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Gibson Fernández / Glen Barboza / Amri Villalobos / Omaira Parra / Geovanny Finol /
Roger A. Ramírez

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ISOLATION AND IDENTIFICATION OF MICROORGANISMS PRESENT IN 53 DOGS SUFFERING OTITIS EXTERNA

Aislamiento e identificación de microorganismos presentes en 53 perros enfermos de otitis externa

Gibson Fernández, Glen Barboza, Amri Villalobos, Omaira Parra, Geovanny Finol y Roger A. Ramírez

University Veterinary Policlinic, Veterinary Sciences Faculty, Zulia State University (Universidad del Zulia).
Maracaibo, Zulia, Venezuela. E-mail: gibfer@hotmail.com.

ABSTRACT

Otitis externa is a multi-factorial etiology disease commonly affecting canines. It represents a complex pathology associated to infections caused by bacteria and yeasts, many times not responding to treatments. The goal of this study was to isolate and identify the pathogenic and non-pathogenic micro-organisms existent in otitis externa patients. This research was performed on 53 canine patients which were attended at the Outpatient Consult Service of the University Veterinary Policlinic at Zulia State University (UVP), and which showed clinical signs such as: auditory canal erythema, increased secretion, pain, continuous head shaking and pruritus, purulent secretions and pruritus being the most frequent. Samples were recollected from auditory canal horizontal portion exudates. Different bacteria groups were isolated in culture media, the most frequent were *Pseudomona aeruginosa* (22.22%), *Proteus mirabilis* (13.89%), *Staphylococcus aureus* (12.50%), *Staphylococcus epidermidis* (8.33%), *Escherichia coli* (5.56%) and *Staphylococcus coagulase-negative* (5.56%). In the control group, *Bacillus* spp. (16.67%), *Staphylococcus epidermidis* (16.67%) and *Staphylococcus coagulase-negative* (16.67%) were the most frequently isolated. Cytological examination showed *Malassezia pachydermatis* to be present in 69.8% of the cases studied. The most affected breeds were Poodles (30.19%), Mongrels (26.42%), Cocker Spaniels (16.98%) and German Shepherd Dogs (9.49%); likewise, a higher incidence of otitis was evidenced in dogs between 2 and 5 months of age (43.40%).

Key words: Otitis externa, canines, isolation, bacteria, yeasts.

RESUMEN

La otitis externa es una enfermedad de etiología multifactorial que comúnmente afecta a los caninos. Representa una patología compleja que se asocia a infecciones causadas por bacterias y levaduras, y que muchas veces no responden a los tratamientos. El objetivo de este estudio fue aislar e identificar los microorganismos patógenos y no patógenos presentes en pacientes con otitis externa. Esta investigación fue realizada sobre 53 pacientes caninos con otitis externa que fueron atendidos en el Servicio de Consulta Externa de la Policlínica Veterinaria de La Universidad del Zulia, los mismos presentaron signos clínicos tales como: eritema del canal auditivo, aumento de las secreciones, dolor, sacudidas constantes de la cabeza y prurito, siendo las secreciones purulentas y el prurito los más resaltantes. Las muestras fueron colectadas de los exudados presentes en la porción horizontal del canal auditivo. Diferentes grupos de bacterias fueron aisladas en medios de cultivos, presentándose con mayor frecuencia *Pseudomona aeruginosa* (22,22%), *Proteus mirabilis* (13,89%), *Staphylococcus aureus* (12,50%), *Staphylococcus epidermidis* (8,33%), *Escherichia coli* (5,56%) y *Staphylococcus coagulasa negativa* (5,56%). Por medio de la evaluación citológica se evidenció que *Malassezia pachydermatis* se encontró presente en un 69,80% de los casos estudiados. Las razas mayormente afectadas fueron Poodle (30,19%), mestizos (26,42%), Cockers spaniel (16,98%) y Pastor alemán (9,49%); así mismo se observó mayor incidencia de otitis en caninos entre los 2 y 5 meses de edad (43,40%).

Palabras clave: Otitis externa, caninos, aislamiento, bacterias, levaduras.

INTRODUCTION

Otitis externa has been reported in canines in a proportion varying from 5% to 20% [1, 9, 25], though in tropical climates prevalence is more near 30% to 40% [1, 25]. Otitis externa has been reported in canine patients between 5 and 8 years of age [1, 25]; it did not find sex related statistics in the literature [1, 9, 15, 34].

The canine auditory canal is an environment vulnerable to any change; anatomic and physiologic alterations can set off the development of a microclimate which favors microorganism proliferation [1, 16, 25, 33].

The most common causes directly inducing otitis externa are atopy, contact allergies, food hypersensitivity, flea-bite allergy, foreign bodies, autoimmune diseases, keratinization disorders [1, 9, 25-28, 33]. Miscellaneous causes include juvenile cellulitis, idiopathic inflammatory otitis externa, and eosinophilic proliferative otitis externa [1, 27, 33]. Among the secondary causes there are bacteria and yeasts [8, 17, 23, 25-27], *Malassezia pachydermatis* is the most common yeast contributing to otitis externa [33], some studies reporting a *M. pachydermatis* isolation of between 20% and 50% in healthy ears and up to 72% in affected ears [1, 9, 21], other mycotic microorganisms that have been occasionally isolated in affected ears are *Candida* spp., *Aspergillus* spp., *Microsporum*, *Trichophyllum* spp., *Sporothrix schenckii* and *Paecilomyces* [1, 23]. It is considered that inflammations, pathologic skin and ear structure reactions become perpetuating factors of this condition [33]. The definitive assessment should be based on physical and clinical evaluation and laboratory assays through cytological analysis, cultures and biopsies [1, 17, 27, 31, 33].

The objective of this study was to isolate and identify the microorganisms mainly present among Zulia state UVP otitis externa canine patients and assess the frequency of these microorganisms in relation to age, sex, breed, clinical signology, appearance duration and factors associated to the disease.

MATERIALS AND METHODS

Animal selection and clinical examination

Fifty three (53) dogs were admitted to the ZSU/UVP outpatient consult due to ear disease; 31 females and 22 males, of different breeds and between 2 months and 13 years of age were clinically evaluated. Diagnosis was based on the medical histories, clinical signs and laboratory assays. A control group of 15 clinically healthy patients of different breeds serving for comparison purposes was equally assessed. Each patient, independent of the fact of forming part of the ailing or healthy group was submitted to the same examination procedures. The individuals of both patient groups were submitted to a thorough physical examination and a detailed revision of the auditory canal.

Sampling procedure and cytological examination

Samples of ear secretions were recollected from all patients (ailing and control) so as to isolate and identify the different microorganisms that may be present in the ear. To recollect the samples, a sterile stainless steel (SS) cone was introduced in the ear canal and through it, a sterile cotton swab was introduced into the horizontal section of the ear canal. The sample was then transferred onto a BBL™ Culture Swab™ transport media and submitted to the laboratory for seeding and isolation of the microorganisms (bacteria and fungi) in the culture media [5, 12, 23]. After recollection for culture purposes, samples were taken for cytological examination, using the same SS cone and cotton swab method to avoid contamination from the external ear lobe area. Once the samples were recollected, each one would be spread over a slide.

Having recollected the samples for culturing, samples were taken for cytological examination, also using the SS cone method to avoid contamination from the ear lobe while introducing the cotton swab down into the horizontal section of the ear canal. The sample having been recollected was spread over the slide, flamed and dyed with Dip Quick Stain® (Jorgensen Laboratories, Inc. Loveland, Colorado, USA) [10, 21] thus allowing the assessment of the amounts and morphology of bacteria, yeasts, parasites, leucocytes, dander tissue or any other type of cells present in the spread.

Isolation and identification of microorganisms

The different culture conditions were as follows: (1) At 37°C in aerobic conditions on Columbia agar + 5% sheep blood, Sabouraud glucose agar, MacConkey agar and Sabouraud glucose agar with gentamicin (0.1g/L) and chloramphenicol (0.05g/L) (2) At 37°C in microaerophilic conditions using the candle jar in Columbia agar + 5% sheep blood and Sabouraud glucose agar [5]. Identification of bacteria was made using macro and microscope observation of the colonies and biochemical tests.

Identification of *Malassezia pachydermatis* was based on characteristics described by Sloof [35] and Blanco et al. [5]. *M. pachydermatis* was most accurately assessed through cytological evaluation applying an evaluation scale according to the number of yeasts found per field. A number of *Malassezia* \geq 4 microorganisms/field viewed under an immersion lens was considered significant and 4 microorganisms/field was considered non-significant [6].

Statistical analysis

Data processing was managed by application of the SAS statistics software package [36]. Results were analyzed and described by way of frequency and percentual distribution expressed in tables and graph charts.

RESULTS

Of 53 dogs diagnosed with otitis externa, 22 were males (42%) y 31 females (58%). Ages were between 3 months and 13 years old. Seventeen patients (32.08%) were between 0 and 2 years old, 23 (43.4%) were between 2 and 5 years old and 13 (24.53%) were over 5 years old.

Different breeds were involved in the study. French Poodles being most frequent (16) representing (30.19%), 14 mongrels (26.42%), 9 Cocker Spaniels (16.98%), 5 German Shepherd Dogs (9.43%), followed by other breeds in lower percentages (FIG. 1).

Seventeen (32.08%) cases of acute otitis externa and 36 (67.92%) cases of chronic otitis externa were diagnosed. The following clinical signs were observed in the physical evaluations: purulent secretions (60.4%); ear pruritus (43.4%), ear canal erythema (37.7%), wax secretions (35.8%) and others in lower percentages (FIG. 2).

The most common bacteria in the ear patient group were: *Pseudomona aeruginosa* (22.22%), *Proteus mirabilis* (13.89%), *Staphylococcus aureus* (12.50%), *S. epidermidis* (8.33%), *Escherichia coli* (5.56%), among other less frequent bacteria. The most common bacteria in the control group were *Bacillus* spp., *S. epidermidis* and *S. coagulasse-negative*, 16.67% of each (TABLE I). In chronic otitis externa cases, the most isolated bacteria were *P. aeruginosa* (26%), *P. mirabilis* (14%) and *S. aureus* (12%), while in the acute cases, *S. epidermidis* was the most frequently isolated (18.18%) and *P. aeruginosa*, *P. mirabilis* and *S. aureus* (13.63% each), and other bacteria in lower percentages (TABLE II).

Among the mycotic microorganisms isolated, *M. pachydermatis* represented 69.85%, *Candida* spp. (20.76%), *Aspergillus flavus* and *A. niger* (3.75% each) and 1.88% was made up of other yeasts, FIG. 3. In those dogs where *M. pachydermatis* was observed (in cytological evaluation) in a number higher than 4 microorganisms per field, 41.5% were in the ear patient group and 26.66% in the control group (FIG. 4).

The factors mostly involved in producing otitis externa were: in 28 patients (52.8%) associated to ear conformation of certain breeds (ear type, hair in ear canal, number of apocrine glands, etc.), 9 canines (17.0%) due to atopia, 4 (7.5%) presented ear canal inflammation due to *Otodectes cynotis*, 4 (7.5%) allergic contact dermatitis and other factors in lower percentages (FIG. 5).

DISCUSSION

The 53 patients showed clinical signs such as: auditory canal erythema, increased secretion, pain, continuous head shaking, purulent secretions and pruritus as reported in other studies [1, 33]. In this study, the most frequent signs were purulent secretions and pruritus.

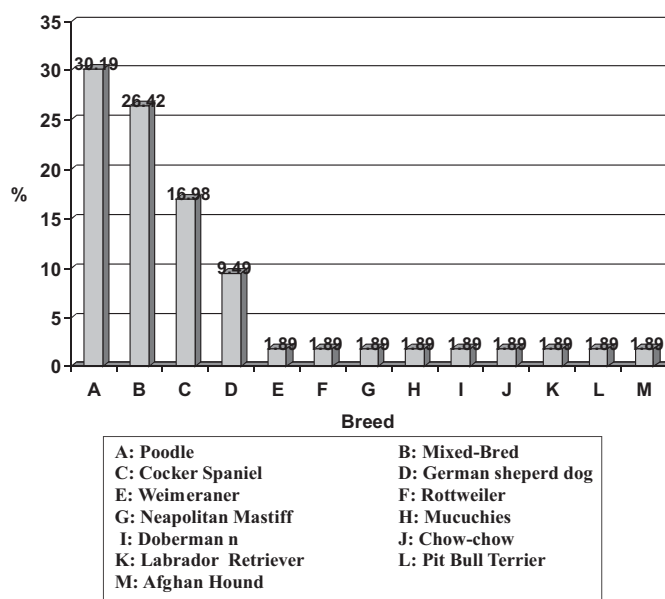


FIGURE 1. DISTRIBUTION OF CANINE OTITIS EXTERNA BY BREED / DISTRIBUCIÓN DE OTITIS EXTERNA CANINA POR RAZAS.

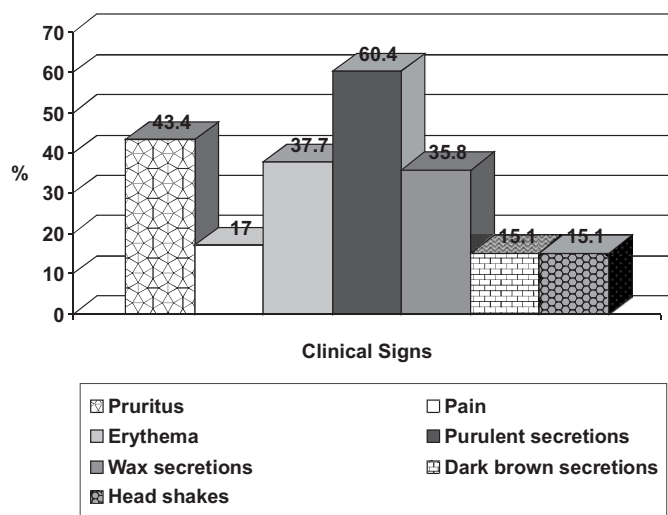


FIGURE 2. DISTRIBUTION OF CANINE OTITIS EXTERNA BY CLINICAL SIGNS IN 53 PATIENTS / DISTRIBUCIÓN DE OTITIS EXTERNA CANINA SEGÚN SIGNOS CLÍNICOS EN 53 PACIENTES.

Most of the otitic affected dogs belonged to the 2 to 5 year old group, which agreed with another study [22], however other authors report a higher incidence in the 5 to 8 year old group [1, 9], the 6 to 8 year old group [20] and the 1 to 4 year old group [34]; these results suggesting that even if otitis externa generally affects older dogs, it may also affect younger ones. The age variation among the different studies may be due to varying methods of handling or use of the dog and hygiene or other factors that may be present in early or middle aged dogs, considering the geographical where the research was made.

TABLE I
MICROORGANISMS ISOLATED FROM EXTERNAL EAR CANAL OF DOGS WITH OTITIS EXTERNA AND HEALTHY DOGS /
MICROORGANISMOS AISLADOS DEL CANAL AUDITIVO EXTERNO EN PERROS CON OTITIS EXTERNA Y DE PERROS SANOS

Bacteria	Clinically Ill Dogs		Clinically Healthy Dogs	
	N	%	N	%
<i>Acinetobacter baumannii</i>	2	2.77	-	-
<i>Bacillus cepaceae</i>	1	1.39	-	-
<i>Bacillus cereus</i>	3	4.17	1	8.33
<i>Bacillus</i> spp.	0	0	2	16.67
<i>Bacillus subtilis</i>	1	1.39	-	-
<i>Citrobacter diversus</i>	1	1.38	-	-
<i>Citrobacter freundii</i>	2	2.77	-	-
<i>Enterobacter agglomerans</i>	1	1.39	-	-
<i>Enterobacter</i> spp.	1	1.39	-	-
<i>Escherichia coli</i>	4	5.56	1	8.33
<i>Klebsiela ozaneae</i>	3	4.17	1	8.33
<i>Pseudomonas aeruginosa</i>	16	22.22	1	8.33
<i>Proteus mirabilis</i>	10	13.89	-	-
<i>Proteus vulgaris</i>	1	1.39	1	8.33
<i>Pseudomonas cepacea</i>	1	1.39	-	-
<i>Sarcina</i> spp.	1	1.39	-	-
<i>Staphylococcus aureus</i>	9	12.50	1	8.33
<i>Staphylococcus β-haemolytic</i>	2	2.77	-	-
<i>Staphylococcus coagulase-negative</i>	4	5.56	2	16.67
<i>Staphylococcus epidermidis</i>	6	8.33	2	16.67
<i>Staphylococcus α-haemolytic</i>	1	1.39	-	-
<i>Staphylococcus intermedius</i>	1	1.39	-	-
<i>Streptococcus viridans</i>	1	1.39	-	-

Poodles represented the most affected breed, this result being similar to those obtained by other researchers [2, 9]; nevertheless, other studies have reported this breed to be the less affected [20, 22]. Mongrels were also highly affected and it is interesting that among mongrels, terrier mixes represented a large number, coinciding with other studies [1, 20]. Cocker Spaniels were also frequently affected and this also coincides with several studies [1, 2, 20, 22]. German Shepherds showed to be another breed with an elevated predisposition to this pathology [2, 25]. Therefore Poodles, Cocker Spaniels and Terrier mixes occupied the first places of incidence of otitis externa, coinciding with other authors [1, 2].

Sex related results contrasted with Grono [19] in that in this study, females were more affected than males. Grono [19] reported a 5.9% affection in males and a 3.5% affection in females; it is important to highlight that some authors state that sexual predisposition is non existent [1, 9, 15, 34].

Isolation of bacteria in ears of healthy dogs revealed a higher percentage of *Bacillus* spp., *S. epidermidis* and even some coliforms such as *E. coli* y *Klebsiella*, which supports the findings of other investigations [1, 9, 19, 25, 28], which also report isolation of *Staphylococcus* (coagulase-positive) in 9.6% of healthy ears. Kowalski [23], reports that neither *Pseudomonas* nor *Proteus* have been isolated in healthy ears and other studies concluded that *Pseudomonas* may be present in 0.4% of healthy dogs [14, 17, 27, 28]. On the other hand, Grono [19] reports the isolation of *Pseudomonas* in 2.4% and *Proteus* in 1.6% of healthy ears, as supported by this study. *Pseudomonas* was isolated in 8.3% of healthy ears.

In this research, the bacteria species most frequently isolated were *P. aeruginosa*, *P. mirabilis*, *S. aureus*, *S. epidermidis*, *Staphylococcus* (coagulase-negative) and *E. coli*, which agrees with the work of some authors [9, 20, 28, 32]. In the present work, frequently were isolated species of *Staphylococcus*, specifically *S. aureus* and *S. epidermidis*, coinciding

TABLE II
MICROORGANISMS ISOLATED FROM EXTERNAL EAR CANAL OF DOGS WITH ACUTE OR CHRONIC OTITIS EXTERNA /
MICROORGANISMOS AISLADOS DEL CANAL AUDITIVO EXTERNO DE PERROS CON OTITIS EXTERNA AGUDA O CRÓNICA

Bacteria	Acute		Chronic	
	N	%	N	%
<i>Acinetobacter baumannii</i>	1	4.54	1	2
<i>Bacillus cepaceae</i>	1	4.54	-	-
<i>Bacillus cereus</i>	-	-	3	6
<i>Bacillus</i> spp.	-	-	-	-
<i>Bacillus subtilis</i>	1	4.54	-	-
<i>Citrobacter diversus</i>	-	-	1	2
<i>Citrobacter freundii</i>	-	-	2	4
<i>Enterobacter agglomerans</i>	-	-	1	2
<i>Enterobacter</i> spp.	-	-	1	2
<i>Escherichia coli</i>	1	4.54	3	6
<i>Klebsiela ozaneae</i>	2	9.09	1	2
<i>Pseudomonas aeruginosa</i>	3	13.63	13	26
<i>Proteus mirabilis</i>	3	13.63	7	14
<i>Proteus vulgaris</i>	-	-	1	2
<i>Pseudomonas cepacea</i>	-	-	1	2
<i>Sarcina</i> spp.	-	-	1	2
<i>Staphylococcus aureus</i>	3	13.63	6	12
<i>Staphylococcus β-haemolytic</i>	1	4.54	1	2
<i>Staphylococcus coagulase-negative</i>	2	9.09	2	4
<i>Staphylococcus epidermidis</i>	4	18.18	2	4
<i>Staphylococcus α-haemolytic</i>	-	-	1	2
<i>Staphylococcus intermedius</i>	-	-	1	2
<i>Streptococcus viridians</i>	-	-	1	2

with the results of other studies [2, 4, 24, 29]; however, different than this study, many researchers have informed of the presence *S. intermedius* in cases of otitis externa [8, 12, 13, 17, 22, 34]; it should express that *S. epidermidis* was the most isolated bacteria in cases of acute otitis in this investigation.

The results herein obtained as to the frequent isolation of *P. aeruginosa* and *P. mirabilis* in chronic otitis externa coincide with others authors [14, 17, 23, 25, 33], and Grono [19] expresses that *Pseudomona* had a high incidence in isolations around the different regions bringing forth a conclusion that this situation could be due to an increased virulence of this bacteria in tropical climates, this explaining the elevated frequency of this microorganism in the present research.

In this study *M. pachydermatis* was the most commonly isolated yeast and this coincided with the findings of other authors [1, 8, 22, 34]. The identification of this yeast was made by the cytological ear exudates, the method recommended by

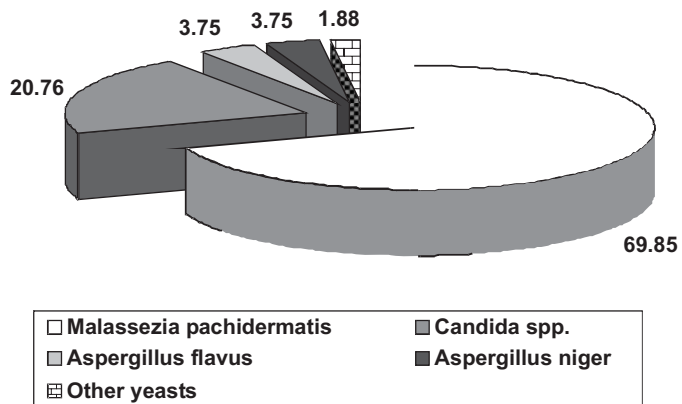


FIGURE 3. PERCENTUAL DISTRIBUTION OF YEASTS ISOLATED FROM OTITIC EARS OF 53 CANINES WITH OTITIS EXTERNA / DISTRIBUCIÓN PORCENTUAL DE LEVADURAS AISLADAS DE OÍDOS OTÍICOS DE 53 PERROS CON OTITIS EXTERNA.

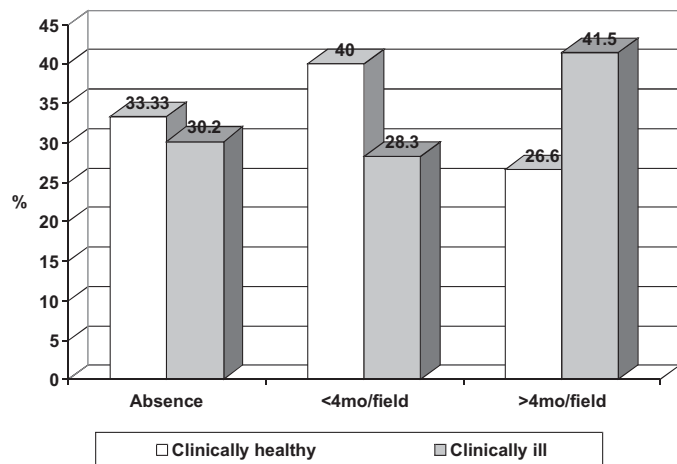


FIGURE 4. PERCENTAGE OF *Malassezia pachydermatis* SHOWN ON CYTOLOGY PERFORMED IN 68 PATIENTS / PORCENTAJE DE MALASSEZIA PACHYDERMATIS MOSTRADO EN CITOLOGÍAS REALIZADAS EN 68 PACIENTES.

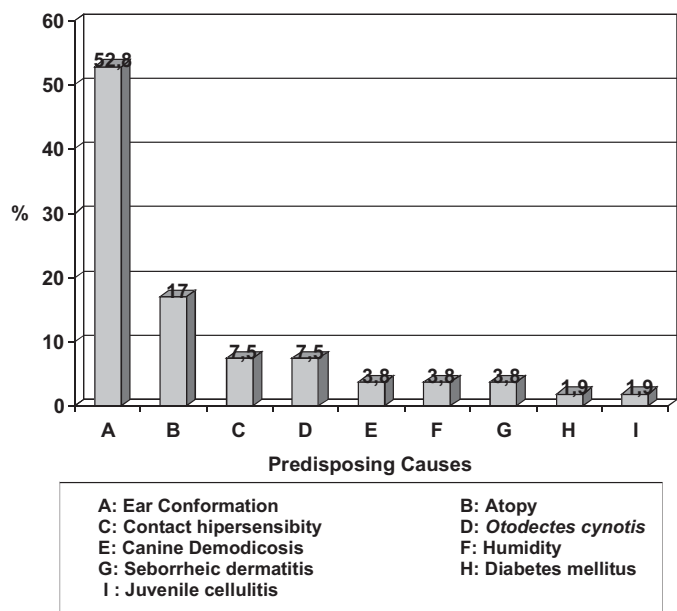


FIGURE 5. PERCENTAGE OF CAUSES ASSOCIATED WITH EXTERNAL OTITIS IN 53 PATIENTS / PORCENTAJE DE CAUSAS ASOCIADAS CON OTITIS EXTERNA EN 53 PACIENTES.

most authors to diagnose *M. pachydermatis* induced otitis externa [7, 11, 25, 31]. On the contrary, a comparative study between cytological examinations and microbiological cultures to indicate quantity and distribution of *Malassezia* on the skin of Basset Hounds, the former procedure was less sensitive than the latter taking into account the isolation frequency of these yeasts [3].

Cytological examination of *M. pachydermatis*, showed more than 4 micro-organisms per field in the most patients with

otitis externa while in the control group, this yeast appeared infrequently. Though some authors use the +/- 4 micro-organism per field as an indicator of over growth of this microbe [11, 12, 30]; others state that even if there is a lower number, being otitis present, it is the most probable cause for the disease [23], hence it may assume that in this study, this could be the main cause for the disease. Some authors recommend such cultural media brain-heart agar with Penicillin G and Streptomycin Sulfate incubated at 37° to grow *Malassezia* [18]; others observed growth of *M. pachydermatis* in modified Malt-Dextrose agar [22]; in the present study was used Sabouraud glucose agar and this yeast did not grow. Colombini [13] isolated 0.6% of *Malassezia* in cultures and explains that if he would have used cytological examination he may have found better results in respect to the detection of *M. pachydermatis*. In this study *M. pachydermatis* was found in clinically ill patients, but also, in a relatively high percentage, in healthy patients. This supports the statements of some authors which consider that this yeast may perpetuate the disease when the conditions in the ear canal are favorable for the action of a secondary pathogen [1, 26, 27, 28].

Other than *M. pachydermatis*, were isolated: *Candida* spp. and *Aspergillus* spp.; which were also isolated in other investigations [1, 32]. Blanco et al. [4] found up to 48% of *Candida* spp. among their isolates.

In this study, there were variable primary causes and predisposing factors associated to the otitis externa patients. Ear conformation was a considerable influence and other studies support the statement that ear type is an important factor which may facilitate the development of otitis externa [1, 25, 33, 34]. The profuse hair in the ear canal of Poodles and Terriers predispose these breeds to otitis externa affection [1, 16]. Cocker Spaniels have a large number of apocrine glands in the ear canal and these glands increase their activity when inflammatory processes are present. Also, the hanging ear lobe of this breed accounts for limited ventilation within the ear canal [16, 25, 27]. It was considered find to erect ears, such as in German shepherd dogs, also predispose dogs to otitis externa due to the hyperactivity of the apocrine glands [1, 20, 25].

Among the primary causes of otitis externa in this study was canine atopy followed by allergic contact dermatitis, the latter also present but less frequent as supported by other authors [2, 17, 25, 33]. Otitis externa caused by *Otodectes cynotis* was found in a considerable percentage, coinciding with several other reports [1, 9, 27]. In this study, it was encountered a case of *diabetes mellitus* associated to otitis externa, it was evidenced that the ear ailment improved in the measure that the patient was treated for diabetes. The patient was monitored after glucose levels were stabilized and condition regression was not observed which agrees with Logas [25] who stated that endocrinal conditions such as hyperadrenocorticism and *diabetes mellitus* may be associated with otitis externa though less frequently.

CONCLUSIONS

In canines, there are generally many bacteria and yeasts associated to otitis externa making the treatment and cure of this disease a somewhat complex matter. Through this study it was acknowledged that the most common microorganisms associated to the disease are: *M. pachydermatis*, *P. aeruginosa*, *P. mirabilis*, *S. aureus*, *S. epidermidis*, *E. coli*, *Staphylococcus* (coagulase negative) and *Candida* spp. The main group of affected patients were dogs 2 to 5 years old. Poodles were found to be the most affected breed, followed by Terrier crossbreds, this being attributed to the ear conformation of these breeds which allows profuse ear canal hair growth.

Cytological assay was found to be the most efficient method for identifying *Malassezia pachydermatis*, and trustworthy data was obtained through samples of ear exudates.

It is important that veterinarians of Zulia state and nationwide understand these results being that this study was performed in this environment and the data obtained provides information accordant to this geographical location.

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