Chapter

Importance of Yeasts in Oral Canine Mucosa

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Abstract

Dentistry science is a new specialty in veterinary medicine that has been growing in recent years, accompanied by the development of professionals who seek to improve the quality of life of pets. Cases related to problems in the oral cavity have gained significant importance in the medical clinic of professionals who treat small animals. Due to lack of professional knowledge or due to animal behavioral problems, such as aggressiveness, the anamnesis of the oral cavity is not performed most of the time, which ends up delaying the diagnosis of the pathology. In addition, an animal with a problem in the oral cavity may take years to show signs of the disease. In general, animals have an oral microbiota composed of various species of fungi, which, under specific conditions, can change from saprophytes to pathogens, compromising their health. Thus, the pre-knowledge of potentially pathogenic yeasts belonging to oral microbiota of dogs and their susceptibility profile compared to the main drugs used in antifungal therapy, is of fundamental importance as it ensures a clinical auxiliary support for the diagnosis and treatment of most diseases of the oral cavity.

Keywords: yeasts, oral cavity, dogs, antifungal, microbial resistance, fungi

1. Introduction

Fungi are eukaryotic, heterotrophic organisms, multinucleated like molds, or only with one nucleus, like yeasts. These organisms can be unicellular, or multicellular, which we call mycelium. Yeasts are unicellular and do not present, in general, morphological differences. The cells are rounded, ovoid or elongated, but some yeast under special conditions may have successive sprouts in a chain, which we call pseudomycelium [1].

The classification of fungi is based on morphological, reproductive and physiological characteristics. The taxonomy of fungi is still varied, but we can classify them in the Kingdom Fungi in the six phylas: Basidyomycota, Ascomycota, Glomeromycota, Chytridiomycota, Blastocladiomycota and Neocallimastigomycota [2, 3].

Approximately 200 out of a total of 100,000 species of yeast are considered pathogenic. Of these pathogenic species, 50 of them are regularly associated with mycoses. Yeasts are the ones that cause the greatest number of mycoses, both in man and in animals and we highlight the genera *Candida*, *Cryptococcus*, *Malassezia* and *Trichosporon* [3].

Figure 1.
Mixed breed dog.

These yeasts can be assexual (anascoporogenous), or sexual (ascoporogenous or basidioporogenous). In general, they are considered opportunists "waiting" for their "opportunity", that is, the drop in the immunity of man and animals, thus causing a case of ringworm.

Among domestic animals, the ones that have the closest proximity to people are dogs. *Canis lupus familiaris* is believed to have emerged approximately 130,000 years ago, from the domestication of the gray wolf. Crossbreeding and selection of characteristics gave rise to different breeds, including Poodle, Yorkshire, Terrier and Labrador Retriever, but mixed breed animals are prevalent in homes around the world (**Figure 1**) [4].

In addition to being mere companions in people's homes dogs have established themselves with essential functions such as security and hunting. These dogs have gained these and other noble functions and thus brought them even closer to human beings in places and situations that would otherwise be dispensed with. Today they also act as guides for the visually impaired, accompanying people to the hospital, monitoring blood glucose levels for diabetic people and even detecting pathogens in hospital environments [5].

These new functions, with consequently greater proximity between dogs and people, also result in a possible greater exchange of microorganisms between these beings, including yeasts. Among these fungi, the most present in the oral mucosa of dogs are the genera *Malassezia* and *Candida* and found less the genus *Cryptococcus* [6].

In the field of public health, these microorganisms have in common the ability to cause disease in both animals and people, and therefore this possible increase in the sharing of microbiota between these beings must be monitored by health specialists.

It is important to emphasize that the exchange of microorganisms occurs in both directions, and that the health of the animals must also be considered in these cases.

The vigilance of the clinical mycologist must be maintained for a better understanding of how future changes can become serious public health problems, especially for yeasts, as we have already seen in several situations.

2. Ecology and sources of yeast infection

Yeasts can be found in plants, soil, air, aquatic environment, in invertebrate and vertebrate animals, that is, in almost all ecosystems. These microorganisms can be in their symbiotic state, in mutualism, or in parasitism. In humans, several species can be part of their natural microbiota, in the gastrointestinal tract, in mucocutaneous tissues and skin. In man, a large part of yeast infections, especially of the genus *Candida*, are of endogenous origin and are linked to risk factors such as old age, prematurity, avitaminosis, antibiotic therapy, cancer, and other diseases that cause immunodepression of the host [7].

Extrinsic factors can also be important, such as the rupture of the natural barrier of the skin and mucous membranes, the use of invasive hospital material and contact with contaminated ecological niches. Direct transmission between people can occur in sexual relations [8].

In dogs, the main yeast found on the skin and mucocutaneous surfaces is *Malassezia pachydermatis*, which easily recovers in the folds of the skin and especially in the various parts of the ear. The prevalence of some types of yeasts in the oral mucosa of dogs is related to several habits, such as licking, sniffing and exploring environments.

The licking of the paws and other areas of the body explains the considerable presence of *Malassezia pachydermatis* in the oral cavity of dogs. Considered a saprophyte in the skin of dogs, this microorganism can cause dermatitis in several situations, and in these cases, there is also an increase in its presence in the oral mucosa. Other relevant yeasts of these animals belong to the genera *Candida*, *Rhodotorula* and *Trichosporon*, which are, in most cases, in balance with the dogs' organism [9].

It is also reported that *Cyniclomyces guttulatus*, present in the stomach, intestine and feces, which in situations of imbalance with the commensal microbiota, may be related to clinical conditions that affect the gastrointestinal tract [10].

The habit of sniffing the soil, in parks and gardens, hunting in forests and dens, favors the sharing of microorganisms among animals linked to these environments. The organic matter present in these places, mainly in the feces of birds and bats, favors colonization by fungi such as *Cryptococcus* spp. and *Histoplasma capsulatum*, which in situations favorable to microorganisms (host immunosuppression; high microbial inoculum load) can cause serious diseases [11].

Advances in veterinary hospital techniques, especially surgical procedures and hospitalizations, also brings new sources of infection for dogs. The ability of microorganisms of the genera *Candida* and *Malassezia* to form biofilms makes equipment such as specula, probes and other surgical materials possible sources of transmission of these microorganisms. For this reason, the correct asepsis and sterilization for handling this equipment is extremely important to avoid mycoses and severe cases of fungemia [12].

3. Predisposing and virulence factors to yeast infections

There are several yeasts that are of interest to the veterinarian, which can cause superficial, subcutaneous, mucosal lesions, and even granulomatous and systemic processes, and, in most cases, suspicion about the fungal etiology of cases is neglected, hence advanced and severe cases of mycosis in dogs are not uncommon [13].

The transition from the yeast stage to commensal to pathogenic will depend both on factors related to the agent's virulence, as well as on the host's own susceptibility [14].

The factors that can predispose humans and animals to a yeast infection are innumerable, resulting from alterations in the defense mechanisms or by compromising the anatomical barriers of protection of the organism [15–17].

Among these factors we can mention: stress; use of broad-spectrum antibiotics or prolonged antibiotic therapy; antineoplastic agents; neutropenia; immunosuppression; age (senility/puppy); inadequate environment (overcrowding); long-term use of corticosteroids; nutritional deficiencies; diets with a high concentration of carbohydrates; pH changes, vitamin A deficiency, trichomoniasis; presence of autoimmune diseases; changes in anatomical barriers due to trauma (maceration); aplastic anemia; hematological infections; periodontal diseases (**Figure 2**) and other concomitant diseases [17, 18].

Prolonged antibiotic therapy and a high concentration of carbohydrates in the diet can lead to the destruction or inhibition of the competitive bacterial microbiota, disrupting its balance with the host organism, thus allowing the accentuated growth of yeasts [15].

Probably due to the poor oral hygiene of dogs throughout their life and associated with the other predisposing factors already mentioned, senility is considered a significant condition for predisposition to periodontal disease. Animals older than 4 years, according to a study with stray dogs, are more likely to develop this disease, ranging from mild gingivitis to severe periodontitis (**Figure 3**).

Virulence factors attributed to microorganisms must also be taken into account, such as production of hydrolytic enzymes, proteases and phospholipases, adhesion, formation of germ tube and biofilms. These factors favor the invasive power and interfere with the host's metabolism. All these factors, from hosts and yeasts, can lead to superficial, or systemic, conditions. It is worth mentioning that the high concentration of viable cells of the microorganism in an ecological niche of the host is another factor that must be considered, as they may be part of the oral microbiota.

Figure 2.
Dog with periodontal disease.

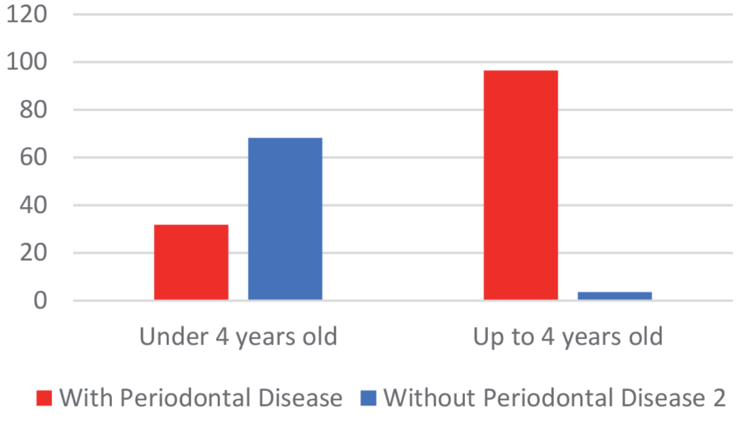

Figure 3.
Connection: Age x Presence of Periodontal Disease in dogs [19].

4. Importance of animals' oral health

Disorders of the oral cavity are of great importance in veterinary medicine due to their high prevalence in dogs and their serious consequences, which can even affect the systemic health of the animal [20]. Abnormalities, injuries or disorders of this organ can cause discomfort and pain, leading the animal to anorexia, due to lack of food, and adipsia, not water intake, predisposing it to conditions of decreased immunity and clinical complications [21].

In addition to this great discomfort and the involvement of other organs, the inflammatory response caused by diseases in the oral cavity can lead to the gingival tissue a progressive loss of tooth fixation to the alveolar bone and, consequently, the loosening and loss of this tooth [22].

The dentistry specialty in veterinary medicine has been evolving in recent years, gaining space in the curriculum of some colleges. Even though the food industry has undergone great advances in the production of diets aimed at improving oral health, the number of professionals who perform an adequate clinical examination is still not significant. In addition to this important factor, the lack of adequate provision of oral hygiene care is worrying [22, 23].

Among dogs over one year of age, 95% have some degree of the disease, and in the clinic, it is believed that 100% of adult animals have varying degrees of periodontal disease [23]. The most common signs associated with periodontal disease are halitosis, dental calculus, inflammation and gingival bleeding, anorexia and the consequent weight loss, ptyalism, difficulty in chewing and grinding food, mobility and migration of teeth, loss of alveolar bone, gingival retraction and behavioral changes [24].

Periodontal disease is, therefore, the most common disease affecting dogs of all breeds, formed from proliferative microorganisms, defense cells (leukocytes and macrophages), epithelial cells, bacterial polysaccharides and salivary glycoproteins, which over time become organize, occurring mineralization and formation of dental calculus [25]. It is believed that this clinical condition is usually caused by the formation of bacterial plaques, but the isolation of yeasts from the oral cavity of dogs with periodontal disease is frequent (**Figure 4**).

The greatest risk in periodontopathic is not only the loss of teeth or the development of local infections, but the possible systemic effects of the pathological agent in the bloodstream.

Thus, the oral health of dogs is extremely important and still needs a greater focus on microbiological research and awareness of those responsible, regarding food, the importance of oral hygiene, and the attention of the tutor and the veterinarian regarding the etiopathogenesis of diseases, such as yeasts.

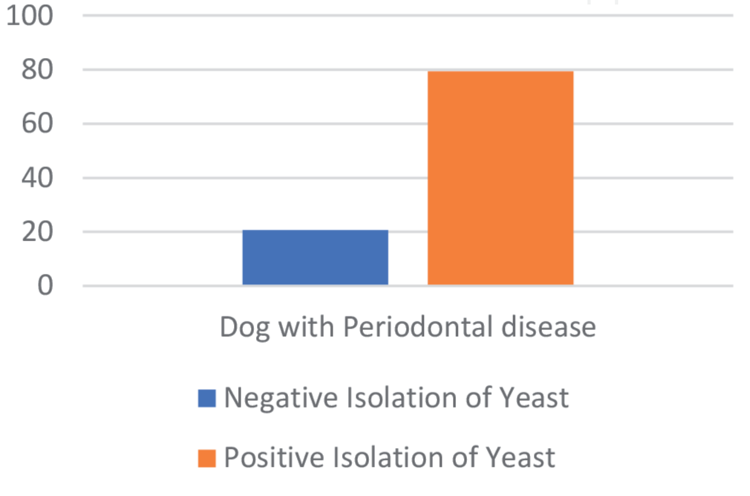

Figure 4.
Connection: Presence of Periodontal Disease x Positive isolation of yeast [19].

5. Main genera and species of yeasts isolated from the oral cavity of dogs and clinical signs

Just like in humans, dogs have a known range of yeasts in their oral mucosa that still requires more studies regarding colonization and pathogenicity. Despite its remarkable importance in the health of dogs, studies involving the isolation and correct identification of yeasts began to be developed in the 20th century [26].

This microbiota is not yet fully described, due to its great complexity and diversity. Fungal colonization of the oral cavity of dogs is associated with yeasts of the genera *Candida*, *Malassezia*, *Trichosporon* and *Rhodotorula*. Less frequently, we can isolate yeasts of the genus *Cryptococcus* [27].

In a recent study conducted with 50 mixed breed dogs, a yeast profile was found, composed of *Candida albicans* (39.5%), *C. parapsilosis* (18.6%), *C. zeylanoides* (13.9%), *C. krusei* (7%), *C. tropicalis* (4.7%), *Trichosporon* spp. (4.7%), *T. asahii* (4.7%), *C. guilliermondii* (2.3%), *T. mucoides* (2.3%) and *Malassezia pachydermatis* (2.3%). The genus *Candida* showed a high prevalence, making up a total of 82.2% of the isolated yeast profile. It is worth mentioning here the isolation of *Candida zeylanoides*, a rare species, even in humans, and thus, the oral mucosa of dogs can harbor a new "ecological niche" of this fungus species, which can also act as an opportunistic pathogen [9].

5.1 Genus *Candida* and Candidiasis

Currently, 317 species of this genus are recognized. Several of these species, more precisely 20, have a pathogenic potential and can thus cause infections in several species of animals, such as dogs [17]. The relationship with the host can be commensal, parasitic or saprophytic. It can also be found in the usual form of a yeast, or in the form of pseudohyphae. *Candida albicans* is the most common colonizer in cases of infections, with a predilection for mucous surfaces and mucocutaneous areas. Other species, such as *C. kefyr, C. lusitaniae, C. guilliermondii, C. tropicalis, C. krusei, C. famata, C. parapsilosis*, can be isolated from animals (**Figure 5**) [15].

There were only few cases found in the literature in small animals, however, reports of candidiasis in various animal species are also increasingly common, described in photos of pyoderma of the lip folds, disseminated and localized mucocutaneous dermatitis, urinary tract infections, gastrointestinal and reproductive system, ear infections, systemic and oral infections [27].

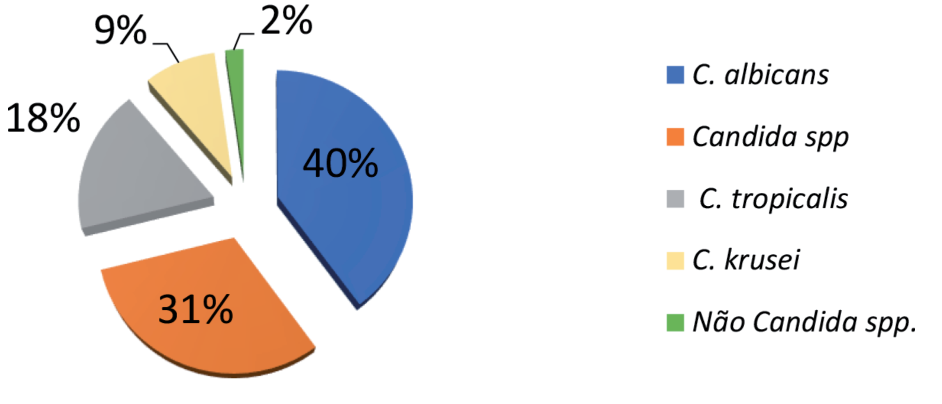

Figure 5.
Presumptive result of Candida *species isolated from the oral cavity of mixed breed dogs according to the CHROMAGAR Candida®.*

Figure 6.
Mixed breed dog with oral candidiasis (glossitis). Friable white and yellowish plates covering the tongue [28].

Candidiasis related to the digestive system of dogs, such as a clinical manifestation of glossitis, is characterized by the formation of pseudomembranous plaques, usually whitish in color, or yellowish beige. Once these plaques were removed, we noticed erythematous regions with the presence of ulcers (**Figure 6**) [28].

5.2 Genus *Trichosporon* and Trichosporonose

The genus *Trichosporon* has 37 species that inhabit different ecological niches, such as water, soil and body and mucous surfaces of humans and animals. They can cause superficial and deep infections, such as *Trichosporon asahii*, *T. mucoides*, *T. ovoides*, *T. inkin*, *T. asteroides* and *T. cutaneum* [29].

No cases of *Trichosporon* infections have been reported in the oral mucosa of dogs, however, several species have already been isolated as colonizers. Clinical cases of nasal granuloma in other animals, cystitis in cats, mastitis in cows and dermatitis in horses and monkeys have already been described (**Figure 7**) [30].

5.3 Genus *Malassezia* and Malasseziosis

The genus *Malassezia* has 15 species, mostly lipophilic yeasts, that can be part of the skin and mucous membranes of humans and dogs. They are opportunistic yeasts, and in certain circumstances, they can lead to clinical manifestations [31]. *Malassezia pachydermatis*, the most frequent in dogs, is not lipophilic and can grow in a culture medium common in mycology [19] (**Figure 8**).

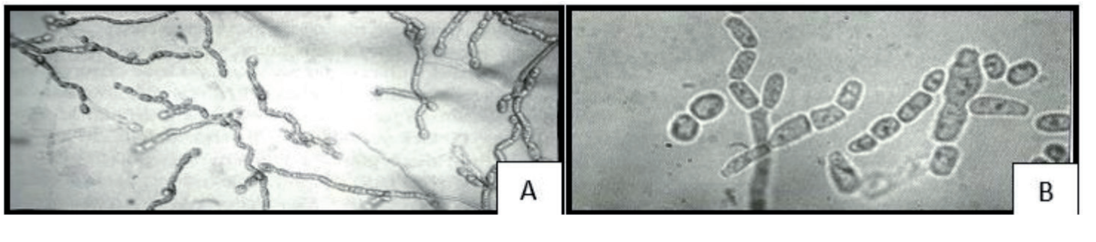

Figure 7.
A and B –Yeasts of Genus Trichosporon *showing oval and rectangular artrospores - (A) 160x and (B) 400x [15].*

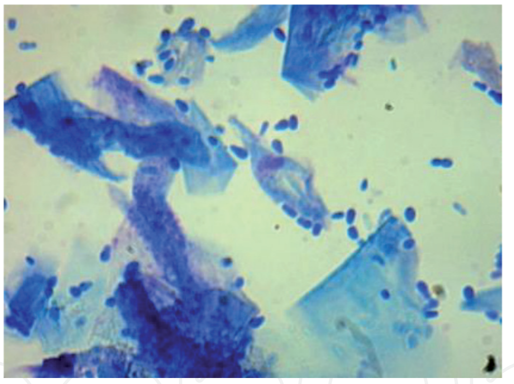

Figure 8.
Yeasts of the Genus Malassezia *spp. - single budding on wide base - Panotic, 1000x.*

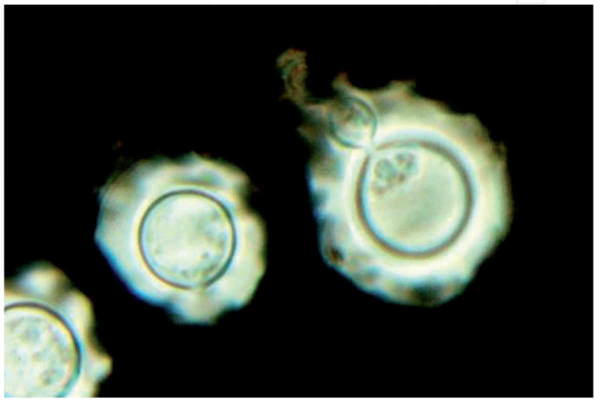

Figure 9.
Cryptococcus *spp. Encapsulated yeasts - Nigrosina, 1000x.*

Several clinical symptoms can be associated with *Malassezia* spp. and, particularly, in cases of otitis and dermatitis in dogs. Cases of otitis by *Malassezia pachydermatis* are frequency, but oral infection caused by this agent have not been described or has not yet been well studied [19].

5.4 Genus *Cryptococcus* and cryptococcosis

In the *Cryptococcus* genus, we found 38 species, with *Cryptococcus neoformans* and *C. gattii* being the most prominent in medical mycology in man and animals (**Figure 9**). The species can be found in different places in the environment, primarily in association with birds' droppings, mainly pigeons, but have an ecological association with trees too, such as eucalyptus [32].

In dogs, can enter the body through the lung causing pulmonary disease, and several clinical signs can be presented, such as skin lesions, nasal mucosa ("clown nose"), and can hit the central nervous system, for its neurotropic nature. These lesions in the nasal mucosa can extend into the oral cavity of the animals [28].

6. Collection of clinical material and identification of yeasts

6.1 Material collection

The collection of the oral cavity of dogs is performed with the aid of a sterile, alginate swab, moistened with sterile saline solution. The swab is introduced,

Figure 10.
Collection with sterile swab of the oral mucosa of a mixed breed dog - City of Campinas, São Paulo - Brazil.

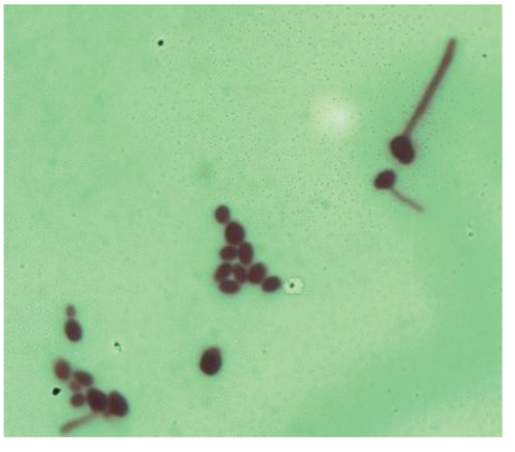

Figure 11.
Yeasts of Genus Candida *in an abdominal dog fluid sample - Fuchsin, 1000x.*

carefully, in the oral cavity, in circular movements, passing through the entire oral mucosa [19] (**Figure 10**).

After this procedure, the collected samples must be sent to the laboratory and sown in Petri dishescontaining basic mycology medium (Sabouraud dextrose agar), plus antibiotics (chloramphenicol - 0.05 g/L concentration). Incubation at 25°C for up to two weeks [19].

There are several procedures that can be used to identify yeasts. Direct examination (fresh), or with Gram stain is also highlighted (**Figure 11**).

6.2 Yeast identification

The identification of yeasts can be performed by means of macro and micromorphological, biochemical, proteome (MALDI-TOF) and molecular tests.
In macromorphological characterization, we studied color, texture and edges (**Figure 12**).

In the more specific micro morphological identification, we must observe the characteristics of the cells (oval, round, unipolar bud, or multiple buds), pseudohyphae, hyphae and structures characteristic of *C. albicans*, such as chlamydoconidia (**Figure 13**).

The formation of a germ tube, another important characteristic of *C. albicans*, originates from blastoconidium when the yeast is sown in fetal bovine serum (**Figure 14**).

Figure 12.
Culture of yeasts on Sabouraud dextrose agar.

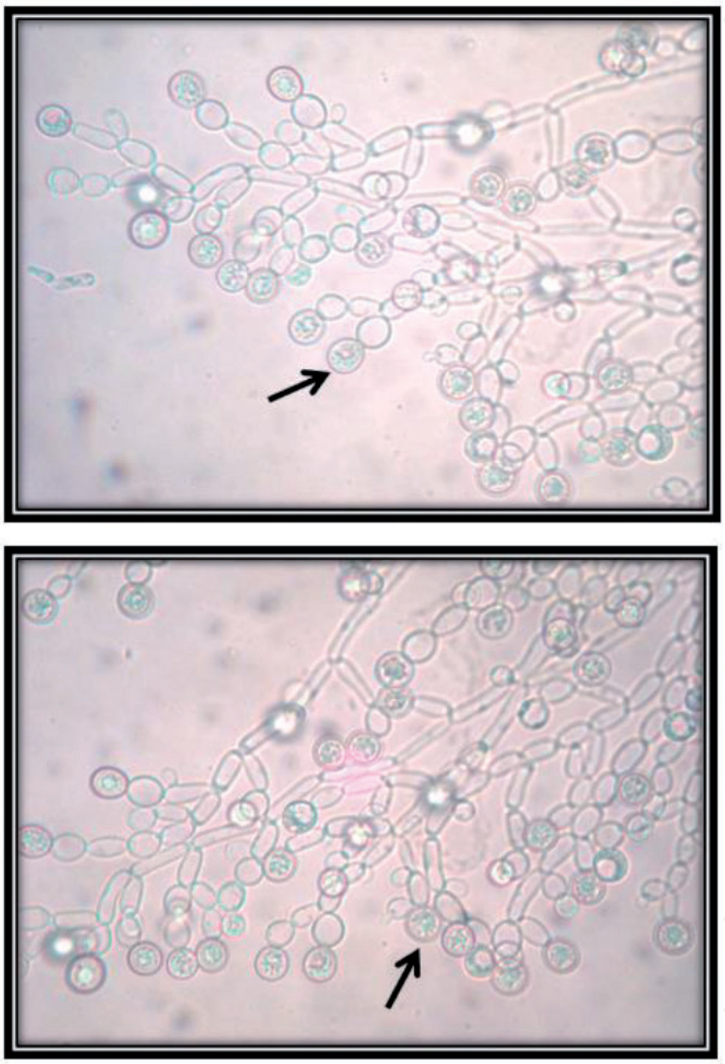

Figure 13.
Candida albicans *in culture broth. Globose, or elongated cells, pseudohyphae, hyphae, blastoconidia and characteristic chlamydoconidia, 1000x.*

Tests of assimilation of sources of nitrogen and carbohydrates can be performed (auxanogram), as well as fermentation tests (zymogram). The protocol followed for these methods is from the manual "The Yeasts: a taxonomic study" (volumes 1, 2 and 3). The MALDI-TOF technique is a mass spectrometry, which determines the protein profile (proteome) of the yeast under study. It is a fast technique (15–20), simple, excellent cost–benefit, however, there are limitations to the use of the laboratory routine, as the device is expensive and requires specialists to use it, as well as a robust base of standard strains.

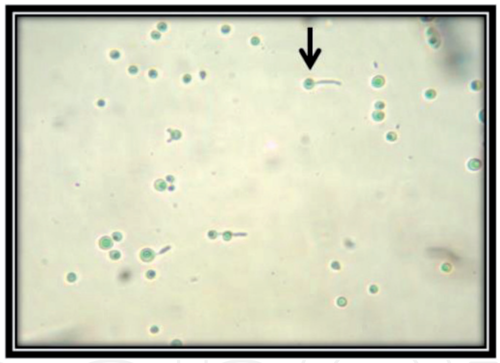

Figure 14.
Germ tube on bovine serum – Candida albicans*, 400x.*

For the identification of yeasts, we also count on molecular biology techniques, which are sensitive and specific. For the differentiation, for example, of *C. albicans* and *C. dubliniensis* is the most accurate technique. There are several methods such as PCR (Polymerase chain reaction), RFLP (Restriction fragment length polymorphism) and RAPD (Random amplified polymorphic DNA). One of the most used is PCR, which detects minimal amounts of DNA, or RNA. But not all laboratories can use these methods, due to the higher costs and the needs of specialized laboratories [33].

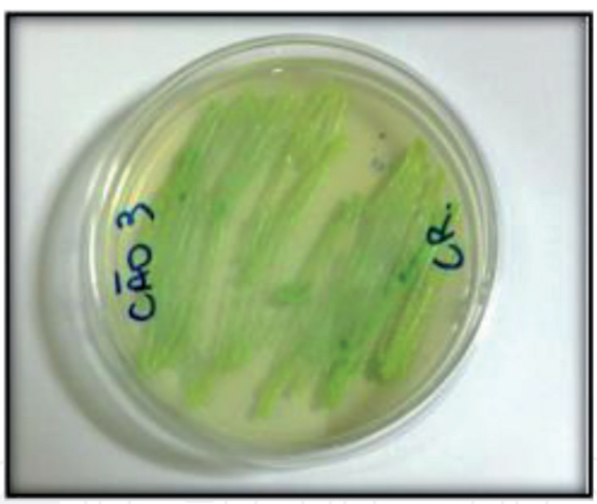

Figure 15.
Yeasts of genus Candida on CHROMAGAR Candida® - Candida albicans *with green color.*

Figure 16.
API20CAUX method (bioMérieux®) –profile of carbohydrates assimilation.

6.3 Chromogenic medium: CHROMagarCandida®

Sowing in chromogenic media, such as CHROMagarCandida®, can provide presumptive identification according to the color developed by the yeast. In this medium, the specie *Candida albicans* develops a light green color; *C. tropicalis* it is blue/green and *C. krusei* light pink, for example (**Figure 15**).

In addition to these identification methods, there are several automated and manual systems that facilitate the laboratory routine, such as Vitek and API20C, as examples (**Figure 16**).

7. Epidemiological markers

As we have already pointed out, yeasts (especially those of the genus *Candida*) have emerged as important pathogens in humans and animals and the interrelation between both is of great relevance, gaining prominence today.

Several markers can be used to detect new yeast species as well as their genotype. Based on the data, we can determine the presence of these microorganisms, the same species/genotype, in one or more anatomical areas of the host, as well as of different ecological niches.

Confirming the colonization/infection area has often been an arduous task. Therefore, the use of these markers shows to be of great importance for the epidemiological study of yeasts.

Among the phenotypic markers, we can highlight those based on colony morphology (morphotyping), enzyme production (enzyme typing), sensitivity to “Killer” toxins and antifungal agents. They are simple and easy to perform techniques.

Genotypic markers are more sophisticated and safer; however, they require more elaborate techniques. The technique is based on short sequential repetition of bases throughout the yeast genome and its reading is performed on a specific sequencing apparatus. The patterns of the visualized DNA bands function as true “fingerprints” of the microorganism, leading us to the recognition of the colonization/infection area of the host. This technique can be used both for use in clinical isolates and for environmental samples (**Figure 17**).

With increasingly interconnected ties between man and his dog, the use of these markers is a valuable technique for detecting epidemiological transmission between

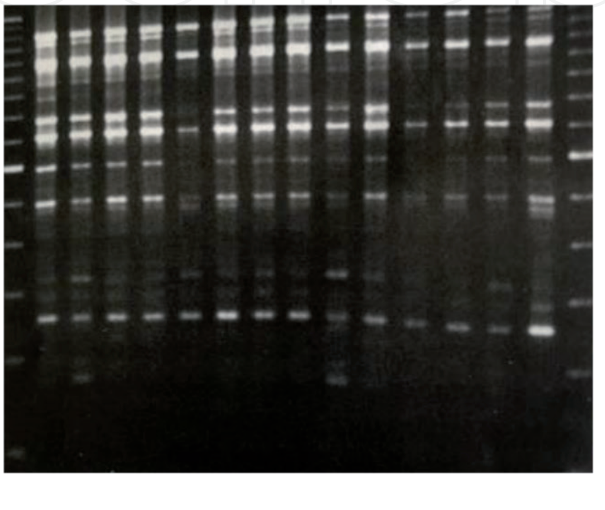

Figure 17.
Chromosomal bands of yeasts obtained by electrophoresis pulsed field - PFGE.

these species and a facilitator for taking therapeutic actions based on the microbiological analysis of the agent's transmitter [33].

8. Antifungals, sensitivity tests and treatment

Currently, yeast mycoses have increased substantially, and it can be considered an important public health problem, especially in systemic clinical conditions and hospital infections. The antifungal drugs used in human and veterinary medicine have special characteristics regarding the chemical structure and the mechanism of action, interfering directly or indirectly in the fungal cell, with fungistatic or fungicidal actions [34].

Among the existing antifungal drugs, the most widely used and known are polyenic, imidazolic, pyrimidine, sulfamide, benzofurenic and other compounds with varying degrees of success, such as iodides, thiosulfates, sulfides and tolnaftates. In the treatment of invasive fungal infections, classes of polyene antifungals (amphotericin B), azoles (fluconazole, voriconazole, ketoconazole, itraconazole, posaconazole), pyrimidines (5-fluorocytosine) and echinocandin, caspofungin, micafungin) are mainly used [35]. The increasing incidence of yeast infections, such as those present in the oral mucosa, has been a target of constant concern in the search for increasingly effective treatments and safer drugs. The use in the treatment and prophylaxis of antifungals such as fewer toxic azoles, especially fluconazole, has given rise to cases of resistance among susceptible yeast species.

The resistance of fungi to antifungal agents can be classified into clinical and microbiological resistance. The concept of clinical resistance is defined when there is a persistence or progression of a fungal infection even with the administration of the drug chosen as appropriate. In this case, "in vitro" tests may indicate the sensitivity of the agent to the antifungal. Usually, the occurrence of clinical resistance is associated with host, iatrogenic, pharmacological factors and factors related to the fungus virulence [36]. Microbiological resistance is a phenomenon in which the etiologic agent can develop in the presence of therapeutic concentrations of antifungals, a capacity verified "in vitro". Resistance can be intrinsic, primary or secondary, or extrinsic. This aspect is of real importance since we are increasingly faced with resistant yeasts, especially the "critical" species, highlighting *C. auris* and *C. haemulori*, whose findings should be immediately reported to the treatment team.

Intrinsic resistance is so called when no member of a species is sensitive to the antifungal, being primary, when in a species normally sensitive to an antifungal we find a resistant strain (without exposure to it) or secondary or acquired, when a previously sensitive strain develops resistance after exposure to a drug, due to phenotypic or genotypic changes [37]. The mechanism of resistance to antifungals by fungi, both for clinical or microbiological resistance, is involved with cellular, biochemical and/or molecular responses.

In the cellular mechanism, strains or sensitive specimens are exchanged for resistant endogenous ones, genetic alteration, a fact that guarantees secondary resistance, transient genetic expression and alteration in the cell type. Regarding the biochemical mechanism, phenotypic changes in fungi occur, allowing the absorption of the drug to be slower, altering the target site and increasing the excretion of the drug. The changes from the molecular point of view causing a genetic amplification to occur, mutations, among other modifications in the gene involved in the defense against the antifungal. In addition to these changes, another molecular alternative of resistance is the ability to form biofilms, an efficient physical barrier [36].

The greater phenotypic variability of *Candida* species, for example, together with the increased resistance of strains to antifungals, has assumed a prominent role as a clinical problem [9]. The different species of this yeast vary in sensitivity to antifungals on the market, a fact that shows the great importance of identifying and determining the minimum inhibitory concentration (MIC) [38].

Due to this aspect, the development of standardized methods of sensitivity "in vitro" is of vital importance and serve as guide to indicate the therapeutic choice, monitor the effectiveness of the antifungal and decrease the formation of resistant strains [39]. The appropriate choice of antifungal agent is, therefore, decisive in the therapeutic response of the animal. To this end, research that aims at determining the antifungal profile of the main yeasts isolated from dogs is of great therapeutic value [9].

The most used parameter for determining sensitivity to antifungals is the minimum inhibitory concentration (MIC), defined as the lowest concentration of an antifungal agent that inhibits the growth of the fungus [40]. From the MIC value, the yeast sample is classified according to the breakpoints established by international committees, which allows the fungus to be characterized as sensitive, intermediate, dose-dependent and resistant sensitivity [38]. For the detection of sensitivity/resistance to antifungals, highlight of use in therapeutic failures, we can count on several techniques, being "Gold standard" the method recommended by CLSI, the microdilution test.

The determination of the sensitivity of a fungus to antifungals can also be determined by commercial methods compatible with the tests recommended by CLSI [17, 37]. Sensitivity tests using a solid medium, such as the commercial method "E-test", are of real interest in several studies and in the laboratory routine. It is an excellent technique for determining the sensitivity to antifungals "in vitro", simple, easy to perform, with fast results, without the need for expensive or specialized equipment [41]. "E-test" is based on a combination of dilution and diffusion test concepts that directly quantify antifungal sensitivity. It consists of tapes containing pre-established concentrations of the antifungal agent, which are placed in a solid medium and with the yeast sample. When the tape is applied to the plate, immediate drug release occurs, thus the MIC is determined by the intersection of the inhibitory hyperbole formed by the growth of yeast in the plate (**Figure 18**).

Because the MIC values of "E-test" are directly proportional to the values referenced by the dilution CLSI, this method has a good correlation with this test. However, it may still present differences inherent to the process.

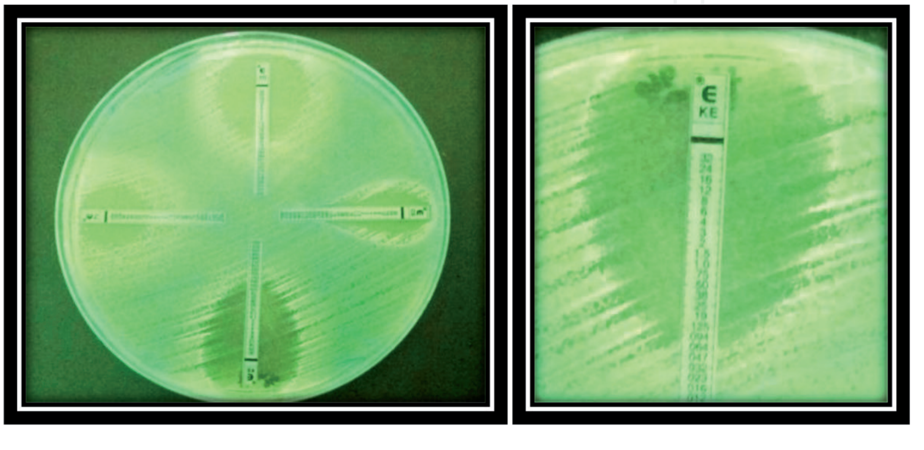

Figure 18.
"E-test" commercial method. MIC is determined by the intersection of the inhibitory area formed by yeast growth.

Antifungals	S (μg/mL)	SDD (μg/mL)	R (μg/mL)
Miconazol*	˂ 8	8–16	≥ 16
Cetoconazol	˂ 16	—	≥ 16
Fluconazol	≤ 8	16–32	≥ 64
Itraconazol	˂ 0,25	0.25–0.5	≥ 1
Voriconazol	≤ 1	2	≥ 4
Caspofungina	≤ 2	—	˃ 2

S: sensitive; SDD: dose dependent sensitivity; R: resistant; NS: not sensitive [38, 42].

Table 1.
Interpretation of the behavior of yeast strains against the concentration of antifungals (μg/ml).

The classification by this method determines the isolate as sensitive, dose-dependent and resistant (**Table 1**).

For tests to determine the antifungal profile, source control strains of the "American Type Culture Collection" (ATCC) are always used under identification, such as, for example, ATCC64548 (*C. albicans*) and ATCC777 (*C. dubliniensis*).

In the treatment of invasive fungal infections, classes of polyene antifungals (amphotericin B), azoles (fluconazole, voriconazole, ketoconazole, itraconazole, posaconazole), pyrimidines (5-fluorocytosine) and echinocandin, (caspofungin, caspofungin, micafungin) are mainly used [35].

For the systemic treatment of yeasts, we can use Amphotericin B, in the most varied forms (liposomal, suspension of lipid complexes). Nystatin can be used orally or in suspension, ointments and creams (as for example, in cases of oral candidiasis). In animals, the use of each of these antifungals is quite varied and their recommendation and dose will depend a lot on the etiological agent in question and the side effects that can be generated.

When analyzing the profile of sensitivity to antifungals compared to isolates from the oral cavity of dogs (mucosa that has greater transmissibility to humans), the best active antifungals found in the veterinary are ketoconazole and voriconazole. Ketoconazole is still widely used in clinics and pet shops, mainly, topically. For the treatment of candidiasis in small animal clinics, ketoconazole is one of the most frequently used drugs, as it has a broad spectrum of activity, encompassing several species of *Candida* spp. and dermatophytes. From isolates from the oral cavity of dogs it shows high sensitivity between yeasts and has several presentations for veterinary use, representing an economically viable alternative, however, due to its toxicity, the trend is disuse [9, 17].

Voriconazole has a broad spectrum of activity and a potent "in vitro" action. Its mechanism of action is like other azole antifungals, inhibiting the enzyme 14 alpha-demethylase, dependent on cytochrome P-450, essential for the ergosterol biosynthesis. It can be indicated as a good alternative to replace ketoconazole, however its cost is high. This drug is also used for the treatment of systemic mycoses, mainly in candidiasis, aspergillosis and cryptococcosis in debilitated, immunosuppressed patients or in cases of resistance to another antifungal [43]. The antifungals fluconazole, itraconazole and miconazole are also routinely applied in the veterinary clinic, used indiscriminately in the treatment of mycosis suggestively diagnosed. However, resistance to these drugs has increased, so their use should be more cautious.

Candida zeylanoides, for example, is a relatively rare yeast in humans and animals. In humans it has been reported from skin, nails and blood isolation, considered an opportunistic pathogen, also involved in cases of endocarditis in an

HIV-positive patient [44]. Samples of this yeast were isolated for the first time in the oral cavity of stray dogs and demonstrated significant resistance to the antifungal fluconazole. In addition to this species, *Candida krusei* also obtained partial results of resistance to this antifungal, as well as yeasts of the genus *Trichosporon* spp. [9].

Itraconazole is a synthetic triazole derivative with a wide spectrum of action, widely used in the treatment of superficial mycoses by candidiasis, malasseziosis and in systemic mycoses. When used orally right after a meal, its bioavailability is maximum, with biphasic elimination. This antifungal has also been used successfully in dogs with mycotic rhinitis and in systemic mycoses, such as blastomycosis. However, its use in dogs can lead to skin rashes and, in high dosages, it can cause anorexia and increased plasma concentration of alkaline phosphatase and aminotransferase enzymes [43].

In addition, species isolated from the oral cavity of dogs (especially *Candida albicans* and *C. tropicalis*) have shown dose-dependent sensitivity to itraconazole. Yeasts of the genus *Trichosporon* also isolated from this active site, show medium resistance to fluconazole and significant resistance to itraconazole, which reveals concern about the use of these drugs in the treatment of candidiasis and triconosporoses in dogs [9, 19].

In the veterinary medical clinic, miconazole is commonly indicated for the treatment of dermatophytosis, malasseziosis and candidiasis. However, yeasts of the genus *Trichosporon* and *Malassezia pachydermatis* isolated from the oral cavity of dogs show important resistance to this antifungal. Different for *Candida* yeasts, in which the antifungal profile demonstrates sensitivity to miconazole [9, 19].

Caspofungin is an antifungal with an inhibitory action on the cell wall of the echinocandin group, important in human medicine as an alternative for the treatment of isolates resistant to fluconazole [45]. Against yeasts isolated from the oral cavity of dogs, yeasts of the genus *Trichosporon* and of the genus *Malassezia* demonstrate significant resistance to this antifungal, resistance also demonstrated to a lesser extent by the species *Candida parapsilosis* [9, 19].

In cases of systemic infections, affecting different species of animals, the use of amphotericin B, a drug that acts on the fungal cell membrane, has efficiency against strains of *Candida* spp. However, due to the high cost and serious side effects, such as hepatotoxicity, nephrotoxicity, myelotoxicity and cardiotoxicity, this medication is seldom used [17].

Due to the great similarity between the fungal cell and the host cell, the action of antifungals presents relatively high toxicity. Thus, there is a need for research for the best choice of antifungal, based on the most appropriate therapeutic response and on the sensitivity profile of yeast against antifungal floodgates, seeking as well to minimize the side effects that can be generated with the use of more drugs needed in cases of therapeutic failure [43].

When information is obtained that a street animal, which in general is a dog that, has never received therapeutic treatment based on antifungal, presents positive isolation for resistant yeasts, it is assumed that environmental yeasts are undergoing an important primary resistance or that the ecological niche in which that animal lives is contaminated by resistant microorganisms originating from direct or indirect human contamination.

Corroborating this fact, we must consider the excessive use of pesticides in the environment and mycoherbicides (placed in plantations, vegetable gardens, and in the soil itself), have a chemical constitution like azoles, thus representing a strong selective pressure for the emergence of strains resistant. This question of possible environmental contamination and fungal resistance is already discussed for other yeast species, such as *Cryptococcus* spp. and medical mycology becomes an important issue.

The growing data on increased resistance of fungi against antifungal drugs have been causing great concern for human and veterinary doctors. Although data on resistance to antifungals from yeasts isolated from dogs are scarce, their importance is notorious, directly associated with the therapeutic success of these animals particularly important for society and human health (physical and mental).

Therefore, the ideal therapeutic choice, for both humans and animals, should be based on prior identification of the agent and, if possible, the use of techniques for determining the sensitivity profile of the etiologic agent against antifungals.

9. Considerations

The oral cavity is an extremely important anatomical area of dogs, considered as one of the determining factors in the longevity of this animal's life. To reduce therapeutic failures and guarantee the perfect health condition of this system, knowledge of the existing microbiota is essential, but it is still scarce.

We can then ask ourselves: Why are recurrent fungal infections more and more frequent in dogs? What is the relationship between the microbiota of dogs and their respective owners? And what is the relationship of resistance to fungal infections between these species?

Possible answer to these questions could be founded in this chapter, as well as the beginning of the knowledge of the main yeasts found in the oral cavity of dogs, their clinical importance and profile of resistance to the main antifungals used in the practical routine of veterinary medicine.

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