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

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Abstract

A total of 25 healthy and 25 dermatitic dogs’ ears were screened for the presence of \textit{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, \textit{Malassezia pachydermatis} was isolated from 36\% per cent of the samples by cultural examination. Further associated flora from ears of dogs with dermatitis included \textit{Staphylococcus} sp. followed by \textit{Candida} spp, \textit{Malassezia} spp., \textit{Pseudomonas} aeruginosa, \textit{Escherichia coli}, \textit{Streptococcus} spp, \textit{Aspergillus} spp., \textit{Rhizopus} sp., \textit{Bacillus} sp, \textit{Rhodotorula} sp., and \textit{Rhizophus} sp. in decreasing order of prevalence. Healthy ears revealed \textit{Malassezia pachydermatis} in 48\% of the cases followed by \textit{Staphylococcus} spp, \textit{Streptococcus} spp, \textit{Candida} spp and \textit{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. \cite{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 \cite{4,5,6}.

Common pathogenic bacterial species in ears include \textit{Staphylococcus}, \textit{Streptococcus}, \textit{Pseudomonas}, \textit{Proteus}, \textit{Corynebacterium} and \textit{Enterococcus} \cite{5,8,7}. The most common fungal pathogen in the aetiology of otitis is \textit{Malassezia} spp., and rarely \textit{Candida} or other saprophytic fungal organisms \cite{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 \cite{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 \cite{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°C and 37°C 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 *Staphylococcus 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 *Rhizopus 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%, *Rhizopus 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.* and 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, *Staphylococcus sp.* was isolated from 41% of the cases, *Pseudomonas sp.* from 24% of the cases, *Escherichia 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 Sabouraud’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. *Escherichia 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.

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