Original Article

Cytological and Microbiological Study of the External Ear Canal in Dogs With Atopic Dermatitis

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ABSTRACT

Background: Otitis externa is the most important disease of the external ear canal. Canine atopic dermatitis (CAD) is a pruritic and inflammatory skin disease with a genetic background. Otitis externa is common in dogs and many of these cases are related to atopy.

Objectives: In this study, by comparing the characteristics of the external ear canal of atopic and non-atopic dogs with otitis, we aimed to determine their cytological and microbiological patterns and guide clinicians in choosing the best diagnostic and treatment plans.

Methods: Twenty atopic and 26 non-atopic dogs were studied. Atopy was diagnosed based on history and clinical examination findings according to Favrot's criteria. Sampling was performed from the external ear canal. The signalment of the cases was recorded and analyzed, along with cytological and microbiological features.

Results: Microscopic and macroscopic studies of the external ear canal showed no statistically significant difference between groups. In addition, the most frequent bacterial isolate cultured was *Staphylococcus pseudintermedius* in both groups, with a frequency of 69.5%. The genera *Pseudomonas*, *Escheria* and *Streptococcus* followed, with frequencies of 17.4%, 10.9% and 7.8%, respectively.

Conclusion: The results of our study are largely consistent with those of previous studies that evaluated the external ear canal of dogs with otitis. According to our findings, the features of the inflamed ear canal did not differ significantly between atopic and non-atopic patients.

Keywords: Atopic dermatitis, Bacteriology, Cytology, Dog, Otitis externa

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Introduction

Otitis externa is an inflammatory condition of the external ear canal in dogs and cats, characterized by symptoms, such as erythema, pruritus, increased ceruminous secretions, and secondary infections (bacterial or fungal). It has a prevalence of 5 to 20% in these animals (Miller et al., 2012). The etiology of otitis externa is categorized into four groups: underlying causes (e.g. structural factors, moisture, and systemic diseases), primary factors (e.g. hypersensitivities and ectoparasites), secondary factors (microorganisms that proliferate due to changes in the ear canal's microenvironment) and perpetuating factors (chronic conditions, like canal stenosis) (Rosser, 2004; Saridomichelakis et al., 2007). Common clinical signs include ear pruritus and head shaking, often indicating atopic dermatitis rather than food allergies (Miller et al., 2012). Cytologic examination of ear discharge aids in diagnosis by identifying the type of organisms involved. In cases related to atopy, cytology typically shows increased squamous epithelial cells and may reveal large numbers of Malassezia or cocci if an infection is present. The normal flora of the canine ear canal includes *Staphylococcus intermedius* and *Malassezia pachydermatis*, with these species frequently isolated in cases of otitis (Lyskova et al., 2007).

Canine atopic dermatitis (CAD) is a complex, pruritic, and inflammatory skin disease characterized by its genetic predisposition and multifactorial etiology. The pathogenesis of CAD predominantly involves type 1 hypersensitivity, where immunoglobulin E (IgE) antibodies play a pivotal role in the clinical manifestations and progression of the disease (Noli et al., 2013). This hypersensitivity reaction is triggered by various environmental allergens, leading to significant inflammation and discomfort in affected dogs.

Genetic and environmental interactions: The etiology of CAD is intricate, stemming from an interplay of genetic factors, environmental influences, and immune system dysregulation. Research indicates that approximately 75% of ear diseases in dogs are associated with atopy, highlighting the significant impact of CAD on overall canine health (Gotthelf, 2004). The genetic basis for CAD is evidenced by breed predispositions; certain breeds, such as terriers and retrievers are more susceptible to developing this condition (Noli et al., 2013; Griffin & DeBoer, 2001). Moreover, environmental factors, such as exposure to house dust mites, dander from various animals, feathers, cockroaches and pollen contribute to the onset and exacerbation of symptoms (Noli et al., 2013).

Clinical presentation: CAD typically presents with signs of pruritus, which is observed in 100% of affected dogs, making it a hallmark symptom of the condition. The absence of pruritus effectively rules out CAD as a diagnosis. The most common age for the onset of clinical signs ranges from six months to three years, with many cases being referred to veterinary clinics within this age range (Griffin & DeBoer, 2001; Favrot et al., 2010). Flares can be seasonal or non-seasonal, indicating variability in allergen exposure or individual sensitivity (Griffin & DeBoer 2001). In addition to pruritus, many atopic dogs exhibit otitis externa, which was present in 43% of cases during initial veterinary visits. Chronic infections of the external ear are frequently observed alongside CAD due to the inflammatory response that compromises the skin barrier (Favrot et al., 2010).

Pathophysiology: The pathophysiology of CAD involves an imbalance in immune responses characterized by a predominance of Th2-type cytokines, such as interleukin-4 (IL-4), IL-5 and IL-13. These cytokines promote IgE production and recruit inflammatory cells, like eosinophils, to the skin (Marsella, 2021; Puchéu-Haston et al., 2016). Histological examinations reveal superficial dermal infiltration by T cells, dendritic cells, eosinophils, and mast cells in affected areas. The chronic nature of CAD often leads to secondary bacterial infections due to skin barrier disruption.

Diagnostic criteria: The diagnosis of CAD has evolved significantly over recent decades. In 2009, Favrot introduced two sets of diagnostic criteria that have gained acceptance within the veterinary community. These criteria emphasize the importance of clinical history, physical examination findings, and exclusion of other differential diagnoses (Favrot et al., 2010). Accurate diagnosis is paramount, as it guides effective management strategies tailored to individual patient needs. In conclusion, CAD represents a multifaceted challenge for veterinarians due to its genetic underpinnings and complex interactions with environmental factors.

Dogs suffering from otitis externa comprise a considerable proportion of cases referring to veterinary centers and many of them are directly or indirectly related to skin reactions taking place in atopic dermatitis. In this study, we aimed to assess the cytologic features of inflamed ear canals in dogs with and without atopy, as well as the microbiological characteristics. Our goal was to determine whether a significant relationship exists between these findings and the phenotypic factors of the patients.

Material and methods

Animals

In this study, dog samples were obtained over a one-year period from 46 dogs with otitis externa (20 atopic dogs [group 1] and 26 non-atopic dogs [group 2]) referred to the Small Animal Teaching Hospital of the Faculty of Veterinary Medicine, University of Tehran. The diagnosis of CAD was made using Favrot's second criteria and the diagnosis of otitis externa was made based on history and clinical examination (including otoscopic examination). Favrot's criteria for diagnosing CAD are a set of clinical features developed to assist veterinarians in identifying the condition in pruritic dogs. These criteria include affected ear pinnae (excluding pinnal margins), affected front paws, age of onset less than three years, chronic or recurrent yeast infections, corticosteroid-responsive pruritus, a mostly indoor lifestyle, a non-affected dorsolumbar area, pruritus without skin lesions at the onset. A diagnosis of CAD is likely if at least five of these criteria are met, provided that other potential causes of pruritus have been ruled out. The sensitivity and specificity of these criteria are reported to be approximately 85% and 79%, respectively (Favrot et al., 2010). Figure 1 shows pictures taken from the cases with signs of atopy.

Sampling

Sampling was conducted from the external ear canal using sterile cotton-tipped swabs. The procedure involved inserting the swab vertically until a blockage was felt, after which it was rolled at that site for approximately 5 seconds before removal. Care was taken to avoid contact with the hair and skin in the ear canal during both insertion and extraction. Each case was sampled with two swabs: one for bacterial culture and another for cytological evaluation. In cases of bilateral otitis, the ear to be sampled was selected randomly.

Cytological evaluation process: The swab designated for cytology was rolled across three microscopic slides: The first slide was prepared for Giemsa staining, the second slide was prepared for Gram staining, and the third slide was kept intact as a backup in case of issues with the first two. All slides were fixed with methanol prior to staining.

Microscopic examination: The gram-stained slides were examined microscopically to identify the presence of bacteria, categorizing them as either gram-positive or gram-negative and further classifying them as cocci or bacilli based on morphology. For Giemsa-stained slides, observations were made in ten randomly selected high-power fields to quantify the presence of yeasts, white blood cells (WBCs), non-nucleated epithelial cells, nucleated epithelial cells, and epithelial cell clumps. The presence and mean counts of cocci or bacilli were recorded using oil immersion at 1000x magnification. Additional information about the dogs involved in the study was gathered through a questionnaire completed by their owners.

Bacteriology

After sampling the ear canal, the swab intended for bacterial culture was sent to the lab in a sterile microtube containing a sterile saline solution. Subsequently, it was utilized for culture in brain-heart infusion (BHI) and kept for 24 hours in a 37 ˚C incubator. Then, bacterial culture was performed on blood agar and MacCanckey agar using the bacteria grown in BHI. These media were kept in a 37 ˚C incubator for 48 hours before being examined.

To evaluate the results of bacterial culture and determine the species, we used morphologic and microscopic studies of the colonies, along with catalase, oxidase, and oxidation-fermentation tests for each colony, as well as differential and complementary tests.

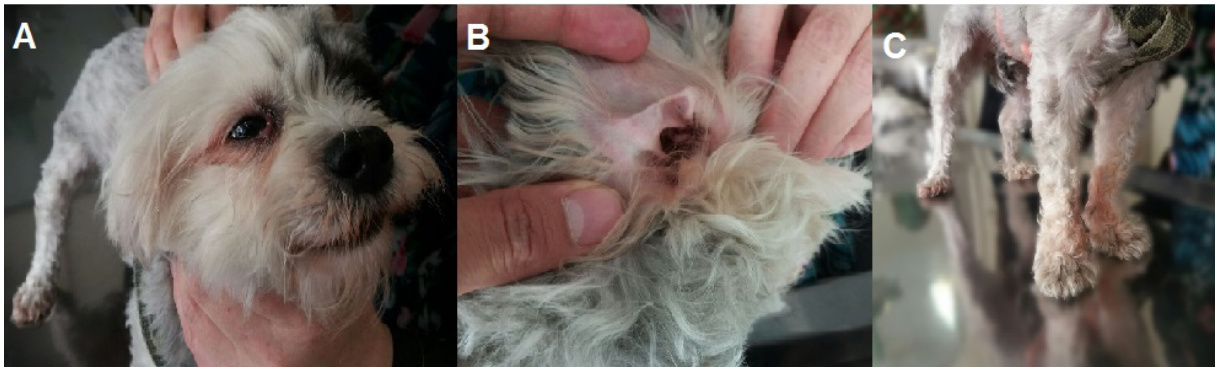

Figure 1. Prominent clinical signs of atopic dermatitis

A) Peri-occular erythema. B: Inflammation of the concave surface of ear pinnae, C) Salivary-stained paws due to severe pruritus

Statistical method

The tests used to evaluate different variables were Fisher's exact test, Mann-Whitney U test, and independent samples t-test (with significant $P<0.05$).

Results

Seventy-eight percent of the studied cases were less than three years old, with a mean age of 29.35 months for the studied dogs with atopy and 25.54 months for those without atopy. No statistically significant difference was observed regarding age ($P\leq0.05$).

Using the chi-square test, it was determined that there were no statistically significant differences in gender, body size, type and shape of the ear, color, odor and type of ear discharge between the two groups. Table 1 shows the clinical characteristics of studied cases in each group.

All cases within the first group (atopic cases) exhibited bilateral otitis, while in the second group, 88% of the cases did.

In gram staining, 60.9% of the cases (28 cases) showed only gram-positive cocci, while 4.3% of the cases (2 cases) showed gram-negative rods. Additionally, 4.3% of our cases (two cases) exhibited both Gram-positive cocci and Gram-positive rods and 30.4% of our cases (14 cases) displayed both gram-positive cocci and gram-negative rods. According to Figure 1, it can be observed that Gram-positive cocci were the most frequent organisms seen in all groups, and the simultaneous observation of gram-positive cocci and gram-negative rods was the second most common occurrence.

The mean number of cocci bacteria, bacillus bacteria, yeasts, nucleated squamous cells, non-nucleated squamous cells, squamous cell clumps, and leukocytes counted per ten microscopic fields (×1000 for cocci and bacillus bacteria and ×400 for the remainder) is shown in Table 2. No significant differences were found in the mean number of the mentioned bacteria, yeasts, and cells between these groups using the independent samples t-test. Moreover, photos of a number of detected organisms and cells can be found in Figure 2.

Bacterial culture results

The most frequent bacteria isolated was *Staphylococcus* spp. In our study, it was isolated from 59% of the cases in the first group and 57.57% in the second group. In both groups, it was the most common genus isolated. *Pseudomonas*, *Escherichia coli* and *Streptococcus* were other frequent genera that were isolated from cases in both groups with differing frequencies. *Pasteurella multocida* and *Klebsiella pneumoniae* were only found once, and both were isolated from the first group. *Arcanobacterium pyogenesis* was found in two cases, one in the first group and the other in the second group. Con-

Table 1. Clinical characteristics of the studied cases

Variables		%	
		Atopic Otitis	Non-atopic Otitis
Gender	Male	60	50
	Female	40	50
Type of ear	Erect	5	19.2
	Pendulous	95	80.8
Color of otic discharge	White or yellow	40	42.3
	Brown or black	60	57.7
Type of otic discharge	Waxy	85	92.3
	Dry	10	0
	Creamy	5	7.7
Odor of otic discharge	Malodorous	85	88.5
	Odourless	15	11.5

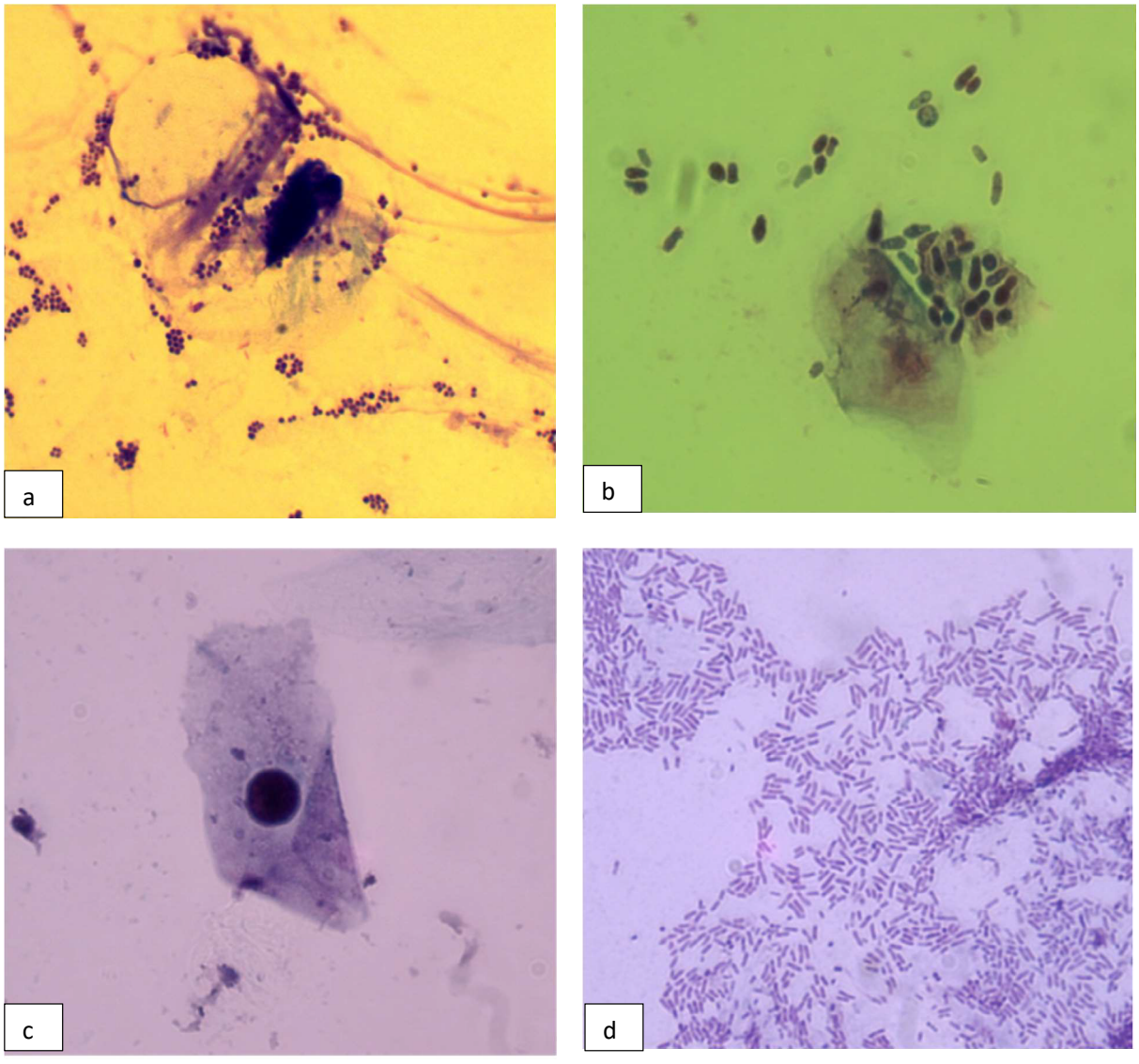

Figure 2. a) Cocci bacteria (Giemsa staining, ×1000 magnification), b) Yeasts around two non-nucleated squamous cells (Giemsa staining, ×400 magnification), C) Nucleated squamous cell (Giemsa staining, ×400 magnification); d) Bacillus bacteria (Giemsa staining, ×1000 magnification)

sidering all bacteria isolated from the two groups, the genus *Staphylococcus* was the most frequent one isolated (69.5%), followed by *Pseudomonas* (17.4%), *E. coli* (10.9%) and *Streptococcus* (8.7%). From the total of 46 samples, no bacteria were isolated from four samples, of which three belonged to the first group and one to the second (Table 3).

All of the isolated *Staphylococcus* spp. were *pseudintermedius*; therefore, all of them were coagulase-positive. All *Streptococcus* spp. were alpha-hemolytic. The only *Proteus* species isolated was *Mirabilis* and the only *Pasteurella* species isolated was *multocida*. All *Pseudomonas* spp. were *aeruginosa*.

Discussion

All atopic cases in our study had bilateral otitis, compared to 88% in the non-atopic group. Gram staining revealed that 60.9% of samples contained only gram-positive cocci, while 30.4% showed both gram-positive cocci and gram-negative rods. The mean counts of bacteria, yeasts, and epithelial cells were similar across groups, with no significant differences detected. Yeasts were present in 89.13% of samples evaluated, indicating comparable cytological features and bacterial presence in the ear canal discharge for both atopic and non-atopic dogs with otitis externa. *Staphylococcus* spp. were the most frequently isolated bacteria, found in 59% of atopic

Table 2. Mean values of items in each group

Group	No.	Mean± SD		
		Cocci	Bacillus	Yeast
Atopic-otitis	20	11.6405±16.96840	3.3745±10.77788	17.2700±31.62759
Non-atopic otitis	26	17.9592±34.79399	33.0812±121.88851	26.1885±55.17903

Group	No.	Mean± SD			
		Nuc. Ep. Cell	Non-nuc. Ep. Cell	Ep. Cell Clump	WBC
Atopic-otitis	20	0.9800±2.10903	11.6850±5.78794	1.4850±0.95105	0.1850±0.73862
Non-atopic otitis	26	0.3577±0.36130	14.5769±5.24239	1.3500±0.79980	1.2692±4.49460

cases and 57.57% of non-atopic cases. Other notable isolates included *Pseudomonas*, *E. coli* and *Streptococcus*. Overall, *Staphylococcus* accounted for 69.5% of all isolates, followed by *Pseudomonas* (17.4%), *E. coli* (10.9%) and *Streptococcus* (8.7%).

The assessment of *M. pachydermatis* in the external ear canal of dogs with and without otitis, as reported by Puig et al. (2019) highlights the utility of cytology as a rapid and cost-effective diagnostic tool. Their findings demonstrate a statistically significant correlation between qPCR quantification, cytologic examination, and colony-forming units (CFUs). This reinforces the value of cytologic evaluation in diagnosing ear conditions in dogs, providing crucial insights regardless of whether the underlying cause is allergic (Puig et al., 2019).

Choi et al. (2018) compared two sampling methods for the cytologic evaluation of the external ear canal—one targeting the vertical portion and the other the horizontal portion. They found a higher prevalence of polymorphonuclear WBCs in samples from the horizontal portion, while no significant differences were noted in the counts of bacteria, yeast, macrophages, or lymphocytes. This finding supports our methodology, suggesting that our sampling technique accurately reflects the microbiological status of the ear (Choi et al., 2018).

In a study by Ngo et al. (2018) a trend was observed indicating increased numbers of *Staphylococcus* spp. in atopic dogs compared to healthy ones, alongside decreased *E. coli* counts in healthy dogs. Our results corroborate this observation; *Staphylococcal otitis* was prevalent among both atopic and non-atopic dogs with otitis. Notably, *E. coli* was isolated from only one atopic dog and four non-atopic dogs. This suggests that while bacterial populations may differ between atopic and non-atopic dogs, *Staphylococcus* spp. and *Pseudomonas* spp. are primarily responsible for inflammatory responses in both groups (Ngo et al., 2018).

Ginel et al. (2002) reported an average of 22.48 epithelial cells in healthy dogs versus 21.20 in those with otitis. The presence of nucleated squamous cells is infrequent; however, when observed in large quantities, it typically indicates severe inflammation—a condition rarely seen even in severe otitis cases. This aligns with our findings, where nucleated squamous cells were seldom detected in significant numbers across samples. Conversely, non-nucleated squamous cells and squamous cell clumps were prevalent in both normal and inflamed ears, explaining the similarity in mean counts across groups.

Thomsen et al. (2023) studied the skin and gut microbiota in Shiba Inu dogs with atopic dermatitis, finding that *Staphylococcus* was the most frequently isolated bacterium from various skin locations. They reported that treatment with a janus kinase (JAK) inhibitor significantly reduced the abundance of this bacterium. This highlights the critical role of *Staphylococcus* in the pathogenesis of this allergic skin condition, suggesting that the data extracted from our study could guide clinicians and inform future research on optimal diagnostic and therapeutic strategies. In addition, Older et al. (2020) similarly found that *S. pseudintermedius* is the most prevalent bacterium cultured from pyoderma lesions in dogs with atopy.

Among samples with negative bacterial cultures, we noted a minimum count of bacilli and cocci at 0 and 0.1, respectively, with maximum counts reaching 0.4 and 14.5. Ginel et al. (2002) established that a mean bacterial count exceeding five should be considered abnormal; however, we identified abnormal counts in two out of four samples from both groups despite negative cultures.

Table 3. Frequency and percentage of different genera of bacteria in each group

Genus	No. (%)		
	Atopic Otitis	Non-atopic Otitis	Total
Staphylococcus	13(59)	19(57.57)	32(69.5)
Pseudomonas	2(9)	6(18.18)	8(17.4)
Escherichia	1(4.5)	4(12.12)	5(10.9)
Strep	3(13.6)	1(3)	4(8.7)
Pasteurella	1(4.5)	-	1(2.2)
Klebsiella	1 (4.5)	-	1(2.2)
Arcanobacterium	1 (4.5)	1 (3)	2(4.3)
Bacillus	-	1 (3)	1 (2.2)
Corynebacterium	-	1	1

This emphasizes the necessity of interpreting bacterial culture results alongside cytological evaluations for a comprehensive understanding of ear conditions.

Lehner et al. (2010) documented mean yeast counts of 53 and 60 from two swabs taken from dogs with otitis externa, while Saridomichelakis et al (2007). found yeast cells present in 66 out of 100 samples evaluated. In our study, mean yeast cell counts were recorded at 17.27 for the first group and 26.18 for the second group, with no significant difference observed between groups; yeasts were identified in 41 out of 46 samples (89.13%).

Our findings regarding bacterial counts align closely with those reported by Lehner et al. (2010) who noted mean bacillus and cocci counts significantly higher than those found in healthy dogs (Ginel et al., 2002). In our study, we recorded mean bacillus and cocci counts of 3.37 and 11.64 for the first group and 33.08 and 17.96 for the second group, respectively; however, statistical analysis did not reveal significant differences between groups.

Zur et al. (2011) evaluated common causes of otitis externa alongside associated pathogens, finding no significant differences between allergic cases and others attributable to otitis externa—a result mirrored in our study, where no notable differences were observed between atopic and non-atopic groups.

Penna et al. (2010) reported that *Staphylococcus* spp. constituted 60.3% of isolated bacteria from their sample population; our findings show comparable results, with *Staphylococcus* accounting for 59% in the first group and 57.57% in the second group. The increased adhesion rates of *S. pseudintermedius* to corneocytes in atopic dogs may explain why secondary staphylococcal infections were present in over 60% of cases within our first group (Paul et al., 2013).

Bugden (2013) identified *Pseudomonas aeruginosa* as a prevalent isolate among a large cohort of dogs with otitis; however, our results indicated that *Staphylococcus* spp. were more commonly isolated than *Pseudomonas* spp., which may reflect regional climatic influences on microbial populations—Bugden's study was conducted in a warm, wet climate conducive to *Pseudomonas* spp. growth, whereas our research took place in Tehran's warm and dry environment.

Maghami et al. (1978) reported outbreaks of mycotic dermatitis in the Iranian sheep population caused by the genus *Dermatophilus*. This emphasizes the importance of evaluating the fungal organisms residing on the skin of small animals in regions where dogs interact with sheep herds, both in healthy dogs and those suffering from dermatologic conditions such as otitis. In a study by Al-Saedi et al. (2022) aimed at evaluating the anti-inflammatory effects of allopurinol gel in a mouse model of dermatitis, it was shown that this product can improve some clinical and paraclinical parameters studied, although the differences were not statistically significant for any parameter. Therefore, we believe that studying the effect of this topical drug on the clinical signs of otitis and otic microbiota can provide beneficial data, considering that this drug, in its oral form, has been widely used in small animal medicine for other non-dermatologic conditions.

Conclusion

In conclusion, while cytological evaluation remains essential for diagnosing otitis externa across all cases, our study found no significant differences in cytological features between atopic and non-atopic dogs with this condition. Future research involving larger sample sizes is warranted to deepen our understanding of similarities and differences regarding ear health between these two groups.

Ethical Considerations

Compliance with ethical guidelines

This study was approved by the Ethics Committee of the University of Tehran, Tehran, Iran (Code: IR.UT.VETMED.REC.1404.003).

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Authors' contributions

Study design: Shahram Jamshidi and Ramak Yahya Raaiat; Samples collection, preparation, and evaluation of the microscopic slides, isolation of the bacteria, and performing complementary tests to determine the species: Hamidreza Jahani; Microbiologic tests interpretation and determination of species of the isolated bacteria: Taghi Zahraei Salehi and Iradj Ashrafi Tamaei; Data analysis: Zahra Boluki; Writing: Shahram Jamshidi, Ramak Yahya Raaiat and Hamidreza Jahani; Final approval: All authors.

Conflict of interest

The authors declared no conflict of interest.

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