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EAR CYTOLOGY - A KEY TEST IN THE DIAGNOSIS AND MANAGEMENT OF CANINE OTITIS EXTERNA

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Abstract

Otitis externa is a common condition in dog with a multifactorial etiopathogenesis including primary factors along with predisposing and perpetuating factors. Therefore, a successfull therapy of otitis externa should target the identification and correction of primary causes as possible as, not only the elimination of secondary infections. In practice, ear swab cytology is considered to be the best choice for the diagnosis and initial treatment of otitis, recommended to be repeated for the therapy evaluation or adjustment. Our study included 20 dogs exhibiting various clinical forms of otitis externa, from mild acute to severe chronic otitis with often associated pruritus and smelly discharge, came to be investigated at the Dermatology Service of Veterinary Medicine Faculty from Bucharest. Cytology showed variable proportion of desquamated keratinocytes, lipid droplets and debris of cerumen, leukocytes, especially degenerated neutrophils and free or phagocytized bacterial and yeast elements. The patients have been reevaluated at every 2-3 weeks after initiating therapy and finally, clinical signs resolution together with decreased cellularity in cytological preps were considered good indicators for patient recovery.

Key words*: cytology, dogs, external otitis.*

INTRODUCTION

Otitis externa (OE) is a common, multifactorial disorder found in 10-20% of dogs and frequently challenging to manage because of treatment failure resulting in progressive or relapsing changes (Cordero, 2015). Successfull therapy of OE requires the identification and correction, as possible as, the *primary causes* (allergies, foreign bodies, ectoparasites, seborrhea, immune-mediated diseases, hypothyroidism) along with the *concurrent perpetuating factors* (bacterial or yeast overgrowth/ infection, excessive moisture, aggresive ear cleaning, wrong medication).

OE is seen in 50-80% of atopic dogs and clinical symptoms may be quite variable including erythema, papules, pustules, crusting, scaling, excoriation, ulceration, lichenification and hyperpigmentation of the pinna, stenosis of ear canal, aural hematomas, malodorous ear exudates with grainy-black, waxy-brown, purulent or mucoid appearance and commonly associated pruritus or pain. OE may be additionally accompanied by other skin sites involvement or systemic illness (such as fever, depression, adenopathy) and can get complicated by otitis media in up to 82% of dogs with chronic disease (Gotthelf, 2005; Medleau, 2006).

The routine evaluation of OE patients consists in detailed history, physical examination and ear swab cytology. Cytology is considered to be the best choice for the diagnosis of secondary infection/overgrowth and initial treatment of otitis that should be repeated at every recheck examination for monitoring response to therapy or medication adjustement (Bajwa, 2019). Instead, culture and sensitivity test is rarely necessary, usually recommeded only for recurrent or resistant otitis even if it cannot accurately determine sensitivity to topical antimicrobials because of susceptibility differences between free and biofilm-forming bacteria (Ghibaudo, 2018; Hensel, 2021) .

Basically, cytological data refer to the presence and abundance of bacteria, yeast and leukocytes, usually neutrophils. The presence

of leucocytes and abundant resident bacteria or yeast is a reliable indicator of true infections which need long-term, high-dose therapy, while the disappearance of leukocytes in otic smears after therapy is considered to be a strong indication of clinical resolution (Gotthelf, 2005). Ear cytology may also reveal ear microbial overgrowth characterized by the presence of abundant or diverse microbiota with lack of leukocytes, being often incriminated in local inflammation that could be ameliorated using only topical therapy. In this regard, a recent study reported that most of canine OE (78,3%) are caused by microbial overgrowth, with predominantly bacterial and less frequently fungal and mixed ear pathogens (Tang et al., 2020).

The most isolated species from otitis were *Staphylococcus pseudintermedius*, *Streptococcus* spp., *Pseudomonas aeruginosa*, *Corynebacterium auriscanis*, *Malassezia pachydermatis* and the newly reported anaerobic organism *Finegoldia magna*, all being recognized as opportunistic pathogens (Kiss et al., 1997; Henneveld et al., 2012; Tang et al., 2020). In clinically affected ears there was also identified a reduced bacterial diversity (dysbiosis) compared to healthy dogs. Surprisingly, allergic dogs were found typically to display a skin and ear dysbiosis with possible implications in increased susceptibility to clinical infections (Tang et al., 2020).

MATERIALS AND METHODS

Patients: 20 dogs of different breeds and ages exhibiting clinical signs of otitis externa were examined at the Dermatology Service of Veterinary Medicine Faculty from Bucharest, in the past year. The affected dogs were 12 males and 8 females with the mean age of 6 years (between 1-12 years) and of the following breeds: Cocker spaniel, German Shepherd, Rottweiler, Labrador, French bulldog, Caniche, Bishon, Pekingese, Dachshund and mixed-breeds. As clinical findings, most patients displayed highly pruritic, bilateral erythemato-ceruminous otitis with greasy or waxy exudate of rancid smel, while five dogs were found with suppurative otitis expressing a malodorous discharge.

Laboratory investigations consisted primarily in ear swab cytology and cultures with susceptibility testing only for chronic or reccurent cases.

Ear cytology sampling. The exudate from each ear (even if of unilateral otitis) was collected from the deeper horizontal canal using a cotton-tipped applicator.Thereafter, the swab was firmly rolled onto 2 microscope slides and stained with Romanowsky (MGG) and Gram stain to be microscopically examined using high-dry (40X) and oil-immersion (100X) objectives. Each specimen was evaluated for the number and morphology of bacteria (cocci, rods), yeast (peanut-shaped *Malassezia* spp.) and leukocytes.

Culture and susceptibility testing. The ear exudates were initially cultured in Mueller-Hinton broth and the 24 h-cultures were used for antibiotic sensitivity testing by the disc diffusion method using a routine antibiotic panel.The plates were incubated at 37°C for 24-48 h.

In all cases, clinical, cytological, microbiological and therapeutic data were recorded and correlated.

RESULTS AND DISCUSSIONS

History and clinical findings

Based on history and physical examination, in most cases we identified highly pruritic, mild acute to severe chronic erythemato-ceruminous otitis, often displaying erythema, lichenification and hyperpigmentation of the pinna with greasy or waxy discharge of rancid smel (Figures 1, 2, 3).

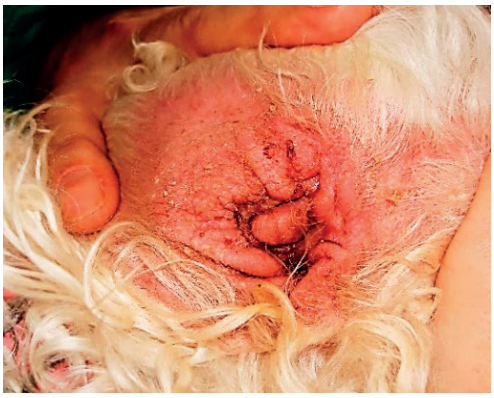

Figure 1. Erythematous otitis in an atopic dog

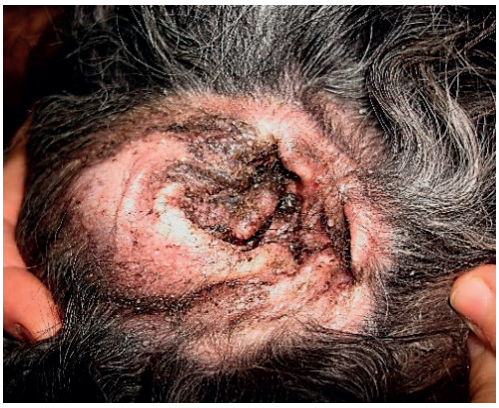

Figure 2. Ceruminous otitis with *Malassezia* (excessive brown waxy discharge)

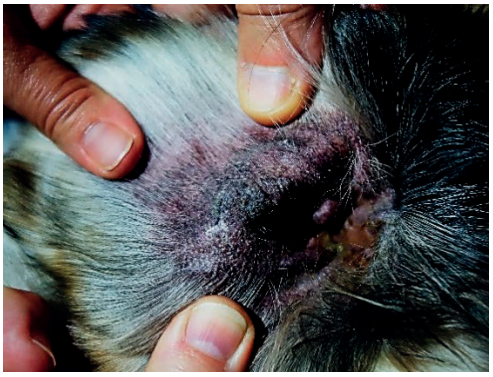

Figure 3. Severe erythemato-ceruminous otitis (pinnal lichenification and hyperpigmentation)

Five dogs were found with persistent or reccurent suppurative otitis characterised by erythema, oedema, ulceration and pain, with mucopurulent or black-colored exudate (Figures 4, 5).

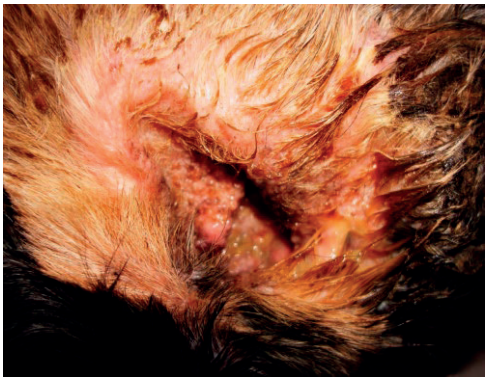

Figure 4. Chronic suppurative otitis with *Pseudomonas* (mucopurulent exudate)

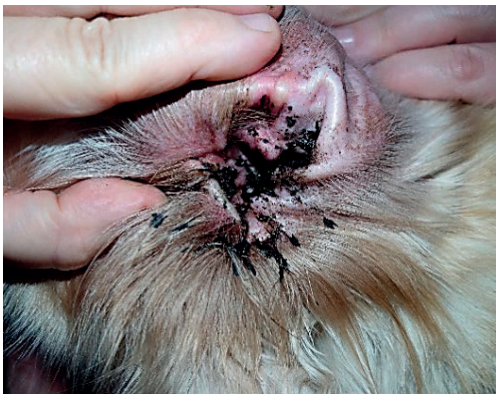

Figure 5. Reccurent otitis with *Pseudomonas* (black-colored exudate)

History data also indicated atopy, food allergy and primary seborrhea as predominant underlying causes of canine OE. In 2 cases, aggresive ear cleaning and overtreatment with oral and topical medication were found to be responsive for persistent, nonodorous exsudative OE. In few cases, OE were associated with facial and interdigital pyoderma and even gingivitis, especially in older patients with *Pseudomonas* infections.

Cytology data

In cytology, the most affected ears contained abundant and more or less diverse microbiota with few or no leukocytes coresponding to microbial overgrowth. Generally, the relative number of resident organisms considered to be normal is of 2 yeasts and 5 bacteria per high-dry field (40x), while more than 5 yeasts and 25 bacteria per field may be interpreted as abnormal, but in correlation with severity of clinical symptoms, past episodes of otitis and previous therapy response (Gotthelf, 2005).

Morphologically, in the most smears we identified a mixed population of rods and cocci (Figure 6) or a combination of rods, cocci and *Malassezia* yeasts (Figure 7), representing normal resident organisms of the ear canal.

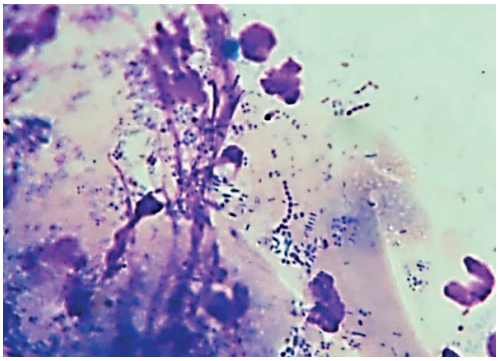

Figure 6. Mixed infection with bacterial rods and cocci together with few degenerate neutrophils (MGG stain, oil immersion objective)

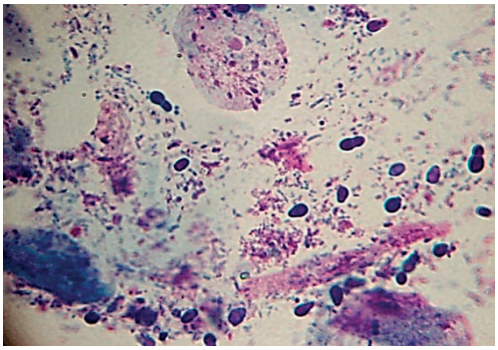

Figure 7. Mixed microbial overgrowth with rods and coccoid bacteria together with *Malassezia* yeasts without any leukocytes (MGG stain, oil immersion objective)

Gram stain was useful to distinguish Gram positive rods (*Corynebacterium* spp.) from Gram negative rods (*Pseudomonas aeruginosa, Proteus mirabilis*) which usually are more resistant to multiple antibiotics (Figure 8).

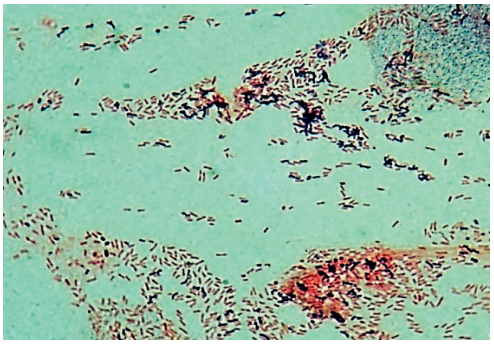

Figure 8. Chronic otitis with severe dysbiosis caused by mixed Gram negative and positive rods (Gram stain, oil immersion objective)

In the five cases of chronic suppurative otitis, cytology showed predominantly rods with variable number of degenerate neutrophils, indicating true infections with severe dysbiosis. In these cases, cytology was also helpful in biofilms detection, looking like an amorphous matrix of variable thickness entrapping microbial cells, leukocytes and other cell debris (Figure 9). Biofilms generally inhibit antimicrobial penetration, being notoriously difficult to eradicate by usual therapeutic schemes (Ghibaudo, 2018; Hensel, 2021).

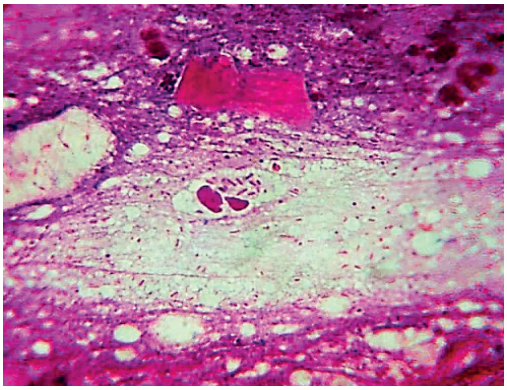

Figure 9. *Pseudomonas* otitis: free and phagocytized rods in a neutrophil within a purple filamentous matrix (biofilm) potentially implicated in antimicrobial resistance (Gram stain, oil immersion objective)

We have also noticed a marked dysbiosis with large numbers of cocci (*Staphylococcus* spp.) in an allergic dog with chronic ceruminous otitis (Figure 10).

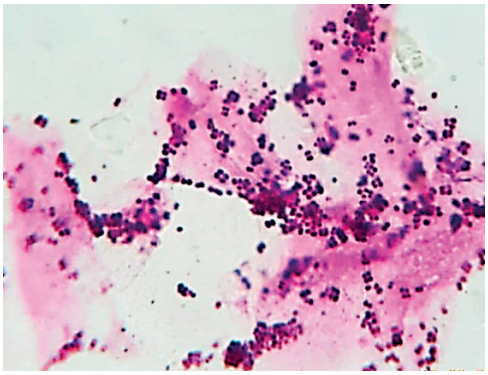

Figure 10. Chronic otitis (bacterial overgrowth) with severe dysbiosis implying Gram positive cocci of *Staphylococcus* spp. (Gram stain, oil immersion objective)

All these patients required cultures and susceptibility testing towards using a long-term, systemic antibiotherapy.
In six cases of *Malassezia* otitis, the ear smears exclusively contained relatively plentiful peanut-shaped elements of *Malassezia pachydermatis* free or adherent to corneocytes, without any neutrophils indicating an yeast overcolonization (Figure 11). *Malassezia* organisms is also commonly found in ear swabs even up to 49% of normal dogs (Cowell et al., 1999).

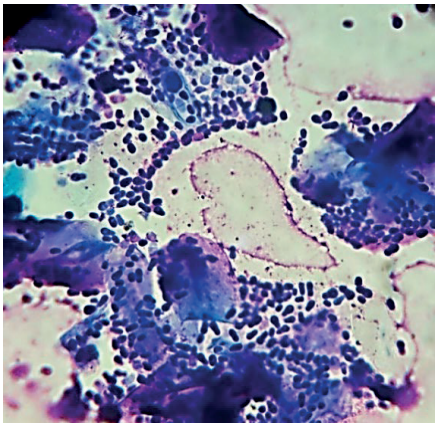

Figure 11. *Malassezia* otitis (yeast overgrowth) with large numbers of budding yeast organisms, free or adherent to corneocytes without any leukocytes (MGG stain, oil immersion objective)

Another particular aspect of ear smears consisted in an intensive desquamation of keratinocytes together with polymorphic resident flora (cocci and rods), secondary to aggresive ear cleaning (Figure 12).

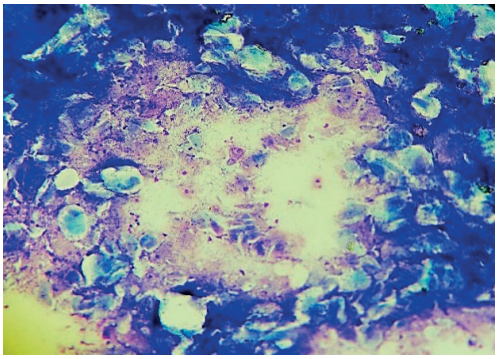

Figure 12. Intensive desquamation of epithelial cells and basophilic debris due to aggresive ear cleaning (MGG stain, magnification 40X)

Occasionally, in ear exudate from a young dog with demodicosis, we have found few adults of *Demodex canis* partially covered by desquamated corneocytes, although *Demodex* is considered a normal inhabitant of the external ear canal (Figure 13). Sometimes, *Demodex* mites may be responsible for chronic ceruminous OE (Hensel, 2021; Medleau, 2006).

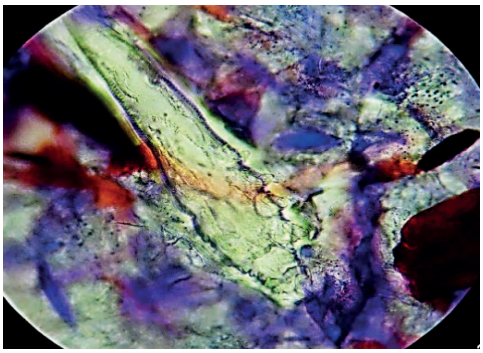

Figure 13. *Demodex* mite in ear exudate from a young dog with demodicosis (MGG stain, oil immersion objective)

Therapeutic data

Most ceruminous otitis clinically evolving for 1-3 weeks and expressing mixed bacterial/yeast overgrowth in cytology were treated with only an ear cleanser with antiseptic properties (Epi-Otic, Otodine). More severe or reccurent ceruminous otitis additionally needed a topical antimicrobial and anti-inflammatory therapy after ear cleaning (Easotic-Virbac, Surolan-Elanco).
Suppurative otitis showing predominantly rods in cytology and cultures (*Pseudomonas, Proteus)* were more difficult to control, combining an ear cleanser with Tris-EDTA (Otodine), topical antimicrobial and antiinflammatory products (Aurizon, Easotic) together with 1-2 systemic antibiotics selected by sensitivity testing (amoxiclav, gentamicin, enrofloxacin) which have been used alternatively for 8-12 weeks to prevent resistance.
Malassezia otitis commonly responded to acid ear cleaners (Epi-Otic, MalAcetic, boric acid) for 2-6 weeks, but in an allergic patient with reccurent otitis we have used topical antifungal medication (Posatex) for 3 weeks to keep it under control.

It also should be mentioned that prescribing of commercial hypoallergenic diets, antihistamines in combination with glucocorticoids together with essential fatty acids supplements visibly helped to ear recovery in allergic patients .

Demodicosis treatment with oral ivermectine and spot-on moxidectin (Advocate) led to otitis and dermatitis remission in Bulldog puppy .

The patients were reevaluated clinically and cytologically at every 2-3 weeks of treatment for therapy monitoring or adjustment.

Clinical improvement was correlated with negative cytology that has been recorded after 2-12 weeks of therapy. Generally, polimicrobial infections/overgrowth have responded better than the monomicrobial ones which needed longer and combined therapy to be controlled.

CONCLUSIONS

Ear cytology has shown to be the most reliable and rapid test for routine diagnosis of otitis externa and therapy monitoring even more accurate than cultures, especially recommended in persistent or reccurent cases.

In most dogs, otitis externa was caused by mixed bacterial overgrowth with underlying allergy or seborrhea. Chronic or recurrent otitis were usually associated with severe ear microbial dysbiosis. Correction of the underlying disorders had a significant impact on ear recovery.

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