International Journal of Advanced Research in Biological Sciences
ISSN : 2348-8069
www.ijarbs.com

Research Article

Comparative study of ear microflora in clinically healthy and dogs with dermatitis

Dr. Anandha Narayanan. S[1*], Dr.Gowri. B[2], Dr.Kavitha[3] and Dr.S.Subapriya[4]

[1]Product Manager Natural Remedies Pvt. Ltd., Bangalore,
[2]Assistant Professor, Department of clinics, [3]Associate Professor, Department of clinical medicine,
[4]Assistant Professor, Centralised clinical laboratory, Madras Veterinary College, Chennai.
*Corresponding author

Abstract

A total of 25 healthy and 25 dermatitic dogs' ears were screened for the presence of *Malassezia pachydermatis* and other organisms on the basis of cultural and microscopic examination. The cases were recorded from March to June, 2014 in Madras Veterinary College teaching hospital. In otitic ears, *Malassezia pachydermatis* was isolated from 36% per cent of the samples by cultural examination. Further associated flora from ears of dogs with dermatitis included *Staphylococcus sp*. followed by *Candida spp, Malassezzia spp., Pseudomonas aeruginosa, Escherichia coli, Streptococcus spp, Aspergillus spp., Rhizophus sp., Bacillus sp, Rhodotorula sp.,* and *Rhizophus sp.* in decreasing order of prevalence. Healthy ears revealed *Malassezia pachydermatis* in 48% of the cases followed by *Staphylococcus* spp, *Streptococcus* spp, *Candida* spp and *Aspergillus* spp,

Keywords: Streptococcus, Malassezia, dermatitis, ear micro flora.

Introduction

Canine otitis externa is one of the most common diseases encountered in veterinary practice and is estimated to affect between 5% and 20% of dogs. [1,2,3] Infectious otitis externa occurs as a secondary complication of primary factors that initiate inflammation within the external ear canal such as hypersensitivity disorders, atopic dermatitis, food reactions, contact dermatitis, foreign bodies, ectoparasites, keratinization disorders, endocrine and autoimmune diseases [4,5,6].

Common pathogenic bacterial species in ears include *Staphylococcus, Streptococcus, Pseudomonas, Proteus, Corynebacterium* and *Enterococcus* [5, 8, 7]. The most common fungal pathogen in the aetiology of otitis is *Malassezia* spp., and rarely *Candida* or other saprophytic fungal organisms [9, 10]. Considering multifactorial pathogenesis of otitis, treatments are varied and include topical therapy with antibiotic, antifungal or corticosteroid medication used alone or in combination [4, 6, 11].

In canine otitis, the clinical diagnosis has an informative value; the adequate selection of a therapeutic method is conditioned by the results of the Para clinical diagnostic tests, imposed by the etiological polymorphism, especially microbiological examination [2, 8]. The purpose of the present study was to evaluate otic micro flora diversity of various clinical forms of otitis as well as to study the antibiogram pattern of ear of dogs in healthy and dermatitic condition.

Materials and Methods

Broad outline of work

Pet dogs presented at Clinics of Madras Veterinary College Teaching hospital for a period of four months between March to June, 2014 were included for the present study. The epidemiological data such as age, breed and sex of the dogs suffering from otitis were recorded. Dogs of all age groups, breed and sex were eligible for enrolment.

25 healthy normal dogs were selected as the control group, if there was no previous history of ear disease and no history of underlying disease (hypersensitivity disorders, keratinization disorders, endocrine and autoimmune diseases) and no clinical signs of ear or skin disease. Dogs in the control group were not currently on any medication. Further those dogs for which ototopical cleansers were used in the previous 2 weeks or systemic antibiotic/antifungal medication was administrated in the previous 4 weeks were excluded.

From dogs with different clinical stages of dermatitis, 25 samples were collected. They had some form of dermatitis (alopecia, pruritus, erythema, parasitic presence) in any part of the body. Complete physical and dermatological examinations were performed prior to collection of otic samples and physical examination findings were categorized to establish dermatological wellness of the dogs. Dogs were not included if any topical or systemic therapy was administrated. Samples were collected with a sterile culture swab introduced into ear canal and submitted to Centralized Clinical Laboratory, Madras Veterinary College for microbiological examination.

Collection of ear swabs

Sterile cotton swab with screw cap poly propylene tube (HI Media®) were used to collect the specimen from the ear. First the pinna was lifted and inserted with the tip of a cotton swab into the vertical portion of the canal [12]. The swab tip was gently rolled against the canal wall to obtain the material and the swab was capped.

Microbiologic examination

The ear swabs were cultured for bacterial and fungal isolation and identification so as to know the prevalence of microorganisms causing otitis and its epidemiology in dogs. For bacterial isolation and identification the samples were cultivated on blood agar. Cultures were incubated aerobically for 24-48 hours at 37°C. Bacterial colonies were then identified based on colony morphologic characters, Gram's stain and various biochemical tests. [13]. For further confirmation of the isolated colonies, the colonies were inoculated in specific or selective media like Edward's medium, Mannitol salt agar, Eosin methylene blue agar etc.,

For the fungal culture, the swabs were inoculated to Sabouraud's Dextrose agar and the Dermatophyte test medium. The plates were incubated at 25^0 and 37^0C for 4 weeks. The fungal growth was identified based on colonial appearance and microscopic appearance by Gram's stain and Lactophenol staining of Tape impression smears [14].

Results and Discussion

In the ears of dogs with dermatitis, 67% of the cases revealed *Escherichia coli,* 41% of the cases revealed *Staphylcoccus sp*., 40% of the cases revealed *Streptococcus sp*. and *Pseudomonas sp*., was isolated from 24% of the cases. Fungal culture studies revealed that 8% of the dogs had *Aspergillus sp*., and 4% had *Rhizophus sp*., and *Rhodotorula sp*. each. Further *Malassezia sp*. was isolated from 32% of the dogs and *Candida sp* from 24% of the dogs. . In a work done by Kumar, (2002)[16], the incidence of *Candida sp*. was 12.8%, *Malassezia sp*. was 7.5%, *Rhizophus sp*. was 70%, *and Rhodotorula sp.* was 11.1%.

In ears of healthy dogs, 60% of the cases revealed *Streptococcus* sp, 55% of the cases revealed *Staphylococcus sp a*nd 33% of the cases revealed *Escherichia coli* in bacterial culture. In a work by Roxana, (2012) [17], the incidence of *Staphylococcus* was 20.8%, and *Pseudomonas* was 12.62%. 20% of the cases revealed *Malassezia sp* and 10% of the cases revealed *Candida sp* in fungal culture. . In a work done by Kumar, (2002)[16], the incidence of *Malassezia* was 17.5% and *Candida* was 15%.

Dogs with dermatitis

25 dogs with signs and history of dermatitis were selected and the following were recorded.

Bacterial inhabitants

In the selected dogs with dermatitis, *Staphylcoccus sp* was isolated from 41% of the cases, *Pseudomonas sp* from 24% of the cases, *Escherechia coli* from 67% of the cases and *Streptococcus sp* was isolated from 40% of the cases. In a work done by Roxana, the incidences were 43.2% of *Staphylococcus sp*., 0.8% of *Escherichia coli*, 12.62% of *Pseudomonas sp*., and 6.93% of *Streptococcus sp*.

Fungal inhabitants

In the selected dogs with dermatitis, *Malassezia sp* was isolated from 32% of the cases, Candida *sp.*, from 24% of the cases, *Aspergillus sp*., was isolated from 8% of the cases and *Rhizophus sp* from 4% of the cases. *Rhodotorula sp*. was isolated from 4% of the dogs

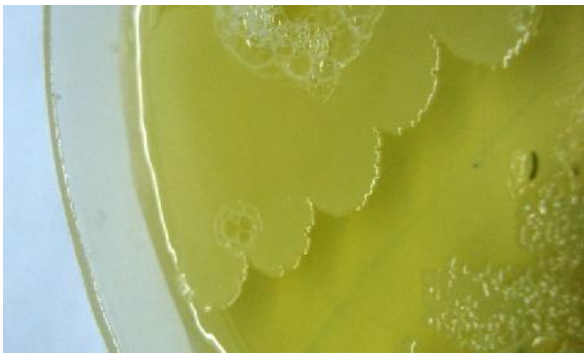
Identification of organisms

Streptococcus sp. are gram positive cocci with chain like colonies (fig. q) [15]. On Mannitol salt agar, pathogenic Staphylococcus *sp.* produces small colonies surrounded by yellow zones [15]. *Bacillus sp.* are gram positive bipolar rods (fig. k) [15]. *Escherichia coli* are gram negative pleomorphic rods (fig.m) [15] and produces metallic sheen in Eosin Methylene blue agar (fig. i) [18]. *Pseudomonas sp.* are gram negative pink unipolar rods (fig. n) [15] .

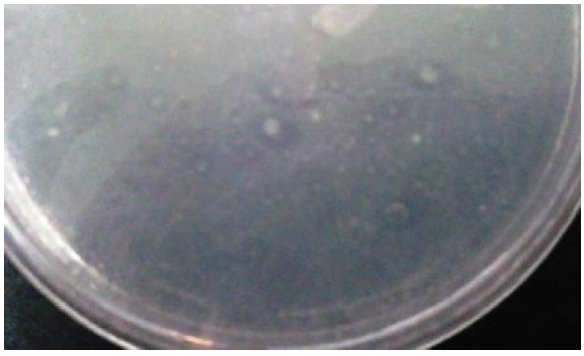
In Sabrouraud's dextrose agar, *Malassezia sp.* produces globose, oblong-ellipsoidal to cylindrical yeast cells [15]. Microscopically, they appear as shoe print like organisms when stained with Leishman and Giemsa (fig. p) [19]. On Sabouraud's dextrose agar, *Candida sp.* colonies are white to cream coloured, smooth, glabrous and yeast-like in appearance [15]. *Rhodotorula sp*. produces coral red, moist, smooth to mucoid, glistening colonies on SDA (fig. j) [20]. *Rhizophus sp*. produces colonies with some tendency to collapse, white cottony at first, becoming brownish grey to blackish-grey and microscopically, Sporangia are globose, often with a flattened base, greyish black, powdery in appearance (fig. h) [21]. *Candida sp.* are seen as budding yeasts on gram's stain (fig. l) [22]

PLATE 1

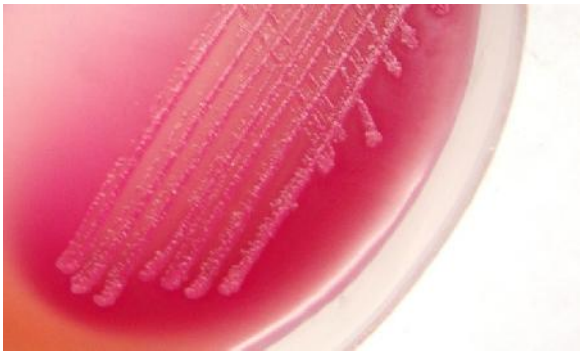
a. *Staphylococcus* sp. In BHI agar



b. *Pseudomonas* sp. In BHI agar



c. *Malassezia* sp. In SDA oil

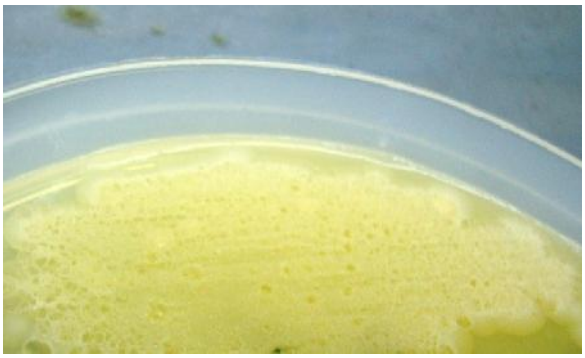


d. *Escherichia coli* in EMB agar

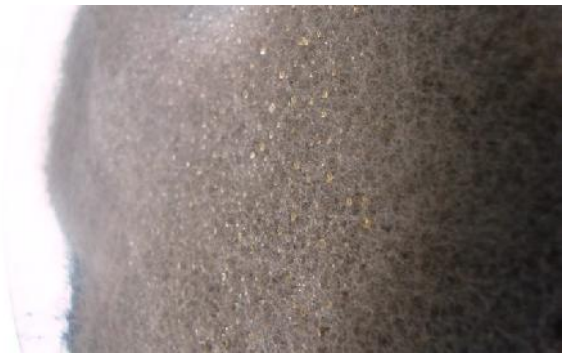
e. *Aspergillus* sp. In SDA

f. *Streptococcus* sp. In Edward's medium

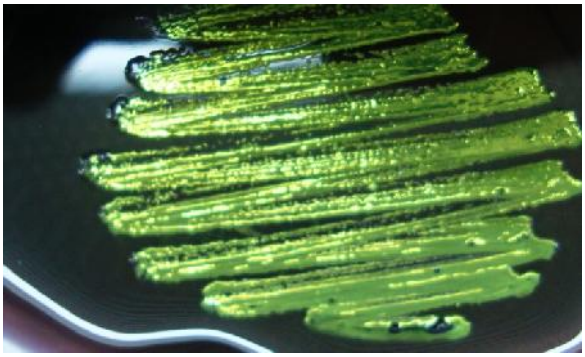
PLATE 2



g. *Bacillus* sp. In BHI agar

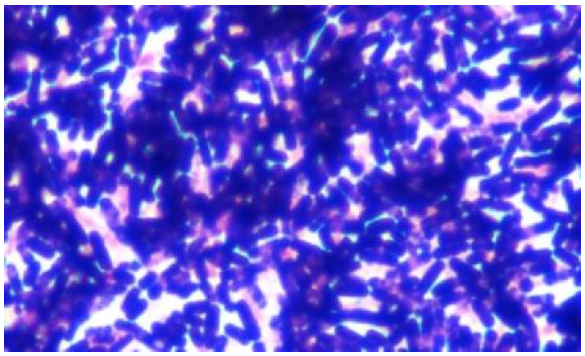


h. *Rhizophus* sp. In SDA

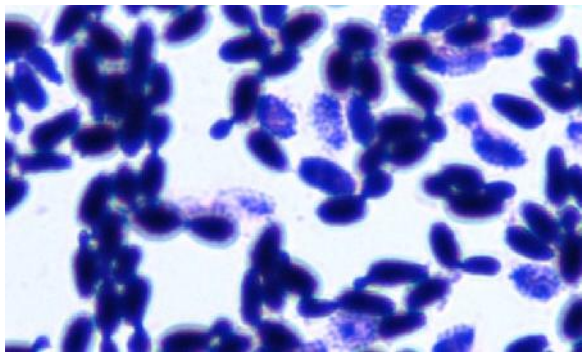


i. *Escherechia coli* in EMB agar

j. *Rodoturella* sp. In SDA

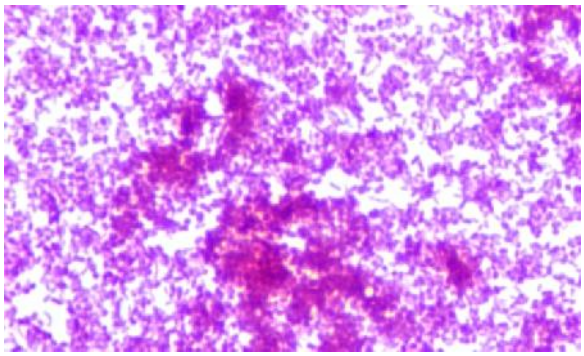


k. *Bacillus* sp.

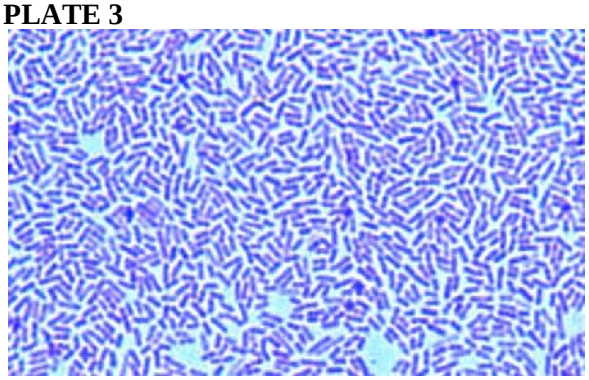


l. *Candida* sp.

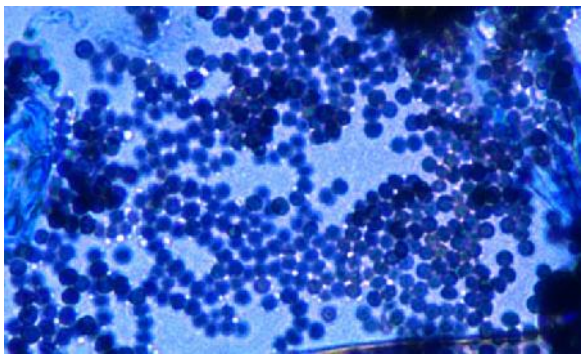
PLATE 3



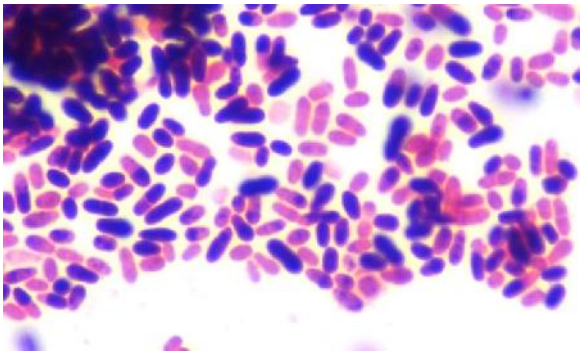
m. *Escherichia coli*



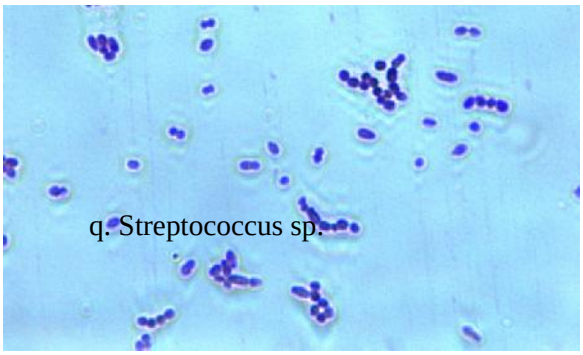
n. *Pseudomonas* sp.



o. *Aspergillus* sp



p.*Malassezia* sp



q. *Streptococcus* sp.

References

1. AUGUST, J. R. Otitis externa. A disease of multifactorial etiology. Vet. Clin. North Am. Small Anim. Pract. 18(4): 731-742. 1988.
2. CARLOTTI, D.N. Diagnosis and medical treatment of otitis externa in dogs and cats. J. Small Anim. Pract. 32: 394-400.1991.
3. LOGAS, D.B. Disease of the ear canal. Vet. Clin. North Am. Small Anim. Pract. 24(5): 905-917. 1994.
4. ANGUS, J., 2004 – Otic cytology in health and disease, Small Animal Practice, nr.34, 411- 424
5. BORNARD, V., 1992 – Bacteriology and mycology of otitis externa in dogs, Schweiz. Arch. Tierhilkd, nr. 143, 341-348
6. CARLOTTI, D.N., ROY, S.T., 1997 – Otitis externa in dog : aetiology and clinical findings, Pratique Medicale and chirurgicale de l`Animal de Compagnie, nr.32, 243-457

7. FERNANDEZ, G., BARBOZA, G., VILLALOBOS, A., PARRA, O., FINOL, G., RAMIREZ, R., 2006 – Isolation and identification of microorganisms present in 53 dogs suffering otitis externa, Revista Cientifica, no. 1, 23-30
8. GINEL, P., LUCENA, R., RODRIGUEZ, J., ORTEGA, J., 2002 – A semi quantitative cytological evaluation of normal and pathological samples form the external ear canal of dogs and cats, Veterinary Dermantology nr.16, 121-127
9. KENNIS, R., ROSSER, E., OLIVER, N., 1996 – Quantity and distribution of Malassezia organisms of the skin of clinically normal dogs, J. Am. Vet. Med. Assoc., nr. 208, 1048-1051
10. MASUDA, A., SUKEGAWA, T., MIZUMOTO, N., TANI, H., MIYAMOTO, T., SASAI, K., MORIKAWA, Y., BABA, E., 2001- Attachement of Malassezia pachydermatis to the ear dermal cells in canine otitis externa, J. Vet. Med. Sci., 63, 667-669
11. GOTTHELF, L.N., 2005 – Small Animal Ear Diseases: An Illustrated Guide, Elsevier Sounders, St. Louis
12. BRYAN, M., FINOLA, L., MARIE, A., ANN, C., DORES, M., 1998- Microbial flora and antimicrobial susceptibility patterns of isolated pathogens from the horizontal ear canal and middle ear in dogs with otitis media, Journal of the American Veterinary Medical Association, 212(4):534-538
13. QUINN,P.J., CARTER, M.E., MARKEY, B.K., CARTER, G.R., - 1994 - Clinical Veterinary Microbiology, Wolfe Publishing, London
14. HUNGERFORD, L.L., CAMPBELL, C.L., SMITH, A. K., - 1998 - Veterinary Mycology Laboratory Manual. First edn. Iowa state University press. Ames, Iowa.
15. MARKEY, B., LOENARD, F., ARCHAMBAULT, M., CULLINANE, A., MAGUIRE, D., - 2013 – Clinical Veterinary Microbiology
16. KUMAR. SINGH, K., SHARMA, A., - 2002 - Prevalence of *Malassezia pachydermatitis* and other organisms in healthy and infected dogs. Vol. 57 (4) 2002
17. ROXANA, T., BURTAN, I., FINTINARIU, M., CIOBANU, S., BURTAN, L. C., BURCOVEANU, I., SINDILAR, E. V., - 2013 - Evaluation of micro flora associated with canine otitis externa
18. JACOBS, M.B., GERSTEIN, M. J – 1960 - Handbook of microbiology. D. Van Nostrand Company Inc., Princeton, NJ
19. CAFARCHIA, C., GALLO, S., ROMITO, D., CAPELLI, G., CHERMETTE, R., GUILLOT, J., OTRANTO, D., - 2005 - Frequency, body distribution, and population size of Malassezia species in healthy dogs and in dogs with localized cutaneous lesions. J Vet Diagn Invest 17: 316–322
20. LO RE V III., FISHMAN, N, O., NACHAMKIN, I., - 2003 - Recurrent catheter - related *Rhodotorula rubra* infection. Clin Microbiol Infect 2003;9:897-900
21. MAHON, C.N., LEHMAN, D.C., MANUSELIS, G.,- 2011 - Textbook of Diagnostic Microbiology
22. AJELLO, L., HAY, R.J., - 1997 - Medical Mycology Vol 4 Topley & Wilson's Microbiology and Infectious Infections