

Feline Otitis caused by *Proteus mirabilis* in Basrah, Iraq

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Abstract

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This study was conducted on 52 local cat breeds, one- and more than one-year-old, of both sexes, from Basrah province, Iraq, showing signs of otitis. Aural cytology is collected and added to the enrichment broth. The diagnosis of the causative organism depended on morphological and molecular techniques. The organisms on MacConkey agar exhibited mucoid, non-lactose fermenters, and colorless colonies; and the Gram's staining showed negative bacilli. Furthermore, all isolates showed positive results in Citrate utilization tests. The result of the triple sugar iron agar of the causative organism was acidic (change of color to yellow) and gas production. Furthermore, several isolates showed a swarming phenomenon on nutrient agar. Results were also validated using DNA sequencing and the PCR technique. The results showed that out of the 52 studied cases, 15 were from *Proteus* species, of which 7 were confirmed to be *Proteus mirabilis*. Seven isolates of the confirmed *Proteus mirabilis* selected were deposited in the gene bank database (accession numbers OR185608, OR185604, OR185605, OR185606, OR082827, OR082828, and OR082829). A phylogenetic tree analysis based on 16S rRNA gene sequence analysis of *Proteus mirabilis* was constructed to show the evolutionary relationships of the obtained sequence with similar sequences in the databases using MEGA11 software. Feline ear infections (especially those caused by *Proteus* species) are important and growing. Therefore, preventive and therapeutic measures are very important to limit the spread of the disease.

Keywords: Feline Otitis; *Proteus mirabilis*; Basrah; Iraq

Introduction

It has been mentioned that the external ear consists of an important anatomical structure called the external acoustic meatus whose function locates and collects the basic sounds (Heine, 2004). Moreover, the Tympanic membrane, auditory tube, the existing ossicles as well as the tympanic cavity are the most anatomical parts of the middle ear (Kennis, 2013). Its main function is to serve as a transducer for incoming sound waves into other waves naturally in liquid media (Oliveira et al., 2008). Furthermore, it also explained anatomically that the main structures of the inner ear are the cochlea,

the vestibule, as well as the semicircular canals. Those important structures will bind the head to gravity, which permits the visual system to work very precisely for movement and to understand the linear as well as the rotational acceleration (Matousek, 2004). It was documented that infections of the ear could be considered as one uncommon clinical problem in feline. However, it could be more serious when it infects a large number of animals and becomes more widespread and common. On the other hand, Otitis externa (A simple outer infection) could soon be extended to the inner ear structures, the middle ear and inner ear. This is what needs to be considered for quick remedial measures to avoid complications

such as hearing loss (Jacobson, 2002; Sari & Sitepu, 2021).

It was confirmed that otitis takes different forms, including otitis externa, which is often a simple form that may respond to treatment quickly, as its main cause is some common bacteria and ear mites, while the inflammation of the inner ear, which is more virulent for the infected animal, is caused by some more dangerous microorganisms that may cause major complications that negatively affect the affected animal health, as the prevalence of ear infections in both dogs and cats has not been accurately determined because of the difficulty of ideal diagnosis sometimes (Rosser, 2004; Zur et al., 2011; Tyler et al., 2020).

It was shown that ear infections could develop when irritation and inflammation arises in the skin of the ear canal reflecting the increase in the production of the wax which could create suitable media for bacterial and fungal growth, causing more itchy and uncomfortable ears which could develop to headshaking, scratching and clawing (Kennis, 2013).

It also documented that, immune system diseases such as Autoimmune diseases, keratinization disorders (such as primary idiopathic seborrhoea and hypothyroidism), Moreover, Wax buildup, Allergies (particularly food allergy and atopy), long and thick fur in the ear, presence of foreign bodies in the ear canal, various bacterial and fungal growth are the most important causes of otitis in cats. Further, incorrect ear cleaning, diabetes mellitus, as well as the presence of tumors or polyps in the ear canal might also play good roles as important predisposing factors for disease occurrence (Oliveira et al., 2008). In addition, accurate diagnostic methods should be used to identify the main etiologies and contributing factors which must be included, disease history, and clinical examinations of the diseased animal with using of the otoscope besides cytology as well as culture and sensitivity test. However, biopsy could be taken in more severe and recurrent cases (Roy et al., 2011).

Different microorganisms were isolated and frequently considered causative agents for otitis in both cats and dogs, such as *Staphylococcus*, *Pseudomonas* spp., and *Proteus* spp. Moreover, *Mycoplasma* spp. as a primary pathogen associated with otitis in cats has also been reported (Amanda et al., 2017; Hiblu et al., 2021). It was mentioned that some infectious microorganisms can be transmitted from animals to humans through direct or indirect contact with contaminated food, surroundings, and the environment (Zhang et al., 2016).

Proteus mirabilis, a Gram-negative bacterium, is an emerging pathogen in veterinary and human medicine (Armbruster et al., 2018). This organism is found in diverse habitats, including soil and animal urinary and digestive tracts, with the most common infection site being the upper urinary

tract (Armbruster & Mobley, 2012; Kwon et al., 2022). *P. mirabilis* infection in the upper urinary tract can cause urolithiasis, permanent kidney damage, bacteremia, and sepsis. Additionally, *P. mirabilis*-induced bacteremia and sepsis had a higher mortality rate than diseases brought on by other pathogens. Moreover, because multidrug-resistant bacteria have been reported, the clinical implications of these bacteria on public health are crucial (Armbruster et al., 2018).

In this study, the causative agents of otitis in local cat breeds in Basrah, Iraq, from a clinical, microbiological, and molecular perspective will be investigated.

Material and Methods

Ethical Approval

The current study has been ethically allowed through the consultant of the animal ethical committee, affiliated of the College of Veterinary Medicine, University of Basrah, Iraq.

Animals and the area of the study

The study was conducted on 52 local cat breeds, one year and more than one-year-old, and of both sexes from different regions of Basrah province/Iraq, showing signs of otitis. Diseased cats are domesticated animals in a free form, eating special cat food. Animal history was taken and clinical signs were registered.

Identification of bacterial isolates

Aural cytology, in the form of samples from the horizontal of the diseased ear canal, is collected onto a clean cotton-tipped swab, which is then directly inoculated onto brain heart infusion broth and incubated at 37°C for 24 h, then inoculated on the MacConkey agar plates and incubated at 37°C for 24 h. The suspected colonies from cultures were purified by subculture onto nutrient agar and incubated at 37°C for 24 h. All isolates were stained with Gram's stain and examined by a light microscope to detect shape and cell arrangements according to Barrow & Feltham (2003). The suspected isolates were further tested for catalase, Oxidase, citrate, and triple sugar iron, according to MacFaddin (2000).

PCR technique

The 16S ribosomal RNA gene was utilized to validate the diagnosis of causative microorganisms. Following the manufacturer's instructions, genomic DNA was extracted from isolates using the Promega Genomic DNA Purification Kit (USA) kit. Using Hot-Start PCR Master Mix (Promega, USA) and the universal primers 27F and 1492R, Frank et al. (2008), the gene encoding 16S rRNA was amplified by PCR (Frank et al., 2008). The 50 µL PCR reaction mixture was

made up of 25 µL of master mix (Promega, USA), 2 µL of template DNA, 2 µL of forward primer, 2 L of reverse primer, and 19 µL of nuclease-free water. The PCR amplification settings were as follows: 96°C for 5 min of initial denaturation, then 35 cycles of 95°C for 30 s, 55°C for 30 s of annealing, 72°C for 60 s of elongation, and 72°C for 10 min of final extension (Frank et al., 2008). After gel electrophoresis, the PCR bands were observed and photographed concerning a DNA ladder (Promega, USA).

Sequencing the PCR products of 16S rDNA

The 16S rDNA PCR products were sent to MacroGen Company in South Korea for sequencing and purification. To determine the sequence homology and identify the bacterial isolates, the acquired 16S rDNA gene sequences of bacterial isolates were obtained in raw format, edited using MEGA-X, and matched with nucleotide sequence databases of NCBI using BLAST tools (<http://www.ncbi.nlm.nih.gov>). MEGA X was used to create the phylogenetic tree (Kumar et al., 2018).

Phylogenetic analysis

Based on the examined 16Sr RNA nucleic acid sequences, a neighbor-joining phylogenetic tree was constructed for 7 isolates in comparison to other isolates from the NCBI database that showed the highest homology to the deposited NCBI *Proteus mirabilis* isolates. The evolutionary distances were calculated using the Maximum Composite Likelihood method and are expressed as the number of base substitutions per site (beneath the branching). Next to each internal node in the tree, the proportion of sites where at least one unambiguous base is present in at least one sequence for each descendant clade is displayed. MEGA11 was used to perform evolutionary analyses (Tamura et al., 2021).

Results

Diseased cats show different clinical manifestations as 25(48%) have a unilateral ear infection and 27(51.9%) have a bilateral ear infection. Further, diseased cats show signs of shaking of the head with feeling pain (48.6%), animals scratching its ear (82.6%). Moreover, (51.9%), of diseased cats tilt its head. Furthermore, the ears discharge are black or yellow exudates with a bad odor (92.3%). On the other hand, the pinna will be detected as red, swollen, and sometimes having sores (78.8%). However, when the infection and the progressed inflammation extended to the inner and middle ears, diseased animals show signs of in-coordination with in-balance movement and orientation (42.3%). Moreover, hematoma (Aural hematoma) due to injured pinna could also but rarely encountered (17.3). (Table 1).

Table 1. Common clinical signs of diseased cats with Otitis

Clinical sings	No. of animals affected	%
Shaking of the head & feeling pain	44	84.6
Animal scratch its ear	43	82.6
Head tilt	27	51.9
Black or yellow discharge with a bad odor	48	92.3
Red, Swollen, and sores pinna	41	78.8
Signs of in-coordination with in-balance, movement, and orientation.	22	42.3
Aural hematoma	9	17.3

On the other hand feline otitis infection in the current study varied according to age, sex, and the location of infection where it was observed in cats more than one-year-old and less than one year old, (42.3%), (57.6%) respectively. Moreover, infection in male animals was (34.6%) and in females was (65.3). Furthermore, (48%) of diseased cats show unilateral infection, whereas, (51.9) show bilateral infection (Table 2).

Table 2. Percentage of diseased animals according to age, sex, location of infection

Animal condition	No. of affected animals	%
More than one year old	22	42.3
Less than one year old	30	57.6
Males	18	34.6
Female	34	65.3
Unilateral infection	25	48
Bilateral infection	27	51.9

On the other hand (Table 3) was showing the biochemical tests of the bacterial isolates

Diseased cats show redness due to congestion of the concave aspect of the ear pinna with brown-blackish ear discharge (Figure 1). However, some diseased animals show more obvious colored discharge (yellow) which might be indicated to *Proteus* spp. *Otitis* (Figure 2)

Diagnosis of the causative organism (*Proteus mirabilis*) depended primarily on the morphological phenotypes of *Proteus* spp. on MacConkey agar which exhibited mucoid, non-lactose fermenters, colorless colonies, and the agar color changed from pink to transparent and yellowish or tan with swarming characterization in nutrient agar medium (Figure 3). Moreover, all isolates were stained with Gram's staining and examined by a light microscope to detect Gram's reaction, shape, and cell arrangements. On the other hand, biochemical tests were used for the detection of *Proteus* isolates (Table 3). These biochemical tests were catalase, Oxidase,

Table 3. Biochemical tests of the bacterial isolates

Code of isolate	Gram stain	Catalase test	Oxidase test	Citrate tests	Triple sugar iron agar
1-	-	+	+	-	+
2-	-	+	+	-	+
3-	-	+	+	-	+
4-	-	+	+	-	+
5-	-	+	+	+	+
6-	-	+	+	+	-
7	-	+	+	+	-
8	-	+	+	+	-
9-	-	+	+	-	-
10	-	+	+	+	-
11	-	+	+	+	+
12	-	+	+	+	-
13	-	+	+	-	+
14	-	+	+	-	+
15	-	+	+	-	+

**Fig. 1. Redness due to congestion of the concave aspect of the ear pinna with brown-blackish ear discharge****Fig. 2. Colored discharge (yellow) in a cat which might indicate *Proteus* spp. otitis**

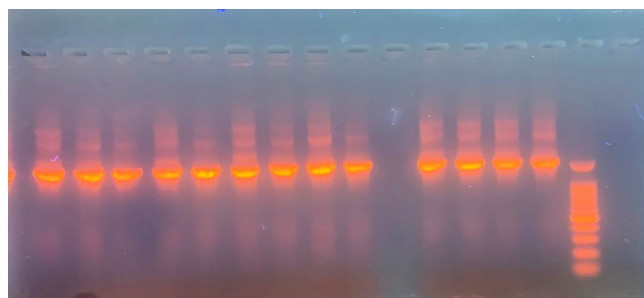
Triple sugar iron test, and citrate test. All *Proteus* spp. isolates from different sources showed positive results to Citrate utilization tests which change the medium from green to blue color. The result of Triple sugar iron agar of *Proteus* spp. was

**Fig. 3. Pink colonies of *Proteus* spp. in MacConkey agar**

as follows: acidic/change color(yellow), gas production.

By using the BLAST (Basic Local Alignment Search Tool) program in the NCBI database to compare the sequences of the 16SrDNA gene of the sequenced isolates to the highly similar DNA sequences in the GenBank database, fifteen isolates related to *Proteus* were found. Seven of these were confirmed to be *Proteus mirabilis* based on their homological sequence identity with global isolates at a 99% matching rate. The *Proteus mirabilis* strains (accession numbers OR185608, OR185604, OR185605, OR185606, OR082827, OR082828, and OR082829) were deposited in the gene bank database.

Moreover, a phylogenetic tree analysis of *P. mirabilis* was constructed based on the 16S rRNA gene sequence analysis (Figure 4), demonstrating the phylogenetic relationship between *Proteus mirabilis* strains isolated from animals and

**Fig. 4. The amplified PCR products of 16S rDNA on 1.5% agarose gel. Lane 1-4, 6, 7 are *P. mirabilis* samples showing 1500 base pairs bands. Lane M, DNA ladder 1500-100 base pair**

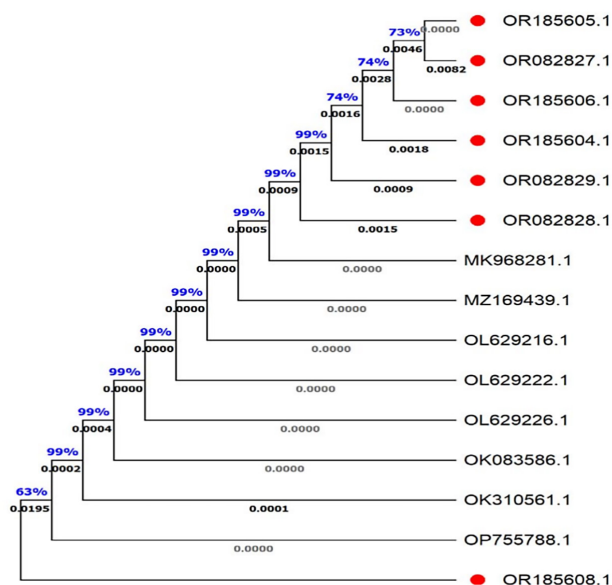


Fig. 5. Phylogenetic tree analysis of a: *P. mirabilis* constructed based on 16S rRNA gene sequence analysis, showing the phylogenetic relationships of strains isolated from animals (Red circles) and closely related *P. mirabilis* strains from the NCBI database. The tree was constructed by the neighbor-joining method

other related genera. The neighbor-joining method was used to construct the tree, and the GenBank ID of each contrast strain is provided (Figure 5).

Discussion

Inflammation of feline ears is considered an important clinical case. However, diagnosis and treatment are rarely approached when applied (August, 1988). Generally, otitis is one of the most common clinical issues in all animals, particularly in dogs and cats. It could also be considered one of the most difficult and disappointing cases for owners, which must be dealt with scientifically and seriously (Sari & Sitepu, 2021).

There are multiple and different causes that might cause and predispose to this disease, and those predisposing factors will allow the inflammation to develop. Moreover, anatomical abnormalities such as pinnal deformity and ear canal stenosis are far more common problems that enhance and play roles in this disease, which is the most common in pets. However, those predispositions do not relate to any breed predisposition to develop otitis in those animals (Moriello &

Diesel, 2010). Of those that predispose to the disease as well as the primary factors, those play an important role in the pathogenesis (Jacobson, 2002; Kennis, 2013). Nevertheless, all of those factors mentioned cannot be separated since they could be involved in a mixed infection that induces a big function covering the perpetuating and/or primary factors for developing otitis (Rosser, 2004).

It is firmly believed that the clinical signs displayed by diseased animals are a response to an underlying and persistent problem. Nevertheless, different agents can induce and exacerbate otitis. Therefore, it must always be remembered that proper monitoring and accurate follow-up of disease cases are the keys to successful management and control (Roy et al., 2011). It must always be taken into account that the basic problems, which always include factors predisposing to the disease, have an ideal role in the development of the disease. Furthermore, it could lead to physiological and anatomical changes to the ear, which may sometimes depend on the breed of the diseased animal, and we must not forget that the presence of thick fur in the ear, abnormal pendulous, and other contributing factors should be known, including swimming for frequent periods with an increase in ear humidity and repeated ear damage or tearing (Roy et al., 2011; Hiblu et al., 2021). It's worth knowing that a healthy ear canal must have a clean, smooth, thin, and pink appearance. On the other hand, a diseased ear appears swollen and contains exudates with a red lining, suggesting acute inflammation. Whereas, if it becomes firm with a fibrinous, indurate texture, it reflects the chronic type of otitis (Kennis, 2013). Furthermore, excessive erythema and congestion of the ear canal indicate allergic otitis (Oliveira et al., 2008). As abrasions and ulcers mixed with purulent exudates indicate pus-producing organisms caused mostly by gram-negative bacteria. Also, a lot of wax and oily secretions in the ear canal can be a sign of hyperplasia, the presence of foreign objects or parasites, or the growth of cancerous tissue (Oliveira et al., 2008; Sari & Sitepu, 2021).

In the current study, diseased cats exhibited different clinical manifestations, which were also mentioned and indicated by (August, 1988; Gotthelf, 2005; Moriello & Diesel, 2010; Kennis, 2013), who explained that, the cat owners always refer to the shaking of the heads of diseased cats with scratching or pawing, which can become severe sometimes, making the animal feel pain, and ceruminous debris being obvious with a bad odor. Nonetheless, diseased animals lose their appetite and appear lethargic. However, clinical examination could confirm that a diseased cat may suffer from clear congestion with abrasions, and an edematous pinna, as well as more obvious ear discharge. Furthermore, diseased cats show a pain reflex when touching the affected ear or

trying to bite, and scratching could start during the examination of the affected ear, which might develop into a hematoma as a result of pruritus. Moreover, Brame & Cain (2021), add that the erythema and the edematous appearance of a diseased ear canal will present with a thickened tympanic membrane. However, if otitis media develops, the tympanic membrane will bulge with obvious tympanum fluid. Nevertheless, the tympanic membrane, or horizontal ear canal, can be more affected by the accumulation of purulent debris and inflammatory exudates. On the other hand, unilateral or bilateral distribution of otitis lesions with synchronized nervous manifestations and systemic illness can assist in identifying the real causes of the disease.

It should also be taken into consideration that normal, healthy cats might show a blackish or ceruminous color, which may give the cat owners and/or the veterinarians the belief that they should clean or treat these ears unnecessarily. However, cleaning or even treating healthy ears is not advised, as it might overlap with the ear's normal clearance mechanisms, cause probable trauma, add moisture to the environment, or finally result in a contact reaction (Hariharan et al., 2011; Perego et al., 2014).

It was documented that erythroceruminous otitis was more obvious, with different degrees of inflammation. Moreover, inflammatory exudates, and chronic alterations were changeable from mild to more severe. However, it was seldom present with ulcers and was most commonly characterized by *Malassezia*, *Proteus spp.*, and staphylococcal infections. However, exudates vary from light dark brown to deep yellow (Roy et al., 2011; Hiblu et al., 2021). On the other hand, Suppurative otitis is also prevalent and shows more ear pain, ulcerations, and malodorous yellow to greenish pus exudates. This form of ear canal infection mostly occurs due to gram-negative bacterial infections (Amanda et al., 2017; Hiblu et al., 2021).

The results of this study showed that the percentage of cases of ear infection was higher in females than in male cats, and this did not agree with many researchers in this field, as the scientific workers did not find any difference in infection depending on the sex of the animal, as they did not find any scientific reason convincingly leads to an increase in infection rates between the two sexes, although females are often exposed to factors that may suppress animal immunity, such as pregnancy, childbirth, lactation, and hormonal changes, and this is consistent with (Hiblu et al., 2021; Sari & Sitepu, 2021). On the other hand, the results of the study showed that the risk of infection in cats was higher in young animals (less than one year) compared to their counterparts in old-aged cats (more than one year), and this is consistent with (Gothelf, 2005; Perego et al., 2014; Brame & Cain, 2021), who attributed the cause to the exposure of these young ani-

mals to increasing stress, especially in unvaccinated animals, and the possibility of exposure to many infectious diseases at this age, which may lead to a negative impact on the body's immunity and predispose the animal to secondary infections, including various types of ear infections.

In the current study, *Proteus mirabilis* was isolated and confirmed using phenotypic and molecular approaches. Bacterial identification was conducted based on morphological and biochemical tests that agree with Abbas et al. (2014). The phenotypic diagnosis of this bacterium is very important in recognizing its characteristics as well as its ability to produce many enzymes. On the other hand, the use of polymerase chain reaction (PCR) allows for fast identification of the causative bacteria using specific primers and sequencing the 16S RNA. This method is a suitable diagnostic test for feline otitis causative bacteria (Armbruster & Mobley, 2012; Amanda et al., 2017; Hiblu et al., 2021; Kwon et al., 2022). Although these isolates were from the same geographic area, the phylogenetic analysis shows some divergences among the isolates inferred from the 16S RNA evolutionary analysis seen in Figure 5. We selected seven isolates and deposited them in the NCBI database, which could help track the recurrence of such isolates in different locations.

The detailed pathology and effects of *Proteus mirabilis* otitis, particularly early on, differ to some extent according to the cause, but in general, changes are rather stereotyped. If acute inflammation and swelling aren't treated, they can turn into chronic inflammation, which is marked by glandular changes, fibrosis, and scarring, and can eventually cause the ear canal to narrow and close off. Moreover, permanent changes such as calcification and later ossification of cartilage can occur. Possible sequelae are otitis media and aural cholesteatoma (The abnormal accumulation of skin cells deep inside the ear canal) (Matousek, 2004; Sari & Sitepu, 2021).

Chronic changes favor the proliferation of bacteria and yeasts, further perpetuating pathology. Ulceration of the ear canal can occur, usually in association with *Pseudomonas* and *Proteus* infections. The secondary lesions of chronic otitis are due to chronic irritation and microbial overgrowth (Forbes et al., 2007; Mauldin et al., 2007). Further, the odor and appearance of the exudates could be helpful, but not very dependable. Therefore, a specific kind of exudates can be used as an indicator of suspicion for the exact type of otitis where the gross examination alone is inadequate (Matousek, 2004; Oliveira et al., 2008).

Conclusions

According to the results of the present work, ear infection in cats is considered as one of the diseases that has begun to

spread significantly among cats now. Therefore, early diagnosis and prompt treatment of diseased animals reduces the negative effects of the disease on infected cats.

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References

- Abbas, Y. A., Khudaier, B. Y. & Salih, H. A. (2014). Detection of multidrug resistant *Pseudomonas* spp. in clinical cases and hospital environments at Thi-Qar Province. *Journal of Education for Pure Science*, 3(2), 26-31.
- Amanda, L., Lenz, J. A., May, E. R. & Frank, L. A. (2017). Mycoplasma infection of the middle ear in three cats. *Veterinary Dermatology*, 28(4), 417-e102.
- Armbruster, C. E. & Mobley, H. L. (2012). Merging mythology and morphology: the multifaceted lifestyle of *Proteus mirabilis*. *Nature Reviews Microbiology*, 10(11), 743–754.
- Armbruster, C. E., Mobley, H. L. & Pearson, M. M. (2018). Pathogenesis of *Proteus mirabilis* infection. *EcoSal. Plus*, 8(1), 10-1128.
- August, J. R. (1988). Otitis externa, a disease of multifactorial etiology. *Veterinary Clinics: Small Animal Practice*, 18(4), 731-742.
- Barrow, G. I. & Feltham, R. K. A. (2003). Cowan and Steel's manual for Identification of Medical Bacteria. 3rd (ed.) Cambridge press., 175-180.
- Brame, B. & Cain, C. (2021). Chronic otitis in cats: Clinical management of primary, predisposing and perpetuating factors. *Journal of Feline Medicine and Surgery*, 23(5), 433-446.
- Forbes, B. A., Sahm, D. F. & Weissfeld, A. S. (2007). Diagnostic microbiology. St Louis: Mosby. 12 ed. 24, 340-350.
- Frank, J. A., Reich, C. I., Sharma, S., Weisbaum, J. S., Wilson, B. A. & Olsen, G. J. (2008). Critical evaluation of two primers commonly used for amplification of bacterial 16S rRNA genes. *Applied and Environmental Microbiology*, 74(8), 2461–2470.
- Gotthelf, L. N. (2005). Diagnosis and management of otitis media. In: Gotthelf L.N. *Small Animal Ear Diseases: an illustrated guide*. 2nd edition. Mosby, St. Louis (MO), 276.
- Hariharan, H., Matthew, V., Fountain, J., Snell, A., Doherty, D., King, B., Shemer, E., Oliveira, S. & Sharma, R. N. (2011). Aerobic bacteria from mucous membranes, ear canals, and skin wounds of feral cats in Grenada, and the antimicrobial drug susceptibility of major isolates. *Comparative Immunology, Microbiology & Infectious Diseases*, 34(2), 129–134.
- Heine, P. A. (2004). Anatomy of the ear. *Veterinary Clinics: Small Animal Practice*, 34(2), 379-395.
- Hiblu, M. A., Ellraiss, O. M., Karim, E. S., Elmishri, R. A., Duro, E. M. Altaeb, A. & Bennour, E. M. (2021). Otodectic and bacterial etiology of feline otitis externa in Tripoli. *Libya Open Veterinary Journal*, 10(4), 377-383.
- Jacobson, L. S. (2002). Diagnosis and medical treatment of otitis externa in the dog and cat. *Journal of the South African Veterinary Association*, 73(4), 162–170.
- Kennis, R. A. (2013). Feline otitis: diagnosis and treatment. *Veterinary Clinics: Small Animal Practice*, 43(1), 51-56.
- Kumar, S., Stecher, G., Li, M., Nnyaz, C. & Tamura, K. (2018). MEGA X: Molecular evolutionary genetics analysis across computing platforms. *Molecular Biology and Evolution*, 35(6), 1547–1549.
- Kwon, J., Yang, M.-H., Ko, H.-J., Kim, S.-G., Park, Ch. & Park, S.-Ch. (2022). Antimicrobial resistance and virulence factors of *Proteus mirabilis* isolated from dog with chronic Otitis Externa. *Pathogens*, 11(10), 1215.
- MacFaddin, J. F. (2000). Biochemical tests for identification of medical bacteria. *Williams and Wilkins*, Philadelphia, 113.
- Matousek, J. L. (2004). Ear disease. *Veterinary Clinics: Small Animal Practice*, 34(2), XI-XII.
- Mauldin, E. A., Ness, T. A. & Goldschmidt, M. H. (2007). Proliferative and necrotizing otitis externa in four cats. *Veterinary Dermatology*, 18(5), 370–377.
- Moriello, K. A. & Diesel, A. (2010). Medical management of otitis. In: *Consultations in Feline Internal Medicine*, 6., Saunders Elsevier, St. Louis (MO), 347-357.
- Oliveira, L. C., Leite, C. A. L., Brilhante, R. S. N. & Carvalho, C. B. M. (2008). Comparative study of the microbial profile from bilateral canine otitis externa. *Canadian Veterinary Journal*, 49(8), 785-8.
- Perego, R., Proverbio, D., De Giorgi, G. B., Della Pepa, A. & Spada, E. (2014). Prevalence of otitis externa in stray cats in northern Italy. *Journal of Feline Medicine and Surgery*, 16(6), 483–490.
- Rosser, E. J. Jr. (2004). Causes of otitis externa. *Veterinary Clinics: Small Animal Practice*, 34(2), 459-468.
- Roy, J., Bédard, C. & Moreau, M. (2011). Treatment of feline otitis externa due to *Otodectes cynotis* and complicated by secondary bacterial and fungal infections with Oridermyl auricular ointment. *Canadian Veterinary Journal*, 52(3), 277–82.
- Sari, W. N. & Sitepu, A. R. (2021). Diagnosis and treatment of otitis media in Persian cat at Central Pet Care. Fanik: *Jurnal Faperta Uniki*, 2(1), 1-6.
- Tamura, K., Stecher, G. & Kumar, S. (2021). MEGA 11: Molecular evolutionary genetics analysis version 11. *Molecular Biology and Evolution*, 38(7), 3022–3027.
- Tyler, S., Swales, N., Foster A., Knowles, T. G. & Barnard, N. (2020). Otoscopy and aural cytological findings in a population of rescue cats and cases in a referral small animal hospital in England and Wales. *Journal of feline Medicine and surgery*, 22(2), 161-167.
- Zhang, X. F., Doi, Y., Huang, X., Li, H. Y., Zhong, L. L., Zeng, K. J., Zhang, Y.-F., Patil, S. & Tian, G. B. (2016). Possible transmission of mcr-1–harboring *Escherichia coli* between companion animals and human. *Emerging Infectious Diseases*, 22(9), 1679.
- Zur, G., Lifshitz, B. & Bdolah-Abram, T. (2011). The association between the signalment, common causes of canine otitis externa and pathogens. *Journal of Small Animal Practice*, 52(5), 254–258.