

Isolation and Identification of Pathological agents in dogs with otitis externa

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ABSTRACT

The present study was conducted to record the suitable cytological diagnostic procedure followed by the isolation and identification of important pathogens causing external otitis in dogs. Out of total 251 dogs presented in Veterinary Hospitals of Durg district, ear swabs were obtained from 51 dogs of 1 to 8 years with clinically diagnosed otitis. Samples collected were grown on special culture media and identification was based on morphological and biochemical characters of cultured for aerobic and anaerobic bacteria, using standard microbiological technique to diagnose isolates. Staphylococcus Spp. was found to be most common bacterial isolate (52.94%) responsible for causation of otitis externa in dogs followed by Pseudomonas spp. (21.56%). However, other bacteria and yeast contributed for less than 10% of cases respectively.

Key words: Dog, Otitis externa, Bacteria, Identification, Diagnosis, Biochemical.

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INTRODUCTION

Otitis externa is defined as an acute or chronic inflammation of the epithelium of the external ear canal which may also involve the pinna. It is one of the most common and multifactorial disorders accounting for up to 10 to 20% of consultations in canine practice [1]. The etiological factors of otitis externa can be parasites, foreign bodies, hypothyroidism and keratinizing diseases those responsible for initiation of the inflammatory process in the ear and are all skin problems [2]. Predisposing factors do change in the microclimate of ear canal [3].

Clinical signs of otitis externa include head shaking, ear scratching and pain on palpation of inner ear pinna and the canal are usually erythematous and swollen. Pinnal alopecia, excoriations and different colored crusts are also noticed. In chronic cases hyper pigmentation, pinnal hyperkeratosis and as well as ear canal stenosis from fibrosis are common [4].

Otitis externa occurs for the first time in middle-aged or older dogs, most frequently observed in dogs of five to eight years of age [5]. Environmental factors may influence the incidence of otitis externa in canines. It is more prevalent in the areas of high temperature and humid environment [6]. Our study aimed to investigate and identify through the bacterial, fungal and yeast agents present in ear swabs of patients with otitis externa.

MATERIAL AND METHODS

A total of 281 dogs irrespective of age, breed and sex both healthy as well as dogs seeking therapeutic intervention presented to the Teaching Veterinary Clinical Complex, College of Veterinary Science and Animal Husbandry Anjora Durg (C.G.) and various Government Veterinary Hospitals in and around Durg were studied for the period of one year from July, 2016, to June, 2017. Fifty one belonging to 24 breeds and aged between 6 months and 12 years, is represented by the pathological ear secretions. The detailed information regarding age, breed, gender, onset of symptoms and other relevant information were collected from the owners. Ear exudates samples were obtained by inserting sterile swabs to the junction of vertical and horizontal external ear canal. Dogs were clinically examined for their normal appearance and diseased condition. Patient's data, owner's complaint and clinical signs of disease were recorded. Restlessness and frequent ear itching were accompanied by otorrhoea with discharge varying from yellowish brown to white purulent in colour. The most common clinical signs observed included head shaking, ear scratching, pain on palpation of the ear, ear rubbing, and tilting of head to one side.

Otic exudates, cerumen, roll swab smears, tape impression smears were collected from the affected dogs for laboratory examination and diagnostic study. Samples were collected, grown on special culture media for isolation of causative agent and identification on the basis of morphological and biochemical characters of microorganisms [7].

Isolation and Identification of Micro organism

Morphology of 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 with Gram's stain 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.

Yeast Identification

For isolation of yeast otic exudates were inoculated on brain heart infusion agar and Sabouraud's dextrose followed by incubated at 30°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 and lacto phenol cotton blue staining.

Identification of bacteria

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., Mumbai) 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 [8].

RESULTS AND DISCUSSION

A total of 51 clinically positive dogs were screened for the presence of microorganisms by cytological examination. In the present investigation the diagnosis of canine otitis externa using tape impression smear (68.62 %) is more as compare to other methods loke roll swab and direct impression smear method. Our findings are in accordance with Shaw, 2016 [9] who also reported that tape impression smear was superior as compared to other tests like collecting surface skin debris and scales for diagnosis of otitis external. Present investigation recorded that with tape impression smear, more number of cocci (71.4%) was observed followed by bacilli (40%) and yeast (11.42%).

The samples found positive by tape impression smears were subjected to microbial isolation and identification. A total of 47 bacterial isolate were obtained which included maximum occurrence of *Staphylococcus* followed by *Pseudomonas*, *Streptococcus*, *E.coli* and *Proteus*

spp. respectively. *Malassezia* organisms were also recorded in 04 samples (7.84%) during this study (Table 1).

In this study, 43.56% of all microbial isolates belonged to the group of coagulase-positive staphylococci, among which *Staphylococcus intermedius* (recently called *Staphylococcus pseudointermedius*) and *Staphylococcus aureus* are considered as common canine pathogens [10]. Petrov, (2013) [11] revealed that the combination *Staphylococcus intermedius* and *Malassezia pachidermatis* are the most common cause of otitis externa. The findings of present study corroborated with the findings of Agnihotri et al. (2014) [12] who have isolated *Staphylococcus aureus* (59.21%), *Pseudomonas aeruginosa* (24.02%), *Proteus* (10.61%) and *Streptococci* (6.15%) from clinical cases of otitis externa in dogs. Lyskova et al., (2007) [13] opined *Staphylococcus intermedius* (58.8%), the most frequently isolated microorganism from otitic ears followed by *Streptococcus canis* (29.9%), *Proteus spp.* (14.4%) and *Escherichia coli* (10.3%) respectively.

Table 1: Microbial isolates from otitis externa in dogs.

Microbial species	Number of isolates (n=51)	Percentage
<i>Staphylococcus spp.</i>	27	52.94
<i>Pseudomonas spp</i>	11	21.26
<i>Streptococcus spp</i>	4	7.84
<i>Escherichia coli</i>	3	5.88
<i>Proteus spp.</i>	2	3.92
<i>Malassezia spp.</i>	4	7.84

Loh et al., [14] reported that coagulase-negative *Staphylococcus* and *Aspergillus niger* were the most common bacteria and fungi cultured respectively in otitis externa patients. They emphasized that self-cleaning of the ears was the most common predisposing factor

It is important to note that bacteria or yeasts associated with cases of otitis externa are only opportunists and are not primary pathogens, in fact they are normally present in low numbers in the external ear canal. When a primary disease damages this anatomic component of the ear, the normal microflora can proliferate and exacerbate or perpetuate inflammatory reactions [15].

Table 2: Biochemical and cultural characteristics of bacterial isolates from otitis externa in dogs.

Test	<i>E. coli</i>	<i>Staph. spp...</i>	<i>Strepto.spp</i>	<i>Pseudo. aeruginosa</i>	<i>Proteus spp</i>
Indole Production	-	-	-	-	-
Methyl red	+	+	+	-	+
Voges Proskauer	-	+	+	-	-
Citrate utilization	-	+	NA	+	+
Catalase Test	+	+	-	+	+
Coagulase Test	-	+	+(6) -(21)	NA	NA
Oxidase Test	-	-	-	+	-
Urease test	-	-	-	-	+

Biochemical testing is based on the metabolic activities of bacteria. Sustained appearance of bubble in catalase test confirms the presence of *Staphylococcus species* and also positive coagulase test. Clear to Blue dark colours produce in oxidase test confirms presence of *Pseudomonas species*. Development of bright red color at the interface of the reagent and the broth indicative of *E coli* and *proteus*. The broth turns a bright pink color in urease test indicate positive for *Proteus species* (Table 2).

Present study concluded that most common bacterial agents isolated from canine otitis externa were coagulase-positive staphylococci. It was also observed that fungi and yeasts

are also frequently involved in the etiology of ear canal inflammations and that in many instances of clinical cases were caused by co-infections.

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