont été prélevés dans les deux culs-de-sac conjonctivaux au moyen d'écouvillons stériles et sur la surface cutanée du canthus médial et du chanfrein au moyen de carrés de moquette stérile. L'identification (à l'échelle du genre) a été réalisée en tenant compte de l'aspect des colonies et des caractéristiques microscopiques des éléments fongiques

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(aspect des conidies, mode de conidiogenèse...). Quatorze genres (plus fréquemment *Alternaria*, *Cladosporium*, *Penicillium* et *Aspergillus*) ont été isolés des culs-de-sac conjonctivaux de sept chiens (soient 14%). Il n'y a pas de différences significatives de la fréquence d'isolement des champignons en fonction du sexe ou de l'âge. Pour 11 chiens (soient 22%), une culture mycologique positive a été obtenue sur au moins un œil. Les organismes fongiques présents dans le cul-de-sac conjonctival pourraient représenter une flore passagère issue de l'environnement, comme suggéré pour d'autres espèces animales, notamment des herbivores. La présence d'organismes fongiques dans le cul-de-sac conjonctival semblait corrélée à la présence de champignons sur la peau à proximité.

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Introduction

Presence and interplay of microbial flora at the ocular surface reveal dynamic interactions with implications for both ocular surface health and disease. Information in this area is scarce or nonexistent in most animal species. Several surveys of the bacterial flora of conjunctival diseases in dogs have been reported but few reports on normal fungal flora have been published so far [6,10,15]. Urban et al. (1972) and McDonald & Watson (1976) collected samples from only one eye of 100 and 135 dogs, respectively, to determine the microbial flora of normal canine conjunctivae but the presence of fungi was not mentioned [11–16]. Gerding (1993) produced a survey of 50 dogs and fungal microorganisms represented only 4.2% of all organisms isolated from the conjunctivae [5]. Ledbetter (2004) showed that preoperative conjunctival and eyelid margin swabs produced positive cultures for at least one fungal organism in 13/22 eyes (59%) [9]. In the survey conducted by Samuelson (1984), conjunctival swab specimens were obtained from both eyes of 43 horses, 25 cows, 50 dogs, and 25 cats without keratitis or other ophthalmologic problems. Fungi were isolated from 95% of the horses, 100% of the cows, 22% of the dogs, and 40% of the cats. *Aspergillus* spp were isolated from 56% of the horses, 12% of the cows, 8% of the cats, and none of the dogs. *Penicillium* spp and *Cladosporium* spp were isolated ubiquitously [15].

The objectives of the present study are to evaluate the conjunctival fungal flora in healthy dogs in southern France and to compare the fungal flora from the conjunctivae with that from adjacent skin areas (medial skin canthus and nose).

Material and methods

Sample population

Between December 2011 and March 2012, 50 privately-owned dogs, which were presented for consultation to the veterinary clinic of Le Muy (south east of France), were included in the present study. Age, gender, breed and living environment were recorded. On the day of sampling, each dog received a thorough clinical examination to rule out signs of disease. The dogs were required to have a normal ophthalmic examination including slit lamp biomicroscopy and indirect ophthalmoscopy by a veterinary ophthalmologist, to be candidates to the study and when the owners accepted to participate. Animals with a recent history of systemic illness or administration of antimicrobial agents whatever the route of administration (injection, pills or eye drops) were

excluded from the study. Many healthy animals were coming to the clinic for vaccination or convenience surgery.

All animals were living in houses with gardens or had access to outside.

Specimen collection

Specimens were obtained from both conjunctivae skin of medial canthus and from the surface of the nose using sterile cotton-wool swabs and a small square of sterile carpet, respectively.

Sabouraud's dextrose agar supplemented with Chloramphenicol (0.05%) was used for fungal isolation in Petri dishes. Plates were incubated at 32 °C for 21 days and examined daily. Filamentous fungi were identified to the genus level by microscopic examination of a wet mount from fungal colonies, using lactophenol cotton blue stain. Fungi, which could not be initially identified because they lacked identifying characters, were subcultured on Sabouraud's dextrose agar and observed for an additional 21 days. Fungi that did not produce any conidia or fruiting structures after subculture were listed as "nonsporulated fungi". Yeasts were identified by microscopic examination and on the basis of urease production and carbohydrate assimilation patterns. The positive identification of a fungal species was defined as the isolation of at least one colony from at least one sampling site.

Statistical analysis

To assess the potential association between mycological results from different sites (right versus left site, eye versus medial canthus...), the Fisher's exact test (presence/absence), the Spearman's rank correlation coefficient test (number of colonies) and the Student's *t*-test (age of the animals versus recovery of fungal colonies) are used. The statistical significance threshold is 0.05.

Results

One hundred eyes from 50 dogs were included in the study. The age of the animals range from 5 months to 13 years with a mean age of 4.8 years. Twenty-nine dogs were females, 22 were males. Twenty-two breeds included Rhodesian ridgeback ($n = 12$), French bulldog ($n = 4$), Visla ($n = 3$), Shih Tzu ($n = 3$), Dachshund ($n = 3$), Cocker ($n = 2$) Spaniel ($n = 2$), German shepherd ($n = 2$), mixed breed ($n = 2$), Labrador Retriever ($n = 2$), Yorkshire Terrier ($n = 2$), Jack Russell

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