SHORT COMMUNICATION

Cytological Evaluation of Otic Exudates of Dogs with Bacterial Otits Externa

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Abstract

This study was conducted on a total of 71 dogs with signs of otitis externa and 10 normal healthy dogs to evaluate and characterize the cytological findings as a diagnostic aid for otitis externa. The cytology of clinical samples (n=71) showed highest percentage (26.8%) of cases with cocci and neutrophils followed by rods and cocci (21.1%), cocci alone (14.1%), rods and neutrophils (12.7%), mixed population with cocci, rods and neutrophils (11.3%), cocci and *Malassezia* (8.5%) and rods alone (5.5%). Cytology of the 10 normal dogs showed presence of non-nucleated keratinised epithelial cells which were found as sheets and 5 samples (50%) had *Malassezia* species.

Key words: Bacteria, Cytology, Dog, Neutrophils, Otitis.

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Introduction

Otitis externa is a commonly encountered condition in dogs and it affects 20% of the dog population (Kumar *et al*., 2010). Stained smears of exudates obtained from the horizontal and external ear canal have traditionally been used for cytologic analysis to obtain prompt diagnostic data regarding the type and number of infectious pathogens and type of inflammatory cells (Ginel *et al*., 2002). Greater diagnostic information on the involvement of bacteria and yeast overgrowth in ear disease is provided by cytological analysis of otic exudates. The normal cytology of external ear is characterised by the presence of squamous epithelial cells, few commensal bacteria which are majorly cocci, *Malassezia* species, and few yeasts. Neutrophils, macrophages, and other inflammatory cells gain access to the lumen of the canal as the result of exudative inflammation. Thus, finding leukocytes on cytology suggests a more severe disease process (Angus, 2004). Based on many studies, a bacterial count of >25 per high power field is considered abnormal in dogs (Ginel *et al.,* 2002) and is used as a standard to differentiate normal ear canal from ears with otitis externa in dogs. The objective of the present study was to evaluate the cytology of otic exudates of dogs with bacterial otitis externa.

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Materials and Methods

The study was conducted on otitic (n=71) and healthy (n=10) dogs presented to Veterinary Clinical Complex, Veterinary College and Research Institute, Namakkal, Tamil Nadu (India) during the months of February, 2022 to November, 2022. Dogs showing clinical signs such as erythema, crusting, scaling, swelling, alopecia, type of otic discharge and pain on palpation of ear were selected for the study. History of duration of illness, symptoms such as head shaking and head tilting and pruritus were noted from the owners. The clinical signs were assessed using OTIS3 scoring system as reported by Nuttall and Bensignor (2014) and Leonard *et al.* (2022). Where appropriate, the scores were ranked from 0 (= none) to the most severe score (2, 3) in each case. Cytology from 10 healthy ears was also studied for comparison.

The samples were collected prior to otoscopic evaluation and cleaning. A cotton-tipped swab was used to target the junction between vertical and horizontal ear canal. The swab was then slowly rolled onto clean glass slide avoiding artifacts, and then allowed to air-dry. Large lumps of wax

were avoided. The smears were fixed immediately with methanol and stained with Field stain [Dip slide in Field B (Eosin) for 5-10 sec and wash in running water, then dip in Field A (Methylene blue) for 15-20 sec, wash in running water and dry]. The dried smears were examined under high power (x400) initially to identify the distribution of inflammatory and epithelial cells. Once the most relevant areas had been identified the cells and organisms were counted under oil immersion (x1000) of the microscope (Shaw, 2016). The mean of number of cells and organism counted from 10 fields were taken (Ginel *et al.,* 2002). The sample was evaluated based on number and morphology of bacterial organisms, number of yeast cells, type of inflammatory cells, number of keratinised epithelial cells per oil immersion field, and the slides were graded semi-quantitatively as 0, 1+, 2+, 3+, 4+ based on the distribution of cells in the fields as reported by Budach and Mueller (2012) and Leonard *et al.* (2022). For isolation of organisms, a sterile swab was collected from the horizontal ear canal and cultured.

Results and Discussion

Cytology of 10 normal dogs showed presence of non-nucleated keratinised epithelial cells mostly found as sheets or appeared rolled up, and 5 samples (50%) had *Malassezia* species showing typical 'footprint' shaped organisms. Neutrophils and rods were not detected in any of the normal samples and their presence was always indicative of infection which corresponds to the findings of various authors (Ginel *et al.,* 2002; Tater *et al.,* 2003).

The clinical samples (n=71) showed majority with cocci and neutrophils (26.8%) (Fig. 1) followed by rods and cocci (21.1%), cocci alone (14.1%), rods and neutrophils (12.7%) mixed population with cocci, rods and neutrophils (11.3%), cocci and *Malassezia* (8.5%) and rods alone (5.5%)(Table 1). Similar observations of a larger percentage of cocci, followed by *Malassezia pachydermatis*, and then rods were reported by Zur *et al.* (2011). The presence of rods (4+) and neutrophils (4+) were commonly associated with chronic infections with pus discharge. Presence of rods without neutrophils was found in acute infections with erythmato-ceruminous discharge. Cocci (4+) with neutrophils (3+) were correlated with both chronic and acute infections with pus discharge. In few cases with mild pus discharge, presence of cocci with epithelial cells was seen. Nucleated keratinocytes were not observed in normal ear cytology, but could be detected in diseased ear/ chronic cases. Otitis due to *Pseudomonas* species was majorly chronic, recurrent infection with pain and ulcerations and pus discharge and cytology showed presence of rods (2+) with neutrophils (1+) in some cases.

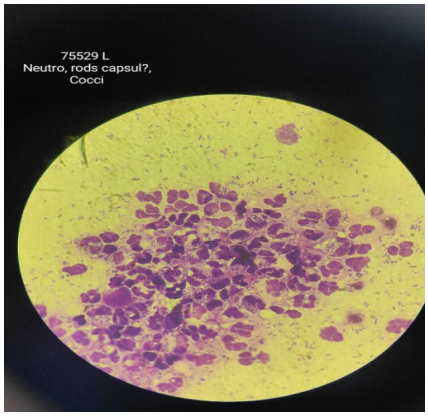

Fig. 1: Cocci with numerous degenerated neutrophils (Field stain, 100x)

Table 1: Cytological findings in clinical cases of otitis externa in dogs

Cytological findings	Number	Percentage
Cocci and neutrophils	19	26.8
Rods and cocci	15	21.1
Cocci alone	10	14.1
Rods and neutrophils	09	12.7
Mixed	08	11.3
Cocci and *Malassezia sp.*	06	08.5
Rods alone	04	05.5
TOTAL	**71**	**100**

The cytological findings, its associated clinical signs and the organisms isolated are presented in Table 2. Clinically the cases of erythmato-ceruminous otitis with whitish yellow pus were maximum (28%) which showed cocci (++) and keratinocytes (+) on cytology and predominant *Staphylococcus* spp. on cultural examination, whereas the cases with excessive ceruminous discharge, crusty lesions and mostly acute were the least (12%) which showed rods (++) and keratinocytes (0/+) with *Klebseilla* spp. on isolation. The other forms were of intermediary with different pathogens.

Cytology was useful to gather clinically relevant information to determine when commensals were likely contributing to the clinical symptoms, the course of treatment and when to stop the antibiotics. Cytological specimens should be assessed for the presence, characteristics and number of yeasts, bacteria, and leukocytes. More than one bacterium per oil immersion field was suggestive of significant microbial activity warranting therapeutic intervention (Millward, 2018). The presence of leukocytes were indicative of true infection especially if it was with phagocytized bacteria; if the disease was of chronic or suppurative discharge was noted, systemic therapy might be indicated.

In brief, the best method to identify bacterial infection was to combine cytology with culture and sensitivity; however, if one of the tests could be carried out, cytology should always be the preferred method of diagnosis. Culture results helped to choose appropriate antibiotic therapy, but cytology determined whether systemic antibiotics were necessary, which organisms were most relevant, and when therapy should be discontinued.

Table 2: Cytological findings and associated clinical signs with organisms isolated from cases of otitis externa in dogs

Clinical signs	Cytological findings	Organism isolated	Percentage of cases
Erythmato-ceruminous otitis, whitish yellow pus in few cases	Cocci- 2+ Keratinocytes- 1+	*Staphylococcus* sp	28%
Excessive ceruminous discharge, crusty lesions, mostly acute	Rods- 2+ Keratinocytes- 0-1+	*Klebseilla* sp	12%
Yellowish white pus, chronic otitis	Rods- 4+ Neutrophils-4+ Keratonicytes-3+	*Proteus* sp	24%
Yellowish brown pus discharge. Can be acute or chronic	Cocci- 4+ Neutrophils- 3+	*Escherichia coli*	16%
Yellowish green pus, chronic recurring otitis sometimes with ulcerations	Rods-2+ Keratinocytes-1+ Neutrophils-1+	*Pseudomonas* sp	20%

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