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Research Article

Epidemiological Investigation of Bacterial, Fungal and Parasitic Factors Causing *Otitis externa* in Dogs and Cats in Northern Iran from 2019 to 2021

Mohammad Asadi Iraee[1]*, Mohsen Peysokhan[1], Mohammad Mehdi Yazdani Rostam[2], Mohammad Javad Mashayekhnia[3], Mohammad Reza Roudaki Sarvendani[4], Shohreh Alian Samakkhah[5], Alireza Farkhakfar[2], Helia Aziziha[2] and Ali Zareh[1]

[1]Department of Veterinary Clinical Sciences, Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz, Iran

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

[3]Department of Veterinary Clinical Sciences, Faculty of Veterinary Medicine, Islamic Azad University, Babol Branch, Tabriz, Iran

[4]Department of Veterinary Clinical Sciences, Faculty of Veterinary Medicine, Tehran University, Tehran, Iran

[5]Department of Food Hygiene, Faculty of Veterinary Medicine, Amol University of Special Modern Technologies (AUSMT), Ahvaz, Iran

***Corresponding Author:** Mohammad Asadi Iraee, Department of Veterinary Clinical Sciences, Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz, Iran.

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Abstract

Otitis externa is a multifactorial disease that has 5 to 20 percent prevalence in small animal medicine which can be diagnosed according to the history and general dermatological and otoscopic examinations. This study aimed to compare the agents that cause *Otitis externa* in dogs and cats, taking into account the breeds, gender, and age differences of these animals. The samples were taken from 63 dogs and cats that were suspected of having external ear infections and referred to veterinary clinics from north of Iran, including Mazandaran, Guilan, and Golestan provinces, within 24 months (from April 2019 to April 2021). After obtaining the consent, sampling was performed from the pinna and the end of the vertical ear canal. The prevalence of infection observed in dogs was: 28.57% for Malassezia, 28.57% for gram-positive bacteria, 11.91% gram-negative bacteria, 16.67% and 14.28% for *Otobius megnini* and *Sarcoptes scabiei* respectively, while in cats, the prevalence of Malassezia infections was 9.52%, 23.80% gram-positive bacteria, 19.04% gram-negative bacteria, 19.04% *Otobius megnini*, and 33.34% *Notoedres cati*. There was no statistically significant difference between the studied dogs and cats with the variables of age, gender, and breed. Identifying the agents causing external ear infections and paying attention to factors such as etiologies, geographical effects, region climate, disease-causing agents, breed differences, gender, and age in Iran is crucial and can improve the rate of success in management and treatment of external ear infections. Therefore, it seems that due to the limited number of these studies in Iran, additional studies in this field are necessary. Among dogs and cats in the north of Iran, this study represents the first assessment of external otitis.

Keywords: Ear; *Otitis externa*; Dog; Cat; Iran

Introduction

Otitis or inflammation of the ear canal can affect all three ear parts, including the external, middle, and internal ear [1]. Approximately 5 to 20% of small animals are affected by *Otitis externa*, which can be detected by examining the animal's history and performing a general dermatologic and otoscopic examination. Even though the process of diagnosing this disease appears to be straightforward, cellular tests, bacterial cultures, audiometry, and radiographs are necessary for the following steps [2]. Predisposing factors, primary factors, and perpetuating factors are the factors responsible for causing this disease. A predisposing factor alone is not sufficient to cause disease, it must be coupled with a primary risk factor to cause disease to occur. These factors include abnormal structures in the ear, temperature and humidity, obstructive ear disease, damage to the epithelium of the ear canal, and systemic diseases. In healthy ears, primary factors are responsible for developing diseases, essentially manifestations of general skin disorders. Diseases related to allergies, external parasites, foreign bodies, keratinization disorders, autoimmune diseases, and juvenile cellulitis can be mentioned among these factors. Finally, perpetuating factors contribute to disease continuation and spread even after the initial factor has disappeared. Bacteria, yeasts, contact with allergens and stimuli, proliferative changes, external otitis, and improper treatment contribute to the persistence of the disease [3,4].

Otitis externa is a multifactorial condition in which only a large number of bacteria of a specific type can be isolated and used to determine the direct effects of the pathogen on complications. *Staphylococcus, Pseudomonas, Escherichia coli*, and *Proteus* species are among the bacteria that are commonly detected in small numbers in healthy ears [5]. Although bacterial and fungal cultures are not required in cases of simple diagnosis, bacterial and fungal cultures are necessary when dealing with resistant cases and recurrent diseases [6]. In parasitic infections, *Otodectes cynotis* is usually the cause of the disease. *Otitis externa* is caused by this parasite in 50% of cats, and it is sometimes responsible for chronic conditions since it is so small that it cannot be detected in a sample taken from a dog's or cat's ear canal. *Sarcoptes scabiei*, *Notoedres cati*, *Eutrombicula Alfreddugesi*, *Demodex Canis*, and *Demodex cati* are some other parasites [4]. Local use of antibiotics to treat bacterial infection may sometimes lead to secondary fungal infection, which is mistaken for a bacterial infection due to severe itching. The yeasts and fungi isolated in these cases include *Aspergillus* and *Candida*. *Malassezia* is another yeast that contributes to chronic skin and external ear conditions [7].

Symptoms of *Otitis externa* in dogs and cats include excessive head shaking, excessive itching, and sometimes alopecia in the ear area. In the case of disruptions in keratinization or secondary fungal or bacterial infections, odoriferous exudates are produced. In addition, itching and pain caused by progressive inflammatory changes may cause the animal to moan when the ear is touched [4]. Treatment should be initiated as soon as the disease is diagnosed, demonstrating the importance of correct diagnosis of *Otitis externa*. As a result of a late diagnosis, this disease usually does not satisfactorily respond to treatment. After the incomplete treatment process, the disease recurs again and appears as a chronic condition [8].

In Iran, little research has been conducted on the causative agents of *Otitis externa*, which is a common cause of clinical visits in dogs and cats. The purpose of this study is to compare the agents responsible for the occurrence of *Otitis externa* in cats and dogs. This study is the first assessment of external otitis in dogs and cats in the north of Iran. The results support a better understanding of the disease.

Materials and Methods

In this study, 63 dogs and cats suspected of having external ear infections were referred to veterinary clinics in the provinces of Mazandaran (36.3994° N, 52.1912° E), Golestan (37.2898° N, 55.1376° E), and Guilan (37.1172° N, 49.5280° E) over the course of two years (from April 2019 to April 2021). The provinces in this region have a specific geographical condition characterized by a moderate to subtropical climate, high relative humidity (70 to 100%), a temperature range of 10 to 35°C, and an average rainfall of 800 to 1200 millimeters per year. Regarding geography, the provinces can be divided into coastal plains and mountainous areas in the Alborz mountain range [9-11]. The cases involved 42 dogs and 21 cats, with 50% females and 50% males and 47% females and 53% males, respectively. The average age of cats in this study was 1.34 years and the average age of dogs was 1.29 years. Of the 42 referred dogs, 22 were mixed breeds, 10 were terriers, 3 were Dobermans, 3 were Shih Tzu, 2 were Pomeranians, and 2 were Golden Retrievers. Among the 21 cats referred for evaluation, 14 were domestic shorthair cats, and 7 were Persian cats.

Clinical signs, such as pain, shaking of the head/tilting to one side, scratching of the ears, and changes in behavior, as well as physical examination (odor, discharge, redness/swelling of the ear), history, and use of specific diagnostic tools (cytology and culture), were used to determine the diagnosis. A complete history of

each animal was obtained, and animals who had been treated with ear infection-related drugs within the past two months were excluded from the study. The data collected included age, breed, sex, and clinical information. Upon receiving the consent form and obtaining the cooperation of the patient's owner, clinical specimens were collected from each healthy and/or diseased ear canal using a sterile dry cotton swab. A new plastic storage tube was used to replace the swab and to label it. After being collected from the veterinary clinics, samples were transferred to the laboratory for further analysis. A direct microscopy examination of the smear was performed as part of the initial analysis to detect *Malassezia* and parasites such as mange. To determine the number of typical *Malassezia* cells per microscopy field, slides were stained with giemsa stain (40× magnification) [12]. In addition, gram staining was performed by the protocol to identify gram-positive and gram-negative bacteria [13]. Furthermore, sterile gloves were used during the sampling process to prevent secondary contamination.

The Fisher exact statistical test was employed to determine the relationship between the different independent variables such as age, gender, breed, and the presence of *Malassezia*, Gram-positive and Gram-negative bacteria, as well as types of mange in the external ears of dogs and cats. The results of qualitative variables were expressed based on absolute and relative frequency. To analyze SPSS version 25 statistical software was used (SPSS Inc., Chicago, IL, USA). All analyses were conducted at a significance level of less than 0.05.

Results and Discussion

In this study, 42 dogs were examined and the rates of infection with *Sarcoptes scabiei*, *Otobius megnini*, Gram-negative and Gram-positive bacteria, and *Malassezia* were 14.28%, 16.67%, 11.91%, 28.57%, and 28.57%, respectively. Furthermore, 33.34% of the 21 cats with *Otitis externa* were diagnosed with *Notoedres cati*, 19.04% with *Otobius megnini*, 19.04% with Gram-negative bacteria, 19.04% with Gram-positive bacteria, and 9.52% with *Malassezia*. Table 1 provides information regarding the prevalence of external ear infections and their relationship to certain variables in the studied dogs.

The incidence of *Malassezia* in dogs less than a year old to that of dogs between 1 and 2 years old, and dogs between 2 and 3 years old were a quarter of all dogs affected by *Malassezia*, indicating no statistically significant difference ($p > 0.05$). Additionally, in this study, there were an equal number of male dogs and female dogs, each with 21 dogs. Although male dogs had a higher percentage of ear involvement with *Malassezia*, the difference between male and female groups was not considered significant ($p > 0.05$). Breed-wise, the Pomeranian showed the same rate of conflict with *Malassezia* as the Golden Retriever with 50%, while about one-third of terrier dogs displayed this type of conflict. Other breeds did not exhibit any conflict with *Malassezia*. Although there were discrepancies between breeds, it was not considered significant ($p > 0.05$).

In regards to bacterial infections, there was a greater rate of gram-positive infections in all three age groups. Although the rate of infection with gram-positive and gram-negative bacteria was higher in the age groups between 2-3 years and under one year, respectively, this difference was not statistically significant ($p > 0.05$). Similarly, the involvement of gram-positive bacteria in both genders was higher than that of gram-negative bacteria. Although the rate of gram-negative bacteria involvement was equal in both genders, the rate of gram-positive bacteria involvement was higher in females than in males, and this difference was not considered significant ($p > 0.05$). In the breed analysis, the Doberman and Terrier showed the highest rate of ear involvement with Gram-positive and Gram-negative bacteria, respectively. However, breed differences cannot be considered an influencing factor in bacterial ear infections ($p > 0.05$).

In the study conducted on mange *Sarcoptes scabiei* and *Otobius megnini*, despite the finding that the age group under one year showed the lowest rate of mange involvement and the highest rate of involvement with *Sarcoptes scabiei* and *Otobius megnini* at the ages of 2 to 3 years and 1 to 2 years, respectively, these differences were not statistically significant ($p > 0.05$). The amount of involvement with *Otobius megnini* was greater in females, whereas *Sarcoptes scabiei* involvement was more prevalent in males. However, statistical significance cannot be attributed to differences between genders ($p > 0.05$). Breed-wise, the Shitzu breed had the highest involvement rate with *Otobius megnini*, while Dobermans and Shitzus were equally involved with *Sarcoptes scabiei*, being more involved than other breeds. Finally, the difference between genders was not considered statistically significant ($p > 0.05$).

Type of infection	Classification	NuManage (n = 42)	Mange		Bacteria		Yeast
Variable			*Sarcoptes scabiei* (%)	*Otobius megnini* (%)	Gram Negative (%)	Gram Positive (%)	*Malassezia* (%)
Age groups (Year) *	<1	21	9.52%	14.28%	19.04%	28.57%	scabiei
	1-2	13	23.07%	15.38%	7.69%	23.07%	30.79%
	2-3	8	12.5%	25%	0	37.5%	25%
Gender*	Male	21	19.04%	14.28%	14.28%	23.80%	28.60%
	Female	21	14.28%	19.04%	14.28%	33.33%	19.07%
Breed*	Mix	22	18.18%	18.18%	18.18%	22.72%	22.74%
	Doberman	3	33.33%	0	0	66.67%	0
	Golden Retriever	2	0	0	0	50%	50%
	Pomeranian	2	0	50%	0	0	50%
	Shih Tzu	3	33.33%	66.67%	0	0	0
	Terrier	10	10%	10%	20%	30%	30%

Table 1: Prevalence of types of infections in the ears of dogs suffering from external ear infections and their relationship with several epidemiological variables.

* $p < 0.05$ indicates a statistically significant difference based on Fisher's exact test.

Table 2 demonstrates the prevalence of external ear infections and their relationship with some epidemiological variables in the studied cats.

The incidence of *Malassezia* in cats between the ages of 2 and 3 years was more than twice that in the youngest group, and the age group between 1 and 2 years, no involvement with this yeast was detected. Despite differences, age cannot be considered a determining factor in the development of *Malassezia* ($p > 0.05$). Furthermore, despite even though the incidence of this complication in female cats was slightly higher than that of male cats, this difference was not considered significant ($p > 0.05$). Moreover, *Malassezia* involvement was equal among both studied cat breeds, so the breed factor cannot play a significant role in the occurrence of this condition ($p > 0.05$).

In the bacterial examination, all three age groups were almost equally contaminated with gram-positive bacteria, while the age group of 1-2 years was slightly more infected with gram-negative bacteria than the others. In spit Despite age-related differences cannot be considered significant ($p > 0.05$). In examining male and female cats, despite the higher rate of involvement of male cats in both types of bacterial infections, the difference was not statistically significant ($p > 0.05$). Moreover, in terms of breed, there was no difference between the two groups regarding the involvement of gram-positive bacteria, while the Persian cats were almost four times more involved in gram-negative bacteria than the DSH cats ($p > 0.05$).

In terms of conflict with mange, cats between the ages of 1 and 2 had the highest number of disputes with both types of mange. In addition, the youngest and oldest age groups had the least amount of conflict with *Otobius megnini* and *Notoedres cati*, respectively. There is, however, no significant relationship between age and infection with these two types of mange ($p > 0.05$). It is also noteworthy that there is no statistically significant difference between males and females, despite the higher rate of involvement among female cats ($p > 0.05$). As a final note, although DSH cats were more likely to develop these two types of mange than Persian cats, the difference was not considered statistically significant ($p > 0.05$).

Veterinary practices frequently encounter *Otitis externa* caused by primary factors and perpetuated by secondary factors such as bacteria and yeast overgrowth [14]. It is one of the most commonly diagnosed diseases in dogs and cats. However, the etiology, various factors, and the role played by various microorganisms in the development of the disease have not been fully established [15-18].

Type of infection	Classification	Number (n = 42)	Mange		Bacteria		Yeast
Variable			*Notoedres cati* (%)	*Otobius megnini*(%)	Gram Negative(%)	Gram Positive(%)	*Malassezia* (%)
Age groups (Year) *	<1	12	33.33%	16.66%	16.66%	25%	8.35%
	1-2	4	50%	25%	25%	0%	0%
	2-3	5	20%	20%	20%	20%	20%
Gender*	Male	11	27.27%	9.09%	27.27%	27.27%	9.10%
	Female	10	50.0%	10%	10%	20%	10%
Breed*	Domestic short hair	14	42.85%	21.42%	7.14%	14.28%	14.31%
	Persian	7	28.57%	14.28%	28.57%	14.28%	14.30%

Table 2: Prevalence of types of infections in the ears of cats suffering from external ear infections and their relationship with several epidemiological variables.

* $p < 0.05$ indicates a statistically significant difference based on Fisher's exact test.

Several characteristics make *Malassezia* yeasts unique, including their reliance on lipids, their cellular ultrastructure, and their presence on the skin of warm-blooded vertebrates. In terms of taxonomy, the genus *Malassezia* is in the process of evolving. So far, 18 species have been described, but many more are most likely present on warm-blooded animals' skin or mucosa [19]. Based on the few studies conducted in Iran, in 2009, 82 cats with external ear damage were referred to the Faculty of Veterinary Medicine at Tehran University for testing, and 95.1% of the cats had *Malassezia pachydermatis* [20]. In addition, a study conducted in Karaj city clinics in 2014 found that *Malassezia Pachydermatis* caused 18 out of 27 ear infections (66.66%) [21]. They found no correlation between patients' age and gender, which is consistent with our study. An analysis of 80 dogs with external ear infections (66 cases of bilateral infection and 14 cases of unilateral infection) conducted in Brazil in 2006 showed that *Malassezia pachydermatis* was the cause of infection in 57.53% of the cultured samples. Most dogs examined in their study were between the ages of 1 and 3, and the poodle breed had the highest number of infections [22]. Another study conducted in Brazil in 2015 examined 70 dogs (44 males and 26 females) in terms of the cause of external ear infections. *Malassezia pachydermatis* was found in 49 dogs studied (70%) [23]. A further study conducted on 616 cases of external ear infections in carnivorous animals (dogs and cats) indicated that *Staphylococcus* hemolytic, *Malassezia pachydermatis*, and Pseudomonas aeruginosa were responsible for 26.27, 12.35, and 8.8% of external ear infections in Brazil [24].

According to a study conducted in 2007, 464 dogs and 105 cats with external ear infections were studied according to their gender, species, breed, and age. The results of this study indicate that dogs are more likely than cats to suffer from external ear infections [25]. This is due to the structure of the ear canal and lobe, as well as less hair in the external ear canal and better ventilation in the cat's ear [26]. The study also found that breeds with drooping ears are more likely to suffer external ear infections. Additionally, external ear infections were higher among dogs aged 5-8 years and cats under 1 year than among other age groups [25]. According to their study, there was no significant difference in external ear infections between males and females, which is by the present study.

Another study conducted in Romania in 2008 examined the causes of external ear infections in 50 dogs. Among the microorganisms, out of 50 cases, 13 cases (26%) parasitic agents, 13 cases (26%) of fungal agents, and 4 cases (8%) bacterial agents were present in the ear. Parasitic infections caused by *Otodectes cynotis* accounted for 9 of 13 (69.23%) cases. Furthermore, *Malassezia Pachydermatis* accounted for 9 out of 13 fungal infections (69.23%). Finally, Gram-positive and Gram-negative bacteria constituted equal proportions of the bacteria in external ears [27]. According to a study of 221 dogs in Northwest China between 2012 and 2016, dogs' most common ear diseases include *Otitis externa*, otitis media, otitis interna, and ear hematomas. Based on the study's results, there were 221 cases of dog ear disease, the most

common of which was *Otitis externa*. *Otitis externa* was caused primarily by bacterial infection, followed by a fungal infection. Males outnumbered females by a ratio of 1:1.5. Teddy was the most common dog breed, followed by Golden Retrievers. The most common ear disorders in puppies were *Otitis externa* and ear canal hyperplasia, while ear canal tumors are more common in older dogs [28]. According to a study of 188 dogs and 17 cats in the Barcelona region, *Malassezia Pachydermatis* was reported in 110 cases (58.51%) of dogs and 9 cases (52.94%) of cats [15]. Additionally, according to the results of a study conducted in 2017 in Canada, bacteria were found in the ears of 60 dogs, with external ear infections in 47% of the cases [29]. In addition, 53 dogs with external ear infections were examined in the United States in 2006 to determine the presence of microorganisms, and 69.8% were found to have a *Malassezia Pachydermatis* infection. Among the observed bacteria, *Pseudomonas aeruginosa* was the most present bacteria, with 22.22%. Additionally, *Proteus mirabilis, Staphylococcus aureus, Staphylococcus epidermidis, Escherichia coli*, and *Staphylococcus coagulase* were detected in the samples in amounts of 13.89, 12.50, 8.33, 5.56, and 5.56%, respectively. The poodle breed had the highest number of contamination cases with 30.19%, out of all breeds. There were also 40.43% of infection cases in dogs aged 2 to 5 months [30]. Also, in a study of 86 cats living free in Italy in 2013, *Otodectes cynotis* was identified as the primary cause of infection in 53.3% of cases [31]. However, according to our statistical analysis, none of the factors of age, gender, or breed had a significant effect on the occurrence of parasitic, yeast, and bacterial ear diseases.

Conclusion

According to our findings, neither age, breed, nor gender plays a significant role in determining the incidence of *Malassezia*, gram-positive and gram-negative bacteria, *Otobius megnini*, *Sarcoptes scabiei*, and *Notoedres cati* in *Otitis externa*. However, further research is required to determine their role in animal skin. Additionally, it appears that increasing the number of cases studied and studying external otitis in different geographical locations will enhance the value of the results. Further case-control studies involving a more significant number of samples may provide insight into the characteristics of dog and cat ear infections in Iran. Therefore, paying attention to these cases can improve the success rate of management and treatment for patients with external ear infections.

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