# STUDIES ON DIAGNOSIS OF OTITIS EXTERNA AND THERAPEUTIC EFFICACY OF OREGANO OIL ON BACTERIAL OTITIS IN DOGS

**BY**

**B.R. BABJI**

**ID No. RVM/2019-23**

THESIS SUBMITTED TO

**P. V. NARSIMHA RAO TELANGANA VETERINARY UNIVERSITY**

IN PARTIAL FULFILMENT OF THE REQUIREMENTS

FOR THE AWARD OF THE DEGREE OF

**MASTER OF VETERINARY SCIENCE**

**(VETERINARY MEDICINE)**

**IN THE FACULTY OF VETERINARY SCIENCE**

**DEPARTMENT OF VETERINARY MEDICINE**

**COLLEGE OF VETERINARY SCIENCE**

**P. V. NARSIMHA RAO TELANGANA VETERINARY UNIVERSITY**

**RAJENDRANAGAR, HYDERABAD - 500 030**

**December, 2021**

# CERTIFICATE

This is to certify that Mr. **B.R. BABJI (ID. No. RVM/19-23)** has satisfactorily prosecuted the course of research and that the thesis entitled **"STUDIES ON DIAGNOSIS OF OTITIS EXTERNA AND THERAPEUTIC EFFICACY OF OREGANO OIL ON BACTERIAL OTITIS IN DOGS"** submitted is the result of original work done and is of sufficiently high standard to warrant its presentation to the examination. I also certify that the thesis or part thereof has not been previously submitted by him for a degree of any University.

Date: 06.12.2021

Place: Hyderabad

**(S. AYODHYA)**

Major Advisor

# CERTIFICATE

This is to certify that the thesis entitled **"STUDIES ON DIAGNOSIS OF OTITIS EXTERNA AND THERAPEUTIC EFFICACY OF OREGANO OIL ON BACTERIAL OTITIS IN DOGS"** submitted in partial fulfillment of the requirements for the degree of **Master of Veterinary Science** of **P.V. Narsimha Rao Telangana Veterinary University** is a record of *bonafide* research work carried out by **Mr. B.R. BABJI (ID. No. RVM/19-23),** under our guidance and supervision. The subject of the thesis has been approved by the Student's Advisory Committee.

No part of the thesis has been submitted by the student for any other degree or diploma. The published part has been fully acknowledged. All assistance and help received during the course of investigations have been duly acknowledged by the author.

The final *Viva Voce* examination was held on 06-12-2021 and the Thesis is approved by the Student Advisory Committee

**Dr. S. AYODHYA**
Associate Professor and Head
Dept. of Veterinary Medicine
College of Veterinary Science,
Korutla, Jagtial– 505 326.

Chairperson ------------------- 6/12/21

Associate Professor & Head
Dep of Venterinary Medicine
College of Veterinary Science, Korutla
Dist: Jagtial. (T.S.) 505 326

**Dr. K. SATISH KUMAR**
Professor and University Head
Dept. of Veterinary Medicine
College of Veterinary Science,
Rajendranagar, 500 030.

Co-Chairperson ------------------- 6/12

Department of Veterinary Medicine
College of Veterinary Science
PVNR Telangana Veterinary University
Rajendranagar, Hyderabad-500030.

**Dr. B. ANIL KUMAR**
Assistant professor
Dept. of Veterinary Pharmacology and Toxicology
College of Veterinary Science,
Rajendranagar,500030

Member ------------------- 6/12

# TABLE OF CONTENTS

# LIST OF TABLES

# LIST OF FIGURES

# LIST OF ABBREVIATIONS

| Abbreviation | | Full form |
|---|---|---|
| % | - | Percentage |
| / | - | per |
| @ | - | At the rate of |
| < | - | Lesser than |
| > | - | Greater than |
| ≤ | - | Lesser than or equal to |
| ≥ | - | Greater than or equal to |
| °C | - | Degree centigrade |
| ± | - | Plus, or minus |
| dl | - | Deciliter |
| DLC | - | Differential Leucocyte Count |
| E. Coli | - | Escherichia Coli |
| EMB | - | Eosin Methylene Blue |
| et al. | - | and others |
| etc. | - | et cetera |
| Fig | - | Figure |
| G | - | Grams |
| g | - | Grams |
| i.e., | - | that is |
| Ltd. | - | Limited |
| M/s | - | Messrs |

| | | |
|---|---|---|
| Mg | - | Milligram |
| MHA | - | Muller-Hinton Agar |
| Ml | - | Milli liter |
| MIC | - | Minimum Inhibitory Concentration |
| Mm | - | Millimeter |
| MR | - | Methyl Red |
| MSA | - | Mannitol Salt Agar |
| ND | - | Non-Descriptive |
| No. | - | Number |
| PBS | - | Phosphate Buffer Saline |
| PCV | - | Packed Cell Volume |
| pH | - | Pouvoir Hydrogen |
| SDA | - | Sabaurouds Dextrose Agar |
| SE | - | Standard Error |
| *Spp.* | - | Species |
| TLC | - | Total Leucocyte Count |
| viz. | - | Namely |
| VP | - | Voges-Proskauer |
| WBC | - | White Blood Cell |
| W/ V | - | Weight by volume |

# DECLARATION

I, **B.R. BABJI (ID. No. RVM/19-23)** hereby declare that the thesis entitled **"STUDIES ON DIAGNOSIS OF OTITIS EXTERNA AND THERAPEUTIC EFFICACY OF OREGANO OIL ON BACTERIAL OTITIS IN DOGS"** submitted to P.V. Narsimha Rao Telangana Veterinary University for the degree of **MASTER OF VETERINARY SCIENCE** is a result of original research work done by me. It is further declared that the thesis or any part thereof has not been submitted for any other degree or diploma.

**(B.R. BABJI)**

**Date:** 06/12/2021

**Place:** Hyderabad

## ACKNOWLEDGEMENTS

*It gives me immense pleasure to express my undoubtful gratitude to my chairperson* ***Dr. S. AYODHYA,*** *Associate Professor and Head, Department of Veterinary Medicine, College of Veterinary Science, Korutla, Jagtial district for his soulful interest, concern, continuous supervision and perfect guidance from the beginning of the research, valuable advices in every situation, endless encouragement and also spending the precious time in correction, execution, compilation and preparation of the manuscript, his great patience and restless efforts of whom I could bring the manuscript into its sensible shape for which I extend my deep sense of reverence and rectitude etiquette.*

*With immense pleasure, I express my deep sense of gratitude to co-chairperson of the advisory committee* ***Dr. K. SATISH KUMAR****, Professor and University Head, Department of Veterinary Medicine, College of Veterinary Science, Rajendranagar, Hyderabad and Principal Investigator and Nodal Officer of NAHEP-IG for inspiring and able guidance, constant encouragement, keen interest, meticulous attention to details, valuable suggestions in planning and execution of research work and in the preparation of thesis*

*I extend my sincere gratitude to* ***Dr. B. ANIL KUMAR,*** *PhD, Assistant Professor, Department of Veterinary Pharmacology and Toxicology, College of Veterinary Science, Rajendranagar and member of my advisory committee for his valuable suggestions and guidance regarding research work.*

*I would not forget to remember* ***Dr. G. Ambica****, Assistant Professor, Department of Veterinary Medicine, College of Veterinary Science, Rajendranagar, Hyderabad and* ***Dr. J. Jyothi*** *Assistant Professor, Department of Veterinary Medicine, College of Veterinary Science, Rajendranagar, Hyderabad for their encouragement and more over for their timely support and guidance till the completion of my research work.*

*I am thankful to and fortunate enough to get constant encouragement, support and guidance from all Teaching staffs of Department of Veterinary Medicine****,*** ***Dr. P. Nagaraj,*** *Professor, Veterinary Clinical Complex, Veterinary Hospital, Bhoiguda who helped me in successfully completing my project work. Also, I would like to extend our sincere esteems to all Nonteaching staff in for their timely support.*

*I am very grateful to my parents* **Sri. B. Ramchander** *and* **Smt. B. Annapurna** *without whose sacrifice, blessings and affection, this study would not have been accomplished. I can't express my feelings, so I would like to dedicate myself to my parents for their unconditional love and support and I owe all my success to my beloved parents. Words cannot express my sentimental gratitude that i owe to my sister* **B. Bhargavi** *and brother* **B. R. Tarun** *for his affection, caring and loving inspiration throughout my research.*

*I use this opportunity to sincerely thank my colleagues* **Dr. K. Rajashekar, Dr. P. Anirudh, Dr. A. Deepika, Dr. Janardhan** *and their friendship, love, help and care and for making the two-year study very much enjoyable and memorable.*

*I owe my deepest gratitude towards my seniors* **Dr. Ranjith Kumar, Dr. Abhinav Kumar Reddy, Dr. Vinay**, **Dr. K. Ramesh, Dr. Shashank** *for their continuous moral support and encouragement throughout my degree life and manuscript preparation, without whose support I could not complete this piece of work intime.*

*I am extremely delighted to extend my thanks to my junior colleagues* **Dr. Suman, Dr. Krishna Chaitanya, Dr. Rajesh Khanna, Dr. Sai Charitha, Dr. Tejaswi, Dr. Bhavitha** *for their timely help rendered during the period of study.*

*I am thankful to* **P. V. Narsimha Rao Telangana Veterinary University, Hyderabad** *for providing financial assistance in the form of stipend during my postgraduate study.*

*I wish to extend my thanks to one and all those who have contributed even in a small way either direct or indirect manner to bring out my work in the present form.*

***(B. R. BABJI)***

| | | |
|---|---|---|
| Name of the Author | : | **B. R. BABJI** |
| Title of the thesis | : | **"STUDIES ON DIAGNOSIS OF OTITIS EXTERNA AND THERAPEUTIC EFFICACY OF OREGANO OIL ON BACTERIAL OTITIS IN DOGS"** |
| Degree to which it is submitted | : | **MASTER OF VETERINARY SCIENCE** |
| Faculty | : | **VETERINARY SCIENCE** |
| Department | : | **VETERINARY MEDICINE** |
| Major Advisor | : | **Dr. S. AYODHYA** |
| University | : | **P.V. NARSIMHA RAO TELANGANA VETERINARY UNIVERSITY** |
| Year of submission | : | **2021** |


## ABSTRACT


The present study "Studies on Diagnosis of Otitis Externa and Therapeutic Efficacy of Oregano oil on Bacterial Otitis in Dogs" was under taken with an aim to estimate the incidence, diagnosis of otitis externa in dogs and to evaluate therapeutic efficacy of two different regimens in dogs presented to Campus Veterinary Hospital, Rajendranagar and Veterinary Hospital Bhoiguda, College of Veterinary Science, Rajendranagar, Hyderabad and also suspected cases referred from private clinics of Hyderabad during period from December 2020 to July 2021.

In the present study screening of 264 dogs which are suspected for otitis externa, 39 dogs were diagnosed with otitis externa by ear cytological examination, and cultural examination forming an overall incidence of 14.77%, age wise incidence was 10.25 percent in the age group of 3 months to 1 year; 48.71 percent in 1 to 5 years and 41.02% in 5-10 years. Breed wise incidence revealed Labrador retriever (21.42%), German shepherd (16.92%), Cocker spaniel (12.00%), Mongrel (12.00%), Doberman (11.11%), Spitz (9.52%), Rottweiler (8.00%), Pug (6.66%) breeds. Sex wise incidence was recorded to be more in the males (58.97%) compared to females (41.02%) and season wise incidence was reported to be highest in summer (43.58%), followed by rainy (30.76%) and winter (25.64%) season respectively.

In the present study, group I dogs suffering with otitis externa showed head shaking (100%), pain (100%), head tilting (100%), ear scratching (71.42%), ulceration (71.42%), erythema (57.14%), inflammation and swelling of ear canal (57.14%), foul smell ear discharge (42.85%), However all the clinical signs subsequently resumed to normal by post treatment (after 15 days). Whereas group II dogs showed clinical signs such as head shaking (100%), pain (71.42%), head tilting (85.71%), ear scratching (42.85%), ulceration (57.14%), erythema (71.42%), inflammation and swelling of ear canal (85.71%), foul smell ear discharge (28.57%) whereas, the post treatment percentages are 0%, 0%, 20%, 0%, 0%, 33.33%, 0%, 25% respectively.

Out of total 39 isolates 31 were bacterial isolates and 08 were yeast isolates which includes *Staphylococcus* spp. (41.02%) followed by *Pseudomonas spp.* (28.20%), *Proteus spp.* (5.12%), *Streptococcus spp*. (5.12%) and *Malassezia spp.* (20.51%). Antibiotic sensitivity test of whole cultures revealed highest sensitivity to enrofloxacin (91.27%), followed by gentamycin (83.34%), amikacin (81.32%), amoxicillin (80.19%) and ofloxacin (74.36%). Whereas, antifungal sensitivity test of whole cultures revealed sensitive to clotrimazole (93.40%) followed by ketoconazole (84.60%) and nystatin (28.40%).

In the present study, the mean values of (Total Leukocyte Count *x* $10^{3)}$ in dogs with otitis externa under group I (11.41 ± 0.29) and group II (10.96 ± 0.19) before treatment that were found significant increase (P<0.01) when compared to that of the apparently healthy control group (9.31 ± 0.58). After therapy the values decreased significantly (P<0.01) to 9.73 ± 0.20 and 9.90 ± 0.15 in group I and II, respectively. The mean values of neutrophils (%) in dogs with otitis externa under group I (75.32± 0.49) and group II (75.48 ± 0.81) before treatment revealed non-significant increase when compared to that of the apparently healthy control group (66.85 ± 3.45). After therapy the values decreased non-significantly to 68.98 ± 0.42 in group I and 70.07 ± 0.52 in group II, respectively. The mean values of lymphocytes in dogs with otitis externa under group I (17.96 ± 0.42) and group II (17.82 ± 0.34) before treatment were found significant decrease (P<0.01) when compared to that of the apparently healthy control group (27.77 ± 1.92). After therapy the values increased significantly (P<0.01) to 28.34 ± 0.45 and 27.15 ± 0.43 in group I and II, respectively. The mean values of monocytes in dogs with otitis externa under group I (0.76 ± 0.03) and group II (0.83 ± 0.05) before treatment were found significant decrease (P<0.01) when compared to that of the apparently healthy control group (1.32 ± 0.02). After therapy the values increased significantly (P<0.01) to 1.18 ± 0.03 and 1.06 ± 0.06 in group I and II, respectively. The mean values of eosinophils in dogs with otitis externa under group I (5.98 ± 0.19) and II (5.86 ± 0.14) before treatment were found significant increase (P<0.01) when compared to that of the apparently healthy control group (1.45± 0.03). After therapy the values decreased significantly (P<0.01) to 1.49 ± 0.14 and 1.72 ± 0.07 in group I and II, respectively.

The treatment protocol which was used in treating the ear infections included cleaning the ear debris using cerumenolytic preparations, salicylic acid (0.2%) which is used to clear the cerumen and wax also to remove the cellular debris in both group I and II and subsequently instillation of ear preparation containing antiseptic and antibacterial properties, oregano oil instillation in group I, which contains carvacrol that has antibacterial effect and pomisol ear drops in group II which contains clotrimazole 1.0%, ofloxacin and glucocorticoids, it inhibits the growth of bacteria. Beside this, effect of immune-modulator was also tried in both group I and II includes immunosky syrup that contain vitamin C, vitamin D3, zinc, curcuma longa and glycyrrhiza, this immune-modulator activates natural killer cells, stimulates lymphocytic proliferation and activates macrophages and help in production of interleukins.

The therapeutic efficacy was assessed based on response to treatment and in the present study it was observed to be comparatively higher in group I (100%) than in group II (71.42%) on day 15 of therapy. It is concluded that oregano oil can be used for efficient treatment of otitis externa in dogs. The therapeutic regimen used in group I could be recommended to achieve good therapeutic response in otitis externa positive dogs.

# INTRODUCTION

CHAPTER I

# INTRODUCTION

The domestic dog (*Canis familiaris* or *Canis lupus familiaris*) of the order carnivora, and member of Canidae family, is a descendant of the wolf. The dog is derived from an ancient extinct wolf and it is the dog's nearest living relative. The dog was the first species to be domesticated, by over 15,000 years ago (Frantz *et al*., 2020). Dogs are considered as one of the most intelligent and loyal pet animals to the mankind. They perform many roles to mankind such as hunting, herding, protection and companionship. Due to loyalty and cooperative behaviour towards the owner they are considered as best animals for companionship. Dog has become the requisite member of the family with more emotional attachment. Hence there is need of regular healthcare for the longer life expectancy in dogs.

Otitis externa is defined as the inflammation of external ear canal and pinna. It is a common and often protracted or recurrent clinical conditions in dog (Bradley *et al.,* 2020). Otitis externa is the one of the most common and multifactorial disorders accounting up to 10 to 20% of consultations in canine practice (Senthil *et al.,* 2010). The maximum incidence of otitis externa was recorded in dogs of age between 1 to 3 years followed by dogs between 3 to 6 years of age (Agnihotri *et al.,* 2014). Certain breeds of dogs such as Cocker Spaniels, Springer Spaniels and Labrador retrievers have more ceruminous or wax glands in their horizontal ear canals that increase their chances of developing otitis externa. Breeds that have an increased number of hairs in the horizontal canal such as poodles, can also be predisposed to otitis externa.

The causes of otitis externa have been divided into three categories primary causes, predisposing factors, perpetuating factors (Miller *et al.*, 2013). Primary causes of otitis externa include allergy/hypersensitivities, auto immune diseases, keratinisation diseases, foreign bodies of which atopic dermatitis is a most common primary cause of otitis externa (Paterson, 2016). The predisposing factors for otitis externa are ear confirmation, excessive moisture, obstructive ear disease, systemic disease and treatment effects (Paterson, 2016). Perpetuating factors are a consequence of the inflammation and if left untreated, can prevent the resolution or worsen an already existing otitis externa. Examples of perpetuating factors include infectious agents such as bacteria and yeast, otitis media, chronic pathological changes of the ear canal, and iatrogenic causes such as contact reactions to medications and excessive cleaning of the ears. Infectious agents such as bacteria and yeast are the most common perpetuating factors of otitis externa. They are present in low numbers in normal ears (Tater *et al.*, 2003). It was stated that bacteria and yeasts are not primary pathogens of otitis externa but opportunistic species that replicated under favourable conditions created by another primary cause. Clinical manifestation of disease is predominantly the result of secondary bacterial or fungal infection, most commonly from the dog's commensal bacterial microbiota and fungal microbiota (Miller *et al.*, 2013).

Indeed, commensal microbes from the external ear canal of normal dogs can proliferate often because of the underlying factors listed above and infections with pathogens such as *Staphylococcus pseudintermedius, Pseudomonas aeruginosa, Proteus mirabilis* and *Malassezia pachydermatis* appear to be the dominant problem (Shaw, 2016).

Diagnosis of the otitis externa is done by otic examination and cytology of the otic discharge and cultural isolation of the pathogens from the specimen collected as swab containing otic exudate from the affected clinical patient. The cytological examination is the quantitative, giving the rapid indication of the relative number of different microorganisms present in the ear, which may aid in empirical selection of therapy. The number and type of bacteria, yeast, and inflammatory cells should be quantified before initiating the treatment.

Topical administration of antiseptics, generally referred to as ear cleaners, could be a useful sole or adjunctive treatment for canine otitis externa (Paterson, 2016). Topical therapy is an important part of the treatment of otitis externa in dogs (Morris, 2004). Some of the acid-containing products (e.g., salicylic acid, acetic acid and lactic acid) have anti-microbial activity against bacteria and *Malassezia* yeasts (Parmar *et al*., 2020).

Some of the herbal medicines like oreganum vulgare, essential oil could be a promising treatment to combat canine cutaneous mixed infections (Ebani *et al.*, 2020).

Keeping in view of the above facts comprehensive studies have been framed incorporating clinical cases of canine otitis externa and with the following objectives.

1. To study the clinical signs of otitis externa and to evaluate the associated haematological alterations.

2. To isolate and identify various causative agents involved and to diagnose the various etiology of otitis externa in dogs.

3. To assess the efficacy of oregano oil against bacterial otitis in dogs.

4. To study the incidence of otitis externa among dogs.

# REVIEW OF LITERATURE

CHAPTER II

# REVIEW OF LITERATURE

## 2.1 INCIDENCE OF OTITIS EXTERNA

Ettinger and Feldman (2000) in their studies on otitis externa, reported an overall incidence of otitis externa as 20 % in clinical cases in canine practice.

Masuda *et al*. (2001) in their study observed that incidence of canine otitis externa to be 4.4% of all canine diseases in the year 1993 which is increased to 8.1% in 1997 in Japan.

Scott *et al*. (2001) in their work opined that otitis externa was a common multifactorial disorder accounting for up to 10 to 20% of cases in canine practice.

Ginel *et al*. (2002) stated that otitis externa is the most commonly diagnosed disorder of the ear canal in dogs with the incidence reported to be between 10 and 20% of total patient admissions.

Angus (2004) opined that otitis externa is a common multifactorial disease with incidence as high as 10 to 20% in dogs.

Cole (2004) reported that the incidence of otitis externa in dogs was 10% to 20%.

Rosser (2004) stated that otitis externa was one of the most common clinical condition, accounting for up to 15% of all dogs presented in veterinary practice.

Fernandez *et al*. (2006) opined that otitis externa is common in small animal veterinary practice with a incidence of between 5% and 20%.

Greene (2006) stated that otitis externa is particularly common in canine pets with incidence rate of 5 to 20%.

Saridomichelakis *et al.* (2007) opined that canine otitis externa is common and represents up to 20% of consultations in small animal practice.

Senthil *et al.* (2010) in his study stated that otitis externa is one of the most common and multifactorial disorders accounting for up to 10 to 20% of consultations in canine practice.

Bartlett *et al.* (2011) recorded that the incidence of otitis externa has been estimated to be between 7.3 and 10.2% of cases presenting to veterinary clinics.

Anthony (2013) in their study observed that the incidence of otitis externa in dogs is 10 to 20%, and also as high as 30 to 40% in tropical and subtropical environments.

Kumar *et al*. (2014) in their study reported that the incidence rate of otitis externa in dogs to be 21.97% in Jammu region.

O'Neill *et al*. (2014) reported that the incidence of otitis externa ranges between 4 and 20 % in different dog population.

Noli *et al.* (2017) opined that otitis externa is a common skin disease in dogs, accounting for 22% of dermatology patients and 13 to 16% of the general hospital population.

Perry *et al.* (2017) stated that otitis externa is one of the most common dermatological diagnoses, affected approximately 20% of the pet dog population worldwide.

Filipo *et al*. (2018) observed that the incidence of otitis externa in dogs was as high as 10 to 20%.

Soares *et al.* (2020) reported that the incidence of otitis externa was 7.30% in United Kingdom.

2.1.1 AGE WISE INCIDENCE

Chaudhary *et al*. (2003) in their study noticed greater incidence of otitis externa in 1 to 14 years old dogs.

Nair (2004) stated that higher incidence of otitis externa was recorded in dogs between 1 to 6 years of age.

Mhatre (2005) noticed higher incidence of otitis in dogs aged between 1 to 3 years (48.14%) followed by dogs older than 3 years (29.63%).

Girao *et al.* (2006) observed that dogs between 1 to 3 years of age group were more susceptible to *Malassezial* otitis externa.

Sapierzynski (2009) in his study opined that the occurrence of otitis externa is most common in 1 to 7 years of age (a mean age of 4.5 years).

Martins *et al.* (2011) observed occurrence of otitis externa in the age group of 0 to 3 years (p=0.059), accounting for 89 cases (32.04%).

Oliveira *et al.* (2012) reported that the cases of canine otitis occurred predominantly in first years of age, in mixed breeds of dogs.

Agnihotri *et al.* (2014) in their study reported that maximum incidence of otitis externa was observed in dogs of 1 to 3 years followed by 3 to 6 years.

Manju *et al*. (2018) reported higher incidence of otitis externa has higher incidence in old age dogs.

Parmar *et al.* (2020) concluded that the highest incidence of otitis externa was found in the age groups of 1 to 5 year (44.77%, n=30), followed by 5 to 10 year (29.65%, n=20), 10 to 15 year (13.43%, n= 9), below one year age group (10.44%, n=7) and above 15 years age (1.49%, n=1).

Hegde *et al.* (2021) in their work observed that out of the total 55 cases studied on age wise analysis 3(5.46%) cases were observed in dogs aged less than one year, 19 (34.55%) in age group less than 5 years, 27 (49.10%) in the age group between 5 to 10 years, and 6 (10.91%) in age group more than 10 years.

2.1.2 BREED WISE INCIDENCE

Ahmed (2000) stated that most of the otitis externa cases were observed in Terriers, Pointers and German Shepherds and cases observed was 16, 10, 9 cases respectively.

Chaudhary and Mirakhur (2002) reported an incidence of otitis in German Shepherd breed followed by Spitz breed as 31.14% & 29.87% respectively.

Kumar *et al.* (2002) opined that the most frequently affected breed with the otitis externa was German Shepherd followed by Labrador Retriever.

Fernandez *et al.* (2006) reported that the most commonly affected breeds with otitis externa are Poodle followed by Mongrel, Cocker Spaniel and German Shepherd and the percentage is 30.19% ,26.42% ,16.98%, 9.49%, respectively.

Topala *et al.* (2007) observed that Cocker Spaniel, Labrador, Setter, German Shepherd had the highest incidence.

Saridomichelakis *et al.* (2007) in their study observed that otitis externa occurred more commonly in breeds like Cocker Spaniel, Poodle, German Shepherd, Brittany's spaniel, Doberman Pinscher, Siberian Husky and Beagle.

Sapierzynski (2009) in his study reported that Yorkshire Terriers, Spaniels, Boxers, Labradors and Golden retrievers were over expressed, on the other hand, in Mongrels, German Shepherds and Dachshund's otitis externa was recognized more rarely than in other breeds.

Sharma *et al.* (2016) reported that Labrador, Beagle and Cocker Spaniel breeds were most commonly predisposed to otitis externa.

Kaimio *et al.* (2017) in their work stated that incidence of otitis externa was highest in Welsh Springer Spaniels (149 out of 468, 31.8%,), followed by American Cocker (89/329, 27.0%,), English Springer (96/491, 19.6%,) and English Cocker Spaniels (231/1467, 15.7%,).

Perry *et al.* (2017) reported that dog breeds such as Spaniels, German Shepherd and Shar-Pei are represented significantly more in otitis externa cases.

Parmar *et al.* (2020) concluded that the breed wise incidence of otitis externa was found to be highest in the Labrador (35.82%, n=24) followed by German Shepherd and Pomeranian (16.41%, n=11, each), Pug (11.94%, n=8) followed by Non-Descript (4.47%, n=3), Doberman, Golden Retriever and Saint Bernard (2.98%, n=2, each), and Rottweiler, Cocker Spaniel, Dachshund and Beagle (1.49%, n=1, each).

Hegde *et al.* (2021) in their work reported that out of the total 55 cases studied, Labrador dogs had highest incidence 15 (27.27%) followed by Spitz 11 (20%), Non-Descript dogs 10(18.18%), German Shepherd dogs 7 (12.72%), Pug 5 (9.09%), Dalmatian 3 (5.46%) and one each (1.82%) in Rajapalyam, Cocker Spaniel, Great Dane and Dobermann.

### 2.1.3 SEX WISE INCIDENCE

Chaudhury and Mirakhur (2002) reported that otitis externa occurs more frequently in male dogs (53.34%) and in female dogs (36.36%).

Kumar *et al.* (2002) concluded in their study that male and female dogs are equally susceptible to otitis externa.

Cunha *et al.* (2003) stated that otitis externa was found more frequently in female dogs (60%).

Fernandez *et al.* (2006) reported that female dogs were more commonly affected than male dogs with otitis externa.

Saridomichelakis *et al.* (2007) in their study stated that among 100 dogs with otitis externa 45 dogs were male and 55 dogs were females.

Topala *et al.* (2007) observed in their research period that there is no apparent sex predisposition to otitis externa in canines

Lehner *et al.* (2010) in their study stated that among 83 dogs with otitis externa 39 dogs were males and 44 dogs were females.

Martins *et al.* (2011) in their study opined that there was no statistical difference between the sex wise occurrence of otitis externa.

Zur *et al.* (2011) in their study opined that no sex predisposition for canine otitis externa.

Agnihotri *et al.* (2014) reported that there is higher incidence of otitis externa in male dogs than that of female dogs that is 68.71% and 31.29% respectively.

Manju *et al*. (2018) reported higher incidence of otitis externa in male dogs.

Parmar *et al.* (2020) concluded that out of total 67 otitis externa cases, 58.20 percent (n=39) cases were male and 41.80% percent (n=28) cases were female.

Hegde *et al.* (2021) reported that out of the total 55 otitis externa cases studied, 29 (52.73%) of affected dogs were males and 26 (47.27%) were females.

### 2.1.4 SEASON WISE INCIDENCE

Conkova *et al*. (2011) in their work reported higher incidence of otitis externa in autumn followed by winter, spring and summer.

Kumar *et al*. (2011) reported that highest incidence of otitis externa among the dogs was observed in summer compared to other seasons.

Balappanavar and Vasant (2013) recorded higher incidence of canine otitis externa in summer season.

## 2.2 ETIOLOGY AND RISK FACTORS

Ahmed (2000) isolated *Staphylococcus intermedius* (14 cases), *Pseudomonas aeruginosa* (10 cases), *Proteus mirabilis* (6 cases), *Candida albicans* (3 cases) and *Actinomyces pyogenes* (2 cases) from canine otitis externa cases.

Colombini *et al.* (2000) recorded that *Staphylococcus intermedius* (26.8%), *Pseudomonas aeruginosa* (23.2%), b-haemolytic *Streptococcus* (12.8%), *Proteus spp*. (11.0%) and *Staphylococcus epidermidis* (8.5%) are the common micro-organisms isolated in canine otitis externa.

Martín Barrasa *et al.* (2000) stated that in chronic otitis externa *Pseudomonas aeruginosa*, either alone or in combination with other microorganisms, is the most frequent gram-negative pathogen.

Mota *et al*. (2000) reported that the commonest organisms isolated from canine otitis externa were *Staphylococcus species* (27.72%) and *Malassezia* (22.77%).

Silva (2001) in his study recorded that *Staphylococcus intermedius* was found in 12.3% of cases and *Staphylococcus aureus* was found in 8.8% of cases in chronic canine otitis externa.

Griffin and Deboer (2001) in their study observed that 55% of dogs diagnosed with atopic dermatitis had showed signs of otitis externa.

Harvey *et al.* (2001) in their study reported *Demodex canis* as a rare cause of otitis externa in dogs.

Scott *et al.* (2001) stated that bacteria and yeasts are the opportunistic organisms of otitis externa, as they need a favorable medium for growth which is provided by the primary cause.

Chaudhary and Mirakhur (2002) observed that the long haired and pendulous eared dogs like Spaniels, Poodles and Terriers were more prone to otitis externa.

Kumar *et al.* (2002) studied otitis externa in Haryana region in a total of 200 dogs and isolated 26.73% of *Pseudomonas aeruginosa*, 69.31% of *Staphylococcus* species and 12.87% cases of *Proteus* species.

Jacobson (2002) in his study opined that primary causes of otitis externa include foreign bodies, hypersensitivity, and keratinization disorder and ear mites.

Petersen *et al.* (2002) recorded that 27.8% of ear swab samples collected from otitis externa were positive for *Pseudomonas aeruginosa*.

Cunha *et al.* (2003) stated that the percentage of dogs with pendulous ears affected with otitis externa was 80 percent.

Tater *et al.* (2003) opined that *Pseudomonas aeruginosa* is not common inhabitant of healthy canine ears and when *Pseudomonas* infection is present it can result in inflammation and ulceration within the external ear canal.

Vikas *et al.* (2003) in their work observed that 40% of dogs with otitis externa had infection with *Pseudomonas* species and *Malassezia* species.

Baksi *et al.* (2004) reported that in 10 cases of otitis externa in dogs, *Streptococcus* species were found from 25% of cases, *Staphylococcus* species from 80% of cases.

Bass (2004) opined that ear diseases were present in over 80% of dogs with food allergy and it may be the only clinical sign in more than 20%-25% of food allergic dogs.

Gotthelf (2004) reported that commonest microbial pathogens associated to otitis externa are members of genera *Staphylococcus, Streptococcus spp., Corynebacterium spp, Pseudomonas aeruginosa, Proteus mirabilis, Escherichia coli* and *Klebsiella pneumoniae.*

Kale and Aher (2004) stated that *Staphylococci*, *Streptococci*, *Pseudomonas*, *Corynebacterium* were the pathogens which were isolated from canine otitis externa.

Rosser (2004) reported that relatively rare causes of otitis externa were auto immune diseases that may affect pinna or the external ear canals include pemphigus erythematosus, pemphigus vulgaris, systemic lupus erythematosus, cutaneous vasculitis, bullous pemphigoid and membrane pemphigoid.

Sarierler and Kirkan (2004) found that *Staphylococcus aureus* (11.53%) was the most commonly isolated pathogen followed by *Corynebacterium spp.* (6.42%), *Staphylococcus* (5.12%), *Pseudomonas species* (3.85%) and *Streptococcus species* (2.56%) in canine otitis externa.

Angus (2005) stated that hypothyroidism results in impaired immune response, increased cerumen production, and alteration of epidermal barrier function. These changes can contribute to overgrowth of *Malassezia* and bacteria resulting in clinical disease, 10% of dogs have clinically significant atopy, of these up to 80% exhibit otitis externa as part of their disease.

Cafarchia *et al.* (2005) observed that dogs with pendulous ears were having high risk of infection with otitis externa than erect eared dogs.

Gotthelf (2005) reported that the most common fungal pathogen in the etiology of otitis externa was *Malassezia spp*. and rarely *Candida* or another saprophytic fungal organism.

Mhatre (2005) reported that among 27 otitic dogs 56% dogs had coagulase positive *Staphylococcus*.

Oliveira *et al.* (2005) stated that *Staphylococcus* species either coagulase negative or positive and *Pseudomonas aeruginosa* were the most common pathogens found in canine otitis externa.

Yamashita *et al.* (2005) reported that, from dogs affected with ear infection *Staphylococcus* species was isolated from 48.3% of cases.

Fernandez *et al.* (2006) in their work cultured 53 ear swabs of canine otitis externa and recorded that most frequently isolated organisms were *Pseudomonas aeruginosa* (22.22%), *Proteus mirabilis* (13.89%), *Staphylococcus aureus* (12.50%), *Staphylococcus epidermidis* (8.33%), *Escherichia coli* (5.56%) and coagulase negative *Staphylococci* (5.55%).

Mahendran *et al.* (2007) opined that dog with erect ears had higher occurrence of otitis externa.

Saridomichelakis *et al.* (2007) observed otitis externa due to otitis media with perforated tympanic membrane in 25% of dogs, atopic dermatitis in 8% of dogs, pemphigus foliaceous in 1% of dogs, grass awns in 12% of dogs, 4% of dogs due to entry of water into the ear canals, allergic dermatitis (atopic dermatitis or food hypersensivity) in 43% of dogs. The ear canal cytological examination revealed cocci (38/100) and rods (22/100) as secondary causative factors of otitis externa.

Schick *et al.* (2007) reported that common organisms isolated from dogs affected with otitis externa include *Staphylococcus spp., Pseudomonas spp., Proteus spp., Streptococcus spp., Escherichia coli, Klebsiella spp., Bacteroides spp., Pasteurella spp.* and *Malassezia spp.*

Mactaggart (2008) stated that grass awns in the ear canal were usually present in the summer months and may be associated with acute signs of otitis externa and also neoplasms, polyps as the primary causative factors of otitis externa.

Turkyilmaz (2008) recorded that percentage of coagulase positive *Staphylococcus* isolated from ear infection in dogs was 24%.

Viorica *et al.* (2008) noticed that causes of otitis externa were (26%) parasitic, (32%) allergic, (8%) bacterial and each of the following were (2%) tumor, (2%) metabolic, (2%) autoimmune and cornification disorders (2%).

Engelen *et al.* (2010) reported that the most frequently isolated pathogenic organisms from otitis externa in dogs were gram positives, with 56% *Staphylococcus spp*., 17% *Streptococcus spp.*, 44% infections with the yeast *M. pachydermatis* and 12.5% cases infected with the gram-negative *Pseudomonas species*.

Lozina *et al.* (2010) observed that the microorganisms isolated from canine otitis externa were *Malassezia pachydermatis* (54.2%), *Staphylococcus aureus* (43.8%), coagulase-negative *Staphylococcus* (25.0%), *Pseudomonas aeruginosa* (20.8%), *Candida albicans* (18.8%), *Proteus mirabilis* (16.7%), *Streptococcus spp.* (16.7%), *Enteroccocus faecalis* (12.5%), *Escherichia coli* (12.5%), *Staphylococcus intermedius* (6.3%), *Klebsiella spp.* (4.2%), and *Candida glabrata* (2.1%).

Malayeri *et al.* (2010) recorded common causes of otitis externa in dogs as *Staphylococcus intermedius, Pseudomonas aeruginosa, Proteus mirabilis, Escherichia coli*, *Pasteurella canis* and six other species of coagulase-negative *Staphylococcus.*

Olivry *et al.* (2010) stated that cause of otitis externa was most often related to allergy such as atopic dermatitis in younger dogs.

Robson *et al.* (2010) observed that *Pseudomonas aeruginosa* was isolated from all ears in canine otitis externa. Other isolated organisms include *Staphylococcus spp, Pseudomonas intermedius, Proteus mirabilis, Streptococcus and Escherichia coli.*

Senthil *et al.* (2010) opined that otitis externa commonly referred to as ear disease, it can occur due to bacteria, fungus or parasites.

Yamamoto *et al.* (2010) recorded that the most frequent microorganisms isolated from animals with otitis externa were *Staphylococcus spp.* and gram-negative bacilli.

Bartlett *et al.* (2011) stated that the common bacterial isolates found in canine ear infection were *Staphylococcus, Pseudomonas intermedius,* Bacillus species, coagulase negative *Staphylococcus spp., Micrococcus species* and *Burkholderia cepacia*.

Coatesworth (2011) stated that the primary causative agents of otitis externa were *Otodectes cyanotes*, *Demodex canis*, *Otobius menigni*, *Sarcoptes scabei*, and *Trombicula autumnalis* and spending more time in water causes maceration inside the ear canal due to impairment in barrier region which predisposes to otitis externa.

Mekic *et al.* (2011) have been recognized that the genera *Corynebacterium* and *Pseudomonas,* as the pathogenic bacteria of otitis externa.

Van duijkeren *et al.* (2011) stated that *Staphylococcus pseudintermedius* is the leading cause of otitis externa in dogs, and is ultimately associated with urinary tract infections.

Zur *et al.* (2011) noticed otitis externa due to endocrinopathies in 6.1% of dogs.

Oliveira *et al.* (2012) stated that the most common microorganisms which were identified from canine otitis externa were *Staphylococcus* (26.27%), *Malassezia pachydermatis* (12.35%), and *Pseudomonas aeruginosa* (8.8%).

Bouassiba *et al.* (2013) studied 75 dogs with otitis externa and stated that it is caused by *Staphylococcus spp*. followed by *Pseudomonas spp*. and *Streptococcus spp*.

Bugden (2013) recorded common isolates of microorganisms in otitis externa which included *Pseudomonas aeruginosa, Staphylococcus, Pseudomonas intermedius, Proteus spp.*, beta-hemolytic *Streptococci* and *Escherichia coli*.

Lakshmi and Rao (2013) opined that otitis externa is caused due to growth of bacteria such *as Staphylococcus aureus, Streptococcus species, Pseudomonas aeruginosa*, *Escherichia coli* and *Proteus spp.*

Petrov *et al.* (2013) in his work showed that *Staphylococci*, *Malassezia pachydermatis* and *Pseudomonas aeruginosa* were the predominating microbial species isolated from dogs with otitis externa.

Pietschmann *et al.* (2013) recorded 17 strains of *Escherichia coli*, 24 strains of *Pseudomonas aeruginosa*, 24 strains of *Proteus mirabilis* and 25 strains of *Staphylococcus and Pseudomonas intermedius* as pathogens of otitis externa in dogs.

Seulgi (2013) reported that causative agents in canine otitis externa were *Staphylococcus, Pseudomonas intermedius, Pseudomonas aeruginosa* and *Malassezia pachydermatis.*

Harvey and Paterson (2014) reported that the presence of compound hair follicles within the Cocker Spaniel's ear canal may be another predisposing factor that is unique to this breed and predisposes it to otitis externa infection.

Nwiyi *et al.* (2014) recorded that among the 42 bacterial organisms isolated from canine otitis externa, *Pseudomonas spp*. was the highest in 18 (42.9%), followed by

*Staphylococcus spp.* 12 (28.7%), *Escherichia coli* 6 (14.3%), *Streptococcus spp*. 3 (7.1%), *Proteus spp*. 2 (4.2%) and *Enterococcus spp.* 1 (2.4%).

Santoro *et al.* (2015) stated that in dogs, a common underlying cause of otitis externa is atopic dermatitis.

Blake *et al.* (2017) reported that common bacterial pathogen associated with the perpetuation of canine otitis externa include *Staphylococcus spp.*, *Streptococcus, Pseudomonas, Proteus* and *Escherichia coli*, with *Staphylococcus pseudintermedius* being the most frequent.

Karlapudi (2017) observed that nearly almost all the ear infections in dog and cat include commensal (*Staphylococc*i and *Malassezia*) or environmental (*Pseudomonas*) pathogens which are many times opportunistic pathogens, Infection with either yeast or bacteria does not occur in a normal ear as the environment inside the external canals of most dogs is sterile. Infection develops because of inflammation produced by primary factors, usually in combination with perpetuating and predisposing factors.

Perry *et al.* (2017) noticed that there is significant increase in diagnosis frequency of otitis externa in dogs with pendulous ears, likely due to the moist, warm conditions facilitating secondary bacterial and fungal growth.

Ngo *et al.* (2018) reported that up to 75% of those diagnosed with otitis externa were also diagnosed with atopic dermatitis.

Terziev and Urumova (2018) collected samples from otitis externa dogs and subjected to bacteriological examination and found 17 coagulase-positive *Staphylococci*, 2 beta-hemolytic *Staphylococci*, 16 *Pseudomonas aeruginosa*, 7 *Proteus mirabilis*, 9 *Malassezia pachydermatis*, and 2 *Candida spp*. Further, the bacterial and fungal infections were most frequently encountered in the summer (26 isolates, 49.06%) succeeded by autumn (13 isolates, 24.54%), and least in winter and spring (7 isolates, 13.21%).

Agnihotri *et al.* (2019) opined that predisposing factors play crucial role in pathogenesis of this disease. Main predisposing factors included anatomical (ear canal stenosis, hair in the ear canal, pendulous ears, haired concave side of ears), environmental [increased humidity, moisture retention], washing, foreign bodies, prolonged antibiotic treatment, obstructive diseases (neoplasms) and systemic conditions causing immunosuppression.

Bourely *et al*. (2019) recorded that the most frequent bacterial genera isolated from dogs with otitis from 2012 to 2016 in France were coagulase-positive *Staphylococci, Streptococcus spp., Pseudomonas spp.* and *Proteus spp.*

Petrov *et al.* (2019) reported that *Staphylococcus pseudintermedius* is a predominant pathogen associated with otitis externa, isolated from 20–94.3% of otitis externa cases in canines.

Nocera *et al.* (2020) opined that otitis externa can be perpetuated by yeasts and bacterial infection, it might be caused due to commensals (*Staphylococcus spp*. and *Malassezia*) or environmental (*Pseudomonas*) organisms.

## 2.3 CLINICAL MANIFESTATIONS

Kumar (2001) reported the most common signs of otitis externa as aural pruritus, head shaking, alopecia, scaling, crusting and erythema of pinna with pruritus, partial drooping of one or both ears and rubbing the ears on the floor.

Noli and Cevidalli (2001) stated that clinical parameters of otitis externa in dogs were discomfort, erythema, oedema, and increased exudates.

Scott *et al.* (2001) noticed that clinical signs of otitis externa caused by *Malassezia* include head shaking, ear scratching, excoriations, foul smelled odour, erythema and swelling of the pinnae and external ear canals with waxy-to-greasy otic discharge.

Ashok *et al.* (2002) reported that clinical signs in canine otitis externa were scales, crust, erosion, redness of tissue pinna, pruritus, pain on palpation of ears, rubbing and scratching of ear, head shaking or scratching of ears and head tilting.

Angus *et al.* (2002) recorded that clinical feature of external otitis were inflammation, pain, bad smell, exudate, pruritus and head shaking. Chronic cases may also showed resistance to antimicrobial agents, ear canal stenosis, polyps and tympanic rupture, which caused chronic pain and lead to deafness.

Hendricks *et al.* (2002) observed nodular swelling along with serosanguineous to purulent discharge, erythema and ulceration of both external ear canals in otitis externa in dogs.

Mishra *et al.* (2003) stated that erythema, pain in ears, itching and otorrhea were the clinical manifestation of otitis externa in canines.

Kale and Aher (2004) reported that the common clinical signs of otitis externa in canines included, pruritus, head shaking, head tilting towards affected ear, dropping of affected ear and presence of serous to purulent discharge.

Dongardive *et al.* (2005) noticed foul smelling discharge from the ear canal, pain to the affected ear, tilting of head to affected side, frequent shaking of head, whining and crying, and off feed in dogs affected with otitis externa.

Mhatre (2005) reported clinical signs like head shaking, scratching of ear pinnae with paws and purulent exudates from external ear canal in dogs suffering with *Staphylococcus* otic infection.

Dixit *et al.* (2006) stated that dog with otitis externa exhibits clinical signs such as foul odour discharge, erythema, pain and loss of appetite.

Fernandez *et al.* (2006) frequently observed clinical signs such as auditory canal erythema, increased wax like secretion, pain, continuous head shaking pruritus and purulent secretions in otitis externa affected dogs.

Kumari *et al.* (2006) noticed symptoms of otitis externa in dogs affected with *Malassezia*, included mild pain upon touching the ear, slight yellow discharge, mild erythema along with grey-colored scales, ear shaking, occasional head shaking, erythema and ear scratching.

Sapierzynski (2009) reported the most common clinical signs in otitis externa in dogs included presence of otic discharge, odour, ear pain and aural pruritus.

Degi *et al.* (2010) stated that clinical signs in dogs with *Pseudomonas* otitis externa included unilateral or bilateral ear damage, head shaking, scratching or rubbing the ear. Vertical portion of the canal was obstructed due to moderate or severe skin hyperplasia, and a greenish yellow discharge, purulent, stinks were found.

Penna *et al.* (2011) observed symptoms of canine otitis externa due to *Pseudomonas aeruginosa* infection which included local pain, pruritus, erythema, ear discharge or desquamations of ear pinna.

Hosseini *et al.* (2012) noticed that clinical manifestations of *Pseudomonas* otitis externa include ear canal erosions and ulcers with frequent bleeding, purulent discharge with severe erythema, pain and discomfort. Tympanic membrane rupture can occur in chronic cases, which is due to inflammatory cells derived lysozymes and proteolytic enzymes secreted by *Pseudomonas aeruginosa*.

Oliveira *et al.* (2012) observed that the presence of itching, foul smell, and secretion from ear canal were the major signs at clinical examination of otitis externa in dogs.

Lakshmi and Rao (2013) observed that clinical signs of otitis externa were aural pruritus, ear pain, head tilt, erythema of pinna, alopecia, scaling, foul odour of ear discharge, crusting of pinna and swelling at the base of the ear canal.

Oegi *et al.* (2013) observed that clinical signs of dogs affected with otitis externa were aural pruritus, local pain, otorrhea and desquamation of the ear tegument.

De Martino *et al.* (2016) collected samples from 122 dogs showing clinical signs of otitis externa like local pain, pruritis, erythema and ear discharge, 74 dogs showing clinical bilateral otitis externa and from 48 dogs showing unilateral otitis externa.

Blake *et al.* (2017) reported that the physical findings indicative of otitis externa may included erythema, swelling, scaling, crusting, discharge, malodor and pain upon palpation of the auricular cartilage.

Karnad *et al.* (2020) stated that the dogs affected with otitis externa exhibit various clinical signs such as erythema of ear pinna, excoriation, foreign bodies, malodorous discharge and pruritis, as evidenced by head shaking and pain.

Hegde *et al.* (2021) stated that dogs affected with otitis externa exhibits symptoms like pruritus (itching) of the ear, head shaking, exudates in and around the ear canal, foul odour from the ear etc.

## 2.4 DIAGNOSIS

### 2.4.1 EXAMINATION OF MITES

Saridomichelakis *et al.* (2007) reported that upon the examination of the ear canal exudate for parasites in 100 dogs; was positive for *Demodex canis* in four dogs and *Otodectes cynotis* in seven dogs.

Mactaggart (2008) documented that mites such as *Otodectes* or *Demodex* can be seen by mixing an exudate sample taken from the ear with liquid paraffin and examining it under a low-power microscope.

### 2.4.2 CYTOLOGICAL EXAMINATION

Noli and Cevidalli (2001) reported that in cytological examination a decreased number of bacteria and yeasts were observed in canine otitis externa.

Hendricks *et al.* (2002) observed that cytological examination of the otic discharge showed scanty small cluster of cocci, no intra-cellular bacteria, many erythrocytes and neutrophils, fewer lymphocytes and macrophages.

Leite *et al.* (2003) in their work found that cytology to be 79 percent specific and concluded that results of direct microscopy were compatible with the results of microbiological culture examination.

Angus (2004) opined that cytology of the ear exudates is indispensable for confirming the presence of an infection and for evaluating the type of infection.

GrahamMize and Rosser (2004) stated that ear cytological examination is reported to be more sensitive in the detection of microorganisms than microbial culture in clinical cases of otitis externa.

Mhatre (2005) recorded that all the 27 swabs containing otic exudates revealed microorganisms along with cellular debris, neutrophils and macrophages and all the four *Malassezia pachydermatis* isolates were pre confirmed on ear cytology.

Fernandez *et al.* (2006) stated that cytological assay was found to be the most efficient method for identifying *Malassezia pachydermatis* and trust- worthy data was obtained through samples of ear exudates.

Saridomichelakis *et al.* (2007) in their work, cytological examination revealed the presence of rod-shaped bacteria in dog suffering with otitis externa.

Mactaggart (2008) reported that cytological examination allows for rapid assessment for the presence of bacterial cocci, rods, *Malassezia* yeast and neutrophils. If rod shaped bacteria were present, a sterile swab should be taken for cultur and isolation for further selective isolation.

Viorica *et al.* (2008) showed that in the cytological examination of the swab collected from infected ears had bacterial polymorphous flora consisting in cocci and bacilli. In severe cases phagocytic cells were seen, especially neutrophils that cleared up the bacteria and presence of macrophages.

Sapierzynski (2009) reported that cytological examination of otitic ear swabs revealed the presence of various pathogens in 88% of the cases, the most common organism observed is yeast *Malassezia*. In some cases, bacterial or mixed infections (yeast+bacteria) were recognized and in 10 cases parasites were found.

Robson *et al.* (2010) recorded that upon ear cytological examination of 20 dogs affected with otitis externa, rods (few to numerous) were detected in all cases of otitis externa, neutrophils (few to numerous) in 19/20 ears, cocci (few to moderate) in 7/20 ears and occasionally yeast (*Malassezia*) in 4/20 ears.

Boehringer (2011) opined that cytological examination of otic exudate confirms *Malassezia* overgrowth in canine otitis externa.

Hosseini *et al.* (2012) reported that cytological examination confirms and quantifies presence of inflammatory cells, rod shaped bacteria, cocci and yeasts in canine otitis externa.

Bosznay (2014) reported that ear cytology should ideally been performed in every case presenting signs of otitis externa, in order to decrease the use of antibiotics and better target specific infections.

Maginn (2016) opined that ear cytology examination of dogs suffering with otitis externa plays a major part in the effective diagnosis and therapeutic plan.

Shaw (2016) stated that cytology gives clear quantitative analysis of organisms present and microbiological analysis helps to identify specific infectious organisms.

Gotthelf (2017) in his work recorded that bacteria alone were found in 7/10 left ears and 7/9 right ears, while yeast was found in 2/10 left ears and 2/9 of right ears.

Neves *et al.* (2018) opined that cytological examination of otitic ear secretion and microbiological culture are excellent forms of diagnosis and cytology also has great use in accessing treatment evolution.

Bajwa (2019) opined that cytological evaluation of otic contents is the single most informative diagnostic test that helps with treatment of otitis externa.

Bradley *et al*. (2020) suggested that cytological evaluation of the ear canal is an informative and cost-effective guide for veterinarians to select pathogen-directed treatment.

2.4.3 HAEMATOLOGY

Lilliehok *et al.* (2000) in their work observed that eosinophilia was found in three (3%) dogs with severe otitis externa and eosinophilic count ranged from 4.6- 6.4 X $10^9$ / litre.

Pratibha *et al.* (2000) in their work noticed that there is significant decrease in total erythrocyte count values indicating anemia in cases of canine otitis externa.

Nair (2004) reported a non-significant difference in hemoglobin, total erythrocyte count, packed cell volume, total leukocyte count and differential leukocyte count in canine otitis externa cases.

Sharma and Gupta (2005) observed that there is leukocytosis in bacterial canine otitis externa.

Kim *et al.* (2009) conducted blood analysis of otitis externa infected dogs prior to treatment, as well as at 1$^{st}$ and 2$^{nd}$ weeks after treatment and concluded that there was significant difference in the total leucocytes counts between the control and experimental group at 1$^{st}$ week and 2$^{nd}$ week after treatment.

Blake *et al.* (2017) reported that in hematological examination, statistically significant differences of hemoglobin, mean corpuscular volume, red blood cell and white blood cell counts was observed in otitic doogs.

Kumar *et al*. (2017) in their work observed that differential leukocyte count revealed marked neutrophilia in bacterial and parasitic otitis externa besides, lymphopenia.

Sandeep (2017) concluded that leukocytosis, neutrophilia, lymphopenia is seen in bacterial canine otitis externa.

2.4.4 ISOLATION AND IDENTIFICATION

Mota *et al.* (2000) reported that ear swabs were cultured on blood agar and levine agar for isolation of bacteria in canine otitis externa.

Yoshida *et al.* (2002) reported that canine ear infections are a common dermatological complaint. *Staphylococcus spp*. and *Malassezia pachydermatis* are more frequently isolated from dogs ears, with or without external otitis, while *Proteus spp.* and *Pseudomonas spp.* are isolated only in dogs with otitis externa.

Kale and Aher (2004) stated that swabs were cultured on nutrient agar and nutrient broth revealed that out of 16 swabs, *Staphylococcus spp*. isolates were highest in 7 swabs (43.75%), *Pseudomonas spp.* from 4 swabs (25%), *Corynebacterium spp.* from 2 swabs (12.5%) and *Escherichia coli* from one (6.23%) swab.

Rosser (2004) in his work reported that *Staphylococcus pseudintermedius* is the commonest pathogen isolated from the dogs affected with otitis externa.

Fernandez *et al.* (2006) in their work recorded that of the different bacteria isolated in culture media, the most frequent were *Pseudomonas aeruginosa* (22.22%), *Proteus mirabilis* (13.89%), *Staphylococcus aureus* (12.50%), *Staphylococcus* *epidermidis* (8.33%), *Escherichia coli* (5.56%) and coagulase-negative *Staphylococcus* (5.56%).

Lyskova *et al.* (2007) reported that the most frequently isolated microorganism from otitic ears were *Staphylococcus intermedius* (58.8%), *Streptococcus canis* (29.9%), *Proteus spp.* (14.4%) and *Escherichia coli* (10.3%).

Petrov and Mihaylov (2008) in their work isolated *Staphylococcus spp.* (70.8%), *Streptococcus spp.*, (14.6%), *Escherichia coli* (8.3%), *Proteus mirabilis* (8.3%) and *Pseudomonas aeruginosa* (6.3%) from otitic ears of dogs.

Aalbaek *et al.* (2010) recorded that *Pseudomonas aeruginosa*, *Escherichia coli*, *Streptococcus spp.*, *Enterococcus spp.*, and *Corynebacterium spp*. have also been associated with otitis externa.

Malayeri *et al.* (2010) performed aerobic bacterial culture on blood agar and MacConkey agar and smears were stained using Gram's and Giemsa staining methods for identification of organisms in otitis externa.

Senthil Kumar *et al.* (2010) mentioned that ear swabs were cultured in Muller Hinton agar medium, MacConkey agar medium and was grown at 37° C and in Sabouraud dextrose agar at 25° C in otitis externa of dogs.

Penna *et al.* (2011) reported that cotton swabs were inoculated into Brain Heart Infusion broth (Difco - New Jersey, USA) and incubated at 37°C. They also stated that samples with morphology of Gram-negative rods were transferred to MacConkey's agar and Pseudomonas agar (Merck - New Jersey, USA) in canine otitis externa.

Zur *et al.* (2011) observed that in their study *Staphylococci* were the most common bacteria cultured from dogs suffering with otitis externa.

Hosseini *et al.* (2012) reported that in canine *Pseudomonas* otitis externa ear swabs were inoculated on blood agar plates (Columbia agar supplemented with 5% sheep blood) and incubated aerobically at 37°C for 48+/-2 hours. After 24-hrs and 48-hrs incubation the plates were examined for the growth of *Pseudomonas spp*. or other pathogenic bacteria. Colonies morphologically consistent with *P. aeruginosa* were sub cultured on fresh blood agar plates for subsequent identification.

Miller *et al.* (2012) recorded that at baseline, microbial cultures, *Malassezia* yeast and *Staphylococcus pseudintermedius*, which are the two most common pathogens isolated in canine otitis externa.

Steen and Paterson (2012) reported that swabs were cultured onto blood agar, colistin-nalidixic acid agar (CNA, which is selective for Gram-positive organisms), MacConkey agar and Saboraud's agar (Oxoid, Basingstoke, UK) in canine otitis externa.

Lakshmi and Rao (2013) stated that in canine otitis externa for isolation of bacteria samples cultured in Nutrient broth and were streaked using the sterile loop on the selective media and incubated at 37°C for 24 hours.

Bugden (2013) stated that *Pseudomonas aeruginosa*, *Staphylococcus spp.*, *Pseudomonas intermedius, Proteus species,* beta-hemolytic *Streptococci* and *Escherichia coli* were the five most frequently isolated microorganisms from cases of otitis externa.

Petrov *et al.* (2013) reported that ear swab samples were cultured on blood agar containing 5% sheep blood (Bul-bio-Base, National Institute of Parasitic and Infectious Diseases, Sofia) and on McConkey agar (Difco). Cultures were incubated aerobically for 24-48 hours at 37°C. Mycological tests included aerobic cultivation of the samples on Sabouraud dextrose agar (supplemented with 0.4 g/L chloramphenicol and 0.5 g/L cyclohexamide) for 2-7 days at 37°C. After incubation, isolates were identified according to conventional microbiological methods.

Agnihotri *et al.* (2014) in their work of otitis externa in canines isolated *Staphylococcus aureus* (59.21%), *Pseudomonas aeruginosa* (24.02%), *Proteus* (10.61%) and *Streptococci* (6.15%).

Subapriya *et al.* (2015) reported that multidrug resistant *Pseudomonas aeruginosa* strains, are frequently isolated from canine otitis externa.

De Martino *et al.* (2016) recorded *Staphylococcus pseudintermedius* as a common pathogen in canine otitis externa with an isolation rate as high as 70 percent.

Blake *et al.* (2017) observed that *Malassezia pachydermatis* was the predominant organism, isolated from 224 (72 per cent) of the 311 ear swab samples. *Staphylococcus pseudintermedius* was isolated from 149 (48 per cent) of the 311 samples, *Pseudomonas aeruginosa* was isolated from 33 (11 per cent) of the 311 samples, *Streptococcus canis* was isolated from 31 (10 per cent) of the 311 samples, and *Escherichia coli*, *Proteus mirabilis* and *Streptococcus dysagalactiae* were isolated from less than 10 per cent of the samples.

Karlapudi (2017) in his work reported that by microscopic examination of stained ear sample (62 samples) affected by otitis externa revealed *Staphylococci* (18/62), *Pseudomonas* (15/62) and mixed infections among 11 of the affected cases. Further, 18/62 (29%) otitis dogs were suspected for *Malassezia* associated ear infection.

Roshan *et al.* (2018) recorded that a total of 47 bacterial isolate were obtained from otitis externa samples which included *Staphylococcus* followed by *Pseudomonas, Streptococcus, Escherichia coli and Proteus spp. Malassezia* organisms were also recorded in 04 samples (7.84%).

Scherer *et al.* (2018) identified Staphylococcus *pseudintermedius* as a common pathogen in canine otitis externa with an isolation rate as high as 70%.

Agnihotri *et al*. (2019) reported that amongst 65 isolated bacteria from otitis externa cases *Staphylococcus spp*. (53.84%) was found to be most commonly isolated followed by *Streptococcus spp.* (18.46%), *Pseudomonas spp.* (12.30%), *Proteus spp.* (7.69%), *Escherichia coli* (6.15%) and least isolated was *Corynebacterium spp.* (1.53%).

Bajwa (2019) reported that other than *Staphylococcus* organism the bacteria commonly associated with otitis included *Pseudomonas, Proteus, Enterococcus, Streptococcus,* and *Corynebacterium.*

Bourely *et al*. (2019) reported that most frequent bacterial genera isolated from dogs with otitis externa from 2012 to 2016 in France were coagulase-positive *Staphylococci, Streptococcus spp., Pseudomonas spp.* and *Proteus spp.*

Demirbilek and Yilmaz. (2019) from his work reported that coagulase-positive *Staphylococcus spp.* were the most frequently isolated bacteria, found in 90 (21.8%) of the samples. 58 samples (14%) were positive for *Staphylococcus aureus*, 51 (12.3%) for *Pseudomonas aeruginosa*, 27 (6.5%) for *Proteus mirabilis*, 27 (6.5%) for *Malassezia pachydermatis*, 21 (5%) for *Corynebacterium spp*., 21 (5%) for β-hemolytic *Streptococcus spp.*, 15 (3.6%) for *Staphylococcus pseudintermedius*, 12 (2.9%) for *Proteus spp.*, 12 (2.9%) for *Escherichia coli*, 9 (2.1%) for *Acinetobacter calcoaceticus*, 7 (1.6%) for *Trichophyton mentagrophytes*, 5 (1.2%) for *Staphylococcus auricularis*, and 46 (11.1%) for different bacteria and yeast.

Manickam *et al.* (2019) isolated 36 *Staphylococcal* strains were isolated and identified from ear canals of dogs with otitis externa of these, 9 were coagulase - positive (CPS 25.00%) and 7 were coagulase negative (CNS 19.44%). the most frequently isolated *Staphylococcus* species were *Staphylococcus intermedius* (30.55%) and coagulase positive *Staphylococcus aureus* (25.00%).

Bradley *et al*. (2020) suggested that cultural examination and cytological investigation are usually correlative and commonly used to guide therapeutic decisions.

Jayalakshmi *et al*. (2020) cultured 25 ear swab samples, a total of 14 (56%) *Staphylococcus pseudintermedius* isolates were recovered, 6 (24%) isolates from healthy and 8 (32%) isolates from diseased dogs.

Parmar *et al.* (2020) subjected 67 otitis externa samples to microbiological evaluation by bacterial culture which revealed 251 isolates from eight bacterial species viz. *Staphylococcus spp.* (56.57%, n=142) followed by *Streptococcus spp.* (17.13%, n=43), gram negative rods (11.15%, n=28), *Escherichia coli* (5.71%, n=13), *Corynebacterium spp.* (4.78%, n=12), *Pseudomonas spp.* (2.39%, n=6), *Klebsiella spp*. (1.59%, n=4) and gram-positive rods (1.19%, n=3).

Hegde *et al.* (2021) in their work reported that of the total 55 otitis externa cases studied, 20(36.36%) isolates were identified as *Pseudomonas spp*. followed by *Streptococcus spp*. 18(32.73%), *Staphylococcus spp*. 10(18.18%), *Escherichia coli* 4 (7.27%) and *Proteus spp.* 3(5.46%).

2.4.4 IN VITRO ANTIBIOTIC SENSITIVITY TEST

Martín Barrasa *et al.* (2000) recorded that in chronic canine otitis externa, *Pseudomonas* strain isolates were susceptible to tobramycin (100%), gentamycin (68%) and enrofloxacin (42%).

Kumar *et al.* (2002) in their work recorded that all the strains of *Staphylococcus spp.*, *Pseudomonas aeruginosa*, *Proteus spp.* and *Streptococcus spp.* showed higher sensitivity to ciprofloxacin and enrofloxacin.

Chaudhary *et al.* (2003) reported that the isolates from otitis externa cases showed sensitivity mostly to ciprofloxacin (86.67%) followed by norfloxacin (56.77%) enrofloxacin (53.33%) gentamycin (41.67%) chloramphenicol (38.33 %) and erythromycin (18.33 %).

Vikas *et al.* (2003) in their work recorded that enrofloxacin and ciprofloxacin were effective against bacteria isolated from canine otitis externa.

Baksi *et al.* (2004) reported that out of the 8-otitis externa positive cases of *Staphylococcus species*, three were highly sensitive to chloramphenicol, two were highly sensitive to ciprofloxacin and the remaining three were highly sensitive to amoxycillin-clavulanic acid.

Kale and Aher (2004) recorded 100 percent sensitivity against bacterial isolates *(Staphylococcus spp., Streptococcus spp., Pseudomonas spp., Corynebacterium spp., Escherichia coli)* to enrofloxacin, gentamycin and ciprofloxacin followed by pefloxacin (87.50%) chloramphenicol (56.25%) neomycin (50 %).

Oliveira *et al.* (2005) observed that the most effective antimicrobials for positive *Staphylococcus coagulase* was cefoxitin, amoxicillin clavulanic acid, imipenem, netilmicin and negative *Staphylococcus coagulase* were sensitive to quinolones, aminoglycoside, netilmicin and the beta-lactams, except ampicillin, penicillin and oxacillin. They also observed that ciprofloxacin, tobramycin and imipenem were the most effective against *Pseudomonas aeruginosa* in otitis externa cases.

Hariharan and Harry (2006) noticed that *Pseudomonas aeruginosa* strain is 38 percent resistance to enrofloxacin and 15% to gentamicin in a clinical otitis externa case.

Lyskova *et al.* (2007) processed 97 samples obtained from otitic dogs and antibiotic sensitivity test revealed piperacillin, ciprofloxacin and gentamicin were the most effective antibiotics against *Pseudomonas aeruginosa.*

Mahendran *et al.* (2007) observed that bacterial isolates (*Staphylococcus, Escherichia coli, Pseudomonas, Proteus, and Klebsiella*) from ear swabs showed higher sensitivity to gentamycin followed by ciprofloxacin and enrofloxacin in some of the in dogs.

McKay *et al.* (2007) reported that bacterial isolates of otitis externa were significantly more sensitive to marbofloxacin than to enrofloxacin or orbifloxacin in antibiotic sensitivity test.

Schick *et al.* (2007) in their work on chronic otitis externa in dogs recorded that isolate of *Pseudomonas* species were susceptible to gentamycin (81%) and enrofloxacin (56%).

Wildermuth *et al.* (2007) recorded that *Pseudomonas aeruginosa* strain in canine otitis externa showed sensitivities as follows enrofloxacin (46.9%), ciprofloxacin (65.7%) and marbofloxacin (75%).

Turkyilmaz (2008) collected samples from 92 dogs suffering from otitis externa and antibiotic sensitivity was performed and observed that *Pseudomonas aeruginosa* strains were found sensitive to (81%) gentamicin and (88%) were resistant to co-trimaxole.

Mansoor *et al.* (2009) isolated from chronic suppurative otitis externa reported that the sensitivity pattern of *Pseudomonas aeruginosa* showed that amikacin was active against 96% of isolates followed by ceftazidime (89%), ciprofloxacin( 85%), gentamicin (81%), imipenem (76%), aztreonam (42%) and ceftriaxone (21%).

Niculae *et al.* (2009) in their study on otitis externa in dogs reported that a high level of antimicrobial resistance is seen for penicillin, erythromycin, gentamicin, kanamycin, streptomycin, lincomycin, sulphamethoprim, cephalotin, polymixin B and amoxicillin.

Degi *et al*. (2010) collected samples from 220 otitic dogs from which 31.25% *Pseudomonas spp.* were isolated and antibiotic sensitivity test was performed and observed that *Pseudomonas* strains tested were found to be most sensitive to fluorfenicol (87.5%), ciprofloxacin (81.25%), enrofloxacin (62.5%) and gentamicin (43.75%).

Malayeri *et al.* (2010) observed that all isolated gram-negative bacteria, from otitic samples were sensitive to amikacin and enrofloxacin and resistant to penicillin, erythromycin, and cephalothin and all isolated *Staphylococcus spp.* were sensitive to amikacin, enrofloxacin, and rifampin, and more than half of gram-positive isolates were resistant to penicillin and ampicillin and *Pseudomonas aeruginosa* strains showed 100% resistance to amoxicillin/clavulanic acid and lincomycin/spectinomycin combinations and 10% to gentamicin and 0% to amikacin.

Senthil *et al.* (2010) recorded that gram positive bacteria were found to be sensitive to amikacin, amoxycillin clauvunic acid, ciprofloxacin and amoxycillin. Gram negative bacterial isolates are highly sensitive to amikacin followed by ciprofloxacin, cephotaxime and cephalexin.

Yamamoto *et al*. (2010) reported that the most effective anti bacterials for otitis externa were enrofloxacin, gentamicin and polymyxin B and antibacterial resistance was greater for penicillin G.

Martins *et al.* (2011) in their work recorded that *Staphylococcus spp*., showed higher sensitivity to cephalexin (60.86%), followed by ciprofloxacin (59.09%), enrofloxacin (59.09%) and trimethoprim-sulfamethoxazole (16.66%).

Penna *et al.* (2011) opined that ciprofloxacin was the best drug among the fluoroquinolones in treating canine otitis externa.

Sanchez *et al.* (2011) reported that, in susceptibility testing bacteria species showed high resistance to penicillin, sulphanamides, tetracyclines, macrolides and lincosamides better antimicrobial effect was showed by quinolones, aminoglycosides, cephalosporins, and penicillins combined with inhibitors of beta-lactamase.

Harada *et al.* (2012) collected 73 *Pseudomonas aeruginosa* isolates from otitic externa dogs and cats in Japan to investigate antimicrobial susceptibility and resistance. Resistance rates was orbifloxacin (34.2%), enrofloxacin (31.5%), ciprofloxacin (20.5%), cefotaxime (17.8%), aztreonam (12.3%) and gentamicin (4.1%).

Oliveira *et al.* (2012) stated that the bacterial isolates were mainly susceptible to norfloxacin (89.62%), followed by gentamicin (83.25%), and ofloxacin (80.16%) in canine otitis externa cases.

Bugden (2013) in his work reported that susceptibility to gentamicin was very high for most of the isolates, from otitis, whereas for polymyxin B, shown high levels of resistance. Beta-hemolytic *Streptococci* had shown high levels of resistance to enrofloxacin, gentamycin, ceftriaxone used in otitis externa.

Lakshmi and Rao (2013) in their work recorded that for whole otitic cultures, ciprofloxacin showed highest sensitivity (91.7%) followed by ofloxacin (79.1%), gentamicin (70.8%), enrofloxacin (58.3%), chloramphenicol (52%), cephotaxime (43.7%) amoxicillin clavulanic acid (27%) and tetracycline (20.8%).

Petrov *et al.* (2013) stated that gram negative bacteria isolated from otitic samples were sensitive to aminoglycoside aminocyclitols, polymyxin B and enrofloxacin. While gram positive bacteria showed high sensitivity to beta-lactams and aminoglycoside-aminocyclitols.

Beier *et al.* (2014) collected otitic samples from 155 dogs, *Pseudomonas aeruginosa* was isolated from them and antibiotic sensitivity testing was done and the highest resistance was recorded to ß-lactams (93.8%) and sulphonamides (93.5%) and percent of resistance to ciprofloxacin, enrofloxacin, sarafloxacin and nalidixic acid was 5%,16%, of 97% and 98% respectively.

Nwiyi *et al.* (2014) analyzed 48 dog samples for otitis externa, antibiotic susceptibility testing showed that gentamicin was the most sensitive (90%) followed by penicillin (80%) while trimethoprim/ sulphurmethoxazole (50%) was the most resistant.

Blake *et al.* (2017) recorded that florfenicol was effective at reducing pathogen growth, with a MIC90 of 4µg/ml towards *Staphylococcus pseudintermedius*, while a MIC90 of 0.06µg/ml was calculated for terbinafine towards *Malassezia pachydermatis.*

Qekwana *et al.* (2017) opined that antimicrobial resistant strains of *Staphylococcus* and *Pseudomonas* otitis externa have been emerged as frustrating and difficult causes of otitis externa because of development of resistance to most common antibiotics.

Filipo *et al.* (2018) demonstrated that for the whole canine otitic cultures the most efficient antibiotics were represented by enrofloxacin, with the average of inhibition diameter area of 22.34 mm, amoxicillin and clavulanic acid with 21.87 mm and marbofloxacin with 21.46 mm, while the least efficient were polymyxin B with 13.76 and cefovecin with 15.29 mm.

Agnihotri *et al*. (2019) reported that the isolates revealed cefoperazone (74.35%) to be the most effective antibiotic for the treatment of otitis externa, followed by gentamicin (61.53%), ciprofloxacin (60.40%), chloramphenicol (60.40%), cephalexin (60%), ceftriaxone (60%), clindamycin (57.69%), amikacin (56.09%), enrofloxacin (54.05%), ampicillin (52.94%), cloxacillin (52.63%), neomycin (51.35%), streptomycin (47.50%), erythromycin (46.66%), tetracycline (38.46%), amoxicillin (37.14%), penicillin G (32.35%), septran (31.25%) and the least effective being oxytetracycline (30.43%).

Bourely *et al*. (2019) during their study period on whole canine otitis externa culture observed that resistance to penicillin was high for *Staphylococci* (68.5% (66.6–70.3) for *Staphylococcus pseudintermedius*, 70.9% (65.1–76.3) for *Staphylococcus aureus*, whereas it was lower for *Proteus mirabilis* (28.9% (26.1–31.9)) and *Streptococci* (14.4% (12.0–17.1). The level of resistance to ceftiofur was low for *Proteus mirabilis* (2.4% (1.6–3.6).

Chan *et al.* (2019) reported that N-Acetylcysteine, Tris-EDTA and disodium EDTA showed extensive *in-vitro* antimicrobial activities against bacterial and yeast isolates associated with canine otitis externa.

Demirbilek and Yilmazo (2019) from their work reported that the isolates from otitic swabs were resistant to amoxicillin clavulanic acid (45%), gentamycin (28%), ampicillin/cloxacillin (69%), tobramycin (28%), amikacin (23%), enrofloxacin (47%), chloramphenicol (58%), doxycycline (65%), lincomycin/spectinomycin (58%) and polymyxin B (62%).

Manickam *et al*. (2019) recorded that coagulase-negative *Staphylococci* isolates from canine otitis *Staphylococcus intermedius* was sensitive to enrofloxacin (90.90%), gentamicin (100%), cephalothin (90.90%) and neomycin (81.81%). *Staphylococcus aureus* (CPS) isolates presented more or less the same sensitivity pattern.

Karnard *et al.* (2020) observed that as a whole sample antibiotic sensitivity assay, the highest sensitivity was noticed for gentamicin (24 cases), followed by ciprofloxacin (21 cases), chloramphenicol (16 cases), ofloxacin (16 cases) and neomycin (12 cases).

Parmar *et al.* (2020) conducted antibiotic sensitivity assay on whole otitic samples revealed gentamycin (17.93%, n=45) to be highly sensitive drug followed by cefotaxim (17.53%, n=44), ceftriaxone (15.53%, n=39), amoxicillin clavulanic acid (11.15%, n=28), enrofloxacin (10.75%, n=27), ampicillin and tetracycline (8.36%, n=21, each), amikacin (7.97%, n=20), chloramphenicol (1.59%, n=4) and streptomycin (0.79%, n=2).

Hegde *et al.* (2021) in their work observed that the highest rate of sensitivity of identified isolates was observed with cefotaxime in 41 cases (74.55%) followed by gentamicin in 36 cases (65.46%), enrofloxacin in 29 cases (52.73%), azithromycin in 21 cases (38.18%), tetracycline in 14 cases (25.46%) and amoxicillin in 3 cases (5.46%).

2.4.5 *IN -VITRO* ANTIFUNGAL SENSITIVITY TEST

Pal and Rao (2001) in their work of otitis externa due to *Candida albicans* reported that the *in-vitro* disc diffusion test showed that the isolate was susceptible to clotrimazole and gentian violet, ketoconazole, miconazole, mercurochrome and nystatin.

Einchenberg *et al*. (2003) recorded that the clinical isolates of *Malassezia pachydermatis from canine otitis externa* to be highly sensitive to itraconazole, and only 2 (2.4%) and 3 isolates (3.7%) have shown resistance against fluconazole and ketoconazole respectively.

Sarierler and Kirkan (2004) in their work observed that miconazole and econazole to be most sensitive for all the fungal agents except *Candida albicans*, whereas nystatin showed the highest antifungal activity for *Candida albicans.*

Nascente *et al*. (2005) reported that fluconazale showed highest antifungal activity (64.3 percent) in clinical cases of otitis followed by ketoconazole (57 percent) and itraconazloe (28.6 percent).

Lyskova *et al.* (2007) recorded that *Malassezia pachydermatis* was susceptible to all antimycotics like nystatin, ketoconazole, amphotericin B tested with the exception of fluconazole (4.4% of resistant strains).

Lakshmi and Rao (2013) in their work recorded that canine otitic isolate showed least sensitivity to nystatin (29.1.%).

## 2.5 TREATMENT

Mckeever and Torres (1997) reported that salicilic acid (0.2%) acts by the removal of wax, bacterial toxins, degenerating cellular debris, and free fatty acid, all of which can act as a focus for infection and stimulate further inflammation.

Cole *et al*. (2003) observed that infected ears treated with an ear cleanser containing 0.1% salicylic acid were free of infection within 2 weeks.

Guardabassi *et al.* (2004) opined that fluoroquinolones (FQs) are often used empirically or as first choice drugs to treat a range of infections, including otitis externa, in small animals.

Rougier *et al.* (2005) opined that topical treatment is the method of choice for treating otitis externa cases since antimicrobial agents come into direct contact with the pathogens.

Coatesworth (2011) stated that therapeutic treatment for otitis externa may increase selection pressure for resistant bacteria and remove the commensal population, leaving an environmental niche for opportunistic invaders such as *Pseudomonas*.

Paterson (2016) reported that the treatment options for secondary bacterial infections in otitis externa may include mechanical removal of debris and microorganisms with ear flushing, use of topical and systemic antibiotics.

Blake *et al.* (2017) opined that in addition to choosing an appropriate antimicrobial agent, successful resolution of otitis externa requires delivery of a sufficient volume of the agent directly into the ear canal to make immediate contact and allow penetration into the cells and fluid within the canal.

Karlapudi (2017) opined that the topical medications are inactivated by exudates and excessive cerumen may prevent medications from reaching the epithelium, so the ears should be gently cleaned with an ear cleaner that will remove the debris in the canal.

Bajwa (2019) reported that topical therapy is the mainstay treatment for otitis externa although systemic use of anti-inflammatory therapy and/or antimicrobial therapy may be indicated for canine otitis externa.

Arisov *et al.* (2020) opined that the drugs used for the treatment of otitis externa include cerumenolytic solutions, analgesics, glucocorticoids, anti-parasitic, antibiotics and antifungals.

Karnad *et al.* (2020) reported that gentamicin had the most effective response against the otic microbes, followed by ciprofloxacin among the anti – microbial drugs tested.

### 2.5.1 OREGANO OIL

Mugnaini *et al.* (2013) opined that appropriately diluted essential oils are suitable for direct application on the skin and can be used in the treatment of several skin diseases both in animals.

Chouhan *et al.* (2017) stated that oregano oil, and its main component have been reported for antimicrobial activities against both bacteria and fungi.

Sakkas *et al.* (2018) recorded that oreganum vulgare essential oil was effective against all *Staphylococcus* strains tested and found high antimicrobial activity against several bacterial species, including *Staphylococcus spp*.

Sim *et al.* (2019) observed that oregano oil exhibited antibacterial activity against all bacterial isolates tested. MIC90 values ranged from 0.015 to 0.03% (146–292 mg/mL) for the gram-positive bacteria and *Proteus mirabilis* and also reported that  oregano oil, thyme oil, carvacrol and thymol could be developed as novel treatments for sensitive and resistant bacteria and fungal organisms involved in canine otitis externa.

Ebani *et al*. (2020) stated that after a proper *in-vivo* evaluation, oreganum vulgare, essential oil could be a promising treatment to combat canine cutaneous mixed infections in canines.

# MATERIALS AND METHODS

CHAPTER III

# MATERIALS AND METHODS

## 3.1 MATERIALS

### 3.1.1 LOCATION AND SOURCE OF ANIMALS

The present study was carried out to study diagnostic and therapeutic aspects of canine otitis externa. The clinical cases that were brought to the Veterinary Clinical Complex, Campus Hospital and Veterinary Hospital, Bhoiguda, College of Veterinary Science, Rajendranagar, Hyderabad during the period of December 2020 to July 2021 were screened for canine otitis externa. In addition, referral cases from the practicing veterinarians in and around Hyderabad and adjoining areas were also included during the study period. The detailed information regarding age, breed, gender, onset of symptoms was collected from the owners.

### 3.1.2 CLINICAL MATERIAL

Otic exudates, cerumen, roll swab smears, tape impression smears and blood samples were collected from the affected dogs for laboratory examination and diagnostic study. Whole blood from dogs with otitis externa as well as healthy animals was collected on day ‘0’ (day of collection) and 15$^{th}$ (post treatment) in blood vacutainers containing heparin as anti-coagulant for hematological estimations**.**

### 3.1.3 LABORATORY MATERIAL

3.1.3.1 Chemicals

1. (Alfa) - Naphthol and liquid paraffin were procured from Hi Media Laboratories/Hyderabad.

2. Hydrogen peroxide (30% W/V) was obtained from Hi Media Laboratories/Hyderabad.

3.1.3.2 Laboratory Media and Reagents

The following culture media and stains were procured from Hi Media Laboratories/Hyderabad used for the bacteriological work.

3.1.3.2.1 Culture media

1. Agar- agar
2. Blood agar base
3. Eosin- methylene blue agar
4. Mac Conkey's lactose agar
5. Mannitol salt agar
6. Nutrient agar
7. Brain heart infusion agar
8. Nutrient broth
9. *Pseudomona*s isolation agar
10. Simon's citrate agar
11. Modified Dixon Agar: Special media for Malassezia species
12. Methyl red - Voges Proskauer medium (MR-VP medium)
13. Peptone water - Sugar fermentation test

14. 10% sugar solution (maltose D)- Sugar fermentation test

15. Glucose phosphate Peptone water – Voges-Proskauer test, Methyl Red test

3.1.3.2.2 Indicators and staining reagents

1. 5 % Alpha- Naphthylamine solution - Voges-Proskauer test
2. Baritt reagent-A - Voges-Proskauer test
3. Baritt reagent-B - Voges-Proskauer test
4. Kovac's indole reagent – Indole test
5. Methyl red indicator - Methyl Red test
6. Oxidase discs – Oxidase test
7. New Methylene blue staining powder
8. Gram stain kit – Gram's staining
9. Lactophenol staining solution – Fungal staining

3.1.3.2.3 Ingredients for morphological identification of bacterial isolates

**Table 3.1: Gram Stains -Kit (Hi Media Laboratories®)**

| S.NO | INGREDIENTS | VOLUME |
|---|---|---|
| 1 | Gram's Crystal Violet | 125ml |
| 2 | Gram's Iodine | 125ml |
| 3 | Gram's Decolorizer | 2x125ml |
| 4 | Safranine 0.5% w/v | 125ml |

3.1.3.2.4 Ingredients for morphological identification of Fungal isolates

**Table 3.2: Lactophenol cotton blue staining kit (Hi Media Laboratories®)**

| S.NO | INGREDIENTS | VOLUME |
|---|---|---|
| 1 | Lactophenol Cotton Blue stain | 125 ml |

3.1.3.2.5 Antibiotic discs for *in-vitro* antibacterial sensitivity testing following standard bio-discs (Hi-Media Laboratory Ltd.) were used

Enrofloxacin (Ex-10 mcg)

Gentamicin (G-10 mcg)

Amikacin (AK-30mcg)

Amoxicillin (AMX- 30mcg)

Ofloxacin (OF-5mcg)

3.1.3.2.6 Antifungal discs for in-vitro antifungal testing, following standard bio-discs (Hi-Media Laboratory Ltd.) were used

Clotrimazole (Cc-10 mcg)

Ketoconazole (Kt-30 mcg)

Nystatin (Ns-50 mcg)

### 3.1.4 GLASS WARE

All the glass ware used in this study were procured from M/s Borosil Hyderabad.

### 3.1.5 THERAPEUTIC AGENTS

The following drugs were used in the management of otitis externa in dogs

1. Ear cleaner containing lactic acid, salicylic acid, chitosanide, propylene glycol, Spherulites, docusate sodium, and dimethicone. (Epiotic ear cleaner, M/S, Virbac)

2. Ear drops containing ofloxacin – 0.3%, clotrimazole 1%, betamethasone dipropionate 0.25%, lignocaine hydrochloride 2%. (Pomisol ear drops, M/S, Intas pharmaceuticals)

3. Essential oil containing cavarcol, thymol, rosamaric acid. (Oregano oil, M/S, Flewby naturals)

4. Immune boosting syrup containing Vitamin C, Vitamin D3, Zinc, Curcuma longa and Glycyrrhiza. (Immuno sky syrup, M/S, Sky-lec pharma)

## 3.2 METHODS

### 3.2.1 INCIDENCE OF OTITIS EXTERNA

The incidence was calculated by taking into account of the otitis externa cases detected out of the total dogs screened. The study was carried on both healthy as well as dog seeking therapeutic intervention for ear affection in relation to age, sex, breed and season wise incidence.

### 3.2.2 DIAGNOSIS

#### 3.2.2.1 Clinical Examination

All the dogs with otitis externa were subjected to detailed clinical examination on the day of presentation, during further period of therapy and observations were recorded.

#### 3.2.2.2 Otic Examination

The ear canals and pinnae were examined for gross abnormalities like erythema, otic exudation, pain on palpation of the ear, excessive ear wax, crusts formation on ear pinna, head shaking, head tilting, constant scratching etc.

#### 3.2.2.3 Sampling Technique

##### 3.2.2.3.1 Collection of materials from ear swabs

Success in recovery of organisms after culturing specimens depends on the careful collection of suitable samples, proper transportation and accurate processing of clinical sample. In the present study cerumen/otic exudate sample is collected aseptically using sterile ear swabs at the junction between vertical and horizontal ear canal of ear. The ear swabs were collected from the dogs with apparently healthy ears (n=6) and also from each clinical case presented with otitis externa at the hospital for cytological evaluation and bacteriological examination as per the method described by (Wilkinson and Harvey, 1994).

#### 3.2.2.4 Cytological examination

##### 3.2.2.4.1 Roll swab cytology

Cerumen discharge from healthy ears as well as otitic ears was collected. An ear swab is inserted at the level of vertical and horizontal ear canal junction, rotated in the single direction and withdrawn. Swabs collected were gently rolled onto two microscopic slides, air dried one slide is for cytology and another for parasitic examination, the slide for cytological evaluation is stained with New Methylene blue and examined under oil immersion objective (100 X) of the microscope which allowed rapid assessment for the presence of cocci, rods and *Malassezia* (Mactaggart, 2008) and for parasitic examination, mineral oil is placed on the slide and examined under low power 4X and 10X (Logas, 2019).

#### 3.2.2.5 Impression smears

##### 3.2.2.5.1 Collection and examination of slide impression smears

Glass slide impression smears were taken from wet lesions on the skin in cases with associated dermatological disorders. Impression smears were then stained with new methylene blue for one minute. The stained smears were then dried and examined under

100X (oil immersion) for the presence of cocci and other inflammatory cells (Scott *et al*., 1995; Greene, 1998).

3.2.2.5.2 Collection and examination of tape impression smears

Sampling was done using strips of tape about 50 per cent longer than microscopic slide. The middle of the tape was pressed several times onto the area to be sampled to collect surface cells and debris. This tape was then placed on the slide with adhesive side up and both ends of the slide was wrapped with another tape to hold it firmly in position (Forsythe, 2007). These smears were stained by using new methylene blue stain for one minute. The stained smears were then dried and examined under 100 X (oil immersion) for the presence of bacteria and *Malassezia* organisms (Rosenkratz, 2008).

3.2.2.6 Preparation of media

All the media used in this study were from Hi media Laboratories Pvt. Limited obtained as dehydrated powders were rehydrated as per the manufacturer's instructions by adding distilled water to dissolve. Then media were sterilized by autoclaving at 15 lb pressure for 15minutes.

3.2.2.7 Preparation of glassware

All the glassware used in this study were dipped into 1% Hydrochloric acid for 24 hours, and then washed under running tap water, again soaked in teepol solution for 24hours, cleaned with brush and washed under running water. The glassware was then rinsed in glass distilled water and dried at 50°C, carefully packed and sterilized at 160°C for one and half hours in hot air oven. The sterilized glassware was used for cultural and biochemical analysis.

## 3.3 ISOLATION AND IDENTIFICATION OF BACTERIAL ORGANISM

The collected otic inoculum was inoculated into the nutrient broth and incubated at 37°C for 24-48 hours. The growth of enriched inoculums from all the samples were streaked on primary media like nutrient agar and blood agar, and incubated at 37°C for 24-48 hours. Colonial morphology on culture media and microscopic morphology of gram-stained smears of the representative colonies were studied. Further the identified colonies were streaked on selective and differential media like Mannitol Salt agar, *Pseudomonas* isolation agar, Mac Conkey agar, Eosin-methylene blue agar, Urea agar, Simmons citrate agar (Hi Media Laboratory Ltd., Hyderabad) and incubated at 37°C for 24 to 48 hours. The inoculated plates were examined for morphological characteristics and growth of bacterial colonies after incubation period. The isolates were then identified on the basis of colony characteristics, staining characteristics (after staining with Gram's stain) microscopic morphology, catalase test, slide coagulase test, and mannitol fermenting ability (Quinn *et al*.,1999).

### 3.3.1 ISOLATION OF THE ORGANISMS

After incubation the plates were thoroughly examined to detect the colonies of the organisms. Single colonies were picked up and sub cultured separately on Nutrient Agar and incubated at 37°C for 24-48 hours. The single colonies were further sub cultured in slant in small tubes to obtain the organism in pure form and preserved at 4°C for further study.

## 3.3.2 MEDIA FOR ISOLATION AND MAINTENANCE OF BACTERIAL CULTURE

The following bacterial culture media or their respective ingredients were procured from Hi Media Laboratory®, India.

## 3.3.3 IDENTIFICATION OF BACTERIA

Identification of the bacteria was carried out based on colony character and microscopic morphology, hemolysin production, coagulase test and biochemical characteristics by standard methods.

### 3.3.3.1 Gram's staining

Gram's Staining was done for all the pure cultured for primary identification and the following characters were considered.

1) Gram positivity and negativity.

2) Shape.

3) Size.

4) Arrangements.

Morphology of bacterial organism

Bacterial smears were prepared by mixing 24-hour old cultures with sterile saline on a clean microscopic slide. The smear was then air dried, heat fixed over flame and then stained by Gram's staining and examined under oil immersion lens. The bacteria were studied and each isolate was recorded as being gram positive cocci or gram negative, cocci, bacilli or coccobacilli.

3.3.3.2 Biochemical tests for identification of bacteria

Sugar fermentation test:

Principle: Acids formed due to fermentation of different specified sugars are detected by a change in the colour of the medium (it is varied according to the use of indicator). Materials:

a) Peptone water.

b) Bacterial culture.

c) 10% sugar solution (Maltose- D)

d) other essentials.

Procedure:

a) Take 4 ml peptone water in the sugar tube.

b) Then bacterial culture is added from the slant.

c) Add 0.4 ml of 10% sugar solution in each tube.

d) Then add 0.4 ml Andrade’s indicator (HiMedia) and incubated for 4-5 days. Observation: Acid production will be indicated by the change of the colour of the medium intense pink or red in positive reaction.

Indole test:

Principle: This test demonstrates the ability of certain bacteria to decompose the amino acid tryptophan to iodole as by-product, which accumulates in the medium. Indole is then tested for by a colour reaction with para-dimethyl amino benzaldehyde.

Material:

a) Peptone water (5ml) without indicator

b) Bacterial culture

c) Kovac's indole reagent (HiMedia)

d) other essentials

Procedure:

a) Inoculate the peptone water with bacterial culture and incubate the tube for at least 48 hours at STC.

b) Add 15 drops of Kovac's indole reagent gently at the side of the tube in slanting way without disturbing.

Observations: In positive cases, a bright red colour ring will develop at the interface of the reagent and the broth within few seconds. A brown or tan colour is considered as negative.

Catalase test:

Principle: This demonstrates the presence of catalase, an enzyme that catalyzes the release of oxygen from hydrogen peroxide ($H_2O_2$).

Materials:

a) Bacterial culture on solid media.

b) Hydrogen peroxide ($H_2O_2$) solution (10 volume)

c) microscopic glass slide

d) other essentials

Procedure:

a) With a sterile inoculation loop take small amount of bacterial culture and place on a clean microscopic glass slide.

b) Pour a drop (or more) of $H_2O_2$ upon the culture and observe the effect.

Observations: In positive case, bubble of oxygen will release from the surface of the culture.

Voges -Proskauer test:

Principle:

Many bacteria ferment glucose with the production of acid and subsequently convert them to a neutral product acetyl-methyl carbinol (CH3CO.CHOH.CH3) or its reduction product 2,3 butylene glycol (CH3.CHOH.CHOH.CH3). The substances can be tested for by a colorimetric reaction between diacetyl and a guanidine group under alkaline conditions.

Materials:

a) Glucose phosphate peptone water (GPPW)

b) Bacterial culture in broth.

c) Voges -Proskauer reagents

d) other essentials

Procedure:

a) Inoculate a tube of GPPW (5ml) with pure bacterial culture and incubate the tube foe at least 24 hours.

b) Add 3 ml of 5% alpha napthol and 1ml of 20% aqueous potassium hydroxide solutions in the tube and mix well by gental shaking.

c) Wait for few minutes to develop intense red colour at or near the surface. Observations: Positive test is indicated by the development of a bright cherry red colour after 5 to 15 minutes or longer.

Methyl Red test:

Principle: This test is employed to detect the production of sufficient acid during fermentation of glucose and the maintenance of conditions such that the pH of an old culture is sustained. It can be detected by a change in colour of the methyl red indicator which is added at the end of the period of incubation.

Materials:

a) Glucose phosphate peptone water.

b) Bacterial culture (broth)

c) Methyl Red reagent

d) other essentials

Procedure:

a) Inoculate a loop full of pure broth to GPPW (5ml) and incubate for at least 48 hours.

b) Add 5 drops of MR reagent directly to the broth and mix well. MR reagents in same amount also add in un-inoculated control tube for comparing the colours.

Observations:

Red colour indicates positive reaction and yellow colour as negative reaction. Intermediate shades may be considered as doubtful.

Oxidase Test:

Principle: Cytochrome oxidase is an enzyme found in some bacteria that transfers electrons to oxygen. Thus, the enzyme oxidizes reduced cytochrome c to make this transfer of energy. Presence of cytochrome oxidase can be detected through the use of an oxidase disc which acts as an electron donator to cytochrome oxidase. If the bacteria oxidize the disk, the disk will turn purple, indicating a positive test. No color change indicates a negative test.

Procedure:

a) Take a loopful of bacterial culture and rub over the disc.

b) Examine for blue colour within 10 seconds.

Observations: If the bacteria oxidize the disk, the disk will turn purple colour, indicating a positive test. No colour change indicates a negative test.

Coagulase test:

Principle:

Some bacteria produce coagulase, which is an enzyme that converts fibrinogen to fibrin, which means that it can coagulate plasma. The ability to produce coagulase is assumed to be associated to the virulence of *Staphylococci.* The test is used to distinguish between coagulase positive and coagulase negative *Staphylococci*.

Method

1. Suspend one colony from the suspected pure culture in 0.5 ml of plasma from horse, rabbit or man.

2. Incubate at 37°C

3. Read the test after 4 h. If the result is negative, continue with the incubation.

4. Perform the final read after 24 h.

Observations:

• Positive reaction if the plasma coagulates and the coagulate is stable. It must not be dissolved upon stirring.

• Negative reaction if the plasma does not coagulate or if the coagulate is dissolved again upon stirring.

### 3.3.4 TEST PROCEDURE OF *IN-VITRO* ANTIBIOTIC SENSITIVITY TEST

A 24-hour old mix culture isolated from dogs were incubated in 5ml of infusion broth and incubated at 37°C for 24 hours. A sterile cotton swab was dipped into each broth culture and the excess broth was removed by pressing the swabs inside the tubes. The swabs were then smeared of the entire surface of Muller Hinton Agar plate and were allow drying in room temperature for 30 minutes. Disc are then taken with sterile forceps and placed gently to the surface of the agar. The plates were incubated at 37°C After 14-24 hours of incubation, the zone of inhibition was measured in mm by means of Vernier - Calipers.

A clear inhibition zones were recorded as (++++)

maximum (>17mm),

(+++) medium (10-17mm),

(++) moderate (5-10mm),

(+) slight (5mm) and

Negative marks denote resistance.

## 3.4 ISOLATION AND IDENTIFICATION OF FUNGAL/YEAST ORGANISMS

### 3.4.1 YEAST INOCULATION

For isolation of yeast otic exudates were inoculated on brain heart infusion agar and Sabouraud's dextrose followed by incubated at 32°C. The identification of yeast was done on colony and microscopic features produced on brain heart infusion and Sabouraud's dextrose agar. To study the microscopic morphology of isolate was studied by Gram's and Lactophenol Cotton Blue Staining.

After 24 hrs enrichment in peptone water each sample was inoculated on Modified Dixon agar for isolation of yeast. The plates were incubated at 32°C for at least 24 hr in cooling incubator. Appearance of discrete and well-developed colony were recorded and employed as criteria for isolation by (Guillot and Bond,1999).

### 3.4.2 MEDIA FOR ISOLATION AND MAINTENANCE OF FUNGAL/YEAST CULTURE

The following bacterial culture media or their respective ingredients were procured from Hi Media Laboratory®, India.

### 3.4.3 IDENTIFICATION OF FUNGAL/YEAST ORGANISMS

#### 3.4.3.1 Lactophenol Cotton Blue staining

The Lactophenol Cotton Blue preparation is used to stain fungal elements, which aids in the microscopic identification of mycotic agents. The cotton blue dye stains the chitin, a nitrogenous substance present in the cell walls of most fungi. The phenol kills any organisms and the lactic acid preserves fungal structures.

Procedure:

a) Place a clean glass slide on a sheet of white paper.

b) Place a small drop of Lactophenol Cotton Blue in the center of the slide.

c) Break a stick in half so that a rough tapered edge is formed.

d) Remove a fragment of a fungal colony (approximately 1-2 mm from the periphery) with the broken stick and place in the Lactophenol Cotton Blue.

e) Gently tease the fragment until it has been separated.

f) Gently lower a coverslip over it. Do not tap or push down as this may dislodge conidia from conidiophores.

g) Examine microscopically for presence of fungal elements.

h) For a permanent preparation, rim the coverslip with clear nail polish or Permount

3.4.4 TEST PROCEDURE OF *IN-VITRO* DRUG SENSITIVITY TEST AGAINST FUNGAL/YEAST ORGANISMS

a) All the fungal isolates recovered on mycological culture examination were subjected to *in-vitro* drug sensitivity testing by disc-diffusion method (Bauer *et al*., 1966).

b) A small amount of growth from isolated colonies of organisms obtained on Sabouraud's dextrose agar (SDA) with chloramphenicol slants was transferred into a tube of nutrient broth with the help of a platinum loop and incubated at 37°C for 24 hours.

c) The broth culture was evenly smeared over the surface of SDA plates with the help of a sterile cotton swab. The standard discs (Hi Media Laboratory Ltd., Mumbai) of antifungal agents were then placed on the agar with sterile forceps keeping uniform spacing between two discs and pressed gently to ensure full contact.

d) The results were recorded after 48-72 hours of incubation at 37°C by measuring the size of zone of inhibition around each disc and compared with the standard chart.

## 3.5 HEMATOLOGY

### 3.5.1 COLLECTION OF BLOOD FOR HEMATOLOGICAL STUDY

Whole blood from dogs suffering with otitis externa as well as healthy animals was collected was collected on day '0' (day of collection) and 15$^{th}$ (post treatment) in blood vacutainers containing heparin as anti-coagulant for hematological estimations like Total Leucocyte Count (TLC in $\times 10^3/\mu L$), Differential Leucocyte Count (DLC in %), were estimated on the same day of collection with the help of Humacount in the Department of Veterinary Clinical complex, College of Veterinary Science, Rajendranagar, Hyderabad.

## 3.6 THERAPEUTIC AGENTS USED FOR THE MANAGEMENT OF BACTERIAL OTITIS EXTERNA IN DOGS

Out of 39 affected dogs, 14 dogs were taken up for the detailed study and divided into 2 groups viz. group I and II and 7 dogs in each group to follow therapeutic protocol as shown in Table 3.3 and 3.4.

## 3.7 STATISTICAL ANALYSIS

The statistical analysis of the data was subjected to one way ANOVA using Statistical Package for Social Sciences (SPSS) version 10. Differences between means were tested using Duncan's multiple comparison test and significance was set at 5 percent ($P<0.01$) and ($p<0.05$). The values were represented as mean ± Standard Error.

**Table 3.3: Therapeutic design for group I otitic dogs.**

| Group | Name of the drugs | Dose rate | Route of administration | Duration of treatment |
|---|---|---|---|---|
| **Group I** | Ear cleaner | Few drops | Topical application (Gently rub the base of the ear and then wipe the interior of the ear flap with cotton or cloth moistened with EPI-OTIC Ear Cleanser). | Once daily for 10 days |
| | Essential Oil | 2 Drops oregano oil + 4 drops of carrier oil (coconut oil) | Topical application | Twice daily for 14 days |
| | Immune booster | 5-10 ml daily | Orally | Twice daily for 21 days |

**Table 3.4: Therapeutic design for group II otitic dogs**

| Group | Name of the drugs | Dose rate | Route of administration | Duration of treatment |
|---|---|---|---|---|
| **Group II** | Ear cleaner | Few drops | Topical application (Gently rub the base of the ear and then wipe the interior of the ear flap with cotton or cloth moistened with EPI-OTIC Ear Cleanser). | Once daily for 10 days |
| | Ear drops | 5-10 drops | Topical application | Twice daily for 14 days |
| | Immune booster | 5-10 ml daily | Orally | Twice daily for 21 days |

# RESULTS

CHAPTER IV

# RESULTS

This study presents the incidence of otitis externa in relation with age, breed, gender, season along with hematobiochemical alterations, isolation of causative agent and different therapeutic regimens in dogs suffering with otitis externa. A total of 3896 dogs were presented to the Veterinary Clinical Complex, Bhoiguda and Campus Veterinary hospital, College of Veterinary Science, Rajendranagar, Hyderabad, Telangana and referral cases from the practicing veterinarians in and around Hyderabad, during the period of December 2020 to July 2021.

Results of the present study are presented here under.

## 4.1 INCIDENCE

### 4.1.1 INCIDENCE OF OTITIS EXTERNA

During the period under study, out of total 3896 cases registered at Veterinary Clinical Complex, Campus Hospital and Veterinary Hospital, Bhoiguda, College of Veterinary Science, Rajendranagar and referral cases from the practicing veterinarians in and around Hyderabad, 264 cases were suspected for otitis externa and 39 dogs were diagnosed for the same. Thus, the overall incidence of otitis externa turned out to be 14.77% (Table 4.1 and Fig 4.1).

### 4.1.2 AGE WISE INCIDENCE OF OTITIS EXTERNA IN DOGS

The age wise incidence of otitis externa was recorded at 3 months to 1 year, 1 to 5 years, 5 to 10 years as 10.25%, 48.71% and 41.02% respectively. Highest (48.71%) incidence was recorded in 1 to 5 years of age group of dogs, followed by (41.02%)

incidence in 5 to 10 years group dogs, and in the age group between 3 months to 1 year (3m to 1Y) the incidence observed was (10.25%). The details are shown in Table-4.2 and Fig 4.2.

**Table 4.1: Showing incidence of otitis externa in dogs**

| | |
|---|---|
| Number of dogs examined | 264 |
| Number of dogs positive for otitis externa | 39 |
| Incidence % of otitis externa | 14.77% |

**Table 4.2: Showing age wise incidence of otitis externa in dogs**

| **Age group** | **Number of cases positive for otitis externa** | **Incidence (%)** |
|---|---|---|
| 3 months- 1 years | 04 | 10.25 |
| 1 to 5 years | 19 | 48.71 |
| 5 to 10 years | 16 | 41.02 |
| Total | 39 | 100 |

### 4.1.3 BREED WISE INCIDENCE OF OTITIS EXTERNA IN DOGS

Otitis externa was recorded in different breeds like Labrador Retriever, German Shepherd, Cocker Spaniel, Mongrel, Doberman, Spitz, Rottweiler, Pug breeds. The highest incidence of otitis externa was recorded in Labrador Retriever (21.42%), followed by 16.92%, 12.00%. 12.00%, 11.11%, 9.52%, 8.00% and 6.66% in German Shepherd, Cocker Spaniel, Mongrel, Doberman, Spitz, Rottweiler and Pug breeds respectively, as presented in Table 4.3 and Fig. 4.3.

**Figure 4.1: Showing incidence of otitis externa in dogs**

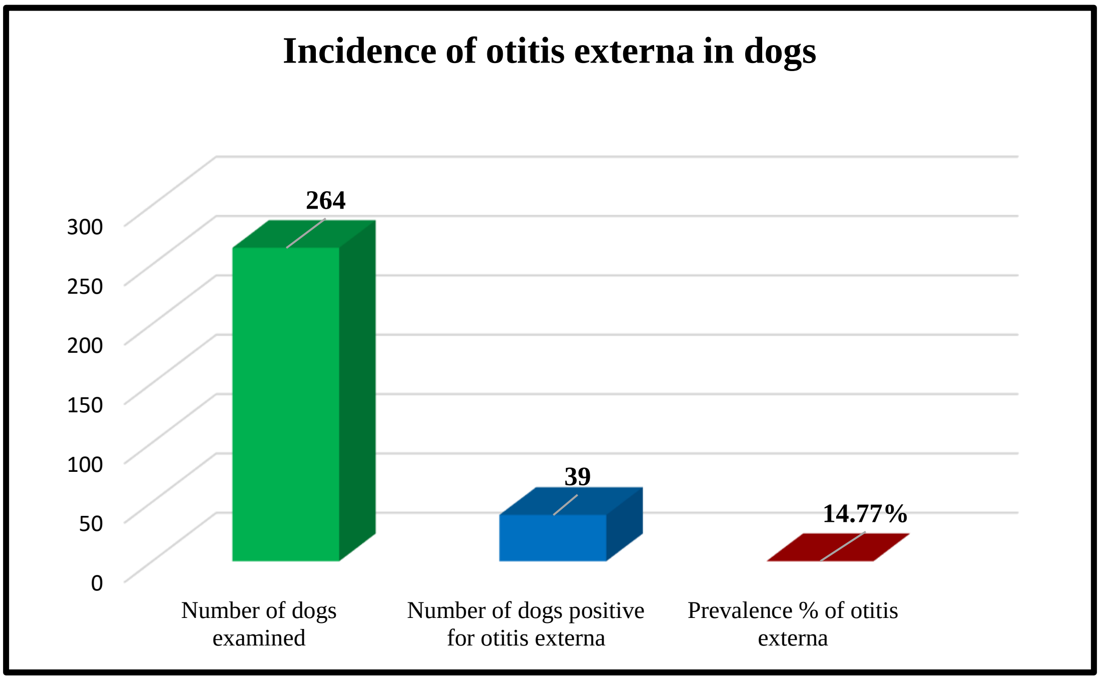



**Figure 4.2: Showing age wise incidence of otitis externa in dogs**

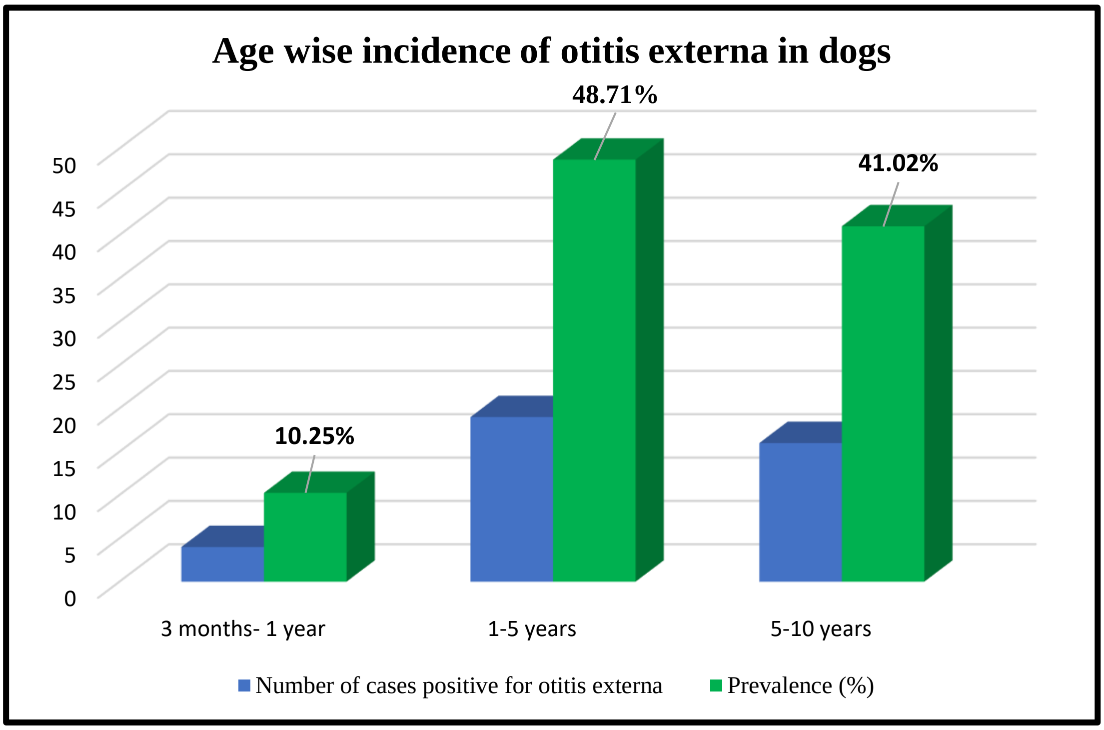
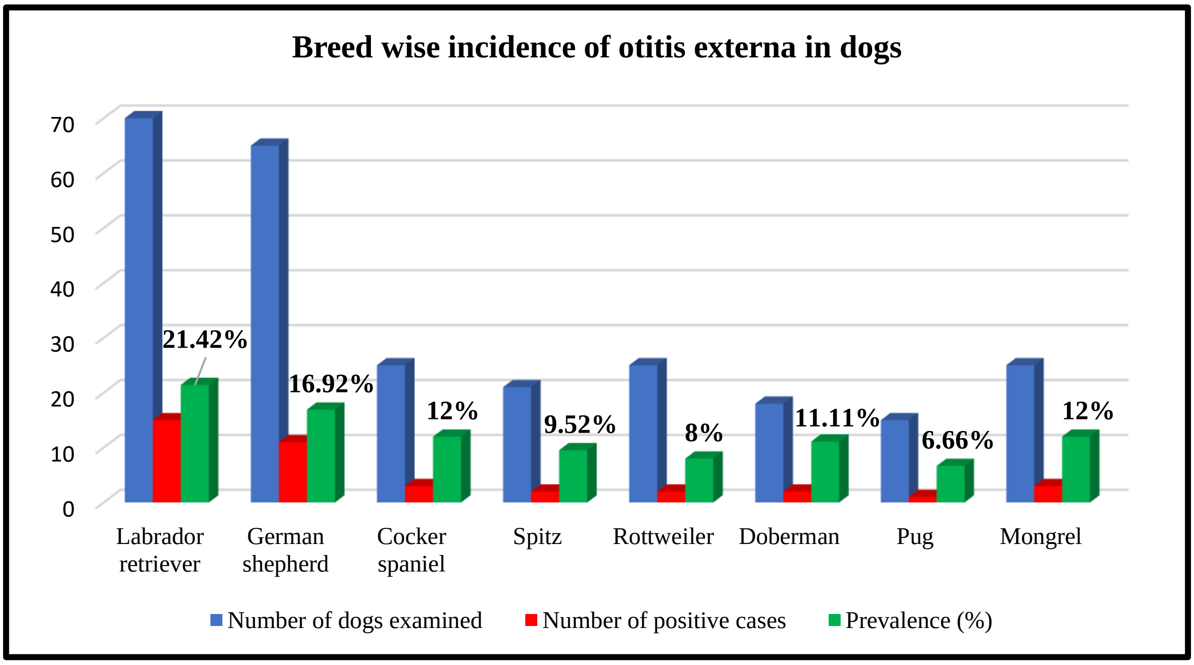


**Table 4.3: Showing breed wise incidence of otitis externa in dogs**

| Name of the breed | Number of dogs examined | Number of positive cases | Incidence (%) |
|---|---|---|---|
| Labrador Retriever | 70 | 15 | 21.42 |
| German Shepherd | 65 | 11 | 16.92 |
| Cocker Spaniel | 25 | 03 | 12.00 |
| Mongrel | 25 | 03 | 12.00 |
| Doberman | 18 | 02 | 11.11 |
| Spitz | 21 | 02 | 9.52 |
| Rottweiler | 25 | 02 | 8.00 |
| Pug | 15 | 01 | 6.66 |
| Total | 264 | 39 | 100 |

**Figure 4.3: Showing breed wise incidence of otitis externa in dogs**



### 4.1.4 SEX WISE INCIDENCE OF OTITIS EXTERNA IN DOGS

Otitis externa was recorded highest (58.97%) among 23 male dogs followed by 16 (41.02%) female dogs and the same is depicted in Table 4.4 and Fig. 4.4.

### 4.1.5 SEASON WISE INCIDENCE OF OTITIS EXTERNA IN DOGS

The season wise incidence of otitis externa in dogs is showed in Table-4.5 and Figure-4.5. The seasons were recorded as summer (March-June), rainy (July-October), and winter (November- February) and the highest incidence was noticed in summer (43.58%), followed by rainy (30.76%) and winter (25.64%) seasons.

**Table 4.4: Showing sex wise incidence of otitis externa in dogs**

| Sex | Number of positive cases | Incidence (%) |
|---|---|---|
| Male | 23 | 58.97 |
| Female | 16 | 41.02 |
| Total | 39 | 100 |

**Table 4.5: Showing season wise incidence of otitis externa in dogs**

| Season | Number of positive cases | Incidence (%) |
|---|---|---|
| Summer | 17 | 43.58 |
| Rainy | 12 | 30.76 |
| Winter | 10 | 25.64 |
| Total | 39 | 100 |

**Figure 4.4: Showing sex wise incidence of otitis externa in dogs**

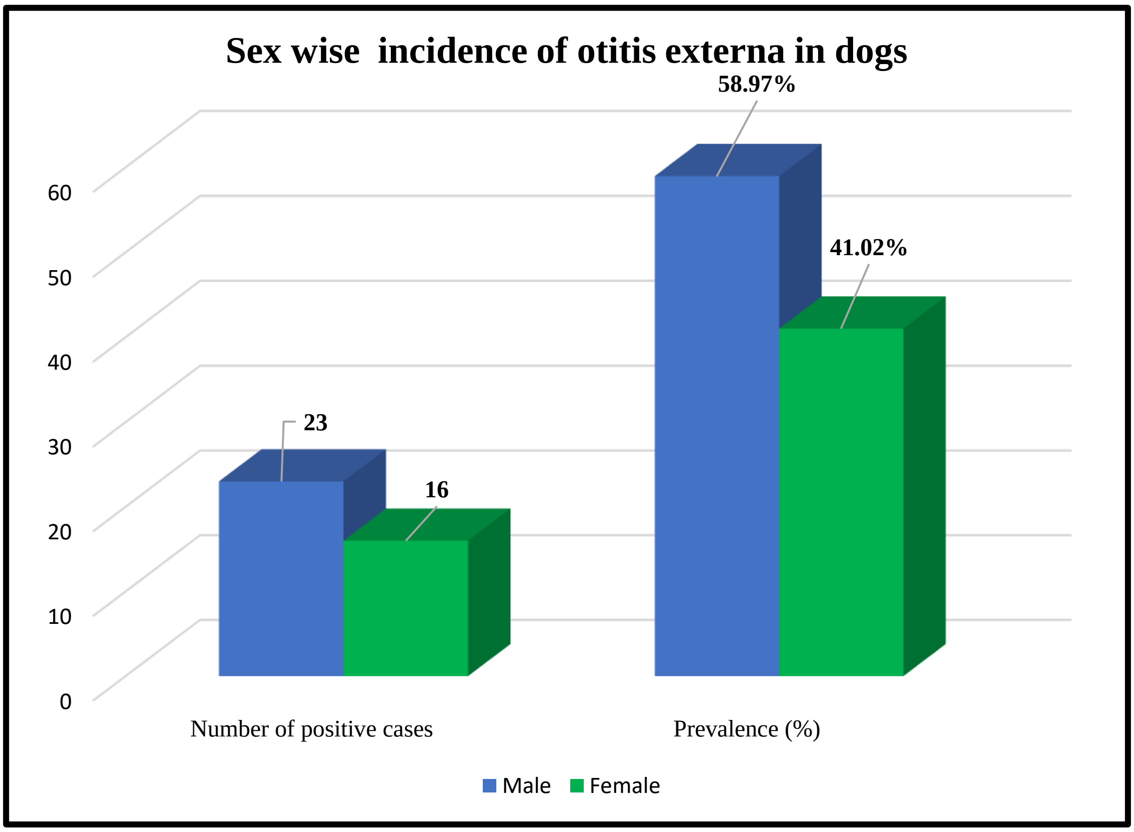
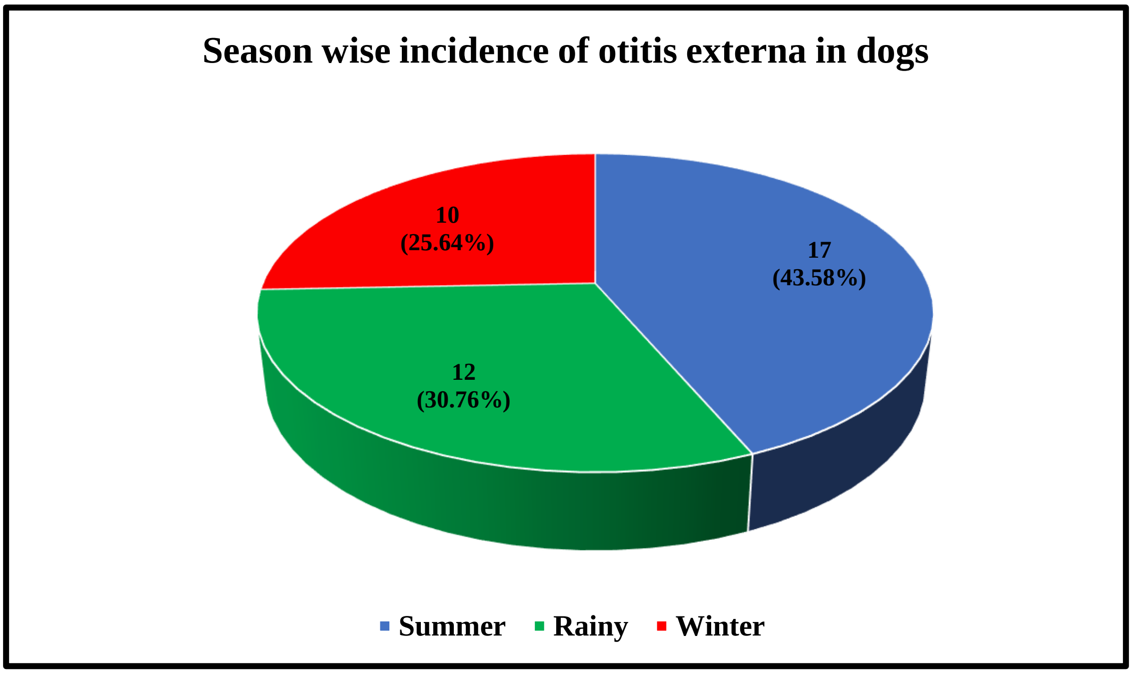



**Figure 4.5: Showing season wise incidence of otitis externa in dogs**



## 4.2 CLINICAL OBSERVATIONS

The dogs suffering from otitis externa showed a wide variety of clinical signs like foul smell from ears, ear exudates, inflammation and swelling of ear canal, shaking of head, ear scratching, head tilting, pain on touching, ulceration and erythema. The assessment of color of exudates was also recorded which in turn helped to diagnose the possible causes of the otitis externa.

In the present investigation group I dogs suffering with otitis externa showed clinical signs such as head shaking (100%), pain (100%), head tilting (100%), ear scratching (71.42%), ulceration (71.42%), erythema (57.14%), inflammation and swelling of ear canal (57.14%), foul smell ear discharge (42.85%), However all the clinical signs subsequently ceased and resumed normally post treatment and the details are given in Table 4.6, Fig. 4.6.

Whereas in group II dogs the common clinical signs were head shaking (100%), pain (71.42%), head tilting (85.71%), ear scratching (42.85%)**,** ulceration (57.14%), erythema (71.42%), inflammation and swelling of ear canal **(**85.71%) and foul smell ear discharge (28.57%). Post treatment clinical signs ceased percentages are 0%, 0%, 20%, 0%, 0%, 33.33%, 0% and 25% respectively as shown in Table 4.7 and Fig. 4.7. The various clinical manifestations are depicted in Fig.4.8 to 4.14.

**Table 4.6: Observations of clinical findings of group I otitic dogs otitis on day 0 (pre treatment) and day 15 (post treatment).**

| | | Pre-treatment (0) day | | Post- treatment (15) day | |
|---|---|---|---|---|---|
| **Group I** | **Clinical signs** | **Number of animals** | **Percentage (%)** | **Number of animals** | **Percentage (%)** |
| | Head shaking | 07 | 100 | 00 | 00 |
| | Foul smell ear discharge | 03 | 42.85 | 00 | 00 |
| | Erythema | 04 | 57.14 | 00 | 00 |
| | Ear scratching | 05 | 71.42 | 00 | 00 |
| | Pain | 07 | 100 | 00 | 00 |
| | Inflammation and swelling of ear canal | 04 | 57.14 | 00 | 00 |
| | Head tilting | 07 | 100 | 00 | 00 |
| | Ulceration | 05 | 71.42 | 00 | 00 |

**Table 4.7: Observations of clinical findings of group II otitic dogs on day 0 (pre treatment) and day 15 (post treatment).**

| | | **Pre-treatment (0) day** | | **Post- treatment (15) day** | |
|---|---|---|---|---|---|
| **Group II** | **Clinical signs** | **Number of animals** | **Percentage (%)** | **Number of animals** | **Percentage (%)** |
| | Head shaking | 07 | 100 | 00 | 00 |
| | Foul smell ear discharge | 02 | 28.57 | 00 | 00 |
| | Erythema | 05 | 71.42 | 01 | 20 |
| | Ear scratching | 03 | 42.85 | 00 | 00 |
| | Pain | 05 | 71.42 | 00 | 00 |
| | Inflammation and swelling of ear canal | 06 | 85.71 | 02 | 33.33 |
| | Head tilting | 06 | 85.71 | 00 | 00 |
| | Ulceration | 04 | 57.14 | 01 | 25 |

**Fig 4.6: Observations of clinical findings of group I otitic dogs on day 0 (pre-treatment) and day 15 (post treatment).**

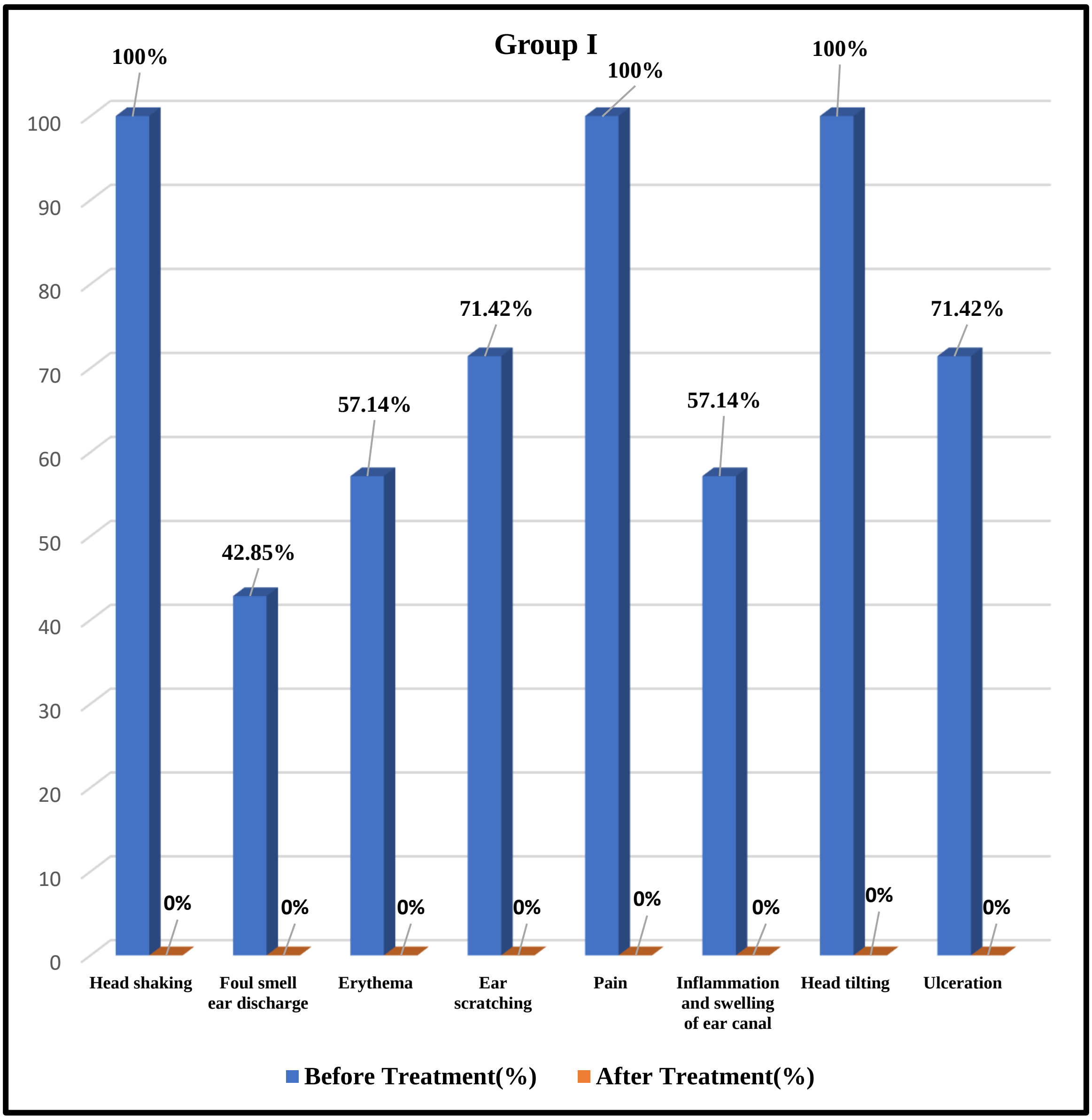


**Fig 4.7: Observations of clinical findings of group II otitic dogs on day 0 (pre - treatment) and day 15 (post treatment).**

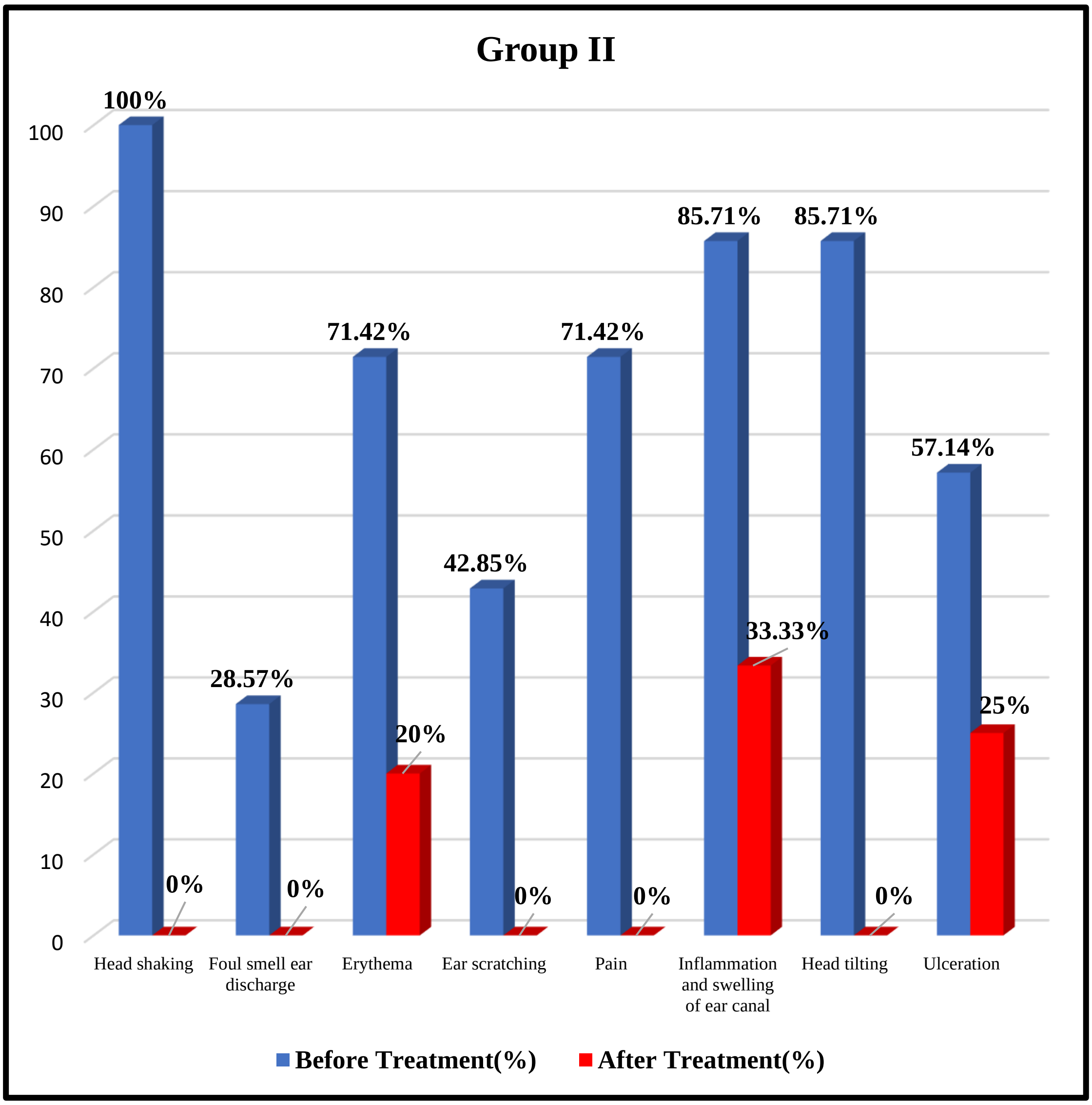


**Fig 4.8: Collection of samples from clinical cases.**

**Fig 4.9: Dog showing excoriations on ear pinna.**

**Fig 4.10: Dog showing scales formation on ear pinna.**

**Fig 4.11: Dog showing brownish greasy ear exudate.**

**Fig 4.12: Dog showing ulceration and excoriation of ear pinna.**

**Fig 4.13: Dog with yellowish purulent ear discharge.**

**Fig 4.14: Dog showing crust formation on ear pinna.**

## 4.3 IDENTIFICATION OF THE ETIOLOGICAL AGENTS

### 4.3.1 DIAGNOSIS

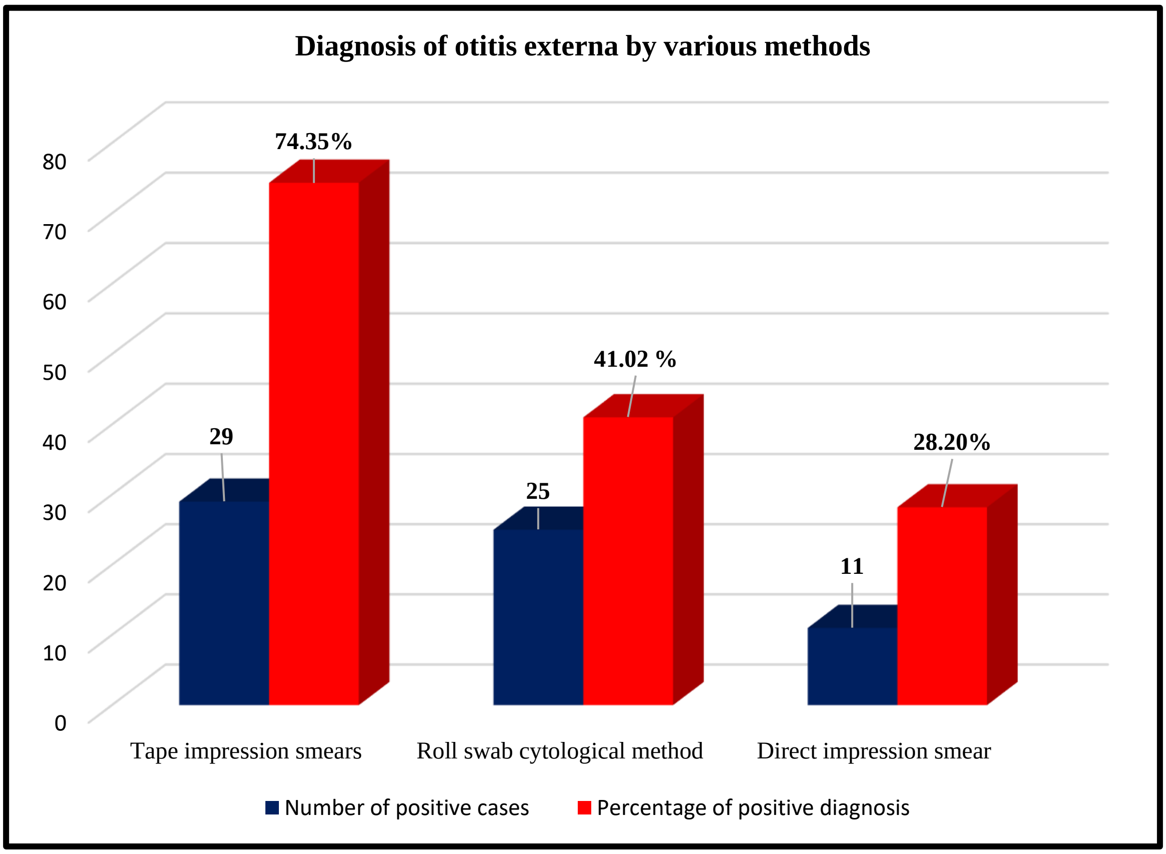
A total of 39 clinically positive dogs were screened for the presence of microorganisms by cytological examination. The percentage of positive diagnosis for tape impression smears, roll swab cytological method and direct impression smear were 74.35%, 41.02% and 28.20 % respectively. The results pertaining to diagnosis of otitis externa is presented in Table 4.8 and Fig 4.15. Diagnosis of otitis externa by various diagnostic methods revealed *Malassezia spp.*, *Pseudomonas spp.*, and gram-positive cocci (Fig. 4.16 to 4.18).

In the present investigation, as shown in Table 4.9 and Fig 4.19 the findings related to diagnosis of canine otitis externa using tape impression smears revealed a greater number of cocci (75.86%) followed by bacilli (55.17%) and yeast (20.68%). From roll swab cytological method, a greater number of cocci (84.00%) followed by bacilli (56.00%) and yeast (20.00%) and from direct impression smear again a greater number of cocci (63.63%) followed by bacilli (45.45%) and yeast (27.27%) were detected.

**Table 4.8: Diagnosis of otitis externa by various methods**

| Cytological method | Number of positive cases | Percentage of positive diagnosis |
|---|---|---|
| Tape impression smears | 29 | 74.35 % |
| Roll swab cytological method | 25 | 41.02 % |
| Direct impression smear | 11 | 28.20 % |

**Figure 4.15: Diagnosis of otitis externa by various methods**



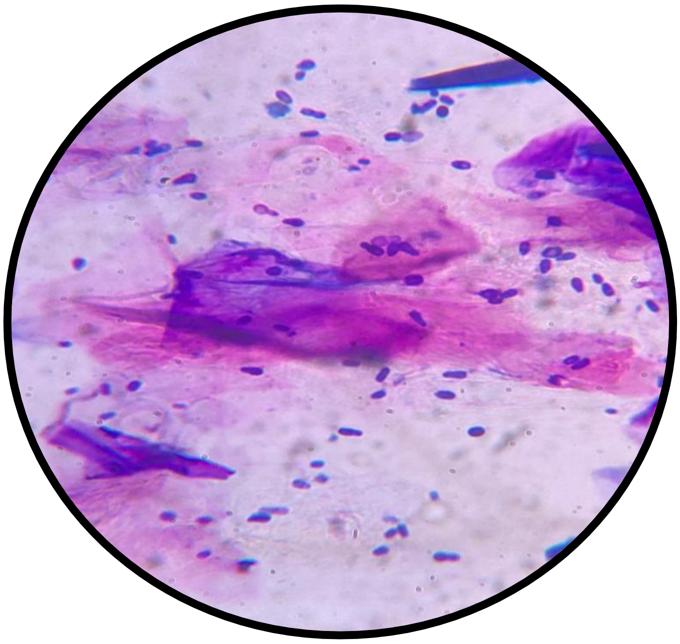



**Fig 4.16:** ***Malassezia*** **yeast in the ear cytology.**

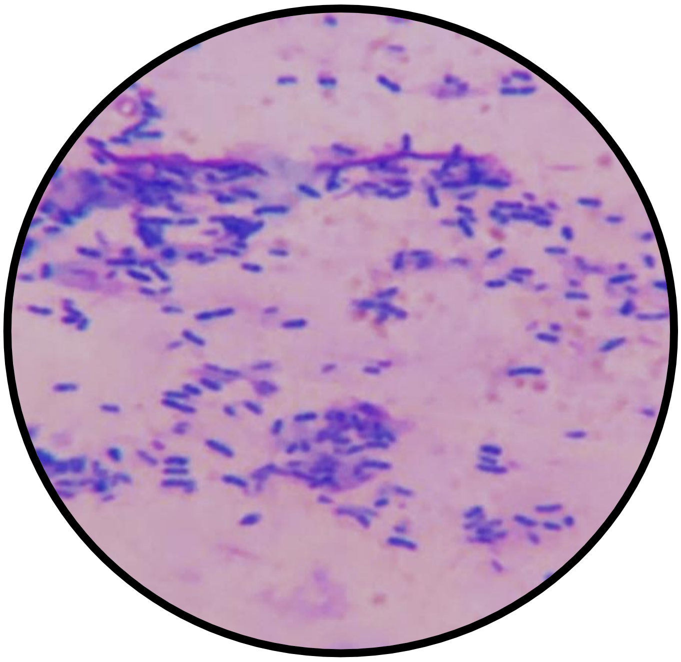


**Fig 4.17:** **Rod shaped (*Pseudomonas*) organism in the ear cytology.**

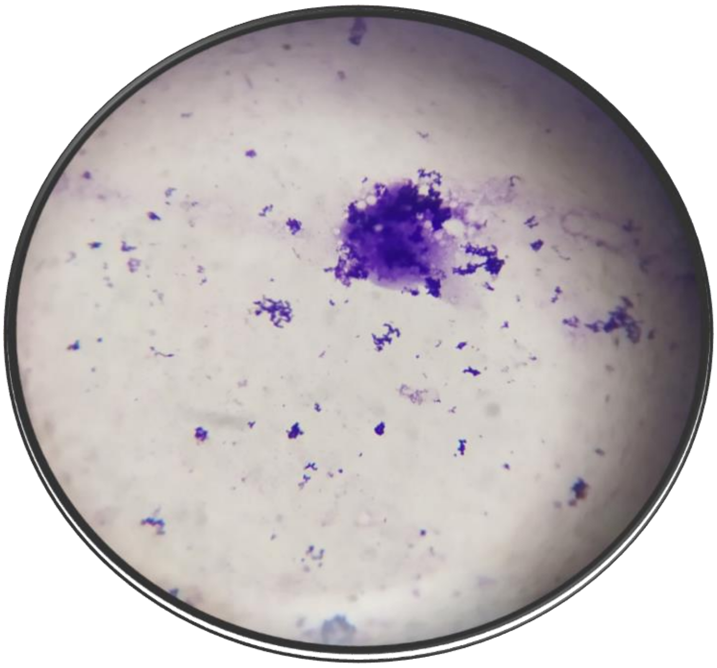
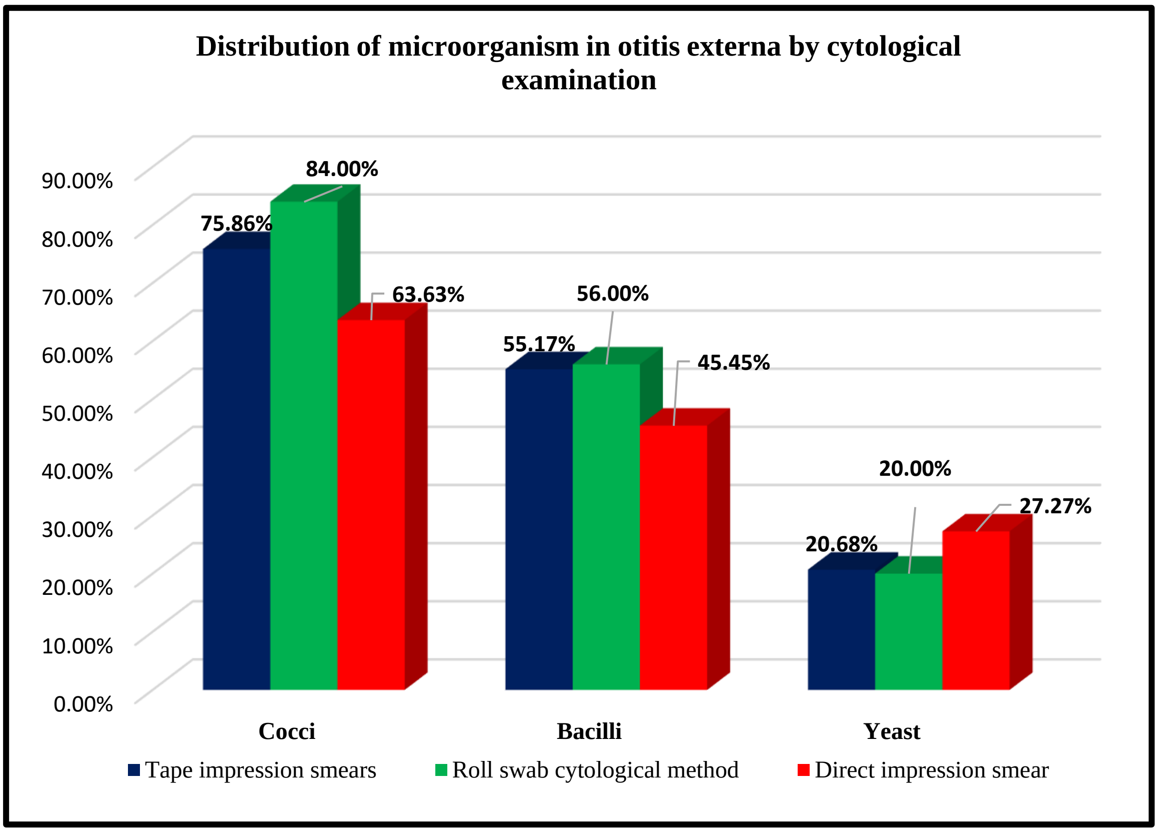


**Fig 4.18:** **Gram positive cocci in ear cytology.**

**Table 4.9: Distribution of microorganism in otitis externa by cytological examination**

| Types of organism | Tape impression smears | | Roll swab cytological method | | Direct impression smear | |
|---|---|---|---|---|---|---|
| | No. of isolates | Percentage | No. of isolates | Percentage | No. of isolates | Percentage |
| Cocci | 22 | (75.86%) | 21 | (84.00%) | 07 | (63.63%) |
| Bacilli | 16 | (55.17%) | 14 | (56.00%) | 05 | (45.45%) |
| Yeast | 06 | (20.68%) | 05 | (20.00%) | 03 | (27.27%) |

**Figure 4.19: Showing distribution of microorganism in otitis externa by cytological examination**



4.3.2 ISOLATION AND IDENTIFICATION OF MICROORGANISM

The samples found positive for cytological examination were further subjected to microbial isolation and identification. Out of total 39 isolates, 31 were bacterial isolates that include *Staphylococcus spp.* (41.02%) followed by *Pseudomonas spp.* (28.20%), *Proteus spp.* (5.12%) and *Streptococcus spp.* (5.12%) and 8 were *Malassezia spp.* (20.51%) and are depicted in Fig 4.21 to 4.26. The details are given in Table 4.10, Fig. 4.20.

**Table 4.10: Microbial isolates from otitis externa in dogs**

| **Organism isolated** | **Number of isolates (n=39)** | **Percentage (%)** |
|---|---|---|
| *Staphylococcus spp.* | 16 | 41.02 |
| *Pseudomonas spp.* | 11 | 28.20 |
| *Proteus spp.* | 02 | 5.12 |
| *Streptococcus spp.* | 02 | 5.12 |
| *Malassezia spp.* | 08 | 20.51 |

**Figure 4.20: Showing Isolation and identification of microorganism**

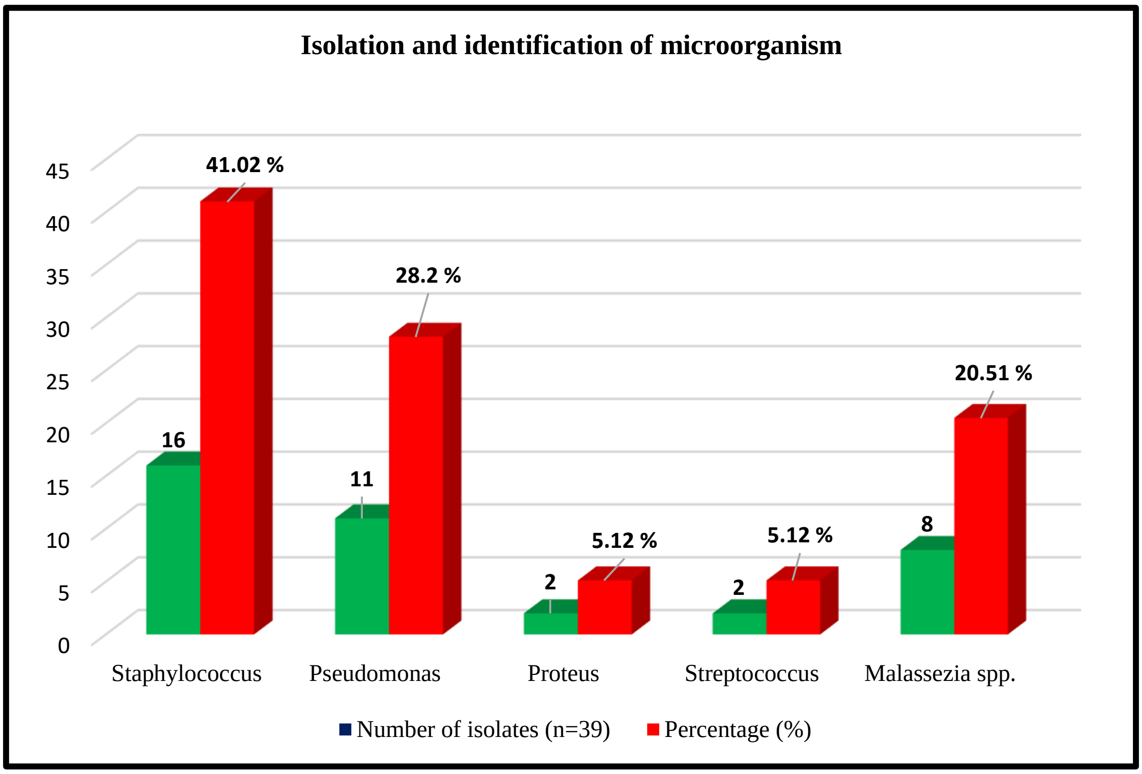



**Fig 4.21: Coagulase positive *Staphylococcus aureus* produced yellow colonies with yellow zones.**

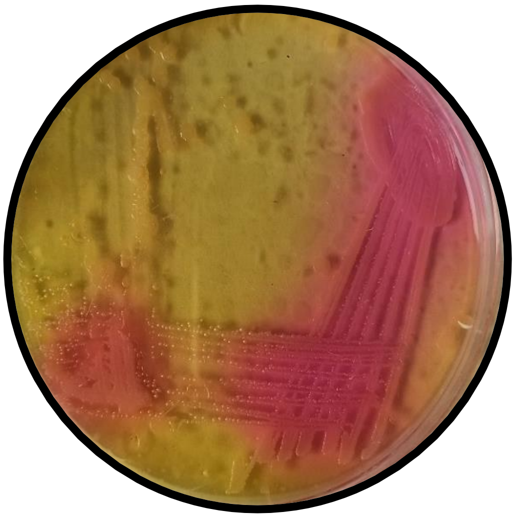


**Fig 4.22: Coagulase-negative *Staphylococci* produce small pink or red colonies with no color change to the medium.**

**Fig 4.23:** **Growth of *Pseudomonas* on cetrimide agar.**

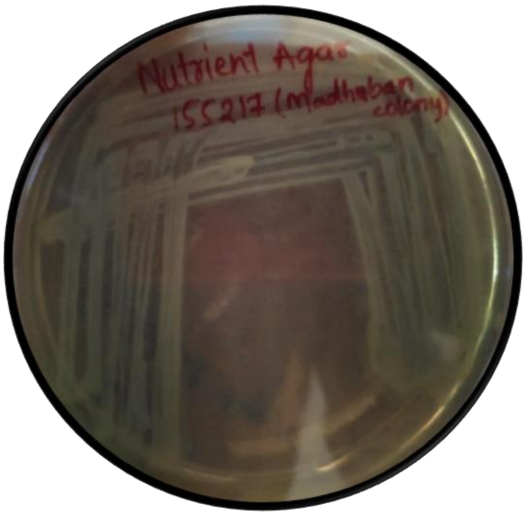



**Fig 4.24:** ***Pseudomonas* on Nutrient agar produced large, opaque, flat colonies with irregular margins and distinctively fruity odour colonies.**

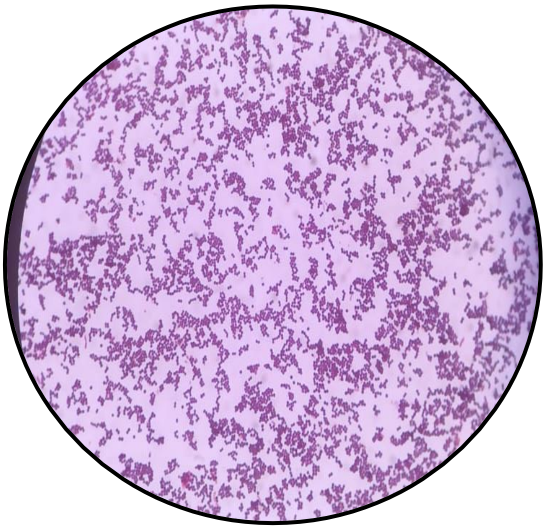


**Fig 4.25:** **Microscopic view showing gram positive *Staphylococcus spp.***

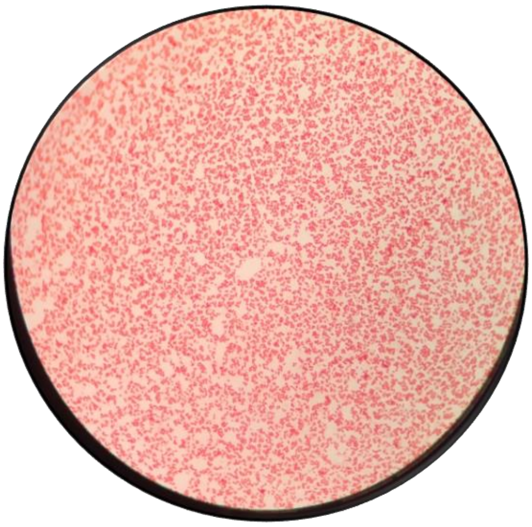


**Fig 4.26:** **Microscopic view showing gram negative *Pseudomonas aeruginosa.***

4.3.2.1 Biochemical analysis of the bacterial isolates

The biochemical tests like Indole test, Methyl Red test, Voges Proskauer test, Citrate Utilization test, Catalase test, Coagulase test, Oxidase test, Urease test were performed to the cultured isolates for identification of bacteria (Table 4.11 and Fig 4.27 to 4.31).

**Table 4.11: Biochemical analysis of the bacterial isolates**

| **Test** | ***Staphylococcus spp.*** | ***Pseudomonas aeruginosa*** | ***Proteus spp.*** | ***Streptococcus spp.*** |
|---|---|---|---|---|
| Indole test | - | - | - | - |
| Methyl Red test | + | - | + | + |
| Voges Proskauer test | + | - | - | + |
| Citrate Utilization test | + | + | + | NA |
| Catalase test | + | + | + | - |
| Coagulase test | + (4) and -(12) | NA | NA | + |
| Oxidase test | - | + | - | - |
| Urease test | - | - | + | - |

4.3.2.2 Fungal isolates recovered from otitis externa affected dogs

In the present investigation, out of total 39 isolates (including bacteria) *Malassezia* otitis externa were shown as 8 positive isolates with percent of 20.51% as mentioned in Fig. 4.32.

**Fig 4.27:** ***Staphylococcus spp.*** **Voges Proskauer test positive.**

**Fig 4.28:** ***Staphylococcus spp.*** **Methyl Red test positive.**

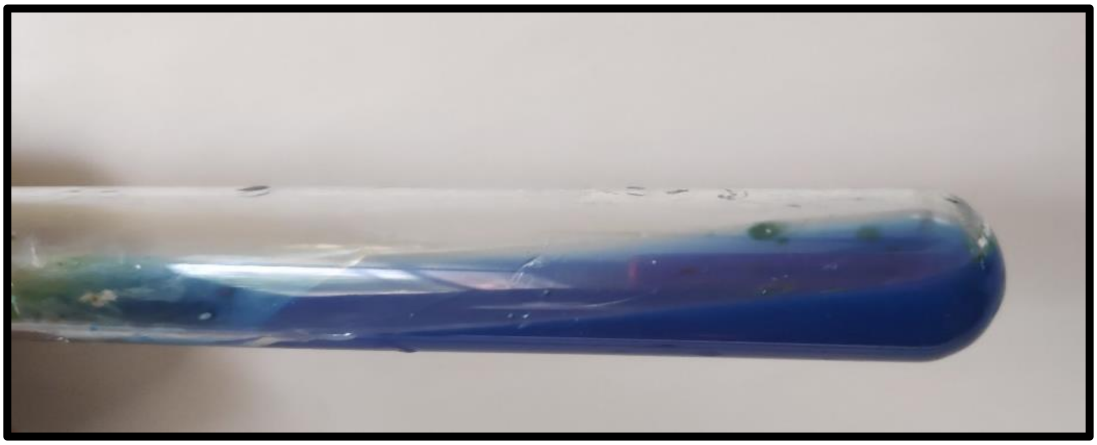


**Fig 4.29: Citrate test positive *Staphylococcus aureus.***

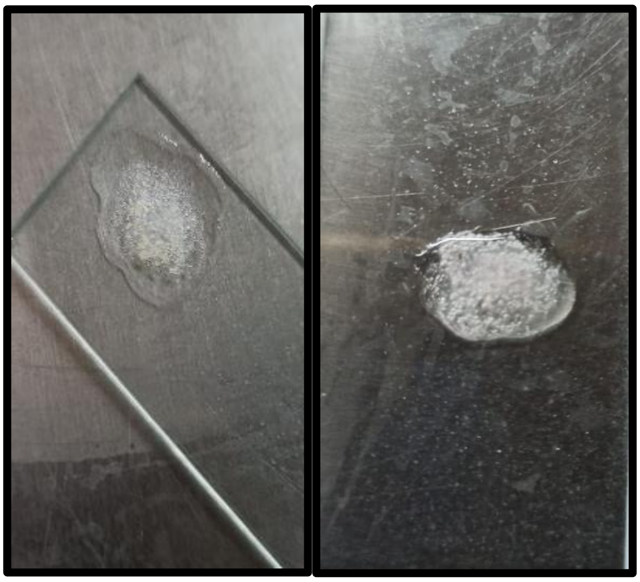


**Fig 4.30:** **Catalase test positive for *Staphylococcus and Pseudomonas.***

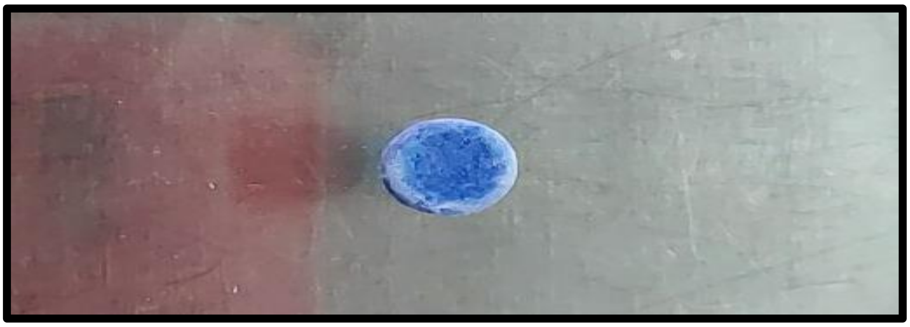


**Fig 4.31:** **Oxidase test positive for *Pseudomonas.***

**Fig 4.32:** **Growth of *Malassezia* on SDA agar.**

## 4.4 *IN-VITRO* DRUG SENSITIVITY TEST

### 4.4.1 ANTIBIOTIC SENSITIVITY TEST OF WHOLE CULTURES

Antibiotic sensitivity test was carried out on the ear discharges (whole cultures) of (18) otitic ears and results revealed a highest sensitivity towards enrofloxacin (91.27%), while the sensitivity pattern of isolates by decreasing order is gentamycin (83.34%), amikacin (81.32%), amoxicillin (80.19%) and the least sensitivity towards ofloxacin (74.36%). The antibiotic sensitivity test of whole cultures is given in Table 4.12 and Fig 4.33).

**Table 4.12: Antibiotic sensitivity test of whole cultures**

| Antibiotic | Sensitivity % |
|---|---|
| Enrofloxacin (Ex-10 mcg) | 91.27% |
| Gentamicin (G-10 mcg) | 83.34% |
| Amikacin (AK-30mcg) | 81.32% |
| Amoxicillin (AMX- 30mcg) | 80.19% |
| Ofloxacin (OF-5mcg) | 74.36% |

### 4.4.2 ANTIFUNGAL SENSITIVITY TEST OF WHOLE CULTURES

Antifungal sensitivity test on the 18 fungal/yeast samples of otitic ears revealed a highest sensitivity of 93.40% to clotrimazole followed by ketoconazole (84.60%) and nystatin (28.40%) the details are shown in Table 4.13 and depicted in Fig 4.34 and 4.35.

**Table 4.13: Antifungal sensitivity test of whole cultures**

| Antifungal disc | Sensitivity % |
|---|---|
| Clotrimazole (Cc-10 mcg) | 93.40% |
| Ketoconazole (Kt-30 mcg) | 84.60% |
| Nystatin (Ns-50 mcg) | 28.40% |

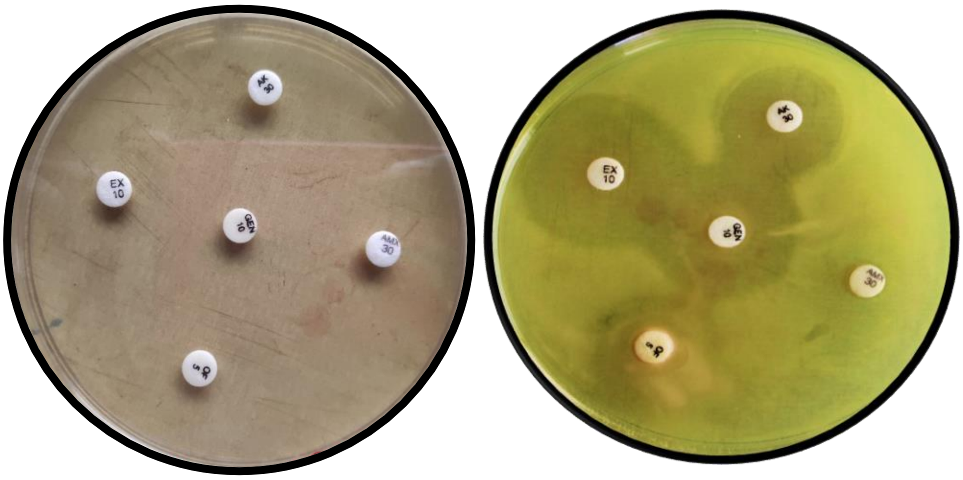



**Fig 4.33:** **Antibiotic sensitivity test showing high sensitivity for enrofloxacin.**

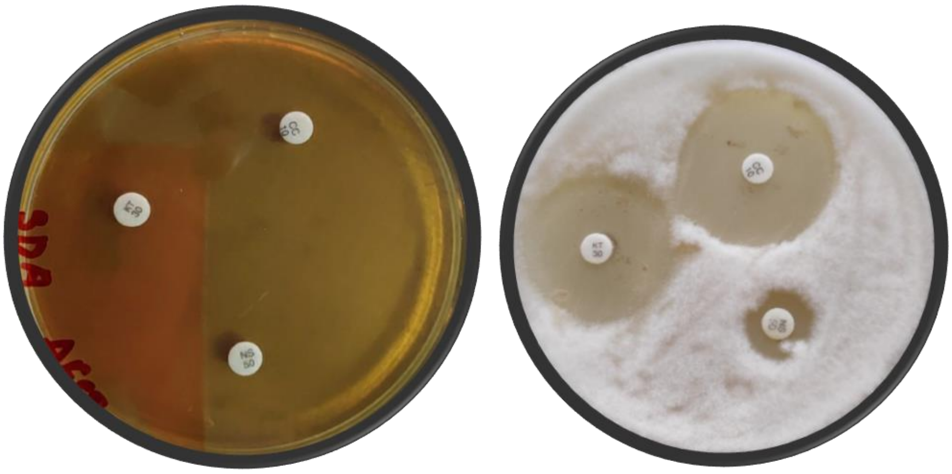



**Fig 4.34:** **Antifungal sensitivity test showing high sensitivity for clotrimazole.**

**Fig 4.35:** **Antifungal sensitivity test showing resistance to nystatin.**

## 4.5 HAEMATOLOGY

The mean hematological values of group I and group II dogs are presented in Table 4.14 and 4.15.

### 4.5.1 TOTAL LEUKOCYTE COUNT (TLC X $10^3$)

The mean values of total leukocyte count (TLC x $10^3$) are presented in Fig.4.36.

The mean total leukocyte count (TLC x $10^3$) of group I otitic dogs on day 0 was 11.41 ± 0.29 which was significantly (P<0.01) higher as compared to the healthy control (9.31 ± 0.58). This value decreased significantly (P<0.01) to 9.73 ± 0.20 following treatment on day 15 as compared to that of day 0.

The mean total leukocyte count (TLC x $10^3$) of group II dogs was 10.96 ± 0.19 that was significantly (P<0.01) higher as compared to the healthy control (9.31 ± 0.58). Following treatment this value decreased non significantly to 9.90 ± 0.15 by day 15 as compared to that of day 0.

However, comparison between groups revealed that the average values of all the treated groups decreased after therapy and maximum restoration was observed in group I than group II by the end of 15 days (post treatment).

### 4.5.2 DIFFERENTIAL LEUKOCYTE COUNT

#### 4.5.2.1 Neutrophils (%)

The mean values of neutrophil (%) are presented in fig.4.37.

The mean values of neutrophil (%) was 75.32± 0.49 in otitis externa affected group I dogs that was non significantly higher as compared to the healthy control (66.85 ± 3.45). Following medication this value decreased non significantly to 68.98 ± 0.42 on day 15 as compared to that of day 0.

The mean neutrophil (%) of group II dogs on day 0 was 75.48 ± 0.81 and was non significantly higher as compared to the healthy control (66.85 ± 3.45). This value decreased non significantly to 70.07 ± 0.52 on day 15 as compared to that of day 0.

A non-significant decrease in the mean neutrophils (%) values was recorded in the animals of group I and II after therapy. However, comparison between groups revealed that the average values of all the treated groups decreased after therapy and maximum restoration was observed in group I than group II by the end of 15 day (post treatment).

4.5.2.2 Lymphocytes (%)

The mean values of lymphocytes (%) are presented in Fig.4.38.

The mean lymphocytes (%) of group I dogs on day 0 was 17.96 ± 0.42 that were affected with otitis externa was significantly ($P<0.01$) lower as compared to the healthy control (27.77 ± 1.92). This value increased significantly ($P<0.01$) to 28.34 ± 0.45 on day 15 as compared to that of day 0.

The mean lymphocytes (%) of group II otitis externa affected dogs on day 0 was 17.82 ± 0.34 that was significantly ($P<0.01$) lower as compared to the healthy control (27.77 ± 1.92). This value increased significantly ($P<0.01$) to 27.15 ± 0.43 on day 15 as compared to that of day 0.

A significant ($P<0.01$) increase in the lymphocytes (%) values was recorded in the dogs of both group I and II after therapy. However, comparison between groups revealed that the average values of all the treated groups increased after therapy and maximum restoration was observed in group I than group II by the end of 15 day (post treatment).

4.5.2.3 Monocytes (%)

The mean values of monocytes (%) are presented in Fig.4.39.

The mean monocytes (%) of group I dogs on day 0 was 0.76 ± 0.03 and was significantly (P<0.01) lower as compared to the healthy control (1.32 ± 0.02). This value increased significantly (P<0.01) to 1.18 ± 0.03 on day 15 as compared to that of day 0.

The mean monocytes (%) of group II dogs on day 0 was 0.83 ± 0.05 that was significantly (P<0.01) lower as compared to the healthy control (1.32 ± 0.02). This value increased significantly (P<0.01) to 1.06 ± 0.06 on day 15 as compared to that of day 0.However, comparison between groups revealed that the average values of all the treated groups increased after therapy and maximum restoration was observed in group I than group II by the end of 15 day (post treatment).

4.5.2.4 Eosinophils (%)

The mean values of eosinophil (%) are shown in Fig.4.40.

The mean eosinophil (%) of group I animals was 5.98 ± 0.19 (day 0) that was significantly (P<0.01) higher as compared to the healthy control (1.45 ± 0.03). This value decreased significantly (P<0.01) to 1.49 ± 0.14 on day 15 as compared to that of day 0.

The mean eosinophil (%) of group II dogs was 5.86 ± 0.14 (day 0) that was significantly (P<0.01) higher as compared to the healthy control (1.45 ± 0.03). This value decreased significantly (P<0.01) to 1.72 ± 0.07 on day 15 as compared to that of day 0. However, comparison between groups revealed that the average monocyte values of all the treated groups increased after therapy and maximum restoration was observed in group I than group II by the end of 15 day (post treatment).

**Table 4.14: Hematology of group I otitic dogs**

| GROUP I | | | |
|---|---|---|---|
| **Parameter** | **Healthy Dogs (n=7)** | **0th day (pre treatment)** | **15th day (Post Treatment)** |
| Total Leukocyte count (TLC x $10^3$) | 9.31 ± 0.58**b | 11.41 ± 0.29**a | 9.73 ± 0.20**b |
| DLC (%) Neutrophils (%) | 66.85 ± 3.45* | 75.32± 0.49* | 68.98 ± 0.42* |
| Lymphocytes (%) | 27.77 ± 1.92**a | 17.96 ± 0.42**b | 28.34 ± 0.45**a |
| Monocytes (%) | 1.32 ± 0.02**a | 0.76 ± 0.03**c | 1.18 ± 0.03**b |
| Eosinophils (%) | 1.45 ± 0.03**b | 5.98 ± 0.19**a | 1.49 ± 0.14**b |

* Significantly different from base values (p<0.05).

****** Significantly different from base values *(P<0.01).*

*The values bearing same or no superscripts does not vary significantly.

**Table 4.15: Hematology of group II otitic dogs**

| GROUP II | | | |
|---|---|---|---|
| | **Healthy Dogs (n=7)** | **0th day (pre treatment)** | **15th day (Post Treatment)** |
| Total Leukocyte count (TLC x $10^3$) | 9.31 ± 0.58**b | 10.96 ± 0.19**a | 9.90 ± 0.15**ab |
| DLC (%) Neutrophils (%) | 66.85 ± 3.45* | 75.48 ± 0.81* | 70.07 ± 0.52* |
| Lymphocytes (%) | 27.77 ± 1.92**a | 17.82 ± 0.34**b | 27.15 ± 0.43**a |
| Monocytes (%) | 1.32 ± 0.02**a | 0.83 ± 0.05**c | 1.06 ± 0.06**b |
| Eosinophils (%) | 1.45± 0.03**b | 5.86 ± 0.14**a | 1.72 ± 0.07**b |

* Significantly different from base values (p<0.05).

****** Significantly different from base values *(P<0.01).*

*The values bearing same or no superscripts does not vary significantly.

**Figure 4.36: Showing changes of total leukocyte count (TLC x $10^{3}$) in group I and II (Mean±S.E)**

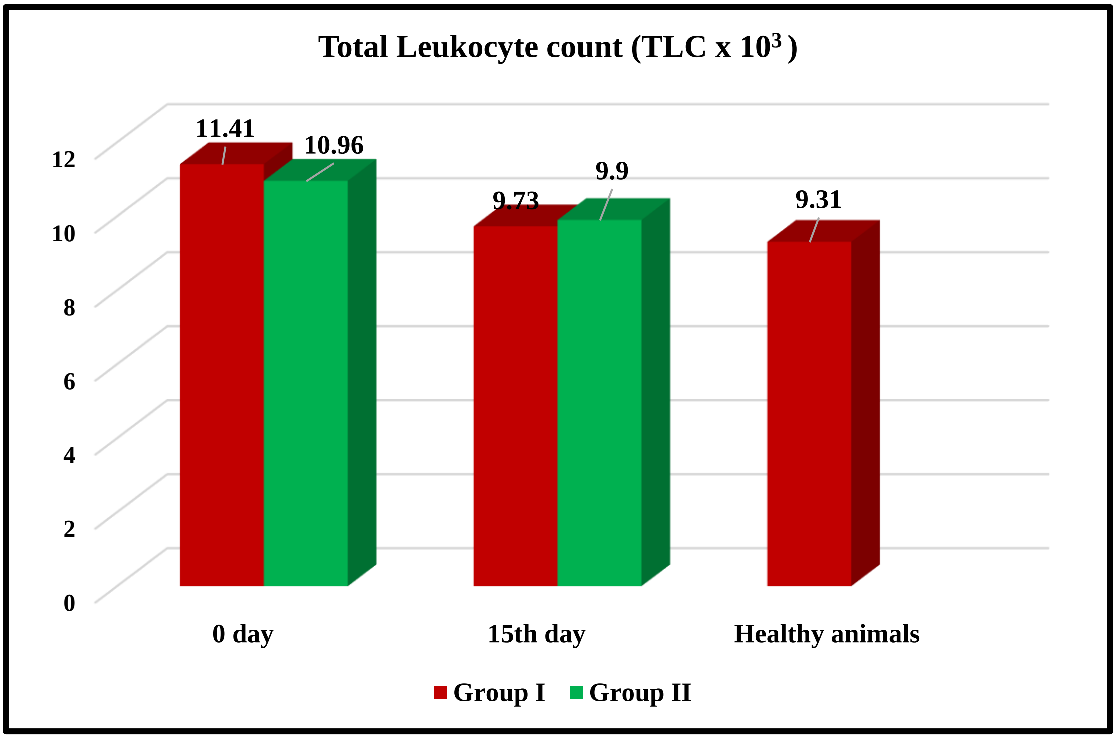



**Figure 4.37: Showing changes of neutrophils (%) in group I and II (Mean±S.E)**

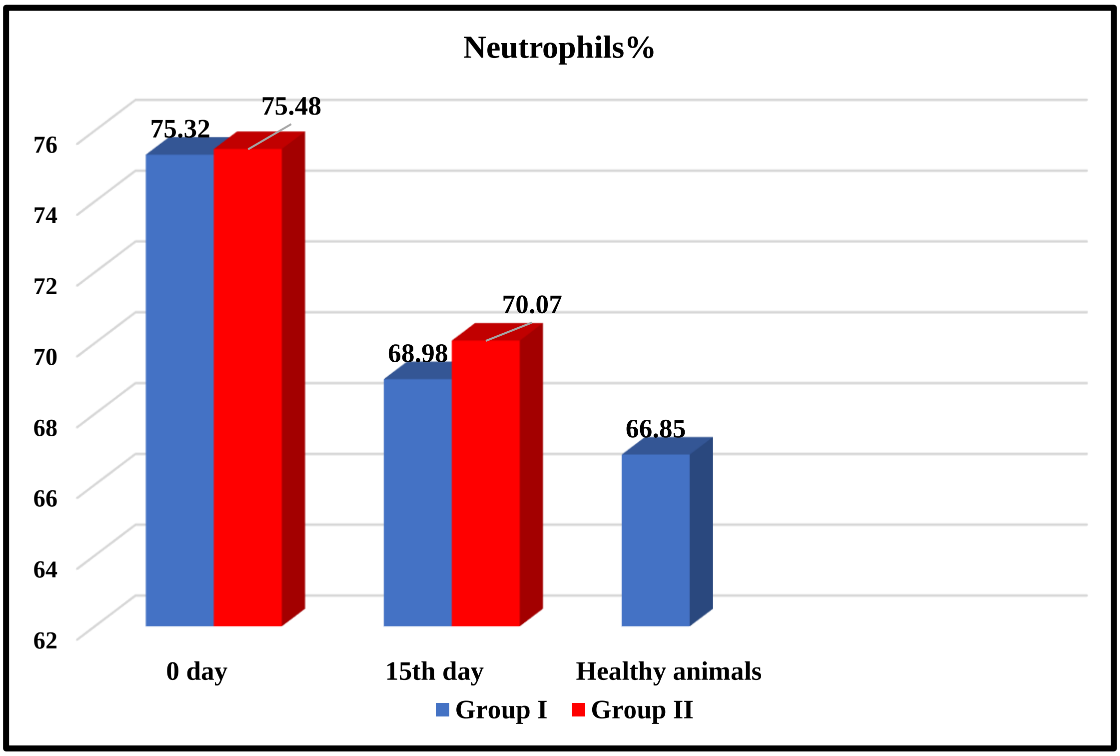


**Figure 4.38: Showing changes of lymphocytes (%) in group I and II (Mean±S.E)**

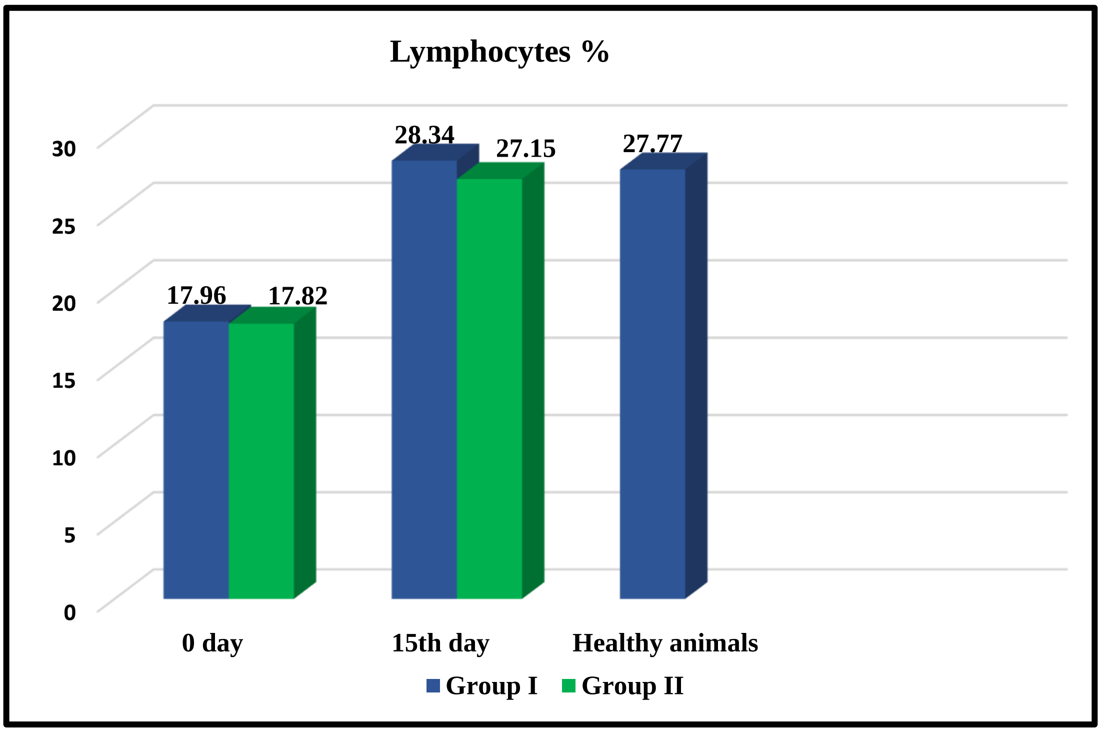



**Figure 4.39: Showing changes of monocytes (%) in group I and II (Mean±S.E)**

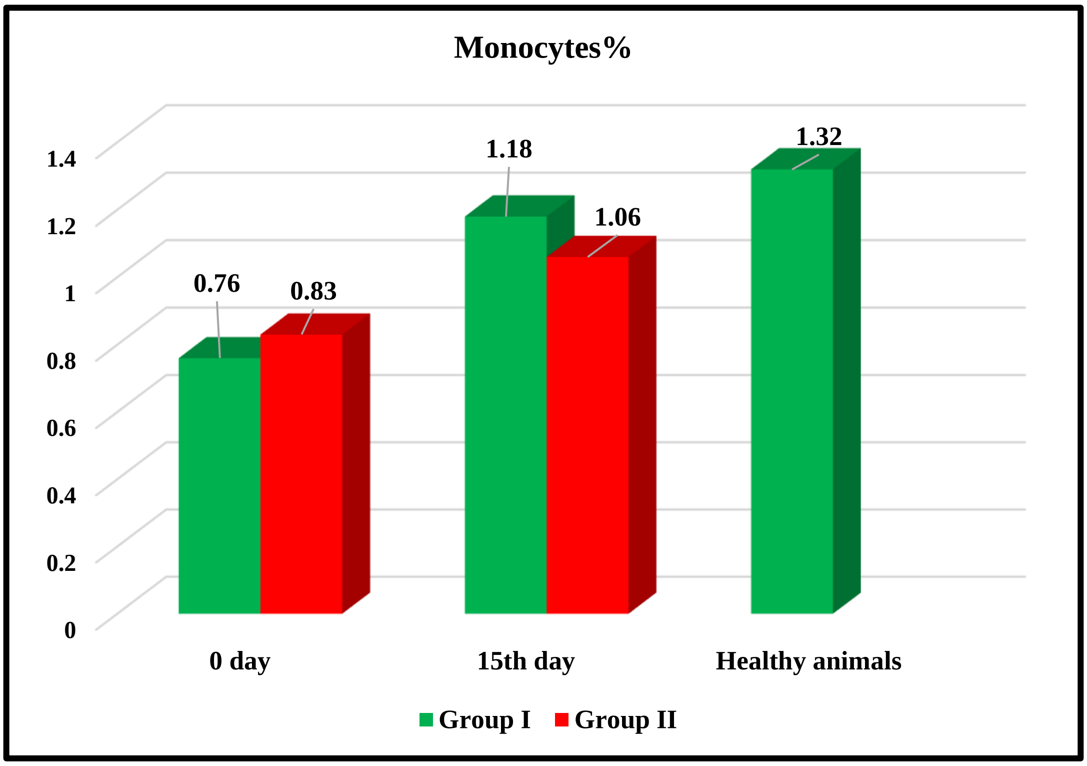
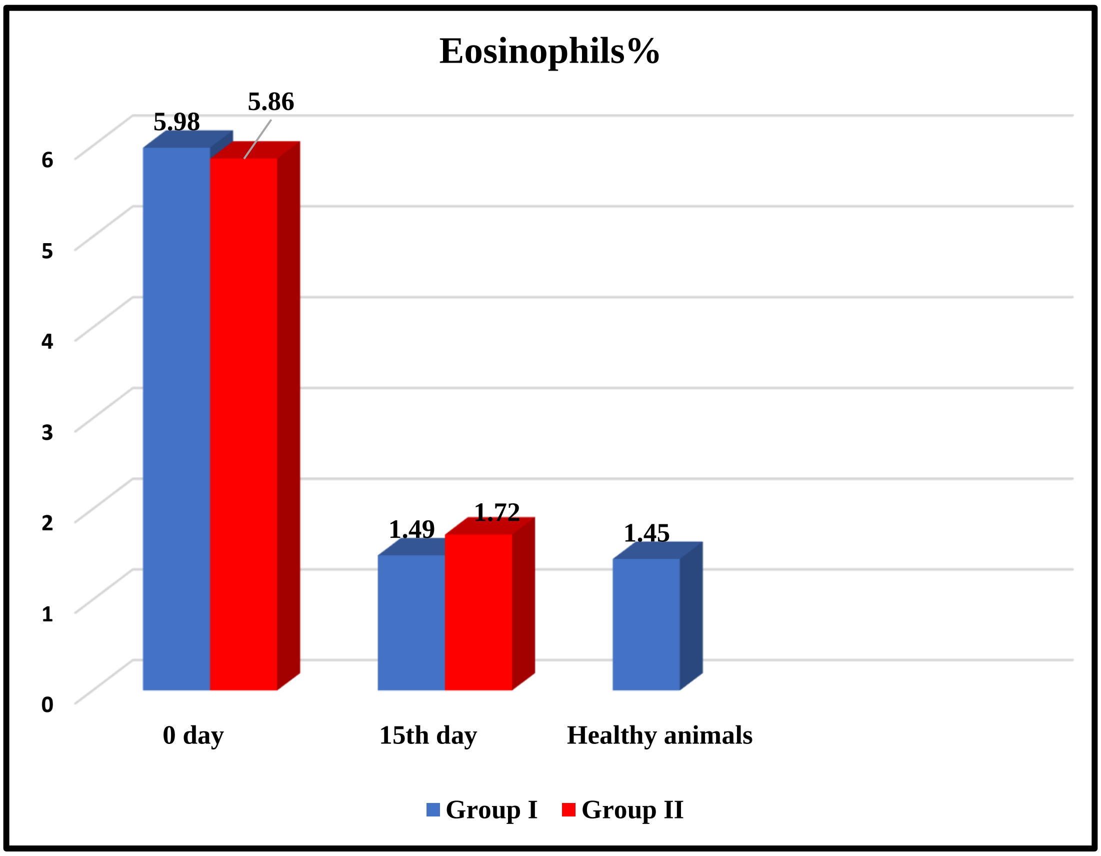


**Figure 4.40: Showing changes of eosinophils (%) in group I and II  (Mean±S.E)**



## 4.6 THERAPY

In the present study, out of 39 dogs (61 ears) affected with otitis externa 14 (28 ears) with bilateral otitis externa were taken for the detailed study. These 14 dogs of having bacterial etiology were divided to form 2 clinical trial groups viz., group I and II, and treatment was initiated for bacterial otitis externa.

In the present investigation, the treatment protocol used in treating the ear infections included cleaning the ear debris using cerumenolytic preparations dissolvent and subsequently instillation of ear preparation containing antiseptic and antibacterial properties. Beside this, effect of immune-modulator was also tried along with topical application.

The clinical recoveries in otitis externa affected canine patients were accessed on the basis of improvement in clinical signs and restoration of the altered hematological parameters following treatment. The best clinical and hematological parameters recovery is recorded in group I dogs compared to group II. (Table 4.16 and Fig.4.41)

The improvement in clinical signs such as resolution of inflammation and swelling of ear canal was seen by 10$^{th}$ day in group I and by day 17 in group II. Reduction in head shaking, foul smell, erythema, ear scratching, pain, head tilting and ulceration was seen by 10, 7, 8, 11, 14, 12, 8, days in group I and 13, 10, 16, 15, 15, 13, 16 days in group II respectively. The details are depicted in Fig. 4.42 to 4.45.

The therapeutic protocol (EPI-OTIC ear cleaner, Oregano oil, and Immunosky syrup) followed in group I dogs of otitis externa showed 100% recovery by day 15. Whereas, recovery was noticed in 71.42% cases that were treated with EPI-OTIC ear cleaner, Pomisol ear drops and Immunosky syrup (group II cases).

**Table 4.16: Evaluation of therapeutic efficacy of different groups of treatment undertaken against otitic dogs related bacterial otitis externa**

| Group | Number of total cases | Number of cases with resolution of condition | Number of cases with incomplete recovery |
|---|---|---|---|
| GROUP I | 07 | 07 (100%) | 00 |
| GROUP II | 07 | 05 (71.42%) | 02 |

**Figure 4.41: Showing evaluation of therapeutic efficacy of group I and group II**

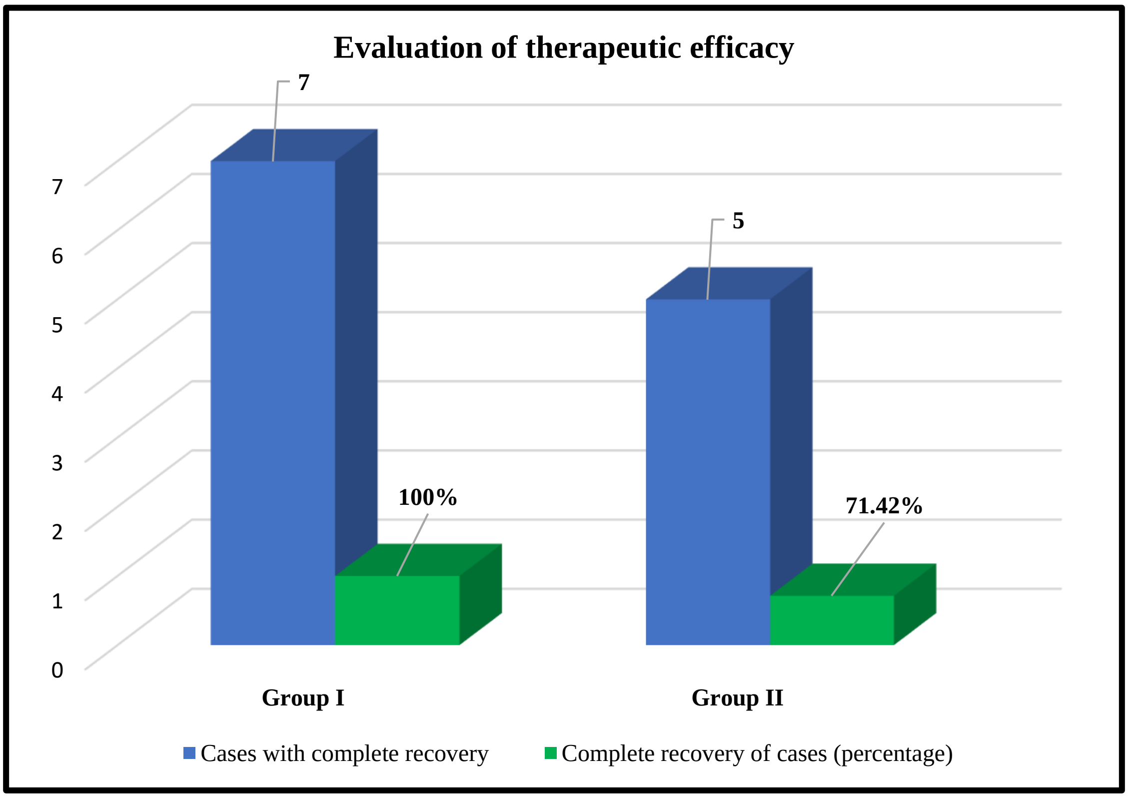


**Figure 4.42: Showing crusts formation on ear pinna on day 0 (Group I).**

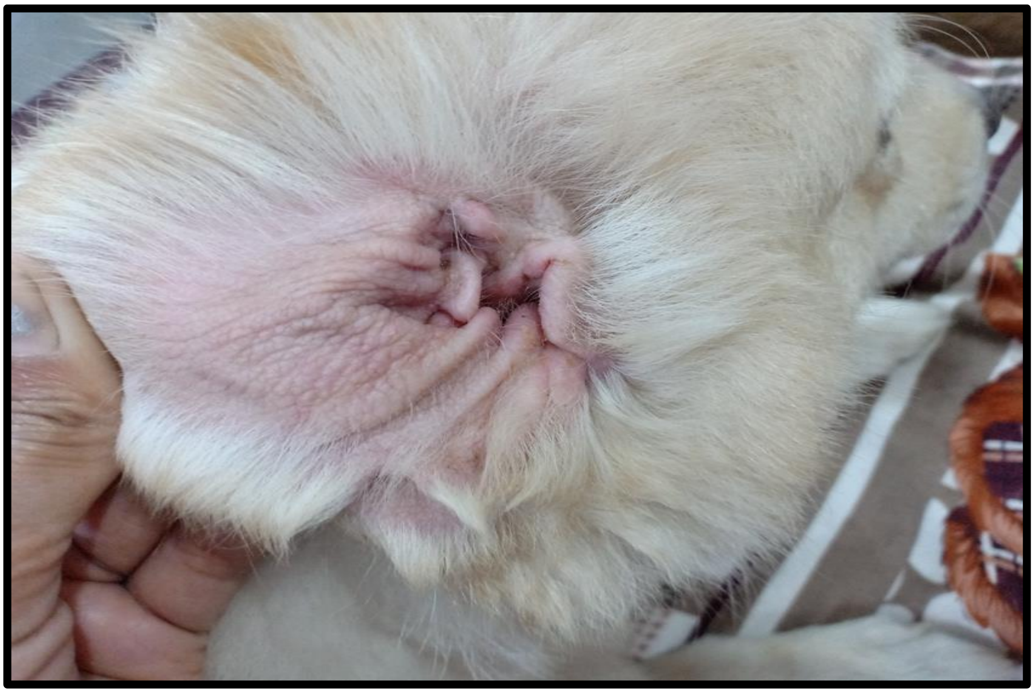


**Figure 4.43: Showing resolution of crusts on ear pinna using oregano oil on day 15 (Group I).**

**Figure 4.44: Showing erythema and excoriations on ear pinna on day 0 (Group II).**

**Figure 4.45: Showing less resolution of erythema and excoriations after using pomisol ear drops on day 15 (Group II).**

# DISCUSSION

CHAPTER V

# DISCUSSION

Skin and Ear infections are most common clinical complaint among dogs but they are not usually fatal and cause discomfort both to the dog and the owner. Otitis externa is acute or chronic inflammation of the ear auricle, horizontal and vertical ear canal and the outer tympanic membrane wall (Tilley and Smith, 2015).

In the present study that was carried out from December 2020 to July 2021, 39/264 dogs were diagnosed with otitis externa forming an occurrence of 14.77 per cent. These findings are in agreement with Ginel *et al*. (2002), Angus (2004), Greene (2006), Senthil *et al.* (2010), O'Neill *et al*. (2014), Noli *et al.* (2017) who reported otitis externa incidence up to 13-16%. Filipo *et al*. (2018) reported incidence of otitis externa is as high as 10-20% and on the contrary, Kumar *et al*. (2014) reported 21.97% incidence of otitis externa in Jammu region, Perry *et al.* (2017) reported 20% of incidence, While Soares *et al.* (2020) reported 7.30% incidence of otitis externa.

In the present investigation the age wise incidence was highest in the age group between 1 to 5 years (48.71%), followed by 5-10 years (41.02%) and in the age group between 3 months to 1 year (10.25%). These findings are in agreement with Chaudhary *et al*. (2003), Nair (2004), Sapierzynski (2009), Agnihotri *et al.* (2014) and Parmar *et al.* (2020) who reported a highest incidence of otitis externa is 1-5 years age group. On the contrary Martins *et al.* (2011) reported highest incidence of otitis externa in 0-3 years age group, Oliveira *et al.* (2012) reported maximum incidence in first years of age, Manju *et al*. (2018) reported highest occurrence of otitis externa in old age dogs and Hegde *et al.* (2021) reported highest incidence in between 5-10 years age group.

However, Zur *et al*., (2011) reported that there is no age predilection in dogs suffering from otitis externa.

With respect to breed, highest incidence of otitis externa was observed in Labrador retriever (21.42%), followed by German Shepherd (16.92%), Cocker Spaniel (12.00%), Mongrel (12.00%), Doberman (11.11%), Spitz (9.52%), Rottweiler (8.00%), and Pug (6.66%) breeds. These findings are in concurrence with Sharma *et al.* (2016), Parmar *et al.* (2020) who reported a highest incidence of otitis externa in Labrador Retriever and on the other hand this was in contrast with Topala *et al.* (2006), Saridomichelakis *et al.* (2007), Kaimio *et al.* (2017), Perry *et al.* (2017) who reported highest occurrence of otitis externa in Cocker Spaniel. Whereas, Chaudhary and Mirakhur (2002), Kumar *et al.* (2002) reported highest occurrence of otitis externa in German Shepherd breed dogs. The higher incidence of otitis externa in Labrador Retriever was probably due to susceptibility of breed and long floppy droopy ears (Kumar *et al.*, 2002).

In the present investigation sex wise incidence of otitis externa accounted relatively high in male dogs (58.97%) compared to female dogs (41.02%) the findings of which are in accordance with Chaudhury and Mirakhur (2002), Agnihotri *et al.* (2014), Manju *et al*. (2018), Parmar *et al.* (2020) and Hegde *et al.* (2021) stated majority of the dogs that were affected with otitis externa were males compared to females. But these findings are in contrary with Cunha *et al.* (2003), Fernandez *et al.* (2006), Saridomichelakis *et al.* (2007), Lehner *et al.* (2010) who reported higher incidence in females compared to males. On the other hand, Kumar *et al.* (2002), Topala *et al.* (2006), Martins *et al.* (2011), Zur *et al.* (2011) reported that there is no sex predisposition for canine otitis externa.

In the present study, the highest incidence was seen in summer season (43.58%), followed by rainy season (30.76%) and (25.64%) winter season. These findings are in accordance with Kumar *et al.* (2011), Balappanavar and vasant (2013) who reported highest incidence of otitis externa in summer season. On contrary, Conkova *et al*. (2011) reported least incidence of otitis externa in autumn. In the present study highest incidence in summer is due to high humidity inside ears that might result in development of ideal environment for growth of bacteria and yeast (Hayes *et al.*, 1987).

In the present investigation dogs suffering with otitis externa showed wide variety of clinical signs such as head shaking, pain, head tilting, ear scratching, ulceration, erythema, inflammation and swelling of ear canal, foul smell ear discharge. These findings are in agreement with Ashok *et al.* (2002), Mishra *et al.* (2003), Kale and Aher (2004), Dongardive *et al.* (2005), Fernandez *et al.* (2006), Sapierzynski (2009), Penna *et al.* (2011), Hosseini *et al.* (2012), Oliveira *et al.* (2012), Lakshmi and Rao (2013), Blake *et al.* (2017), Karnad *et al.* (2020) and Hegde *et al.* (2021).

The otitic dogs instinctively shake their heads to relieve discomfort, itchiness or irritation as it is an effective way to clear the ear canal. Ear scratching might be due to the exacerbated pruritus and discomfort. Carter *et al*. (1991) reported that the color of exudate indicates the microflora involved, the presence of yellow color discharge suggests might be an involvement of bacteria in higher numbers, *Pseudomonas aeruginosa* produces pyocyanin, which imparts a greenish color to exudates and pain in otitis infection was due to the proteolytic enzymes secreted by bacteria and inflammatory cells derived lysozymes.

In the present study percentage of positive diagnosis for tape impression smears, roll swab cytological method and direct impression smear was 74.35%, 41.02%, 28.20 % respectively. The highest percentage for positive diagnosis was by tape impression smears and the findings are in accordance with Curtis (2001), Sai Prasanna *et al*. (2006); reported that the tape impression was superior to roll swab method and direct impression smear for diagnosing canine otitis externa.

In the present study, *Staphylococcus* species was identified as the predominant pathogen accounting for 41.02% and second predominant bacteria was *Pseudomonas spp*. (28.20%) followed by *Proteus spp.* (5.12%) and *Streptococcus spp.* (5.12%). These findings of present study are in accordance with Agnihotri *et al.* (2014) who isolated *Staphylococcus aureus* (59.21%), *Pseudomonas aeruginosa* (24.02%), *Proteus* (10.61%) and *Streptococci* (6.15%). Lyskova *et al.* (2007) opined that the most frequently isolated microorganism from otitic ears was *Staphylococcus intermedius* (58.8%), followed by *Streptococcus canis* (29.9%), *Proteus spp.* (14.4%) and *Escherichia coli* (10.3%). *Staphylococcus spp.* being the predominant pathogen for causation of otitis externa has also been reported by De Martino *et al.* (2016), Karlapudi (2017), Filipo *et al*. (2018), Roshan *et al.* (2018), Scherer *et al.* (2018), Bajwa (2019), Demirbilek *et al.* (2019), Jayalakshmi *et al*. (2020), Parmar *et al.* (2020). While *Pseudomonas spp.* as causative agent for otitis externa in dogs has also been reported by Fernandez *et al.* (2006), Aalbaek *et al.* (2010), Subapriya *et al.* (2015) and Hegde *et al.* (2021). The distribution of other microorganism reported during present investigation is more or less similar to the findings of Lyskova *et al.* (2007) and Agnihotri *et al.* (2014).

In the present investigation, among the 39 isolates by mycological cultural examination of 16 ear swabs suspected for *Malassezia* otitis externa showed 8 positive isolates showing percent of isolation 20.51 %. These findings are in accordance with Karlapudi (2017) who isolated 29% otitis dogs that were suspected for *Malassezia* associated ear infection. Whereas, Yoshida *et al.* (2002)**,** Miller *et al.* (2012), Blake *et* *al*. (2017) reported that *Malassezia spp.* is the predominant organism isolated from otitis externa in dogs.

The present study revealed highest (91.27%) sensitivity to enrofloxacin followed by gentamycin (83.34%), amikacin (81.32 %), amoxicillin (80.19%) and ofloxacin (74.36.%), These findings are in agreement with Kumar *et al*. (2002), Vikas *et al*. (2003) Kale and Aher (2004), Yamamoto (2010), Sanchez *et al*. (2011), Filipo *et al*. (2018), who stated that enrofloxacin was the first ideal choice of antibiotic in the treatment of otitis externa in dogs. Manickam *et al*. (2019) recorded 90.90% sensitivity of enrofloxacin towards the bacterial isolates. In contrary, Hariharan and Harry (2006) reported that 38% of isolates were resistant to enrofloxacin. Schick *et al*. (2007), Parmar *et al*. (2020) reported that the clinical isolates were highly susceptible to gentamycin. Similarly, fungal isolates were highly sensitive (93.40%) to clotrimazole followed by ketoconazole (84.60%) and nystatin (54.40%), which is in agreement with Lakshmi and Rao (2013).

The leukocytosis noticed on day 0 of the present study have also been reported by Sharma and Gupta (2005), Dadhich and Khanna (2008), Kim *et al*. (2009), Reddy *et al*. (2016). Leukocytosis following primary or secondary bacterial infection or due to disease stress in cases of otitis externa in dogs has been documented by Suresh (2016). Sandeep (2017) in his study concluded that there is leukocytosis on day 0 of treatment. Leukocytosis could be due to cellular and hormonal immune response in otitis externa. The leukocytosis could also have resulted from toxins released due to tissue damage or necrosis produced by inflammation or from secondary bacterial infection (Kumar *et al.*, 2017). However, the reduction in TLC values following treatment might be due to control of infection in the patients during our study period.

The mean neutrophil per cent in otitis externa affected dogs was elevated non significantly on day 0 when compared to the healthy control. Similar findings have also been reported by Nair (2004), Pradhan *et al*. (2012), Reddy *et al*. (2016), Suresh (2016), Sandeep (2017) and Kumar *et al*. (2017). The findings of neutrophilia in the present study on day of presentation might be due to bacterial infection of ear or due to stress induced by the disease. Bacterial infection can lead to mobilization of marginal and bone marrow granulocytic pool thereby leading to neutrophilia. The reduction in neutrophil count following treatment on day 15 might be due to control of infection in our treated patients. Neutrophilia observed in otitic dogs was due to persisting infection and inflammatory changes in otitis externa (Pradhan *et al.* 2012).

The mean lymphocyte per cent in otitis externa affected dogs was decreased significantly (P<0.01) compared to the healthy control, is in agreement with Reddy *et al*. (2016), Kumar *et al*. (2017), Sandeep (2017) and a contrary finding of lymphocytosis was reported by Suresh (2016). The findings of reduced lymphocyte count in the present study might be attributed to the fact that neutrophilia in canine patients leads to concurrent lymphopenia. The values of lymphocytes were found to be gradually increased over a period of time as the treatment continued. This might be due to reduction in stress or due to concurrent decrease in neutrophil count following treatment. Lymphopenia could be considered as concomitant change observed along with neutrophilia.

The mean monocyte per cent of otitis externa affected dogs was decreased significantly (P<0.01) compared to the healthy control. These findings are in agreement with Kumar *et al*. (2017), Whereas, Nair (2004), Reddy *et al*. (2016), reported non-significant change in monocyte values in dogs suffering from otitis externa.

The findings of eosinophilia in the present otitis dogs is in accordance with Lilliehok *et al*. (2000). The bacteria or yeast irritates and stimulates the mast cells for release of more histamine and since histamine is chemotactic for eosinophils from the bone marrow to the circulation leading to eosinophilia. Besides, eosinophilia is assumed to be also due to liberation of protein or secretary products of the parasite (Dimiri *et al*., 2000).

In the present study the clinical recovery was noticed in two groups but earlier and best response to therapy was recorded in the animals of group I followed by group II.

The various therapeutic drugs used in the treatment of bacterial otitis dogs of the present study is in accordance with Kim *et al*. (2009). There are numerous ear cleansers containing antibiotics or disinfectants with antibacterial activity. However, the most commonly used ear cleansers are incorporated with salicylic acid, chlorhexidine, EDTA-Tris and propylene glycol-based products. A single or combination of these agents is mostly used for topical ear cleaning in ear affections in dogs (Paterson, 2016).

The topical application of salicylic acid-based ear cleanser for clinical management of otitis externa in dogs has been recommended by various workers (Nuttall and Cole, 2004; Pushpa *et al*., 2015; Paterson, 2016 and Singh, 2016). In addition to antibacterial activity, salicylic acid has also been proved to exert anti yeast activity (Wilke, 1988). Epi-Otic ear cleaner contains salicylic acid (0.2%) which is used to clear the cerumen and to remove the ear wax and cellular debris. These findings are in agreement with Cole *et al*. (2003) who reported that infected ears treated with an ear cleanser containing 0.1% salicylic acid were free of infection within 2 weeks this might be due to salicylic acid (0.2%) which acts by the removal of wax, bacterial toxins, degenerating cellular debris, and free fatty acid, all of which can act as a focus for infection and stimulate further inflammation (Mckeever and Torres, 1997).

Oregano oil upon application in group I dogs showed 100% recovery after 15 days of treatment; there was no complaint regarding recurrence after 15 days. Sim *et al.* (2019) investigated in vitro efficacy of oregano oil, thyme oil and their main phenolic constituents against bacterial and fungal isolates associated with canine otitis externa, and documented that both oregano oil and thyme oil showed good antimicrobial activity against gram-negative and gram-positive bacteria as well as *Malassezia pachydermatis* with MIC90 values ranging from 0.02 to 0.25% (200–2,292 μg/mL) and suggested oregano oil could be developed as novel treatment for sensitive and resistant bacteria and fungal organisms involved in canine otitis externa.

Oreganum vulgare could be a promising treatment to combat canine cutaneous mixed infections (Ebani *et al.,* 2020). Oregano oil contains cavarcol and it has antibacterial effect (Burt, 2004, Barros *et al.,* 2009) and their main components cavarcol and thymol shows antimicrobial activities against both bacteria and fungi (Chouhan *et al.,* 2017). This might be due to exposure of bacterial cells to carvacrol has resulted in increase in the membrane fluidity and leakage of hydrogen ions and potassium ions by dissipating proton motive force, leading to a decrease in pH gradient across the cytoplasm membrane, a collapse of the membrane potential, an inhibition of ATP (adenosine triphosphate) synthesis, and ultimately cell death (Ultee *et al.,* 1998).

Ear drops (Ofloxacin, Clotrimazole, Betamethasone and Lignocaine) upon application in group II dogs showed 71.42% recovery after 15 days of treatment. Dogs showed clinical improvement but recovered completely after 21 days of treatment and there was no complaint regarding recurrence after 21 days; similar results were obtained by Sharma *et al*. (2016) where there was complete resolution of clinical signs after 21 days of therapy.

The ingredients of ear drops (clotrimazole 1.0%, ofloxacin and glucocorticoids) reduces the inflammation and in inhibition of growth and multiplication of bacterial and fungal organisms and reduction in inflammation and pruritus (Merchant, 1995 and Jacobson, 2002). Ofloxacin is a fluoroquinolone with fast bactericidal action against gram positive and gram-negative bacteria. Betamethasone is a mild gluco-corticosteroid and it act as antipruritic, anti-inflammatory and decreases the glandular secretions, pain and also restores the normal barrier function of epithelium of the ear canal according to (Reddy and Sivajothi 2014).

The rational for immune-modulator therapy includes the stimulation of enhanced immune surveillance and altered response to bacterial allergens leading to diminish recurrence (Ihrke, 2005). The immune-modulatory activity of immune booster liquid might be due to the synergistic action of various ingredients (vitamin C, vitamin D3, zinc, Curcuma longa and glycyrrhiza). Vitamin C is an immune system booster, has antioxidant activity can decrease inflammation, also boosts the activity of phagocytes, immune cells that can swallow harmful bacteria and other particles, it promotes the growth and spread of lymphocytes and increases circulating antibodies. Vitamin D plays a critical role in promoting immune response. It has both anti-inflammatory and immunoregulatory properties and is crucial for the activation of immune system defense. Zinc is known to play a central role in the immune system, it helps in multiple aspects of the immune system, from the barrier of the skin to gene regulation within lymphocytes. Glycyrrhiza glabra root is called “Licorice”. The principal constituent is glycyrrhizin, it enhances immune stimulation and increases macrophage (white blood cells that ingest antibodies) which combats infection.

Curcuma longa (Turmeric)'s principal constituent is curcumin, it has strong anti-inflammatory and antioxidant activities and it is a potent immunomodulatory agent that can modulate the activation of T cells, B cells, macrophages, neutrophils, natural killer cells, and dendritic cells. Turmeric helps to bolster the immune system by increasing the immunomodulating capacity. Phyllanthus Emblica (Indian Gooseberry), is a very useful antioxidant to support normal oxidative stress levels during the body's natural aging process. It enhances immunity and rejuvenates skin. It helps in building the body's immune system (Nashine *et al.*, 2019).

In the present study, animals of group I which were treated with topical application of salicylic acid-based ear cleanser and instillation of oregano ear drops showed better and early recovery. It might be due to the additive action of topical application of salicylic acid containing ear cleaner and oregano oil.

# SUMMARY

CHAPTER VI

# SUMMARY

The clinical cases that were brought to the Veterinary Clinical Complex, Bhoiguda and Campus Veterinary Hospital, College of Veterinary Science, Rajendranagar, Hyderabad, Telangana during the period of December 2020 to July 2021 were screened for canine otitis externa. In addition, referral cases from the practicing veterinarians in and around Hyderabad and adjoining areas were also included. The major clinical signs recorded in otitis externa affected dogs included head shaking, ear scratching and pain on palpation of the ear, erythema etc.

Based on the results of ear cytology 14 dogs with bacterial otitis externa were selected and randomly divided into 2 groups namely group I and group II with 7 dogs each and were subjected to therapeutic trail and haematological changes were recorded before initiation of therapy (day 0) and after therapy (day 15).

Dogs belonging to group I and group II received therapy with epiotic ear cleaner and immunosky syrup along with oregano oil in group I and pomisol ear drops in group II. The response to the treatment was evaluated based on the improvement in the clinical signs and haematological changes.

In the present study after screening 264 dogs of 39 were diagnosed with otitis externa forming an overall incidence of 14.77%. Highest incidence was recorded in the age group of 1 to 5 years (48.71%) followed by 5 to 10 years (41.02%) and 3 months to 1 year (10.25%). Breed wise incidence was highest in Labrador Retriever (21.42) and least in Pug (6.66%) breed. Similarly, the incidence of otitis externa was relatively more in the males (58.97%) compared to females (41.02%), The same thing was highest in summer (43.58%), followed by rainy (30.76%) and winter (25.64%) seasons.

The common clinical signs of otitis dogs were head shaking, pain, head tilting, ear scratching, ulceration, erythema, inflammation and swelling of ear canal and foul smell ear discharge, resumed to normal following 15 days of treatment in group I and 21 days in group II.

The findings related to diagnosis of canine otitis externa using tape impression smears, roll swab cytological method and direct impression smear revealed a greater number of cocci followed by bacilli and yeast.

*Staphylococcus spp., Pseudomonas spp., Proteus spp.,* and *Streptococcus spp*. were the common bacterial isolates, and whereas, *Malassezia spp.* is the only fungal isolate of the present study.

Antibiotic sensitivity test revealed highest sensitivity to enrofloxacin and whereas antifungal sensitivity test revealed highest sensitive to clotrimazole followed by ketoconazole and nystatin.

In group I there was significant increase ($P<0.01$) in TLC and eosinophils, significant decrease ($P<0.01$) in lymphocytes and monocytes, non-significant increase in neutrophils on day 0 when compared to healthy control group. A significant ($P<0.01$) better improvement of haematological parameters in group I was noticed in the mean values of TLC, lymphocytes, monocytes and eosinophils on day 15 after therapy as compared to group II. Whereas, non-significant improvement was observed in neutrophils of group I dogs on day 15 after therapy as compared to group II.

The treatment protocol which was used in treating the ear infections included cleaning the ear debris using cerumenolytic preparations and subsequently instillation of ear preparation containing antiseptic and antibacterial properties. Beside this, immune-modulator were also tried along with topical application. Treatment included cleaning of ears with salicylic acid (0.2%) ear cleaner which is used to clear the cerumen and wax removal also to remove the cellular debris and oregano oil instillation which contains carvacrol which has antibacterial effect. An immune booster syrup containing vitamin C, vitamin D3, zinc, Curcuma longa and glycyrrhiza was also used in both the groups but with ear drops which contains clotrimazole 1.0%, ofloxacin and glucocorticoids in group II dogs.

Dogs in group I achieved good results compared to group II suggesting oregano oil for effective treatment of otitis externa. Hence therapeutic regimen used in group I could be recommended to achieve good therapeutic response in otitis externa dogs.

# LITERATURE CITED

# LITERATURE CITED

Aalbaek B, Bemis D A, Schjaerff M, Kania S A, Frank L A and Guardabassi L. 2010. Coryneform bacteria associated with canine otitis externa. *Veterinary Microbiology* **145** (3-4): 292-298.

Agnihotri D, Sharma A and Khurana R. 2014. XXXII Annual Convention of ISVM and International Symposium on the 21st Centuary Road map for Veterinary Practice. *Education and Research in India and Developing Countries* **6**: 21-91.

Agnihotri D, Charaya G, Chabbra R, Kumar T and Jain V K. 2019. Antibiogram of bacteria isolated from dogs suffering from otitis externa. *Indian Journal of Comparative Microbiology, Immunology and Infectious Diseases* **40** (1):15-20.

Ahmed L M. 2000. Medical and surgical management of canine otitis externa. *Iraqi Journal of Veterinary Sciences* **13** (2): 403-408.

Amalendu Chakrabarti. 2014. *Text Book of Clinical Veterinary Medicine*. Kalyani Publishers.

Angus J C, Lichtensteiger C, Campbell K L and Schaeffer D J. 2002. Breed variations in histopathologic features of chronic severe otitis externa in dogs: 80 cases (1995–2001). *Journal of the American Veterinary Medical Association* **221** (7):1000-1006.

Angus J C. 2004. Otic cytology in health and disease. *The Veterinary Clinics of North America. Small Animal Practice* **34** (2): 411-424.

Angus J C. 2005. Pathogenesis of otitis externa: understanding primary causes. In *Proceeding of the North American Veterinary Conference*, Orlando, Florida, USA. pp 807-809.

Anthony Yu. 2013. Therapeutic approach to otitis in veterinary dermatology. Western Veterinary Conference.

Arisov M V, Indyuhova E N and Arisova G B. 2020. The use of multicomponent ear drops in the treatment of otitis of various etiologies in animals. *Journal of Advanced Veterinary and Animal Research* **7** (1): 115.

Ashok K, Kitab S, Anshu S and Parven G. 2002. Microbiology of otitis externa in dogs. Proc. National Symposium and XX ISVM Convention, Bikaner 14-16 Feb, 2002, pp. 183.

Bauer A W. 1966. Antibiotic susceptibility testing by a standardized single disc method. *American Journal of Clinical Pathology* **45** :149-158.

Baksi S, Jana P S and Chakrabarti A. 2004. Bacterial otitis externa in dogs and its treatment. *Indian Veterinary Journal* **81** (12):1402-1403.

Bass M 2004. Canine otitis externa: Causes and predisposing factors. *Veterinary Medicine*.

Barros J C, da Conceiçao M L, Neto N J G, da Costa A C V, Junior J P S, Junior I D B and de Souza E L. 2009. Interference of Origanum vulgare L. essential oil on the growth and some physiological characteristics of Staphylococcus aureus strains isolated from foods. *LWT-Food Science and Technology* **42** (6):1139-1143.

Bartlett S J, Rosenkrantz W S and Sanchez S. 2011. Bacterial contamination of commercial ear cleaners following routine home use. *Veterinary Dermatology* **22** (6):546-553.

Balappanavar B R and Vasanth M S. 2013. Clinico-diagnostic and therapeutic management of canine malasseziosis. *Intas Polivet* **14** (2): 353-357.

Bajwa J. 2017. Canine Malassezia dermatitis. *The Canadian Veterinary Journal* **58** (10): 1119.

Bajwa, J. 2019. Canine otitis externa Treatment and complications. *The Canadian Veterinary Journal* **60** (1): 97.

Beier R C, Foley S L, Davidson M K, White D G, McDermott P F, Bodeis-Jones S and Nisbet D J. 2014. Characterization of antibiotic and disinfectant susceptibility profiles among Pseudomonas aeruginosa veterinary isolates recovered during 1994–2003. *Journal of Applied Microbiology* **118** (2): 326-342.

Blake J, Keil D, Kwochka K, Palma K and Schofield J. 2017. Evaluation of a single administration ototopical treatment for canine otitis externa: a randomised trial. *Veterinary Record Open* **4** (1).

Boehringer S I. 2011. Diagnostic value of the cytological exam in canine otitis externa. *Revista Veterinaria* **22** (1): 38-42.

Bouassiba C, Osthold W and Mueller R S. 2013. In-vivo efficacy of a commercial ear antiseptic containing chlorhexidine and Tris-EDTA. A randomised, placebo controlled, double-blinded comparative trial. *Tierarztliche Praxis Ausgabe K, Kleintiere/heimtiere* **40** (3): 161-170.

Bosznay J. 2014. Ear cytology in otitis externa: when, why, how? *The Veterinary Nurse* **5** (2): 70-75.

Bourely C, Cazeau G, Jarrige N, Leblond A, Madec J Y, Haenni M and Gay E. 2019. Antimicrobial resistance patterns of bacteria isolated from dogs with otitis. *Epidemiology & Infection* **147**.

Bradley C W, Lee F F, Rankin S C, Kalan L R, Horwinski J, Morris D O and Cain C L. 2020. The otic microbiota and mycobiota in a referral population of dogs in eastern USA with otitis externa. *Veterinary Dermatology* **31** (3): 225-249.

Bugden D L. 2013. Identification and antibiotic susceptibility of bacterial isolates from dogs with otitis externa in Australia. *Australian Veterinary Journal* **91** (1-2):43-46.

Burt S. 2004. Essential oils: their antibacterial properties and potential applications in foods a review. *International Journal of Food Microbiology* **94** (3):223-253.

Carter G R, Chengappa M M. 1991. Haemophilus and Taylorella. *Essentials of Veterinary Bacteriology and Mycology*. 4th Ed. *Philadelphia*. pp 187-190.

Cafarchia C, Gallo S, Capelli G and Otranto D. 2005. Occurrence and population size of Malassezia spp. in the external ear canal of dogs and cats both healthy and with otitis. *Mycopathologia* **160** (2): 143-149.

Chaudhary M and Mirakhur K K. 2002. Studies on occurrence of canine otitis. *Indian Veterinary Journal* **79**: 748-749.

Chaudhary M, Mirakhur K K and Jand S K. 2003. Antibiogram and microbiological patterns of external ear canal of dogs with reference to otitis. *Indian Veterinary Journal* **80** (9): 951-952.

Chouhan S, Sharma K and Guleria S. 2017. Antimicrobial activity of some essential oils-present status and future perspectives. *Medicines* **4** (3): 58.

Chan W Y, Hickey E E, Page S W, Trott D J and Hill P B. 2019. Biofilm production by pathogens associated with canine otitis externa, and the antibiofilm activity of ionophores and antimicrobial adjuvants. *Journal of Veterinary Pharmacology and Therapeutics* **42** (6): 682-692.

Colombini S A R A H, Merchant S R and Hosgood G I S E L L E. 2000. Microbial flora and antimicrobial susceptibility patterns from dogs with otitis media. *Veterinary Dermatology* **11** (4): 235-239.

Cole L K, Kwochka K W, Kowalski J J, Hillier A and Hoshaw-Woodard S L. 2003. Evaluation of an ear cleanser for the treatment of infectious otitis externa in dogs. *Veterinary Therapeutics Research in Applied Veterinary Medicine* **4** (1): 12-23.

Cole L K. 2004. Otoscopic evaluation of the ear canal. *The Veterinary clinics of North America. Small Animal Practice* **34** (2): 397-410.

Coatesworth J. 2011. Causes of otitis externa in the dog. *Journal of Companion Animal* **16**: 35-38.

Conkova E, Sesztakova E, Palenik L, Smrco P and Bilek J. 2011. Prevalence of Malassezia pachydermatis in dogs with suspected Malassezia dermatitis or otitis in Slovakia. *Acta Veterinaria Brno* **80** (3): 249-254.

Curtis C F. 2001. Diagnostic techniques and sample collection. *Clinical Techniques in Small Animal Practice* **16** (4): 199-206.

Cunha F M, Coutinho S D, Matera A, Fiorio W A, Ramos M C C, Silveira L M G. 2003. Clinical and cytological evaluation of the external ear canal of dogs with otitis. *Revista de Educagao Continuada do CRMV-SP* **6** (1/3): 7-15.

Dadhich H and Khanna R. 2008. Pathological, haemato-biochemical and immunological studies of cutaneous ectoparasitoses in dogs. In *Proceedings of the 15th Congress of FAVA. FAVA-OIE Joint Symposium on Emerging Diseases. Bangkok, Thailand*.

Degi J, Cristina RT, Stancu A. 2010. Otitis externa caused by bacteria of the genus pseudomonas in dogs. *Lucrari Stiinlific Medicine Veterinara* (1).

De Martino L, Nocera F P, Mallardo K, Nizza S, Masturzo E, Fiorito F and Catalanotti P. 2016. An update on microbiological causes of canine otitis externa in Campania Region, Italy. *Asian Pacific Journal of Tropical Biomedicine* **6** (5): 384-389.

Demirbilek S K and YILMAZ O. 2019. Identification and antimicrobial susceptibility of microbial agents of otitis externa in dogs1. *Med. Weter* **75** (2): 107-110.

Dimiri U, Sharma M C, Kalicharan R and Dwivedi P. 2000. Clinicobiochemical and Histopathological alterations in demodectic mange in canines with special reference to ivermectin therapy. *Indian Journal of Veterinary Pathology* **24**: 23-25.

Dixit A A, Rao M L V, Roy K, Sharma I J and Malik Y P S. 2006 a A Comparative efficacy of therapeutics regime in canine otitis. Compendium, XXIV Annual Convention of the Indian Society for veterinary medicine, Bangalore, Karnataka. Abstract No. 4.44. P.P.97.

Dongardive K M, Gahlod B M, Panchbhai V S, Patil S N and Dhakate M S. 2005.Acute otitis media in canine. *Intas Polivet* **6** (l): 110-113.

Ebani V V, Bertelloni F, Najar B, Nardoni S, Pistelli L and Mancianti F. 2020. Antimicrobial activity of essential oils against Staphylococcus and Malassezia strains isolated from canine dermatitis. *Microorganisms* **8** (2): 252.

Einchenberg M L, Appel C E, Berg V, Muschner A C, de Oliveira Nobre M, da Matta D and Ferreiro L. 2003. Susceptibility of Malassezia pachydermatis to azole antifungal agents evaluated by a new broth microdilution method. *Acta Scientiae Veterinariae* **31** (2): 75-80.

Engelen M, Bock M D, Hare J and Goossens L. 2010. Effectiveness of an otic product containing miconazole, polymyxin B and prednisolone in the treatment of canine otitis externa: multi-site field trial in the US and Canada. *International Journal of Applied Research in Veterinary Medicine* **8** (1): 21-30.

Ettinger S J and Feldmen E C. 2000. Disease of the ear. *Textbook of Veterinary Internal Medicine*, 5$^{th}$ Ed. W.B. Saunders Philadelphia. pp 19-93.

Fernandez G, Barboza G, Villalobos A, Parra O, Finol G and Ramirez R A. 2006. Isolation and identification of microorganisms present in 53 dogs suffering otitis externa. *Revista Cientifica-Universidad Del Zulia Facultad De Ciencias Veterinarias Division De Investigacion* **16** (1): 23.

Filipo C D, Fiţ N, Bouari C. M, Buzura-Matei and Nadaş G. 2018. Microbiological study of external otitis in dogs from North Rhine-Westphalia, Germany. *In Medicină Veterinară* **49**: 115-117.

Forsythe P. 2007. Collection and interpretation of clinical samples for dermatological analysis. *In Practice* **29** (3): 158-164.

Frantz L A, Bradley D G, Larson G and Orlando L. 2020. Animal domestication in the era of ancient genomics. *Nature Reviews Genetics* **21** (8): 449-460.

Ginel P J, Lucena R, Rodriguez J C and Ortega J. 2002. A semiquantitative cytological evaluation of normal and pathological samples from the external ear canal of dogs and cats. *Veterinary Dermatology* **13** (3): 151-156.

Girão M D, Prado M R, Brilhante R S N, Cordeiro R A, Monteiro A J, Sidrim J J C and Rocha M F G. 2006. Malassezia pachydermatis isolated from normal and diseased external ear canals in dogs: a comparative analysis. *The Veterinary Journal* **172** (3): 544-548.

Gotthelf L N. 2004. Diagnosis and treatment of otitis media in dogs and cats. *Veterinary Clinics: Small Animal Practice* **34** (2): 469-487.

Gotthelf L. 2005. Small animal ear diseases: an illustrated guide, 2nd Ed. St Louis, Saunders Elsevier. pp 434.

Gotthelf L N. 2017. Efficacy of Apoquel® for the control of otitis externa secondary to allergic skin disease in client-owned dogs. *International Journal of Veterinary Health Science and Research* **5** (7): 208-212.

Greene C E. 1998. Otitis externa In Infectious Diseases of the Dog and Cat, 2nd Ed. W.B. Saunders, Philadelphia, USA. pp 542.

Griffin C E and Deboer D J. 2001. The ACVD task force on canine atopic dermatitis (XIV): clinical manifestations of canine atopic dermatitis. *Veterinary Immunology and Immunopathology* **81**: 255-269.

Graham-Mize C A and Rosser Jr E J. 2004. Comparison of microbial isolates and susceptibility patterns from the external ear canal of dogs with otitis externa. *Journal of the American Animal Hospital Association* **40** (2): 102-108.

Greene C E. 2006. Otitis externa. *Infectious diseases of the dog and cat.* 3rd Ed. *Saunders Elsevier, St. Louis, Missouri*. pp 815-817.

Guardabassi L, Loeber M E and Jacobson A. 2004. Transmission of multiple antimicrobial-resistant Staphylococcus intermedius between dogs affected by deep pyoderma and their owners. *Veterinary Microbiology* **98** (1): 23-27.

Guillot J and Bond R. 1999. Malassezia pachydermatis: a review. *Medical Mycology* **37** (5): 295-306.

Hayes Jr H M, Pickle L W and Wilson G P. 1987. Effects of ear type and weather on the hospital prevalence of canine otitis externa. *Research in Veterinary Science* **42** (3): 294-298.

Harvey R G, Harari J and Delauche A J. 2001. Etiopathogenesis and classification of otitis externa in ear diseases of the dog and cat. London Manson publishing pp: 81-122**.**

Hariharan and Harry. 2006. Update on antimicrobial susceptibilities of bacterial isolates from canine and feline otitis externa. *The Canadian Veterinary Journal* **47** (3): 253.

Harada K, Arima S, Niina A, Kataoka Y and Takahashi T. 2012. Characterization of Pseudomonas aeruginosa isolates from dogs and cats in Japan: current status of antimicrobial resistance and prevailing resistance mechanisms. *Microbiology and Immunology* **56** (2): 123-127.

Harvey R G and Paterson S. 2014. Medical management of ear disease. Otitis externa: an essential guide to diagnosis and treatment. CRC Press, Boca Raton, Florida.

Hendricks A, Schuberth H J, Schueler K and Lloyd D H. 2002. Frequency of superantigen-producing Staphylococcus intermedius isolates from canine pyoderma and proliferation-inducing potential of superantigens in dogs. *Research in Veterinary Science* **73** (3): 273-277.

Hegde G, Subapriya S and Vairamuthu S. 2021. Update on microbial profile and drug sensitivity of canine otitis. *The Pharma Innovation* **10**(3): 549-551

Hosseini J, Zdovc I, Golob M, Blagus R, Kušar D, Vengušt M and Kotnik T. 2012. Effect of treatment with Tris-EDTA/chlorhexidine topical solution on canine Pseudomonas aeruginosa otitis externa with or without concomitant treatment with oral fluoroquinolones. *Slov Vet Res* **49**: 133-40.

Ihrke P J. 2005. Recurrent canine pyoderma. *Compendium on Continuing Education for the Practicing Veterinarian* **27** (4): 15-19.

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.

Jayalakshmi V, Tamilarasu S, Srinivas V V, Barbuddhe S, Antony P and Mukhopadhyay H. 2020. Isolation and Molecular Characterization of Methicillin-Resistant Staphylococcus pseudintermedius in dogs. *The Indian Journal of Veterinary Sciences and Biotechnology* **15** (4): 71–74.

Kale S D and Aher V D. 2004. Studies on the symptomology and diagnosis of otitis in clinical cases of canine. *Intas Polivet* **5** (1): 103-107.

Kaimio M, Saijonmaa-Koulumies L and Laitinen-Vapaavuori O. 2017. Survey of otitis externa in American Cocker Spaniels in Finland. *Acta Veterinaria Scandinavica* **59** (1): 1-9.

Karlapudi S K. 2017. Diagnosis and management of Malassezia otitis in dogs. *The Pharma Innovation* **6** (9): 36.

Karnad V V, Jeyaraja K, Vijayarani K, Vairamuthu S, Subapriya S and Ronald B S M. 2020. Cytological and microbiological analysis of canine otitis externa. *Indian Journal of Animal Research* **54** (10): 1309-1313.

Kim S H, Kim S, Jun H K and Kim D H. 2009. Efficacy of aromatherapy for the treatment of otitis externa in dogs. *Korean Journal of Veterinary Research* **49** (1): 85-89.

Kowalski J J. 1988. The microbial environment of the ear canal in health and disease. *Veterinary Clinics of North America: Small Animal Practice* **18** (4): 743-754.

Kumar A. 2001. Studies on the prevalence, Characterization and therapy of Malassezia pachydermatitis associated with otits externa and dermatitis in dogs. Abstract No.194.

Kumar A, Singh K and Sharma A. 2002. Prevalence of Malassezia pachydermatis and other organisms in healthy and infected dog's ears. *Israel Journal of Veterinary Medicine* **57** (4): 145-148.

Kumari K N, Srilatha C H, Prameela D R and Sreedevi B. 2006. Malassezia associated otitis in canines. Compendium, XXIV annual convention of the Indian society for veterinary medicine, Bangalore, Karnataka. Abstract No: 47. pp.158

Kumar K S, Selvaraj P, Vairamuthu S, Nagarajan B, Nambi A P and Prathaban S. 2011. Survey of fungal isolates from canine mycotic dermatitis in Chennai. *Tamil nadu Journal of Veterinary Animal Science* **7**: 48-50.

Kumar S, Hussain K, Sharma R, Chhibber S and Sharma N. 2014. Prevalence of canine otitis externa in Jammu. *Journal of Animal Research* **4** (1): 121-130.

Kumar S, Hussain K, Batoo A S, Chaudhary S, Najar A A and Kour S. 2017. Effect of Enrofloxacin and Enrofolxacin in Combination with Fish Oil on Hematobiochemical and Otic Cytological PA. *Veterinary Practitioner* **18** (1): 27-30.

Lakshmi K and Rao D S. 2013. Clinico-microbiological and therapeutic studies on canine otitis externa. *International Journal of Pharma and Bio Sciences* **4** (3): 1209-1214.

Leite C A L, Abreu V L V and Costa G M. 2003. Freqüência de Malassezia pachydermatis em otite externa de cães. *Arquivo Brasileiro de Medicina Veterinária e Zootecnia* **55** (1): 102-104.

Lehner G, Sauter Louis C and Mueller R S. 2010. Reproducibility of ear cytology in dogs with otitis externa. *Veterinary Record* **167** (1): 23-26.

Lilliehöök I, Gunnarsson L, Zakrisson G and Tvedten H. 2000. Diseases associated with pronounced eosinophilia: a study of 105 dogs in Sweden. *Journal of Small Animal Practice* **41** (6): 248-253.

Lozina L A, Peichoto M E, Boehringer S I, Koscinczuk P, Granero G E and Acosta O C. 2010. Efficacy of Argentine propolis formulation for topical treatment of canine otitis externa. *Arquivo Brasileiro de Medicina Veterináriae Zootecnia* **62**: 1359-1366.

Logas D. 2019. Otitis Externa. *Small Animal Dermatology for Technicians and Nurses*. pp 53-64.

Lyskova P, Vydrzalova M and Mazurova J. 2007. Identification and antimicrobial susceptibility of bacteria and yeasts isolated from healthy dogs and dogs with otitis externa. *Journal of Veterinary Medicine Series* **54** (10): 559-563.

Martín Barrasa J L, Lupiola Gomez P, Gonzalez Lama Z and Tejedor Junco M T. 2000. Antibacterial susceptibility patterns of Pseudomonas strains isolated from chronic canine otitis externa. *Journal of Veterinary Medicine Series B* **47** (3): 191-196.

Masuda A, Sukegawa T, Tani H, Miyamoto T, Sasai K, Morikawa Y and Baba E. 2001. Attachment of Malassezia pachydermatis to the ear dermal cells in canine otitis externa. *Journal of Veterinary Medical Science* **63** (6): 667-669.

Mahendran K, Bhat M N, Murallidhara A and Suryanarayana T. 2007. Study on the antibiogram pattern in otiits of dogs. Compendium, XXIV Annual convention of the Indian Society for veterinary Medicine Bangalore, Karnataka Abstract. No.4.47. pp. 99.

Mactaggart D. 2008. Assessment and management of chronic ear disease. *In practice* **30** (8): 450-458.

Mansoor T, Musani M A, Khalid G and Kamal M. 2009. Pseudomonas aeruginosa in chronic suppurative otitis media: sensitivity spectrum against various antibiotics in Karachi. *J Ayub Med Coll Abbottabad* **21** (2): 120-123.

Malayeri H Z, Jamshidi S and Salehi T Z. 2010. Identification and antimicrobial susceptibility patterns of bacteria causing otitis externa in dogs. *Veterinary Research Communications* **34** (5): 435-444.

Mactaggart D. 2008. Assessment and management of chronic ear disease. *In practice* **30** (8): 450-458.

Martins E A, Momesso C S, de Nardo C D D, de Castro K F, Atique T S C, Netto H A and Furini A D C. 2011. Clinical and microbiological study of canine otitis in a veterinary hospital in the northwest of São Paulo state, Brazil. *Acta Veterinaria Brasilica* **5** (1): 61-67.

Manju R, Roshan K and Suhsovan R. 2018. Prevalence of canine otitis externa, etiology and clinical practice in and around Durg District of Chhattisgarh State, India. *International Journal of Current Microbiology and Applied Sciences* **7** (3): 269-274.

Maginn K. 2016. Management of otitis externa and the veterinary nurse's role. *The Veterinary Nurse* **7** (1): 25-32.

Manickam R, T Reetha L and Puvarajan B. 2019. Antibiotic Resistance Level of Staphylococcus Spp. Isolated from Dogs with Otitis Externa. *International Journal of Science Environment and Technology* **8** (5): 978-983.

McKeever P J and Torres S M. 1997. Ear disease and its management. *Veterinary Clinics of North America: Small Animal Practice* **27** (6): 1523-1536.

McKay L, Rose C D S, Matousek J L, Schmeitzel L S, Gibson N M and Gaskin J M. 2007. Antimicrobial testing of selected fluoroquinolones against Pseudomonas aeruginosa isolated from canine otitis. *Journal of the American Animal Hospital Association* **43** (6): 307-312.

Merchant S. 1995. Pathogenesis and clinical management of otitis externa in the dog. October 14-15, 83. 19th Annual Proceedings, Philadelphia, PA: WB Sunders; 351-357, 970-87.

Mekic S, Matanovic K and Seol B. 2011. Antimicrobial susceptibility of Pseudomonas aeruginosa isolates from dogs with otitis externa. *Veterinary Record* **169** (5): 125-125.

Mhatre M D. 2005. *Studies on etio-pathology of bacterial and mycological infections of skin and ear in canines and their clinical management* (Doctoral dissertation, AAU, Anand).

Mishra G S, Mehta N and Pal M. 2003. Chronic bilateral otomycosis caused by Aspergillus niger. *Mycoses* **47** (2): 82-84.

Miller W H, Griffin C E and Campbell K L. 2012. Diseases of eyelids, claws, anal sacs, and ears. *Muller & Kirk's Small Animal Dermatology.* 7$^{th}$ Ed. *St. Louis: Elsevier Inc*. pp 724-73.

Miller W H, Griffin C E, Campbell K L and Muller G H. 2013. Diseases of eyelids, claws, anal sacs, and ears. *Muller and Kirk's Small Animal Dermatology*, 7$^{th}$ Ed. Elsevier Health Sciences. pp 724–774.

Mota R A, Farias J K O, da Silva L B G, de Lima E T, Oliveira A A F and de Moura R T D. 2000. Efficacy of Otomax for the treatment of bacterial and fungal otitis in dogs. *A Hora Veterinária* **19** (113): 13-16.

Morris D O. 2004. Medical therapy of otitis externa and otitis media. *Veterinary Clinics: Small Animal Practice* **34** (2): 541-555.

Mugnaini L, Nardoni S, Pistelli L, Leonardi M, Giuliotti L, Benvenuti M N and Mancianti F. 2013. A herbal antifungal formulation of Thymus serpillum, Origanum vulgare and Rosmarinus officinalis for treating ovine dermatophytosis due to Trichophyton mentagrophytes. *Mycoses* **56** (3): 333-337.

Nair S S. 2004. *Studies on clinico-etiopathology and therapeutic management of various canine dermatoses* (Doctoral dissertation, AAU, Anand).

Nascente P da S, Cleff M B, Faria R Ode, Nobre M de O, Xavier M O, Meireles M C A and Mello J R De B. 2005. Canine otitic Malasseziosis, experimental inoculation and treatment. *Clinica Veterinaria* **10** (55): 54-60.

Nashine S, Kanodia R, Nesburn A B, Soman G, Kuppermann B D and Kenney M C. 2019. Nutraceutical effects of *Emblicaofficinalis* in age-related macular degeneration. *Aging* **11** (4): 1177–1188.

Neves R C, Makino H, Cruz T P, Silveira M M, Sousa V R, Dutra V and Belli C B. 2018. In vitro and in vivo efficacy of tea tree essential oil for bacterial and yeast ear infections in dogs. *Pesquisa Veterinária Brasileira* **38**: 1597-1607.

Ngo J, Taminiau B, Fall P A, Daube G and Fontaine J. 2018. Ear canal microbiota–a comparison between healthy dogs and atopic dogs without clinical signs of otitis externa. *Veterinary Dermatology* **29** (5): 425-140.

Niculae M, Spînu M, Șandru C D, Brudașca F, Cadar D, Ungvari A and Tauțan M. 2009. Antibiotic resistance level in Staphylococcus spp. strains isolated from dogs with otitis externa. *Lucrari Stiintifice-Universitatea de Stiinte Agricole a Banatului Timisoara, Medicina Veterinara* **42** (1): 176-180.

Noli C and Cevidalli A E. 2001. Efficacy of an otological product containing gentamicin, clotrimazole and betamethasone for the treatment of external otitis in dogs. Veterinaria (Cremona) **15** (l): 61-71.

Noli C, Sartori R and Cena T. 2017. Impact of a terbinafine–florfenicol–betamethasone acetate otic gel on the quality of life of dogs with acute otitis externa and their owners. *Veterinary Dermatology* **28** (4): 386-e90.

Nocera F P, Addante L, Capozzi L, Bianco A, Fiorito F, De Martino L and Parisi A. 2020. Detection of a novel clone of Acinetobacter baumannii isolated from a dog with otitis externa. *Comparative Immunology, Microbiology and Infectious Diseases* **70**:101471.

Nuttall T and Cole L K. 2004. Ear cleaning: the UK and US perspective. *Veterinary Dermatology* **15** (2): 127-136.

Nwiyi P, Okonkwo C and Enwere S. 2014. Isolation of pathogenic bacteria and antibiotic susceptibility testing of dogs with otitis externa in Aba, Abia state, Nigeria. *Sky Journal Microbiology. Research* **2**: 59-62.

Oegi J, Imre K, Catana N, Morar A, Sala C and Herman V. 2013. Frequency of isolation and antibiotic resistance of Staphylococcal flora from external otitis of dogs. *Veterinary Record* **l73** (2): 42.

Oliveira L C. 2005. Antimicrobial sensitivity of bacteria from otitis externa in dogs. *Arquivo Brasileiro de Medicina Veterinária e Zootecnia (Brazil)*. **57**:

405-408.

Oliveira L C, Medeiros C M O, Silva I N G, Monteiro A J, Leite C A L and Carvalho CBM. 2005. Antimicrobial sensitivity of bacteria from otitis externa in dogs. *Arquivo Brasileiro de Medicina Veterinaria e Zootecnia* **57** (3): 405-408.

Oliveira V B de, Ribeiro M G, Silva Almeida A C da, Paes A C, Condas L A Z, L ara G H B, Franco M M J, Fernandes M C, Listoni F J P. 2012. Etiology, antimicrobial susceptibility profile and epidemiological aspects in canine otitis; a retrospective study of 616 cases. *Semina: Ciencias Agrarias, Londrina* **33** (6): 2367-2374.

Olivry T, DeBoer D J, Favrot C, Jackson H A, Mueller R S, Nuttall T and International Task Force on Canine Atopic Dermatitis. 2010. Treatment of canine atopic dermatitis: 2010 clinical practice guidelines from the International Task Force on Canine Atopic Dermatitis. *Veterinary Dermatology* **21** (3): 233-248.

O′ Neill D G, Church D B, McGreevy P D, Thomson P C and Brodbelt D C. 2014. Prevalence of disorders recorded in dogs attending primary-care veterinary practices in England. *Plos one* **9** (3): e90501.

Pal M and Rao N M. 2001. Canine otitis due to Candida albicans. *Indian Veterinary Journal* **78**: 150-151.

Paterson S. 2016. Discovering the causes of otitis externa. *In Practice* **38**: 7-11.

Parmar J J, Rao N, Shah A I, Sadhu D B, Bhanderi B B and Patel D M. 2020. Clinical Studies on Ear Infections, Microbiological Evaluation and Therapeutic Management in Canines. *International Journal of Current Microbiology and Applied Sciences* **9** (1): 1496-1501.

Petersen A D, Walker R D, Bowman M M, Schott H C and Rosser Jr E J. 2002. Frequency of isolation and antimicrobial susceptibility patterns of Staphylococcus intermedius and Pseudomonas aeruginosa isolates from canine skin and ear samples over a 6-year period (1992–1997). *Journal of the American Animal Hospital Association* **38** (5): 407-413.

Penna B, Thome S, Martins R, Martins G and Lilenbaum W. 2011. In vitro antimicrobial resistance of Pseudomonas aeruginosa isolated from canine otitis externa in Rio de Janeiro, Brazil. *Brazilian Journal of Microbiology* **42**: 1434-1436.

Petrov V and Mihaylov G. 2008. Malassezia pachydermatis–Etiology and clinical findings in canine external otitis–therapeutic approaches. *Trakia Journal of Sciences* **6** (1): 123-126.

Petrov V, Mihaylov G, Tsavhev I, Zhelev G, Marutsov P and Koev K. 2013. Otitis externa in dogs: microbiology and antimicrobial susceptibility. *Revue Med. Vet* **164** (l): 18-22.

Perry L R, MacLennan B, Korven R and Rawlings T A. 2017. Epidemiological study of dogs with otitis externa in Cape Breton, Nova Scotia. *The Canadian Veterinary Journal* **58** (2): 168.

Petrov V, Zhelev G, Marutsov P, Koev K, Georgieva S, Toneva I and Urumova V. 2019. Microbiological and Antibacterial Resistance Profile in Canine Otitis Externa--A Comparative Analysis. *Bulgarian Journal of Veterinary Medicine* **22** (4).

Pietschmann S, Meyer M, Voget M and Cieslicki M. 2013. The joint in vitro action of polymyxin B and miconazole against pathogens associated with canine otitis externa from three European countries. *Veterinary Dermatology* **24** (4): 439-497.

Prathibha S, Ashok Kumar, Sharma S D and Saxena S C. 2000. Dermatological disorders in canines. *Indian Journal of Veterinary Research* **9**: 59-60.

Pradhan N R, Chatterjee S and Lodh C. 2012. Demodicosis in dogs and its therapeutic management. *Indian Journal of Canine Practice* **4** (1): 17-20.

Pushpa Y, Anand R K and Anand G. 2015. Diagnosis and Management of Pseudomonas Associated Canine Otitis. *Intas Polivet* **16** (2).

Qekwana D N, Oguttu J W and Sithole F. 2017. Burden and predictors of Staphylococcus aureus and S. pseudintermedius infections among dogs presented at an academic veterinary hospital in South Africa (2007-2012). *PeerJ* **5**: e3198.

Quinn P J, Carter M E, Markey B K and Carter G R. 1999. *Clinical Veterinary Microbiology*, 9$^{th}$ Ed. Mosby yearbook Limited, London. pp 95-126.

Reddy B S and Sivajothi S. 2014. Notoedric mange associated with malassezia in cats. *International Journal of Veterinary Health Science & Research* **2** (1): 18-20.

Reddy C B K, Kumari N, Sundar T and Kumar V. 2016. A study on Prevalence of Canine Otitis Externa – A Report of 42 Cases. *Livestock Research* **7** (3): 197-201

Rosser E J. 2004. Causes of otitis externa. *Veterinary Clinics: Small Animal Practice* **34** (2): 459-468

Rougier S, Borell D, Pheulpin S, Woehrle F and Boisrame B. 2005. A comparative study of two antimicrobial/anti-inflammatory formulations in the treatment of canine otitis externa. *Veterinary Dermatology* **16** (5): 299-307.

Rosenkrantz W. 2008. Cutaneous cytology: a quick review of an indispensable test. *Veterinary Medicine*: 20-21.

Robson D, Burton G and Bassett R. 2010. Correlation between topical antibiotic selection, in vitro bacterial antibiotic sensitivity and clinical response in 16 cases of canine otitis externa complicated by Pseudomonas aeruginosa. *Dermatology Chapter of the ACVSc Science Week Proceedings, Gold Coast*: 2-3.

Roshan K, Manju R and Sushovan R. 2018. Isolation And Identification of Pathological Agents in Dogs with Otitis Externa. *International Journal of Agricultural Science and Research* **8** (2): 19-22

Sarierler M and Kirkan S. 2004. Microbiological diagnosis and therapy of canine. *Veteriner Cerrahi Dergisi* **10** (3-4): 11-15.

Sai Prasanna J, Madhavi Latha S and Satish Kumar K. 2006. Therapeutic studies on seborrheic dermatitis in dogs associated with Malassezia pachydermatis. *Indian Veterinary Journal* **83** (2): 162-164.

Saridomichelakis M N, Farmaki R, Leontides L S and Koutinas A F. 2007. Aetiology of canine otitis externa: a retrospective study of 100 cases. *Veterinary Dermatology* **18** (5): 341-347

Sapierzynski R. 2009. Otitis externa in dogs. *Medycyna Weterynaryjna* **65** (8):552-556.

Sanchez Ch R, Calle E S, Falcon P N and Pinto J C. 2011. Bacterial isolation in canine ear infections and its antimicrobial susceptibility. *Revista de Investigaciones Veterinarias del Perú* **22** (2): 161-166.

Santoro D, Marsella R, Pucheu-Haston C M, Eisenschenk M N C, Nuttall T and Bizikova P. 2015. Pathogenesis of canine atopic dermatitis: skin barrier and host–microorganism interaction. *Veterinary Dermatology* **26**: 84-94.

Sandeep Kumar. 2017. Effect of enrofloxacin and enrofloxacin in combination with fish oil on haemato-biochemical and otic cytological parameters in canine bacterial otitis externa. *Veterinary Practitioner* **18** (1): 27-30.

Sakkas H, Economou V, Gousia P, Bozidis P, Sakkas V A, Petsios S and Papadopoulou, C. 2018. Antibacterial efficacy of commercially available essential oils tested against drug-resistant gram-positive pathogens. *Applied Sciences* **8** (11): 2201.

Scott D W, Miller W H and Griffin C E. 1995. Diseases of eyelids, claws, anal sacs and ear canals. *Small Animal Dermatology,* W.B. Saunders Co, Philadelphia.

Scott D W, Miller W H and Griffin C E. 2001. Diseases of the eyelids, claws, anal sacs and ears. *Small Animal Dermatology*, 6th Ed. W B Saunders, Philadelphia. pp 71 - 1235.

Schick A E, Angus J C and Coyner K S. 2007. Variability of laboratory identification and antibiotic susceptibility reporting of Pseudomonas spp. isolates from dogs with chronic otitis externa. *Veterinary Dermatology* **18** (2):120-126.

Scherer C B, Botoni L S, Coura F M, Silva R O, Santos R D D, Heinemann M B and Costa-Val A P. 2018. Frequency and antimicrobial susceptibility of Staphylococcus pseudintermedius in dogs with otitis externa. *Ciência Rural* **48**.

Senthil K K, Selvaraj P, Vairamuthu S, Mala S and Kadiresan D. 2010. Antibiogram patterns of microbes isolated from otitis externa of dogs. *Tamil Nadu Journal Veterinary & Animal Sciences* **6** (3): 145-147.

Seulgi B, Sungwon C, Byeongmok K, Youngju L, Taeho 0. 2013. Efficacy of enrofloxacin and silver sulfadiaznine topical otic suspension for the treatment of canine otitis externa. *Journal of Veterinary Clinics* **30** (3): 172-177.

Sharma J and Gupta G C. 2005. Serum proteins profiles in naturally occurring dermatological disorders in dogs. *Indian Journal of Veterinary Medicine* **25** (1): 33-34.

Sharma A K, Sood N K, Sharma S and Filia G. 2016. Epidemiology and diagnosis of mycotic infections in canine otitis externa. *Intas Polivet* **17** (2): 302-303.

Shaw S. 2016. Pathogens in otitis externa: diagnostic techniques to identify secondary causes of ear disease. *In Practice* **38**: 12-16.

Silva N. 2001.Identification and antimicrobial susceptibility patterns of Staphylococcus spp. isolated from canine chronic otitis externa. *Arquivo Brasileiro de medicina veterinaria ezootecnia* **53** (2): 1-5.

Sim J X F, Khazandi M, Pi H, Venter H, Trott D J and Deo P. 2019. Antimicrobial effects of cinnamon essential oil and cinnamaldehyde combined with EDTA against canine otitis externa pathogens. *Journal of Applied Microbiology* **127** (1): 99-108.

Singh R. 2016.*A Study on Surgico-therapeutic Management of Various Dermatological Emergencies in Dogs* (Doctoral dissertation, Rajasthan University of Veterinary and Animal Sciences, Bikaner-334001).

Soares T S, Brodbelt D C, Church D B, O'Neill D G and Pegram C L. 2020. Do pendulous ears predispose dogs to otitis externa? In *BSAVA Congress Proceedings 2020*. pp 389-390.

Steen S I, and Paterson S. 2012. The susceptibility of Pseudomonas spp. isolated from dogs with otitis to topical ear cleaners. *Journal of Small Animal Practice* ***53*** (10), 599-603.

Subapriya S, Senthil N R, Vairamuthu S, Nagarajan B, Jayanthi C and Thirunavukkarasu P S. 2015. Opportunistic fungi as etiologic agents of dermatitis–A case of Alternaria fungal infestation in canines. *International Journal of Livestock Research* **5**: 24-28.

Suresh R. 2016. Diagnosis and therapeutic management of Malasseziosis in dogs. Ph.D. thesis submitted to P.V.N.R. Telangana Veterinary University, Hyderabad, India.

Tater K C, Scott D W, Miller Jr W H and Erb H N. 2003. The cytology of the external ear canal in the normal dog and cat. *Journal of Veterinary Medicine Series* **50** (7): 370-374.

Tamilarasu S, Jayalakshmi V, Srinivas V V, Barbuddhe S B, Antony P X and Mukhopadhyay H K. 2020. Isolation and Molecular Characterization of Methicillin-Resistant Staphylococcus pseudintermedius in dogs. *The Indian Journal of Veterinary Sciences and Biotechnology* **15** (4): 72.

Terziev G and Urumova V. 2018. Retrospective study on the etiology and clinical signs of canine otitis. *Comparative Clinical Pathology* **27** (1): 7-12.

Tilley L P and Smith Jr F W. 2015. Otitis externa. *Blackwell's five-minute Veterinary consult canine and feline*. John Wiley and sons.

Topala R, Burtan I, Fantanaru M, Ciobanu S and Burtan L C. 2007. Epidemiological studies of otitis externa at carnivores. *Lucrări Ştiinłifice Medicină Veterinară* **40**: 247-251.

Türkyilmaz S. 2008. Antibiotic susceptibility patterns of Pseudomonas aeruginosa strains isolated from dogs with otitis externa. *Turkish Journal of Veterinary and Animal Sciences* **32** (1): 37-42.

Ultee A, Gorris L M G, Smid E J. 1998. Bactericidal of carvacrol towards the food borne pathogen Bacillus cereus. Health Services and Regional Inspectorates for Health Protec. *Journal Applied Microbiology* **85**: 211–218.

Van Duijkeren E, Kamphuis M, Van der Mije I C, Laarhoven L M, Duim B, Wagenaar, J A and Houwers D J. 2011. Transmission of methicillin-resistant Staphylococcus pseudintermedius between infected dogs and cats and contact pets, humans and the environment in households and veterinary clinics. *Veterinary Microbiology* **150** (3-4): 338-343.

Vikas K, Pal D and Aggarwal A. 2003. Antibiogram of microorganisms isolated from ears of dogs having Otitis externa. *Indian Veterinary Journal* **80** (12): 1316-1317.

Viorica M, Mircean M, Gavrea Raluca, Cozma V. 2008. Epidemiological aspect of otitis externa in dogs. *Lucrari Stiiniifice Medicina Veterinara* **41**: 427-436.

Wilkinson G T and Harvey R G. 1994. *Color atlas of small animal dermatology, a guide to diagnosis,* 2nd Ed. Mosby-Wolfe Publishers.

Wilke J R. 1988. Otopharmacology. *The Veterinary Clinics of North America. Small Animal Practice* **18**: 783-797.

Wildermuth B E, Griffin C E, Rosenkrantz W S and Boord M J. 2007. Susceptibility of Pseudomonas isolates from the ears and skin of dogs to enrofloxacin, marbofloxacin, and ciprofloxacin. *Journal of the American Animal Hospital Association* **43** (6): 337-341.

Yamashita K, Shimizu A, Kawano J, Uchida E, Haruna A and Igimi S. 2005. Isolation and characterization of staphylococci from external auditory meatus of dogs with or without otitis externa with special reference to Staphylococcus schleiferi subsp. coagulans isolates. *Journal of Veterinary Medical Science* **67** (3): 263-268.

Yamamoto D M, Colino V C M, Leal C R B and Babo-Terra V J. 2010. Canine external otitis in Campo Grande, *Mato Grosso do Sul State, Brazil PUBVET* **4** (27).

Yoshida N, Naito F and Fukata T. 2002. Studies of certain factors affecting the microenvironment and microflora of the external ear of the dog in health and disease. *Journal of Veterinary Medical Science* **64** (12): 1145-1147.

Zur G, Lifshitz B and 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.

# APPENDICES

# APPENDIX - I

Proforma for collection of epidemiological data for Bacterial otitis externa in dogs

**I. Case No**: Name of the owner:

Address:

**II. Signalment**

Age:

Breed:

Sex:

**III. Ears affected**: unilateral / bilateral

**Conformation of the ear:** Erect / Semi Erect / Dropped

**IV. History of the animal:**

**V. Clinical signs:**

Head tilt: Aural pruritus:

Ear pain: Ear shaking:

Foul odour ear discharge: Erythema of pinna:

Scaling and crusting of ear pinna: Swelling at the base of the ear:

**VI. Otoscopic examination**

Nature of ear discharge:

**VII. Cultural examination of ear samples**

a. Bacterial isolates:

b. Fungal isolates:

**VIII. Invitro antibiotic sensitivity:**

Gentamycin Amikacin

Ofloxacin Enrofloxacin

Amoxicillin

**XI. Invitro Fungal sensitivity:**

Ketoconazole Clotrimazole

Nystatin

**X. Therapy Given:**

**XI. Remarks:**

**Signature of Major Advisor**

# APPENDIX – II

## A. Reagents for Biochemical tests:

### 1. Indicator for Methyl red test:

| | | |
|---|---|---|
| Methyl red | - | 0. I gm |
| 95% alcohol | - | 300ml |
| Distilled water | - | 200ml |

Dissolve indicator in alcohol and add water.

### 2. Reagents for Voges-Proskauer test:

Solution A.

| | | |
|---|---|---|
| Alpha naphthol | - | 5gm |
| Ethanol | - | 100ml |

Dissolve alpha-naphthol in alcohol

Solution B.

| | | |
|---|---|---|
| Potassium hydroxide | - | 40gm |
| Distilled water | - | 100ml |

Dissolve potassium hydroxide in70ml distilled water in boiling flask.

Allow cooling and making the volume upto 100 ml with distilled water.

## B. Media for Biochemical and Sugar Fermentation test:

### 1. Peptone water:

It is used for the detection of the presence of indole and ammonia by bacterial enzymatic reactions.

Composition

| | | |
|---|---|---|
| Peptone | - | 1 gm |
| NaCl | - | 0.5gm |
| Distilled water | - | 100ml |
| pH | - | 7.2 |

Procedure:

Dissolve all the ingredients, dispense in 5ml quantity to each tube and sterilize at 121°C, for 15 minutes.

### 2. Glucose phosphate peptone water:

It is used for methyl red (MR) and Voges-Proskauer (VP) test of bacterial cultures.

**Composition:**

| | | |
|---|---|---|
| Peptone | - | 0.5gm |
| Potassium phosphate(K2HP04) | - | 0.5gm |
| Glucose | - | 0.5gm |
| Distilled water | - | 100ml |
| pH | - | 7.4 |

**Procedure:**

1. Dissolve peptone and $K_2HPO_4$ in warm water.

2. Adjust the volume to 100ml.

3. Dissolve glucose and filter.

4. Dispense in 5 ml quantities to each tube.

5. Sterilize at 115°C (10 lb pressure) for 30 minutes

# APPENDIX III

## COMPOSITION AND PREPARATION OF MEDIA (HI-MEDIA)

### 1. NUTRIENT BROTH (M002)

| Composition | | Grams / Litre |
|---|---|---|
| Peptone | - | 5.0 |
| Sodium Chloride | - | 5.0 |
| Beef Extract | - | 1.5 |
| Yeast Extract | - | 1.5 |
| PH at 25oC | - | 7.4+0.2 |

Preparation:

To dehydrate this medium 13 grams of the medium was suspended in one liter of distilled water, heated to boiling temperature to dissolve the medium completely and sterilized by autoclaving at 15 lbs pressure for 15 minutes.

### 2. NUTRIENT AGAR (M001)

| Composition | | Grams / Litre |
|---|---|---|
| Beef extract | - | 10 |
| Peptone | - | 10 |
| Sodium Chloride | - | 5.0 |
| Agar | - | 12 |
| PH at 25 °C | - | 7.4+0.2 |

Preparation

32 grams of the medium was dissolved in one lit. of distilled water heated to boiling temperature and sterilized by autoclaving at 15 lbs pressure (121 °C) for minutes.

**3. MANNITOL SALT AGAR (M118)**

| Composition | | Grams / Litre |
|---|---|---|
| Beef extract | - | 1.0 |
| Protease Peptone | - | 10 |
| Sodium Chloride | - | 75.0 |
| D-Mannitol | - | 10.0 |
| Phenol Red | - | 2.025 |
| Agar | - | 15.0 |
| PH at 25 °C | - | 7.4+0.2 |

Preparation

To rehydrate the medium, 111 grams of this medium was dissolved in one lit. of distilled water boiled to dissolve and sterilized by autoclaving at 15 lbs pressure (121 °C) for 15 minutes.

**4. SABAURAUDS DEXTROSE BROTH**

| Composition | | Grams / Litre |
|---|---|---|
| Dextrose | - | 140 |
| Peptone | - | 10 |

Distilled water - 100 ml

Preparation:

Dissolve ingredients, in 10 ml. amounts in 18 by 150 mm tubes, and autoclave at 121 °C for 10 minutes.

**UREASE TEST**

The decomposition of urea is an outstanding characteristic of the organism. Proteus hydrolyze urea rapidly producing marked alkaline reaction (pink colour) 4 hours of inoculation

**GRAMS STAINING**

Composition Gram's modification of lugols solution

Iodine 1g

KI 2g

**Counter stain**

Saffranin 0(2.5% sol in 95% ethyl alcohol) 10 ml

Distilled water 100ml

**Amonium Oxalate Crystal Violet (Hucker)**

**Solution A**

Crystal violet 2g

Ethyl.alcohol 95% 20ml

**Solution B**

| | |
|---|---|
| Ammonium oxalate | 0.8g |
| Distiled water | 80ml |

**Procedure**

1. Stain smear with ammonium oxalate crystal violet for 1 minute

2. Wash in tap water for not more than 2 seconds

3. Immerse I minute in iodine solution

4. Wash in tap water and blot dry.

5. Decolorize 30 seconds with gentle agitation in 95% ethyl alcohol. Blot dry.

6. Counterstain 10 seconds in the above safranin solution

7. Wash in tap water.

8. Dry and examine