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Bacteriological Profiles of Conjunctivitis in Neonatal and Juvenile Cats: A Comparative Analysis

Abstract

Conjunctivitis is an inflammatory condition of the conjunctiva caused by infectious or noninfectious factors that disrupt or stimulate the local defense system. It is commonly observed in animals of all ages, with bacterial pathogens from the normal microbiota or external sources being the primary cause. This study investigates bacterial alterations in the conjunctival microbiota of neonatal and juvenile cats (under 1 year) presenting with conjunctivitis and evaluates the influence of animal-related and environmental factors using various statistical methods. Fifty cats were included: 25 neonates and 25 juveniles of mixed breeds and sexes. Each cat underwent a detailed anamnesis and ophthalmic examination, including direct ophthalmoscopy, slit-lamp biomicroscopy, Schirmer test, and fluorescein staining. Conjunctivitis severity was scored from 0 to 4. Conjunctival swabs were cultured, and bacterial identification was performed. Neonates showed more severe clinical signs and significantly higher bacterial growth ($P<0.05$). Staphylococcus spp. and Pseudomonas spp. were isolated in juveniles, while neonates exhibited a broader bacterial spectrum, including Staphylococcus spp., Streptococcus spp., Pasteurella spp., Corynebacterium spp., and Actinobacillus spp. Age was the most significant factor influencing bacterial growth. The findings suggest that neonates are more susceptible to conjunctival bacterial microbiota, likely due to immature immune defenses. This study highlights the need for age-specific considerations in the diagnosis and management of feline conjunctivitis.

Keywords: Bacteria, feline conjunctivitis, microbiota, pathogens

Yeni Doğan ve Genç Kedilerde Konjunktivitis'in Bakteriyolojik Profilleri: Karşılaştırmalı Bir Analiz

Öz

Konjunktivitis, enfeksiyöz ya da enfeksiyöz olmayan etkenlerin konjunktival savunma sistemini bozması veya uyarması sonucu gelişen inflamasyondur. Farklı hayvan türlerinde ve yaş gruplarında yaygın olarak görülür. En sık nedeni, doğal mikrobiyotada bulunan ya da çevreden edinilen bakteriyel patojenlerdir. Bu çalışmada, klinik olarak konjunktivitis belirtileri gösteren neonatal ve juvenil (1 yaş altı) kedilerin konjunktival mikrobiyotasındaki bakteriyel değişimlerin değerlendirilmesi amaçlanmıştır. Ayrıca, hayvana ve çevreye bağlı etkenlerin bu yapıya etkisi çoklu istatistiksel yöntemlerle analiz edilmiştir. Oftalmoloji kliniğine getirilen, farklı ırk ve cinsiyette toplam 50 kedi (neonatal n= 25, juvenil n= 25) çalışmaya dahil edilmiştir. Her bireyden anamnez alınmış; oftalmoskopi, slit-lamp biyomikroskopi, Schirmer testi ve fluorescein boyama ile muayeneleri yapılmıştır. Konjunktivitis şiddeti 0-4 arası skorlanmış, konjunktival sürüntü örneklerinden bakteri kültürü ve tanımlaması gerçekleştirilmiştir. Neonatal kedilerde klinik belirtiler daha şiddetli olup, bakteri üreme oranı anlamlı şekilde daha yüksektir ($P<0.05$).

Juvenil kedilerde Staphylococcus spp. ve Pseudomonas spp. izole edilirken; neonatal grupta Staphylococcus spp., Streptococcus spp., Pasteurella spp., Corynebacterium spp. ve Actinobacillus spp. tespit edilmiştir. Sonuç olarak, konjunktivitisli kedilerde yaşa bağlı olarak konjunktival bakteriyel mikrobiyota anlamlı düzeyde farklılık göstermektedir. Neonatal kedilerdeki yüksek üreme oranı, immun sistemin yeterince olgunlaşmamış olmasına bağlı artmış duyarlılığı düşündürmektedir.

Anahtar kelimeler: Bakteri, kedi konjunktivitisi, mikrobiyota, patojenler

Introduction

The conjunctiva is a mucous membrane that lines the inner surface of the eyelids and, depending on the species, extends to the inner and outer surfaces of the third eyelid. After forming the fornix, it continues over the sclera and thins as it approaches the limbus, the corneoscleral junction. This tissue contributes to tear production through numerous secretory glands and goblet cells, and plays a critical role in ocular immunity via its lymphoid follicles and resident immune cells. Its smooth surface also facilitates eyelid movement over the globe (Maggs et al., 2007; Mitchell and Oliver, 2015). Structurally, the conjunctiva consists of a non-keratinized squamous epithelial layer in direct contact with the external environment, and an underlying double-layered substantia propria. The outer epithelial layer functions as a protective barrier, while the conjunctival microbiota harbors opportunistic bacteria and fungi that help prevent pathogenic colonization (Sebbag et al., 2013; Mitchell and Oliver, 2015).

Feline conjunctival microbial communities differ from other domestic animals, with Gram-positive bacteria predominating in healthy cats. *Staphylococcus* spp. are most frequently isolated, followed by *Streptococcus* spp. and *Corynebacterium* spp., whereas Gram-negative species such as *Pseudomonas* spp., *Pasteurella* spp., and *Escherichia coli* are less common (Espínola and Lilenbaum, 1996; Kiełbowicz et al., 2015). Disruption of this microbial balance, either by external pathogens or compromised epithelial barriers, predisposes cats to conjunctivitis (Büttner et al., 2019; Arteaga et al., 2021).

Conjunctivitis refers to inflammation resulting from disruption or overstimulation of the conjunctival defense system by infectious or non-infectious factors (Davari et al., 2024). It is one of the most frequently encountered ocular disorders in cats. Viral and bacterial agents commonly cause infectious conjunctivitis, while non-infectious causes include trauma, anatomical abnormalities, allergies, and autoimmune diseases (Hartmann et al., 2010; Sainkaplan et al., 2022; Ergin et al., 2025). Clinically, it manifests as conjunctival hyperemia and swelling, often accompanied by serous to mucopurulent discharge (Davari et al., 2024).

Although conjunctivitis can affect cats of any age, cases arising during the first 2-3 weeks of life-commonly defined as the neonatal period-may lead to more severe outcomes (Casal, 2010; Mitchell and Oliver, 2015). During this critical developmental phase, pathogens such as *Staphylococcus* spp. and feline herpesvirus are the primary etiological agents, often compromising both ocular and systemic health (Willoughby, 2008; Veronesi and Fusi, 2022).

The present study aimed to investigate bacterial alterations in the conjunctival microbiota of neonatal and juvenile cats (under 1 year of age) presenting with conjunctivitis and to evaluate the influence of animal-related and environmental factors using various statistical methods.

Materials and Methods

Ethical Statement

Ethical approval for this study was obtained from the Animal Experiments Local Ethics Committee of Ankara University (Approval No: 2023-21-183). Prior to the examination, written informed consent was obtained from the animal owners.

Animals

In this study, 50 cats presenting with conjunctivitis at the Ophthalmology Clinic between December 2023 and September 2024 were evaluated. The cohort consisted of neonatal (n= 25) and juvenile (under one year of age, n= 25) individuals of various breeds and sexes. Only cats within the defined age range and exhibiting clinical signs of conjunctival inflammation were included. Animals with concurrent disorders affecting the eyelids or conjunctiva were excluded. In cases of bilateral conjunctivitis, one eye was randomly selected for analysis. Written informed consent was obtained from all owners prior to examination. The number of animals included in the study was not determined by a priori power analysis. Instead, it was based on the total number of eligible cases that met the predefined inclusion criteria within the study period. Although no formal sample size calculation was performed, the resulting sample was deemed adequate for applying non-parametric statistical tests and detecting group-level differences with interpretative value.

Study Design

A detailed anamnesis was obtained for each cat, followed by ophthalmic examinations, including direct ophthalmoscopy (Welch Allyn, REF 11710, USA), slit-lamp biomicroscopy (Shin-Nippon, SL-65, Japan), Schirmer tear test (ERC, Schirmer Test Strip, Turkiye), and fluorescein staining (PharmARGUS, Fluosine, Turkiye). Clinical symptoms of conjunctivitis were assessed based on the clinical appearance of the eyelids and conjunctiva, as well as the characteristics of ocular discharge. The severity of conjunctivitis was assessed using a scoring system ranging from 0 to 4, as previously described (Hartmann et al., 2010). After the clinical evaluation, conjunctival samples were collected from the included cases and sent to the laboratory for microbiological analysis.

Herpesvirus antigen detection was performed on tear samples from cats. Based on the anamnesis provided by the owners, recommendations were given to mitigate environmental factors contributing to ocular irritation. Throughout the procedure, all animals were fitted with Elizabethan collars, and hyaluronic acid eye lubricants were prescribed for twice daily application. Additionally, topical antibiotic eye drops were administered to all cats according to their bacteriological results.

Collection of conjunctival samples from the eye

Conjunctival samples were collected under aseptic conditions using a dry sterile swab (True Line, China). During sampling, the lower eyelid was gently retracted to expose the conjunctival tissue fully. The swab tip was positioned at a 45-degree angle to the conjunctival fornix and moved from the medial to the lateral aspect, ensuring uniform contact along the entire fornix. The procedure was performed in a single attempt, with strict precautions to prevent contact with the eyelid margins and cornea. Collected samples were immediately placed into sterile tubes and transported to the laboratory at +4°C (Arcelik, 5070 NF, Türkiye) to preserve sample integrity.

Bacteriological Analyses

Swab samples transported to the laboratory under a cold chain were inoculated onto 5% sheep blood agar for isolation and incubated aerobically at 37°C (Heraeus, B5060 EK/C02, Germany) for 24-48 hours. Colony morphology, including shape, size, color, odor, and hemolytic activity, was assessed. To obtain and propagate pure cultures, colonies were subcultured onto 5% sheep blood agar and re-incubated under the same conditions.

Pure isolates were identified through Gram staining (Olympus, CX21FS1, China) followed by biochemical characterization. Catalase, oxidase (Bactident Oxidase, Merck), oxidation-fermentation (OF), urease, and nitrate reduction tests were applied to all isolates. MacConkey (MC, Oxoid) and Triple Sugar Iron (TSI, Oxoid) agars were used for Gram-negative bacteria. *Staphylococcus* species were further differentiated using Mannitol Salt Agar (MSA, Oxoid) and DNase Test Agar (Oxoid), and a tube coagulase test. *Streptococcus* species were additionally tested on Bile Aesculin Agar (BAA, Oxoid) (Quinn et al., 2011; Markey et al., 2013).

Statistical Analysis

The bacteriological status of neonatal and sub-one-year-old cases included in the study was proportionally analyzed based on age, breed, sex, season and living environment. The statistical significance of differences in bacterial growth rates among these variables was tested using the chi-square test. At the same time, the effect sizes of factors were examined via univariate logistic regression analysis. The impact of age and bacterial species on bacterial growth was evaluated and classified using the Classification and Regression Tree (CRT) method. A significance level of P<0.05 was applied for all statistical analyses. Data were analyzed using the SPSS 30 statistical package

Results

The most common clinical signs observed in both groups were conjunctival hyperemia, conjunctival thickening and chemosis, with eyelid redness and edema also being prominent findings in all cats. Fluorescein staining was negative in all eyes, indicating an intact corneal surface. Ocular signs were observed to be more severe in the neonatal group, as reflected by the conjunctivitis scoring system. The neonatal group had a higher prevalence of severe scores (S3: 12, S4: 7) compared to the juvenile group (S3: 5, S4: 2), while mild cases (S1 and S2) were more frequent in juveniles (S1: 5, S2: 13) than in neonates (S1: 1, S2: 5). This distribution indicates a greater severity of ocular signs in neonates (Figure 1).

Figure 1. Ocular signs observed in eyes with conjunctivitis during the neonatal (a, b, c, d) and juvenile periods under one year of age (e, f, g, h). a: Neonatal Case 14, S2 conjunctivitis, mucoid discharge. b: Neonatal Case 18, S3 conjunctivitis, serous discharge. c: Neonatal Case 10, S4 conjunctivitis, purulent discharge. d: Neonatal Case 8, S4 conjunctivitis, mucoid discharge, conjunctival thickening. e: Juvenile Case 18, S2 conjunctivitis, serous discharge, eyelid hyperemia. f: Juvenile Case 19, S3 conjunctivitis, mucoid discharge. g: Juvenile Case 15, S3 conjunctivitis, mucopurulent discharge, conjunctival chemosis. h: Juvenile Case 8, S4 conjunctivitis, severe eyelid hyperemia and chemosis.

All cats exhibited ocular discharge, which varied in character and included serous, seromucous, mucoid, mucopurulent, or purulent. Schirmer tear test results were recorded as 15.64 ± 1.64 mm in the neonatal group and 16.36 ± 2.07 mm in the juvenile group. In cases of bilateral conjunctivitis, measurements were taken from a randomly selected eye only. Notably, herpesvirus antigen positivity was more prevalent in neonatal cats (13/25, 52%) compared to juvenile cats (5/25, 20%). Furthermore, environmental assessment revealed that the juvenile group had a history of increased exposure to ocular irritants (15/25, 60%) (Table 1a,b).

Table 1a. Comprehensive clinical and environmental profile of neonatal kittens with conjunctivitis

Case	Breed	Age (week)	Sex	Season	History	Living Environment	Ocular Signs	Localization	STT (mm/min)	Bacterial Species
1	Mix	3	M	W	N/A	outdoor	S4, mucopurulent discharge	Left eye	14	No bacterial growth
2	Mix	2	F	SP	eye irritants	indoor	S3, mucoid ocular discharge, conjunctival thickening	Bilateral	13	*Staphylococcus* spp.
3	Mix	2	M	SP	eye irritants	indoor	S4, mucopurulent discharge, eyelid chemosis	Right eye	15	*Streptococcus* spp.
4	Mix	3	M	SP	N/A	outdoor	S3, purulent discharge	Right eye	15	*Staphylococcus* spp.
5	Mix	3	M	S	FHV	indoor	S3, serous discharge, eyelid edema	Bilateral	14	*Pasteurella* spp.
6	Mix	3	M	S	FHV	outdoor	S3, mucoid discharge, eyelid redness	Bilateral	17	*Corynebacterium* spp.
7	Mix	3	M	S	N/A	outdoor	S3, purulent discharge	Right eye	15	*Corynebacterium* spp.
8	Mix	2	F	W	N/A	outdoor	S4, mucoid discharge, conjunctival thickening	Bilateral	16	*Streptococcus* spp.
9	Mix	3	M	W	FHV	outdoor	S3, mucopurulent discharge, blepharitis	Bilateral	15	*Pasteurella* spp.
10	BS	3	M	A	eye irritants	indoor	S4, purulent discharge, eyelid hyperemia	Left eye	14	*Staphylococcus* spp.
11	BS	3	F	A	FHV	indoor	S4, mucopurulent discharge, blepharitis	Left eye	16	*Streptococcus* spp.
12	SF	3	F	S	eye irritants	indoor	S2, mucoid discharge	Bilateral	16	*Actinobacillosis* spp.
13	Mix	2	M	S	N/A	outdoor	S1, serous discharge	Bilateral	18	No bacterial growth

14	Mix	2	F	S	FHV	indoor	S2, mucoid discharge, eyelid hyperemia	Bilateral	15	*Actinobacillosis* spp.
15	Mix	2	M	S	FHV	indoor	S3, purulent discharge, blepharitis	Bilateral	16	*Actinobacillosis* spp.
16	BS	2	M	A	N/A	outdoor	S4, purulent discharge, eyelid hyperemia	Bilateral	16	No bacterial growth
17	Mix	1	F	A	FHV	outdoor	S2, purulent discharge	Right eye	18	*Staphylococcus* spp.
18	Mix	3	M	A	N/A	outdoor	S3, serous discharge	Bilateral	19	*Corynebacterium* spp.
19	Mix	1	M	A	FHV	outdoor	S3, mucopurulent discharge, blepharitis	Right eye	15	*Corynebacterium* spp.
20	Mix	2	M	A	FHV	outdoor	S4, purulent discharge, blepharitis	Right eye	14	No bacterial growth
21	SF	3	M	W	eye irritants	indoor	S3, mucopurulent discharge, eyelid edema	Bilateral	14	No bacterial growth
22	Mix	3	M	W	FHV	outdoor	S2, serous discharge	Bilateral	15	*Corynebacterium* spp.
23	SF	3	F	W	FHV	outdoor	S2, seromucous discharge	Bilateral	20	*Actinobacillosis* spp.
24	Mix	2	F	SP	FHV	outdoor	S3, mucoid discharge, blepharitis	Bilateral	16	*Corynebacterium* spp.
25	BS	2	F	A	FHV	outdoor	S3, purulent discharge	Left eye	15	*Corynebacterium* spp.

M: Male, F: Female, W: Winter, S: Summer, A: Autumn, SP: Spring, N/A: Not Available, BS: British Shorthair, SF: Scottish Fold, FHV: Feline Herpesvirus, w: week, d: day, S1: Score 1, S2: Score 2, S3: Score 3, S4: Score 4, STT: Schirmer Tear Test.

Table 1b. Comprehensive clinical and environmental profile of juvenile cats under one year of age with conjunctivitis

Case	**Breeds**	**Age (month)**	**Sex**	**Season**	**History**	**Living Environment**	**Ocular Signs**	**Localization**	**STT (mm/min)**	**Bacterial Species**
1	Mix	4	M	W	eye irritants	indoor	S2, seromucous discharge, eyelid chemosis	Bilateral	16	*Pseudomanas* spp.
2	Mix	3	F	W	FHV	outdoor	S1, serous discharge	Bilateral	14	No bacterial growth
3	Mix	8	F	SP	N/A	indoor	S2, purulent discharge, eyelid hyperemia	Right eye	12	*Staphylococcus* spp.
4	Mix	11	M	SP	eye irritants	indoor	S1, serous discharge	Bilateral	17	No bacterial growth

5	Mix	3	M	A	FHV	outdoor	S2, purulent discharge, blepharitis	Left eye	15	*Staphylococcus* spp.
6	Persian	11	F	W	eye irritants	indoor	S3, purulent discharge, eyelid hyperemia	Right eye	18	*Staphylococcus* spp.
7	Persian	11	M	W	eye irritants	indoor	S1, mucoid discharge, eyelid chemosis	Bilateral	15	*Staphylococcus* spp.
8	BS	11	M	SP	FHV	outdoor	S4, mucopurulent discharge	Bilateral	16	*Staphylococcus* spp.
9	Mix	11	F	A	N/A	indoor	S3, mucoid ocular discharge,	Bilateral	20	No bacterial growth
10	BS	5	M	W	eye irritants	indoor	S2, seromucous discharge	Bilateral	19	No bacterial growth
11	BS	5	M	A	eye irritants	indoor	S2, mucopurulent discharge, eyelid edema	Bilateral	14	*Staphylococcus* spp.
12	Mix	11	F	S	N/A	outdoor	S2, serous discharge	Bilateral	16	No bacterial growth
13	Mix	10	M	S	FHV	outdoor	S4, purulent discharge, eyelid hyperemia	Left eye	20	*Staphylococcus* spp.
14	BS	11	M	S	FHV	outdoor	S3, mucoid discharge	Bilateral	15	*Staphylococcus* spp.
15	SF	10	F	S	eye irritants	indoor	S3, mucopurulent discharge, blepharitis	Right eye	14	*Staphylococcus* spp.
16	Mix	11	M	A	eye irritants	indoor	S2, serous discharge	Bilateral	18	No bacterial growth
17	Mix	4	F	A	eye irritants	indoor	S2, seromucous discharge	Bilateral	17	No bacterial growth
18	SF	3	M	SP	N/A	outdoor	S2, serous discharge	Bilateral	15	No bacterial growth
19	BS	11	M	A	eye irritants	indoor	S3, mucoid discharge, eyelid hyperemia	Left eye	15	*Staphylococcus* spp.
20	Mix	11	F	S	eye irritants	indoor	S2, mucoid discharge, blepharitis	Left eye	17	*Staphylococcus* spp.
21	BS	11	M	A	eye irritants	indoor	S2, mucoid discharge, eyelid hyperemia	Right eye	19	No bacterial growth

22	BS	8	F	A	eye irritants	indoor	S2, mucoid discharge, eyelid hyperemia	Bilateral	16	*Staphylococcus* spp.
23	SF	11	F	W	eye irritants	indoor	S1, serous discharge	Bilateral	20	No bacterial growth
24	BS	11	F	W	eye irritants	indoor	S1, mucoid discharge	Right eye	16	No bacterial growth
25	Mix	11	M	W	N/A	outdoor	S2, mucoid discharge	Left eye	15	*Staphylococcus* spp.

M: Male, F: Female, W: Winter, S: Summer, A: Autumn, SP: Spring, N/A: Not Available, BS: British Shorthair, SF: Scottish Fold, FHV: Feline Herpesvirus, w: week, d: day, S1: Score 1, S2: Score 2, S3: Score 3, S4: Score 4.

The effect of age, breed, gender, season, and living environment type on bacterial growth was not statistically significant (P>0.05). However, univariate logistic regression analysis revealed that age significantly impacted on bacterial growth (P<0.05). Specifically, bacterial growth was 3.14 times higher in neonatal cats compared to juvenile cats under one year of age (Table 2).

Table 2. Environmental and animal-related factors influencing bacterial growth

		Bacterial Growth			
		Positive n%	**Negative n%**	***p***	**OR (95% C.I.)**
Age	Under 1 Year Period	14 (56%)	11 (44%)	0.069	Reference
	Neonatal Period	20 (80%)	5 (20%)		3.14 (0.89-11.06)
Breed	Hybrid	22 (70.97%)	9 (29.03%)	0.561	Reference
	British	7 (63.64%)	4 (36.36%)		0.72 (0.17-3.06)
	Scottish	3 (50%)	3 (50%)		0.41 (0.07-2.42)
	Persian	2 (100%)	0 (0%)		0
Gender	Male	20 (66.67%)	10 (33.33%)	0.804	Reference
	Female	14 (70%)	6 (30%)		1.17 (0.34-3.96)
Seasons	Winter	8 (57.14%)	6 (42.86%)	0.483	Reference
	Spring	6 (75%)	2 (25%)		2.25 (0.33-15.33)
	Summer	10 (83.33%)	2 (16.67%)		3.75 (0.59-23.87)

	Autumn	10 (62.5%)	6 (37.5%)		1.25 (0.29-5.41)
Housing Type	Indoor	17 (65.38%)	9 (34.62%)	0.68	Reference
	Outdoor	17 (70.83%)	7 (29.17%)		1.29 (0.39-4.25)

OR: Odds Ratio, C.I.: Confidence Interval, p: p value

The findings were considered clinically relevant. In this regard, using the 0-1 week period as a reference, conjunctivitis symptoms in neonatal kittens were most frequently reported during the 2-3 week period.

Bacteriological analysis of conjunctival swabs showed no bacterial growth in 32% of the collected samples. Among the 34 culture-positive samples, bacterial identification, irrespective of group differences, revealed that *Staphylococcus* spp*.* was the most frequently isolated bacterium, accounting for 50% (17/34) of the cases. This was followed by *Corynebacterium* spp. (20.59%), *Actinobacillus* spp*.* (11.76%), *Streptococcus* spp*.* (8.82%), *Pasteurella* spp*.* (5.88%), and *Pseudomonas* spp*.* (2.94%).

According to the classification and regression tree method, the most effective factor on bacterial growth was identified as age. Bacterial growth rate was calculated as 80% in the neonatal period, which was 68% in total, and the most identified bacterial species in this period were *Staphylococcus* spp*, Streptococcus* spp*, Pasteurella* spp*, Corynebacterium* spp. and *Actinobacillosis* spp*.* However, in the period under 1 year of age, the bacterial growth rate was 56% and the bacterial species detected were *Staphylococcus* spp. and *Pseudomonas* spp. (Figure 2).

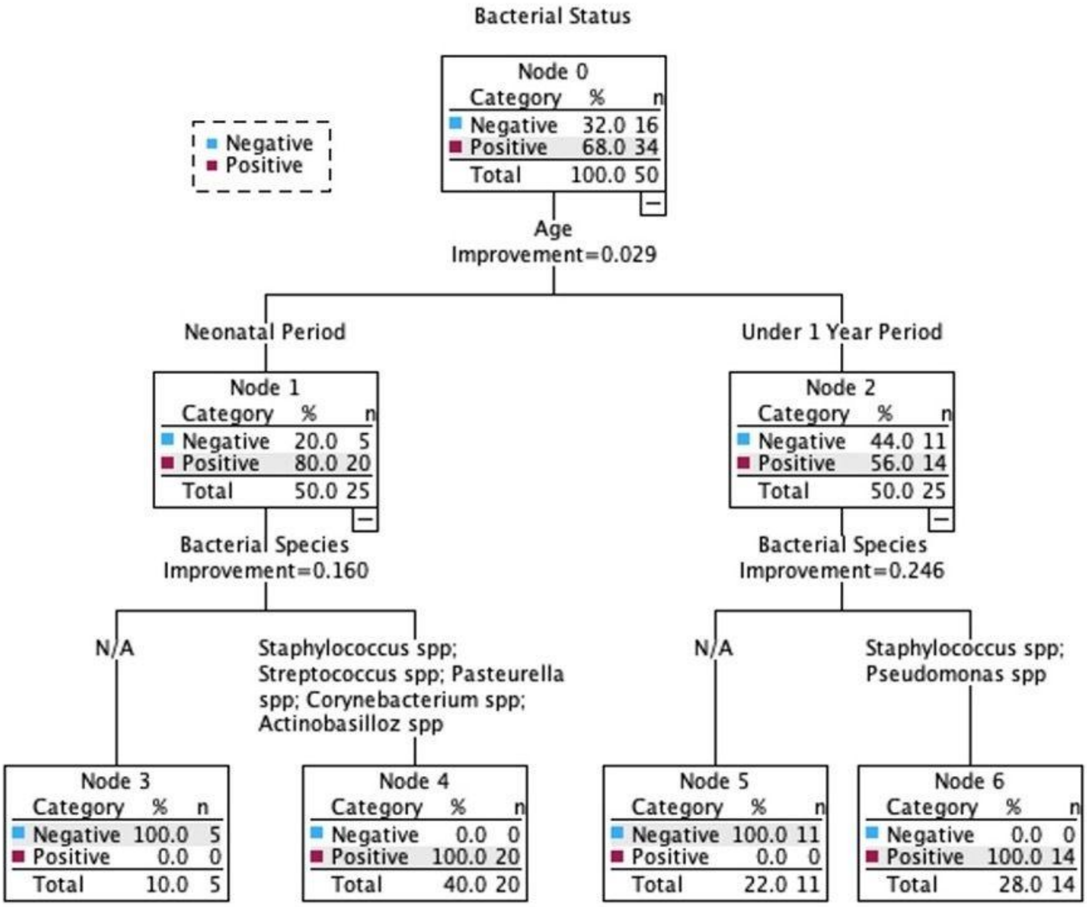

Figure 2. Analysis of bacterial growth using the Classification and Regression Tree (CRT) Method

The isolates were identified based on colony morphology, microscopic features, and biochemical test profiles. The characteristic results for each genus are summarized in Tables 3a and 3b.

Table 3a. Gram-positive bacterial isolates and their biochemical test profiles

Species	Gram Staining & Microscopic Morphology	Hemolysis	Catalase	Oxidase	Motility	Oxidation-Fermentation (O/F)	Urease	DNase	MSA (Mannitol)	Koagulase	Bile Esculin	Nitrate Reduction
Staphylococcus spp.	Gram (+) cocci in clusters	± (β or γ hemolysis)	+	-	-	F	±	±	±	±	-----	-----
Streptococcus spp.	Gram (+) cocci in chains	+ α or β	-	-	-	F	-----	-----	No growth observed	-----	-	-----
Corynebacterium spp.	Gram (+) coccoid rods, pleomorphic; arranged in Y, V, or L shapes	- γ	+	-	-	F	±	-----	-----	-----	-----	±

(+): Positive reaction, (-): Negative reaction, (±): Variable among species, α: Alpha hemolysis, β: Beta hemolysis, γ: No hemolysis, F: Fermentative, (-----): Not applicable

Table 3b. Gram-negative bacterial isolates and their biochemical test profiles

Species	Gram Staining & Microscop	Hemolysis	Catalase	Oxidase	Motility	Oxidation-Fermentat	Urease	MacConkey/ Lactose	TSI	Indol	Nitrate Reduction
Pseudomonas spp.	Gram (-) rods	+ β	+	+	+	O	-	Growth + Lactose -	K/K H_2S - Gas -	-	+
Actinobacillus spp.	Gram (-) rods, pleomorphic arrangement	- γ	+	+	-	F	+	Growth + Lactose -	K/A H_2S - Gas -	-	+
Pasteurella spp.	Gram (-) coccoid rods	- γ	+	+	-	F	-	Growth -	A/A H_2S - Gas -	+	+

(+): Positive reaction, (-): Negative reaction, α: Alpha hemolysis, β: Beta hemolysis, γ: No hemolysis; O: Oxidative, F: Fermentative, K/K: Alkaline/alkaline (no sugar fermentation), K/A: Alkaline/acidic (only glucose fermentation positive), A/A: Acidic/acidic (glucose and lactose/sucrose fermentation positive).

Among Gram-positive bacteria, *Staphylococcus* spp. were recognized as Gram-positive cocci in clusters, catalase positive, with variable coagulase activity, mannitol fermentation, DNase production, and β or γ hemolysis. *Streptococcus* spp. appeared as cocci in chains, catalase/oxidase negative, with α or β hemolysis, and bile esculin negative. *Corynebacterium* spp. were pleomorphic Gram-positive rods (Y-, V-, or L-shaped), catalase positive, non-hemolytic, with variable urease and nitrate reduction activity.

Among Gram-negative bacteria, *Pasteurella* spp. were small coccobacilli, catalase/oxidase positive, indole and nitrate positive, fermentative with A/A reactions on TSI. *Actinobacillus* spp. were pleomorphic rods, catalase/oxidase positive, urease positive, fermentative with K/A TSI reactions, and indole negative. *Pseudomonas* spp. were motile rods, catalase/oxidase positive, non-fermentative with K/K reactions on TSI, indole negative, and produced pigmented colonies with a characteristic odor.

Together, these findings confirmed the identity and distribution of bacterial species isolated from conjunctival swabs in neonatal and juvenile cats.

Discussion and Conclusion

In cats, conjunctivitis is the most frequently observed ocular disorder and can occur at any age due to bacterial, viral, fungal, or allergic etiologies. Bacterial conjunctivitis is the most commonly encountered type, occurring as a primary infection or secondary complication associated with various inflammatory conditions (Çatalkaya et al., 2023; Krivenko et al., 2023). In the present study, Gram-positive bacteria were the predominant isolates in both neonatal and juvenile conjunctivitis cases. Neonates exhibited five different species, whereas juveniles under one year showed only two species. In both age groups, isolates were primarily opportunistic *Staphylococcus* spp., suggesting that age-related shifts in the conjunctival microbiota may influence bacterial diversity. This observation is consistent with previous reports highlighting the predominance of opportunistic pathogens in feline ocular infections.

Inflammation of the ocular surface is a common feature of conjunctivitis regardless of etiology. Limited studies in cats have reported a positive correlation between increased tear levels of IL-6 and TNF-alpha and disease severity, highlighting a potential role of cytokines in pathogenesis (Davari et al., 2024). Similar patterns have been observed in humans with allergic conjunctivitis (Varandas et al., 2020).

Anamnesis data indicated that neither the season nor the animal's living environment had a significant impact on the occurrence of conjunctivitis. However, an analysis of the factors to which animals were exposed at different life stages revealed that neonatal kittens were most frequently affected by feline herpesvirus, whereas ocular irritants were the predominant factor in juvenile cats. Considering that conjunctivitis can develop due to one or multiple etiological factors, definitive conclusions regarding its exact cause cannot be drawn from the obtained results. However, the predominant factors identified in different age groups likely contribute to the development of conjunctivitis within these groups. These factors, despite their role in conjunctivitis, did not appear to have a significant impact on bacterial growth in cases of bacterial conjunctivitis.

Notably, the age at which conjunctivitis develops appears to correlate with the severity of the inflammatory response. Bacterial conjunctivitis, which often arises due to shifts in the density of commensal bacterial microbiota at different life stages, tends to present more severely in neonates. In this period, commonly referred to as the neonatal stage, conjunctivitis can lead to more severe complications, including vision loss (Martin et al., 2019).

In line with these observations, the findings of the present study indicate that ophthalmologic manifestations of neonatal conjunctivitis were more severe compared to those observed in the juvenile period. Furthermore, the significantly higher bacterial growth detected in samples from neonates compared to juveniles suggests that the conjunctival microbiota in neonates is more vulnerable, potentially due to an underdeveloped conjunctival immune system. Supporting this hypothesis, *Corynebacterium* spp., the second most frequently identified bacterial genus following *Staphylococcus* spp., was exclusively detected in neonates within a narrow timeframe of 2-3 weeks of age. This finding strengthens the notion that neonatal conjunctival microbiota exhibits greater susceptibility to bacterial colonization. Indeed, previous studies have also reported an increased prevalence of *Corynebacterium* spp. in the conjunctival microbiota of immunocompromised cats (Elmenshawy et al., 2021), further reinforcing the association between immature immune defenses and altered bacterial colonization in neonatal conjunctivitis.

The diagnostic accuracy of bacterial culture depends on the identified bacterial group. As a general rule, Gram-positive species are more commonly associated with the normal microbiota, whereas Gram-negative species are considered more likely to be true pathogens. Results of the bacterial culture must be carefully interpreted. A high concentration of Gram-positive bacteria can be clinically significant in diagnosing bacterial conjunctivitis (Hamor, 2001). In the present study, although pathogenic Gram-negative bacteria were detected, the predominance of Gram-positive species suggests that, in both age groups, bacterial conjunctivitis is largely associated with opportunistic microorganisms. This finding aligns with previous research, reinforcing the notion that conjunctivitis in both neonatal and juvenile periods is primarily driven by disruptions in the conjunctival microbiota rather than overtly pathogenic bacteria.

A limitation of this study is that the identification of bacterial isolates was performed using only phenotypic and biochemical methods. Although these approaches are widely applied in routine diagnostic microbiology and provided reliable genus- and species-level information, they cannot achieve the same taxonomic resolution as molecular or bioinformatic analyses. Future studies incorporating sequencing and advanced chemotaxonomic tools will be essential to validate and expand upon the present findings.

This study revealed age-related differences in the conjunctival microbiota of cats with conjunctivitis. Neonatal cats showed higher bacterial growth and a broader spectrum of bacterial species compared to juveniles under one year of age, suggesting increased susceptibility due to immature immune defenses. Age was identified as the main factor influencing bacterial growth, while the effects of other animal-related and environmental factors require further investigation. These findings may inform age-specific approaches for the management and prevention of feline conjunctivitis.

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1. Statement of Originality:
This work is original.

2. Author Contributions:
Concept: BUÇ,SS,EK,AAS,İE; ***Conceptualization:*** BUÇ,SS,EK,AAS,İE; ***Literature Search:*** BUÇ,SS,EK,AAS,İE; ***Data Collection:*** BUÇ,SS,EK,AAS,İE; ***Data Processing:*** BUÇ,SS,EK,AAS,İE; ***Analysis:*** BUÇ,SS,EK,AAS,İE; ***Writing – original draft:*** BUÇ,SS,EK,AAS,İE; ***Writing – review & editing:*** BUÇ,SS,EK,AAS,İE.

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The authors declare no competing interests.

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No GenAI tools were used at any stage of the study.

7. Sustainable Development Goals:

☐ 1 NO POVERTY ☐ 2 ZERO HUNGER ☐ 3 GOOD HEALTH AND WELL-BEING ☐ 4 QUALITY EDUCATION ☐ 5 GENDER EQUALITY ☐ 6 CLEAN WATER AND SANITATION ☐ 7 AFFORDABLE AND CLEAN ENERGY

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