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PRELIMINARY STUDY REGARDING THE PREVALENCE OF BACTERIA AND YEAST SPECIES ISOLATED FROM DOGS WITH OTITIS EXTERNA

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• Abstract

Occurrence of Malassezia and bacteria was evaluated through cytology, culture and Vitek 2 Compact system from samples collected from 40 dogs with otitis externa. M. pachydermatis was isolated in pure culture from 30% dogs (12/40). Bacteria were detected in all sampled dogs and species Staphylococcus pseudintermedius, Staphylococcus aureus, Staphylococcus intermedius, Staphylococcus schleiferi, Staphylococcus lentus, Staphylococcus haemolyticus, Staphylococcus warneri, Staphylococcus saprophyticus were identified. Among all the identified species, S. pseudointermedius had the highest prevalence 30% (12/40) followed by S. aureus 15% (6/40) and S intermedius 12.5% (5/40). Others bacteria species that cultures allowed the identification were: Escherichia coli (4 dogs) and Pseudomonas aeruginosa (3 dogs).

• Introduction

Otitis externa in pets, dogs and cats is one of the most common diseases encountered in veterinary clinics, being sometimes difficult to treat. Yeasts belonging to *Malassezia* genus and bacteria, mainly *Staphylococcus* spp., *Streptococcus* spp., *Proteus* spp., *Pseudomonas* spp., *Escherichia coli*, are frequently involved in the occurrence of otitis externa of small animals (19).

M. pachydermatis is a commensal organism that is frequently found on the skin, mucous membranes, especially in the ear of healthy dogs (12). Favorable growth conditions in the local environment allow the overgrowth of this organism, which may then function as an opportunistic secondary agent (3).

The Staphylococcus genus is consisted of two groups that are differentiated by their coagulase enzyme production, constituting coagulase-positive and coagulase-negative staphylococci (7). The colonization with *Staphylococcus spp* of healthy people and animals occurs at different sites as ears, conjunctival sacs, nares, mouth, skin, and anus (2, 9, 27).

The greatest threat to human health is posed by the coagulase-positive S. aureus. In dogs, a similar risk is associated with S. pseudintermedius (2).

• Materials and methods

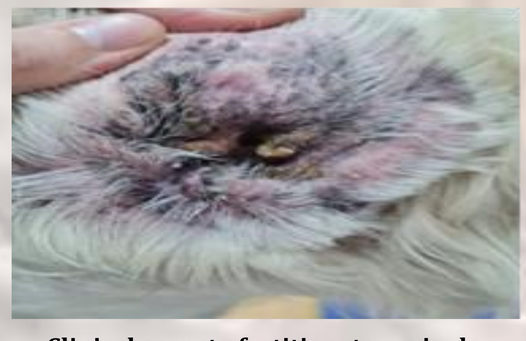
Adult dogs (more than 1 year old) referred for otitis externa to private veterinary practitioners were enrolled in the study. Dogs presented clinical signs such as ear scratching, swelling and pain, head shaking, auricular discharge.

Samples were performed from 40 dogs. All dogs were examined for *Malassezia* by cytology. Ears smears were stained by Diff Quick and examined at 400X and 1000X.

The highlighting of *Malassezia* cells, with a characteristic champagne cork appearance, confirmed the presence of yeast at the otic level. For cultivation and isolation, in the laboratory, inoculations were carried out by streaking on the surface, on the Sabouraud medium, without added lipids, poured into Petri dishes. The plates were incubated at 25 °C for 4-5 days.

Colonies with a characteristic appearance were then passaged on Sabouraud medium without the addition of lipids to obtain pure cultures. Colonies of *Malassezia pachydermatis* are convex and smooth initially, but their appearance changes, becoming dry and shriveled, and creamy white color. Each pure culture developed was examined microscopically to confirm the presence of the microorganism (yeast) in a pure state. For the examination, smears were made, which after drying and fixation were stained by the Giemsa method. These were examined under a cedar oil immersion microscope and 100 objective.

For the bacteriological examination from both ears of all examined animals, samples of ear wax and secretions were collected by means of sterile ear swabs. Each sample was cultivated on nutritive broth for 24 h at 37°C. After that, inoculations were performed on E.M.B. *Levine* Agar (Eosin. Methylene Blue Agar), Chapman and Baird-Parker Medium (Oxoid) and *Pseudomonas* CFC Selective medium (Oxoid). Plates were incubated aerobically at 37°C for 24 hours. The isolates obtained on selective medium and that gives characteristic colonies were submitted to Gram staining and then cultured on plates containing Blood Agar and incubated aerobically at 37°C for 24 hours. For the identification of *Staphylococcus* species were used Vitek 2 Compact system using identification cards for Gram positive bacteria



Clinical aspect of otitis externa in dog

***M. pachydermatis* macroscopic and microscopic view**

• Results and Discussions

From this study it could be observed that in the ears of dogs with otitis the species from the genus *Staphylococcus* had the highest prevalence.

Among all the species of this genus, *S. pseudointermedius* had the highest prevalence 30% (12/40) followed by *S. aureus* 15% (6/40) and *S intermedius* 12.5% (5/40). The prevalence of other *Staphylococcus* species identified were *Staphylococcus schleiferi* 7.5% (3/40), *Staphylococcus lentus* 5% (2), *Staphylococcus haemolyticus* 1 (2.5%), *Staphylococcus warneri* 2 (5%) and *Staphylococcus saprophyticus* 2 (5%).

Other species that were identified were *Escherichia coli* 10% (4/40) and *Pseudomonas aeruginosa* 7.5% (3/40).

As found by other authors (8, 21, 29), staphylococci were the most commonly isolated bacteria in the ears of dogs with otitis.

The isolation rate obtained in this study was lower than that reported by other previously published studies (13, 22).

Even so, our results indicated that *S. pseudintermedius* is an important bacterium causing otitis in dogs and should be considered when treating this disease, together with *Malassezia* (5, 8, 19).

In addition, all bacterial species here isolated were already reported in dogs with otitis externa (17, 29, 30).

Table 1. Prevalence of bacteria species isolated from 40 dogs

Species	No of isolates	Percent
Staphylococcus pseudintermedius	12	30
Staphylococcus intermedius	5	12.5
Staphylococcus schleiferi	3	7.5
Staphylococcus aureus	6	15
Staphylococcus lentus	2	5
Staphylococcus haemolyticus	1	2.5
Staphylococcus warneri	2	5
Staphylococcus saprophyticus	2	5
Escherichia coli	4	10
Pseudomonas aeruginosa	3	7.5

• Conclusions

Malassezia pachydermatis, *Staphylococcus pseudintermedius Staphylococcus aureus and Staphylococcus intermedius* were found in high frequency on dog ears with otitis.

Other species of *Staphylococcus* such as *Staphylococcus schleiferi, Staphylococcus lentus, Staphylococcus haemolyticus, Staphylococcus warneri, Staphylococcus saprophyticus,* identified in this study, allow proper understanding of pathology in otitis since the specie *Staphylococcus schleiferi* has recently emerged as a common cause of canine pyoderma.

Isolation of other bacterial species as *Pseudomonas aeruginosa* and *Escherichia coli* is associated with chronic and recurrent otitis induced by a primary disease of abnormalities conformation of the ear in dog and respectively a poor care that ensured a fecal contamination.

In dogs with external otitis can be considered to establish a correct etiological diagnosis through cytological and bacteriological examinations for effective treatment and reduction of relapses.