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Otodectic and bacterial etiology of feline otitis externa in Tripoli, Libya

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Abstract
Background: Feline otitis externa is a dermatological disorder with a multifactorial complex etiology.
Aim: This study aimed to investigate the prevalence of different etiological agents, particularly the parasitic and bacterial, responsible for the cases of feline otitis externa in Tripoli, Libya, and to assess the antimicrobial susceptibility of the bacterial isolates from those cases.
Methods: Cerumen and otic discharges of the suspected cats were collected for parasite detection and bacterial culture. Kirby–Bauer's disk diffusion method was used for antimicrobial susceptibility testing.
Results: The results showed that otodectic mites and bacterial causes were equally the most prevalent in those cases, with a prevalence of 47.1% each. *Otodectes cynotis* infestation was more frequently bilateral and severe. *Staphylococcus* spp. were the most prevalent among bacterial causes (75%), followed by *Proteus* spp. (16.6%) and *Pseudomonas* spp. (8.4%). Norfloxacin and gentamicin were the most effective antimicrobials against bacterial isolates, as they were effective against 83.3% and 70.8% of isolates, respectively.
Conclusion: *Otodectes cynotis* infestation and staphylococcal infections constituted the most common etiology of feline otitis externa in Tripoli, Libya, and norfloxacin represented a cogent antibacterial for the treatment of otitis externa.
Keywords: Antimicrobial susceptibility, Bacteria, Cats, Ear mite, Otitis externa.

Introduction

Feline otitis is etiologically a complex disease, which can be clinically challenging (Shokri *et al*., 2010; Kennis, 2013). Otitis externa is the inflammation of the external ear canal, outside of the tympanic membrane, and sometimes the pinna (Rosser *et al*., 2004; Kennis, 2013; Bollez *et al*., 2018). This dermatological disorder may be acute or chronic (Moriello, 2013).

Causes of otitis externa could be primary or secondary, with a variety of predisposing and perpetuating factors that contribute to/or promote the disease. Collectively, these causes and factors are referred to as the primary secondary predisposing perpetuating (PSPP) classification system (Jacobson, 2002; Moriello, 2013). Primary causes of otitis externa affect normal ear and include foreign bodies, parasites, fungi (e.g., *Aspergillus*, *Sporothrix*, and *Stephanoascus*), allergy, autoimmune and immune-mediated diseases, endocrine and glandular disorders, viruses, and miscellaneous (Dion and Speckmann, 1978; Jacobson, 2002; Shokri *et al*., 2010; Kennis, 2013; Moriello, 2013; dos Reis Gomes *et al*., 2014; Mascarenhas *et al*., 2019).

These primary causes are usually unnoticed clinically, but they alter the ear environment and, therefore, facilitate secondary infections. Secondary causes of otitis externa affect abnormal ear and include bacteria, fungi, medication reactions, over-cleaning, and yeast overgrowth (Crespo *et al*., 2000; Jacobson, 2002; Hariharan *et al*., 2006; Shokri *et al*., 2010; Kennis, 2013; Moriello, 2013; Kittl *et al*., 2018).

Predisposing factors contributing to otitis externa include excessive local moisture and increased pH, trauma, obstruction of the ear canal, primary otitis media, systemic diseases, and treatment effects (Jacobson, 2002; Moriello, 2013). Perpetuating factors include changes in the ear epithelium, ear canal edema and stenosis, tympanic membrane dilatation and rupture, and sebaceous gland hyperplasia (Jacobson, 2002; Moriello, 2013).

Clinically, otitis externa can be unilateral or bilateral, acute or chronic and mild to severe. The common clinical findings of otitis externa include headshaking, aural pruritus, pain, and discomfort upon ear palpation and swelling, fibrosis, or calcification of the ear canal or pinna (Jacobson, 2002; Moriello, 2013).

Because of the diversity of the PSPP system contributing to the etiology and pathogenesis of otitis externa, the successful treatment depends on the identification of the causes and factors implicated in a given case. Accordingly, the treatment protocol of otitis externa may include ear cleaning, management of pain

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and pruritus, and the administration of topical and/or systemic drugs. Generally, drugs used for the treatment of otitis externa include ceruminolytic solutions, analgesics, glucocorticoids, anti-parasitics, antibiotics, and antifungals (Hariharan *et al*., 2006; Roy *et al*., 2011; Moriello, 2013; Momota *et al*., 2016; Yang and Huang, 2016; Arisov *et al*., 2020).

The prognosis of otitis externa is variable. Cases of feline otitis externa may be amenable, nonrecurring, and curable with treatment for weeks or via surgery. However, long-term management, which may take months, could be required in other recurrent cases that are unresponsive to routine therapy (Jacobson, 2002; Moriello, 2013).

Antimicrobial-resistant strains of *Staphylococcus* and *Pseudomonas* otitis have been emerged as frustrating and difficult causes of otitis because of the development of resistance to most common antibiotics (Hariharan *et al*., 2006; Qekwana *et al*., 2017). Antimicrobial-resistant strains are capable of causing a large nosocomial outbreak that is difficult to control in veterinary healthcare facilities (Gronthal *et al*., 2014).

Feline otitis externa is a multifactorial dermatological disorder that has not been fully elucidated and studies about it are lacking (Perego *et al*., 2014; Bollez *et al*., 2018; Tyler *et al*., 2019). In this regard, the objective of this study was to investigate the prevalence of different etiological agents responsible for feline otitis externa in Tripoli, Libya, particularly the bacterial and parasitic organisms, and to assess the antimicrobial susceptibility of bacterial isolates from such cases.

Materials and Methods

Clinical examination and sampling

Fifty-one cats presented to Al-Helal Al-Azraq central veterinary clinic in Tripoli, Libya, during the period from August 2016 to November 2019, with a complain of discomfort, otic pruritus, head rubbing, headshaking, ear scratching, and sometimes a head tilt, with the affected ear evidently tilted down. A thorough clinical examination of the ear pinnae and canals was carried out with the help of otoscope whenever possible.

Samples of maximum possible quantity of cerumen and discharges were collected from the affected ear(s) for further microscopic ear mite detection, as well as for bacterial isolation and antimicrobial susceptibility testing. Cytology sterile ear swabs, for bacterial cultures, were taken from the horizontal ear canal before otoscopy was completed and before any cleaning process. Collected samples were transported immediately to the lab for culturing.

Laboratory examination

Ear mite examination

Smears were made by combining cerumen and otic discharge with a small quantity of mineral oil on a microscopic glass slide. A clean glass coverslip was put on the top surface of the smear and examined microscopically (40X and 100X magnification) on the same day, for the presence of adult mites, nymphs, larvae, or ova. The total mite number was determined and ear mite genus was identified through morphological characteristics. The severity of infestation was scored according to the adult mite count on the smear; as mild (less than three mites), intermediate (three to five mites) or severe (more than five mites).

Bacteriological culture

The collected specimens were cultured onto 5% sheep blood agar and MacConkey agar (Oxoid, UK). Plates were incubated aerobically at 37°C for 24–48 hours. Subculture plates were made from primary cultures. Bacterial growth on culture plates were identified using standard biochemical tests as previously described (Markey *et al*., 2013). API bacterial identification strips (BioMérieux, Marcy L'Etoile, France) were also used for the identification of isolates.

Antimicrobial susceptibility testing

For investigating the susceptibility of bacterial isolates to antibiotics, isolates were subjected to Kirby–Bauer's disk diffusion method. Briefly, antibiotic disks including norfloxacin (10 µg), gentamicin (30 µg), oxytetracycline (30 µg), amoxicillin (30 µg), penicillin (10 units), and colistin (10 µg) were diffused onto Mueller–Hinton agar (Oxoid, UK) culture plates seeded with the bacterium to be tested. The plates were then incubated at 37°C for 18–24 hours. The inhibition zones of all drugs were interpreted as described previously (Markey *et al*., 2013).

Statistical analysis

Collected data were analyzed using the Statistical Package for Social Science (SPSS) v20 software (IBM). Frequency and percentage were used to describe the data.

Ethical approval

This study was approved by the Department of Internal Medicine of the Faculty of Veterinary Medicine at the University of Tripoli, Libya. Clinical examination and sampling procedures were carried out as per animal welfare protocols.

Results

General characteristics of feline subjects

In total, 51 cats (aged 2–48 months; mean ± SD of 15.26 ± 13.32 months) showing signs of otitis externa were included in the current study. Of these, 41.2% (n = 21) were males and 58.8% (n = 30) were females (Table 1). Also, 51% (n = 26) of the cases were local breed (Table 2). Other breeds were Persian, Mixed, Siamese, and Himalayan, which constituted 17.6% (n = 9), 15.7% (n = 8), 11.8% (n = 6), and 3.9% (n = 2) of the cases, respectively (Table 2).

Common causes of feline otitis externa in Tripoli, Libya

Otitis externa is multifactorial with a diversified PSPP classification system. The aim of this study was to determine the causes responsible for otitis externa in cats in Tripoli, Libya, particularly those of parasitic and bacterial origin. The results revealed that the parasitic

and bacterial causes were equally the most prevalent (Table 3), with a prevalence of 47.1% (24/51) each. *Otodectes cynotis* was diagnosed alone in 25.5% (13/51) of cats with otitis externa and in 21.6% (11/51) of the cases together with bacterial infection (Table 3). Similarly, feline otitis externa was exclusively of bacterial origin in 25.5% (13/51) of the cases and together with *O. cynotis* in 21.6% (11/51) of the cases (Table 3). The results also revealed that 27.4% (14/51) of otitis externa cases were attributed to other causes, viz foreign body, ringworm, and *Malassezia* yeast (Table 3).

Frequency and severity of otodectic feline otitis externa in Tripoli, Libya

In order to focus more clinically on feline otodectic otitis externa, some aspects like ear involvement and degree of infestation were investigated. In terms of ear involvement, *O. cynotis* more frequently infested both ears, as it was bilateral in 75.0% (18/24) of the cases and unilateral in 25.0% (6/24) of the cases: 16.7% (4/24) in the left ear and 8.3% (2/24) in the right ear (Table 4).

Concerning the degree of infestation, otodectic feline otitis externa cases were mostly seriously affected. *O. cynotis* infestation was severe in 70.8% (18/24), intermediate in 25.0% (6/24), and mild in 4.2% (1/24) of cases (Table 4).

Prevalence of bacterial organisms associated with feline otitis externa in Tripoli, Libya

Bacteria represent an important element in the PSPP classification system of otitis externa. One part of the study was to determine the prevalence of bacterial organisms isolated from cases of feline otitis externa in Tripoli, Libya. The results showed that *Staphylococcus* spp. were the most prevalent among bacterial causes, with a prevalence of 35.3% (18/51) among all feline otitis externa cases and 75% (18/24) among bacterial feline otitis externa cases (Table 5). *Staphylococcus* spp. were followed by the *Proteus* spp., with a prevalence of 7.8% (4/51) among all feline otitis externa cases, and 16.6% (4/24) among bacterial feline otitis externa cases (Table 5). *Pseudomonas* spp. were the least isolated, with a prevalence of 3.9% (2/51) among all feline otitis externa cases and 8.4% (2/24) among bacterial feline otitis externa cases (Table 5).

Antimicrobial susceptibility of bacteria isolated from feline otitis externa cases in Tripoli, Libya

Effective treatment of cases with bacterial otitis externa requires antimicrobial therapy. Recently, many antibiotic-resistant bacterial strains have emerged. Therefore, an antimicrobial susceptibility assay was carried out for determining the antibiotic(s) of choice for the diseased cats.

Table 1. Gender-wise distribution of cats with otitis externa.

Gender	Number	%
Male	21	41.2
Female	30	58.8
Total	51	100.0

Table 2. Breed-wise distribution of cats with otitis externa.

Breed	Number	%
Local	26	51.0
Persian	9	17.6
Mixed	8	15.7
Siamese	6	11.8
Himalayan	2	3.9
Total	51	100.0

Table 3. Epidemiology of feline otitis externa in Tripoli, Libya.

Cause of otitis external	Number	%
O. cynotis	13	25.5
Bacteria	13	25.5
Both mite and bacteria	11	21.6
Others	14	27.4
Total	51	100.0

Table 4. Distribution and severity of feline otodectic otitis externa in Tripoli, Libya.

Ear Mite		Number	%
Ear involved	R	2	8.3
	L	4	16.7
	R+L	18	75.0
	Total	24	100.0
Degree of severity	Mild	1	4.2
	Intermediate	6	25.0
	Severe	17	70.8
	Total	24	100.0

Table 5. Prevalence of bacterial organisms isolated from cats with otitis externa in Tripoli, Libya

Isolated Bacteria	Among all		Among bacteria	
	Number	%	Number	%
Staphylococcus spp.	18	35.3	18	75.0
Proteus spp.	4	7.8	4	16.6
Pseudomonas spp.	2	3.9	2	8.4
No bacteria	27	52.9	////	////
Total	51	100.0	24	100.0

Table 6. Antibiogram profile of bacteria isolated from cats with otitis externa in Tripoli, Libya.

	Staphylococcus spp.		*Pseudomonas* spp.		*Proteus* spp.		Total
	Number[a]	%[b]	Number	%	Number	%	
Norfloxacin	16 (2)	80.0	2 (0)	10.0	2 (2)	10.0	20
Gentamicin	12 (6)	70.6	2 (0)	11.8	3 (1)	17.6	17
Oxytetracycline	5 (13)	62.5	1 (1)	12.5	2 (2)	25.0	8
Amoxicillin	1 (17)	33.3	0 (2)	0.0	2 (2)	66.7	3
Penicillin	3 (15)	100.0	0 (2)	0.0	0 (4)	0.0	3
Colistin	0 (18)	0.0	1 (1)	100.0	0 (4)	0.0	1

[a]Susceptible (resistant).
[b]Among the total isolates susceptible to the respective antibiotic.

Table 7. Antimicrobial susceptibility of bacteria isolated from feline otitis externa cases in Tripoli, Libya.

		Staphylococcus spp.	*Pseudomonas* spp.	*Proteus* spp.	Total
		Number (%[a])	Number (%)	Number (%)	Number (%)
Norfloxacin	+	1 (5.0)	0 (0.0)	0 (0.0)	1 (5.0)
	++	15 (75.0)	2 (10.0)	2 (10.0)	19 (95.0)
Gentamicin	+	10 (58.8)	2 (11.8)	2 (11.8)	14 (82.4)
	++	2 (11.8)	0 (0.0)	1 (5.9)	3 (17.6)
Oxytetracycline	+	4 (50.0)	1 (12.5)	2 (25.0)	7 (87.5)
	+++	1 (12.5)	0 (0.0)	0 (0.0)	1 (12.5)
Amoxicillin	+	1 (33.3)	0 (0.0)	2 (66.7)	3 (100.0)
Penicillin	+	1 (33.3)	0 (0.0)	0 (0.0)	1 (33.3)
	++	2 (66.7)	0 (0.0)	0 (0.0)	2 (66.7)
Colistin	+	0 (0.0)	1 (100.0)	0 (0.0)	1 (100.0)

+ = Mildly susceptible; ++ = Intermediately susceptible; +++ = Highly susceptible.
[a]Among the total isolates susceptible to the respective antibiotic.

The results showed that norfloxacin was the most effective antimicrobial against bacterial isolates (83.3%, 20/24) (Table 6). *Staphylococci* isolates were most susceptible to enrofloxacin, (80%, 16/20), whereas *Pseudomonas* spp. and *Proteus* spp. isolates constituted only 10% (2/20) each (Table 6). The majority of isolates, 95% (19/20), were intermediately susceptible to norfloxacin (Table 7).

Gentamicin was following enrofloxacin in effectiveness, as 70.8% (17/27) of bacterial isolates were susceptible to it (Table 6). Furthermore, staphylococcal isolates were the most susceptible to gentamicin (70.6%, 12/17), followed by *Proteus* spp. (17.6%, 3/17) and then by *Pseudomonas* spp. (11.8%, 2/17) (Table 6). The majority of isolates (82.4%, 14/17) were mildly susceptible to gentamicin (Table 7).

Oxytetracycline was less effective against 33.3% (n = 8) of bacterial isolates when compared to norfloxacin and gentamicin (Table 6). *Staphylococci* species isolates were the most susceptible to oxytetracycline (62.5%, 5/8), followed by *Proteus* spp. (25%, 2/8) and *Pseudomonas* spp. (12.5%, 1/8) (Table 6). The majority of these bacterial isolates (87.5%, 7/8) were mildly susceptible to oxytetracycline (Table 7).

The results also showed that amoxicillin, penicillin, and colistin were the least effective antibiotics against the bacterial isolates with a percentage of 12.5% (3/24), 12.5% (3/24) and 4.1% (1/24), respectively (Table 6). Amoxicillin exhibited a mild effect mostly against some *Proteus* isolates (66.7%, 2/3), whereas penicillin showed an intermediate effect against staphylococcal isolates (100%, 3/3) (Tables 6 and 7). Colistin was mildly effective only against *Pseudomonas* spp. isolate (100%, 1/1) (Tables 6 and 7).

In terms of bacterial isolates susceptibility, *Staphylococcus* isolates were most susceptible to norfloxacin (88.8%, 16/18), followed by gentamicin (66.6%, 12/18), oxytetracycline (27.7%, 5/18), penicillin (16.6 %, 3/18), and amoxicillin (5.5%, 1/18), but resistant to colistin (Table 6). *Proteus* isolates were more susceptible to gentamicin (75%, 3/4), followed by norfloxacin, oxytetracycline, and amoxicillin (50%,

2/4 each), but resistant to penicillin and colistin (Table 6). However, *Pseudomonas* isolates were highly susceptible to norfloxacin and gentamicin (100%, 2/2 each) (Table 6).

Discussion

Feline otitis externa is a dermatological disorder with a complex etiology. It is multifactorial with a diversified PSPP classification system. In this context, this study was conducted to investigate the prevalence of different etiological agents, in particular the parasitic and bacterial organisms, responsible for the cases of feline otitis externa in Tripoli, Libya, and to assess the antimicrobial susceptibility of the bacterial isolates.

The current study involved 51 cats presented with a history and clinical findings compatible with otitis externa irrespective of the gender, age, and breed.

The results revealed that most of feline otitis externa in Tripoli were due to otodectic mites and bacterial causes with a prevalence of 47.1% each. In addition, 25.5% of feline otitis externa was caused by either *O. cynotis* or bacterial infection. However, co-infection with *O. cynotis* and bacteria was seen in 21.6% of cases. The remaining cases of otitis externa (27.4%) were due to other causes. These findings are in agreement with other studies' results (Griffin, 1993; Topala *et al*., 2007; Roy *et al*., 2011; Perego *et al*., 2014; Harvey and Paterson, 2014; Nardoni *et al*., 2014; Yang and Huang, 2016), which reported that otodectic otitis externa was prevalent in more than 50% of cats with otitis externa.

These observations are also in the line with the findings of Perego *et al*. (2014) who reported that bacterial causes were highly prevalent (>70%) in feline otitis externa cases. However, other studies reported a low prevalence of otodectic and bacterial otitis externa, but a more prevalent *Malassezia* species microorganism in otitis externa or healthy subjects (Bollez *et al*., 2018; Tyler *et al*., 2019).

In the present study, otodectic otitis was more frequently bilateral and severe. Generally, *O. cynotis* is considered to be very contagious (Roy *et al*., 2011). Roy *et al*. (2011) and Sotiraki *et al*. (2001) reported aural pruritus and abnormal auricular secretions in 41.5% and 85.4% of infested cats, respectively. In addition, *O. cynotis* can cause a hypersensitivity reaction in some individuals (Powell *et al*., 1980; Roy *et al*., 2011). Furthermore, the severity of otodectic otitis in the current study could be also attributed to the delayed presentation of the cases and negligence of therapy.

The results also showed that *Staphylococcus* spp. infection was the most prevalent among the causes of bacterial otitis externa with a prevalence of 35.3% (75% of bacterial otitis externa), followed by *Proteus* spp., with a prevalence of 7.8% (16.6% of bacterial otitis externa) and *Pseudomonas* spp., with a prevalence of 3.9% (8.4% of bacterial otitis externa). Bacterial organisms play an important role in otic diseases as a secondary or perpetuating factor and are usually opportunistic (Greene, 1998; Jacobson, 2002). Bacteria isolated from cases of feline otitis externa include *Staphylococcus*, *Pseudomonas*, *Escherichia*, *Proteus*, *Enterococcus*, and *Corynebacterium* species (Carter and Chengappa, 1993; Jacobson, 2002; Hariharan *et al*., 2006; Qekwana *et al*., 2017; Kittl *et al*., 2018). *Staphylococcus* species were the most commonly isolated bacterial organisms from dogs and cats with otitis externa and *Pseudomonas* infection was prevalent in chronic-resistant cases (Jacobson, 2002; Hariharan *et al*., 2006), which is in accordance with the observations reported in this study.

Recently, many antibiotic-resistant bacterial strains have emerged (Hariharan *et al*., 2006; Gronthal *et al*., 2014; Qekwana *et al*., 2017). Therefore, an antimicrobial susceptibility assays are required to determine the susceptibility to extended antimicrobial classes and for monitoring this phenomenon.

Accordingly, our results revealed that bacterial organisms, which caused feline otitis externa, were most susceptible to norfloxacin and gentamicin when compared to other antimicrobials. These observations are in accordance with Hariharan *et al*. (2006), who noted that ≥70% of bacterial isolates of canine and feline origin were more susceptible to gentamicin and enrofloxacin and, more particularly, 90% of isolates were susceptible to gentamicin.

The present study showed that staphylococcal isolates were most susceptible to norfloxacin, followed by gentamicin and less susceptible to oxytetracycline, penicillin, and amoxicillin, but resistant to colistin. Hariharan *et al*. (2006) also reported that staphylococcal isolates of feline otitis externa were susceptible to gentamicin, enrofloxacin, and clavulanated amoxicillin, but more frequently resistant to penicillin and ampicillin.

Proteus isolates were also more susceptible to gentamicin and less susceptible to norfloxacin, oxytetracycline, and amoxicillin, but resistant to penicillin and colistin. Hariharan *et al*. (2006) and Jacobson (2002) similarly reported that *Proteus* isolates were more susceptible to enrofloxacin and gentamicin in cases of canine otitis externa. Our study also showed that *Pseudomonas* isolates were more susceptible to norfloxacin and gentamicin, and less susceptible to oxytetracycline and colistin, but resistant to amoxicillin and penicillin. Hariharan *et al*. (2006) and Jacobson (2002) also reported that *Pseudomonas* isolates were susceptible to gentamicin and polymyxin B, but more resistant to penicillin in canine otitis externa cases.

In conclusion, this is the first study to provide novel data on the status of feline otitis externa and antimicrobial susceptibility and determine the most common etiologies responsible for feline otitis externa in Tripoli, Libya. Otodectic mites and bacterial organisms constituted equally the most prevalent

causes of otitis externa in affected cats. Ear mite (*O. cynotis*) infestation caused frequently severe bilateral otitis externa. *Staphylococcus* spp. was the most prevalent cause of feline otitis externa among bacterial organisms, followed by *Proteus* spp. and *Pseudomonas* spp.. Norfloxacin and gentamicin were the most effective antimicrobials against bacterial isolates.

Conflict of interest

The authors declare that there is no conflict of interest.

Authors' contribution

Omar Ellraiss examined the patients and collected the samples. Elfurgani Karim and Rabia Elmishri carried out the laboratory examination. Abdulnasser Altaeb contributed to consultation and revising the manuscript. Enass Duro carried out the statistical analysis of data. Emad Bennour and Murad Hiblu equally contributed to designing the study, interpreting the results, and writing the manuscript.

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