S6.5c
The overgrowth of Malassezia spp.in canine atopic dermatitis - the reason or the result of disease?

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Objective: Yeasts of the genus *Malassezia* are well known as the members of normal skin mycobiome in animals or human and usually colonize the skin as in small population. *Malassezia pachydermatis* is a species for which dogs are a natural host. Both *Staphylococcus pseudintermedius* and *Malassezia pachydermatis* microorganisms may colonize a healthy skin if their population size is proper to maintain the skin equilibrium. In dogs with allergic skin disorders the number of *M. pachydermatis* usually increase dramatically.

Atopic dermatitis (AD) is a chronic inflammatory skin disorder that results from interaction between genetic predisposition, host environment, chemical skin abnormalities, barrier and immunological defects. It is hypothesized, that in dogs the atopic dermatitis is a risk factor for *M.* pachydermatis infection, but in our opinion, on contrary, abundant colonization by these yeasts determine increased reactivity against members of the normal skin microbiota. Many factors which stimulate the atopic dermatitis stimulate also a proliferation of *Malassezia* and *Staphylococcus*. The growing bacteria and fungi release a lot of extracellular products which results in itchiness that makes the dog scratch, which further damages the skin, leading to a bigger area of infection. Also *Malassezia* cell wall components, as in other yeast may have immunomodulating potency and support an inflammation progress. The factors which have influence on skin fatty acid coat composition play also role as an inflammation starter and are correlated with *Malassezia* skin population.

The objective of this work was to provide an update on recent advances in the explanation of the role of *Malassezia* in acute phase of AD lesions in dogs.

Methods: experimentally studies, review data

Results: Our studies have shown that administration of *Malassezia pachydermatis* cell walls increase proliferation of lymphocytes, enhances the respiratory burst and increases the killing potential of immunocompetent cells. The stimulatory effect on lymphocyte culture was comparable to Con A. We conclude that cell wall of *M. pachydermatis* may have immunomodulating properties, what should be consider in mechanisms of *M. pachydermatis* infection pathogenesis. The response of the host to the overgrowth of yeast includes non-specific defense mechanisms (phagocytosis by neutrophils) as well as cell-mediated specific defense mechanisms. Langerhans cells presents the antigen which activates T-cells. These T-cells multiply and produce lymphokines that stimulate phagocytosis and multiplication of epidermal basal cells. This leads to their mechanical removal yeast via scaling. Alterations of the cutaneous microclimate or host defense mechanisms allow *Malassezia pachydermatis* to multiply and realizing their stimulatory effect on immunocompetent cells.

Conclusion: The changing of Canine Skin Microbiome in association with leucocytes responses, including the large spectrum of cytokine and non-cytokine factors that appear in *Malassezia* overgrowth are in our opinion a potential etiological agent of the development of canine AD.

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S6.5d
Characterization of growth of lipid-dependent Malassezia yeast species, members of the skin mycobiome

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Objective: *Malassezia* species are lipid-dependent due to the lack of cytosolic fatty acid synthase required for *de novo* synthesis of fatty acids (FAs) and these yeasts are part of the skin mycobiome. Pathogenicity of *Malassezia* has been related to several factors including the ability to produce enzymes such as esterases, lipases, lipoxygenases and proteases which enable growth on the host skin and lead to changes in sebum (skin fat) composition.

The skin functions in the innate defense against pathogens due to its low water content, acidic pH, its microbiota, and antimicrobial compounds like free fatty acids. Understanding lipid dependency of *Malassezia* will help to understand how these yeasts establish themselves as part of the skin microbiota, which adaptation mechanisms are involved, and how, and whether, lipid metabolism impacts the shift to pathogenicity. The complex nutritional requirements of *Malassezia* have delayed the full comprehension of its lipid metabolism.

Reconstruction of the lipid-synthesis pathways of *Malassezia* species *in silico* predicted amongst others a defect in the assimilation of palmitic acid in *M. globosa*, *M. sympodialis*, *M. pachydermatis* and an atypical isolate of *M. furfur*, but not in *M. furfur*. This prediction was validated by physiological characterization in chemically defined media (MM) using different lipid sources.

Methods: **Growth on FAs in liquid MM:** Strains were first grown for 7 days at 33°C in lipid-rich mDixon medium. To prevent subsequent growth in MM due to the presence of residual lipids we performed a two-phase growth in MM. First, cells were diluted into MM containing specific lipids. After 3 days, these cells were diluted again in fresh MM with the same lipids. Growth was monitored for 7 days by determining OD600 nm and CFU by plating on mDixon plates.

Results: *M. furfur* could assimilate palmitic acid or oleic acid as well as all Tween variants tested. The atypical *M. fufur* strain could assimilate only Tween 80, Tween 20, and oleic acid. *M. pachydermatis, M. globosa*, and *M. sympodialis* were able to grow in the first step in MM but not in the second step in MM with any of the lipid sources tested. Only *M. furfur* was able to maintain growth in MM with palmitic acid in the second growth step. Both *M. pachydermatis* and atypical *M. furfur* could sustain growth in MM with a mixture of palmitic acid and oleic acid.

Conclusion:

1. A new culturing method for *Malassezia* spp. in chemically defined media was developed.
2. *In silico* predicted assimilation defects of palmitic acid for *Malassezia* spp. was confirmed.
3. Palmitic acid is fungicidal for a subset of *Malassezia* spp. but not for *M. furfur*.
4. FAs that induce lipid toxicity and do not affect the skin cells and microbiome harmony might have a therapeutic use.

S6.6
Surveillance azole resistance
S6.6c
First report of azole-resistant Aspergillus fumigatus harboring TR34/L98H and M220R in Brazil

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Objective: Despite the high use of triazole fungicides in Brazilian agriculture, azole-resistant Aspergillus *fumigatus* has not been documented in Brazil. The objective of this study was to characterize the Cyp51A-gene of two phenotypic resistant *A. fumigatus* isolates.

Methods: One *A. fumigatus* isolate was cultured from a patient with chronic necrotizing pulmonary aspergillosis (CNPA), following tuberculosis. The patient was treated with itraconazole (ITZ) followed by voriconazole (VCZ) and finally, amphotericin B (AmB). The other resistant isolate was isolated from maize harvested from a crop in southern Brazil by the in-house plating method in surface with Dichloran Rose Bengal Chloramphenicol Medium (DRBC, Himedia®). Both strains were identified by macro and micro morphology, sequencing of ITS1-5.8s-ITS2 region and the ß-tubulin gene. *In vitro* susceptibility testing was performed using the EUCAST reference methods. To detect resistance mutations full sequencing of the *Cyp*51A gene and promoter region was performed.

Results: R_{34}/L98H was found in the clinical *A. fumigatus* isolate and M220R in the environmental isolate. Both strains showed high MICs to ITZ (>16 mg / L); the clinical isolate was resistant to the other medical azoles, while the environmental strain was resistant only to isavuconazole and showed intermediate susceptibility to VCZ (Table 1).

Conclusion: This is the first report of azole-resistant *A. fumigatus* isolates harbouring TR_{34}/L98H or M220R mutations in Brazil. Although the detection of resistance mechanisms in two strains of *A. fumigatus* does not represent great concern regarding the general context of azole resistance in Brazil, we believe that our finding underscores the need for resistance surveillance in clinical and environmental isolates to further determine the frequency of resistance.

Picture 1: https://www.eventure-online.com/parthen-uploads/89/8ISH/add_1_420288_98f02d45-7932-497a-bd0a-cc80a989ecc3.png

Caption 1: Table 1. Susceptibility of A. fumigatus isolates harboring azole-related mutations.

S6.6d
Status and Surveillance of Azole Resistance in North America

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With the emergence of drug resistant fungal pathogens comes a threat to the utility of the most commonly used antifungals, such as the triazole drug class. By and large, the most pressing current issues relating to azole resistance in North America involve two fungal pathogens – *Aspergillus fumigatus* and *Candida auris*. The environmentally derived TR_{34}/L98H and TR_{46}/Y121F/T289A mutations in the azole target gene of *Aspergillus fumigatus* have recently been identified in isolates from the US. Likewise, the emerging fungal pathogen, *Candida auris*, has been reported in the US, Canada, and Panama and is novel among *Candida* in terms of antifungal resistance. This presentation will address both of these organisms, detail the azole resistance for each, discuss existing surveillance efforts for each, and very briefly describe considerations for other relevant fungal species.

S7.1
Fungal biofilms
S7.1a
Candida auris and non-albicans biofilms

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The role of *Candida* biofilms in human disease has become increasingly recognized over the past 20 years. Much of this work has focused on *Candida albicans*, where the molecular basis of development and resistance is in an advanced state. However, clinically it is clear that non-albicans species such as *C. glabrata, C. tropicalis and C. parapsilosis* are capable of exhibiting biofilm characteristics, which also includes the emergent fungal pathogen *Candida auris*. This presentation will examine the contribution that these non-albicans species play in human disease, the basic and molecular characteristics that drives their biofilm formation, and therapeutic considerations for biofilm management. Our early understanding of *C. auris* biofilms will also be examined, and how this lifestyle may contribute its persistence in the healthcare environment.

S7.1b
Insights into new antibiofilm molecules

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Candida albicans is a common opportunistic pathogen and the most frequent causative agent of candidiasis, the main invasive fungal infection in immune- and medically-compromised patients. Candidiasis is often associated with the formation of biofilms, which contribute to virulence and further complicate treatment due to the high level of resistance to conventional antifungal agents. Clearly, new anti-biofilm strategies and therapeutics are urgently needed. Our group has taken a multipronged approach to tackle this problem. First, we have performed large-scale screening of over 50,000 small molecule compounds present in different commercial chemical libraries to identify inhibitors of *C. albicans* biofilm formation. This resulted in the identification of several leading series of anti-virulence compounds displaying potent *in vitro* and *in vivo* activity against *C. albicans* biofilms. They represent promising candidates for the development of novel antifungal agents, with new molecular structures, as well as new targets and modes of action. Second, unlike the tortuous and costly path of de novo drug discovery, drug repurposing (finding new uses to existing drugs) is gaining traction as an alternative path to accelerated drug development. Thus, we have also embarked in a "repurposing" campaign, in order to identify already existing drugs with activity against fungal biofilms. Third, we have determined the activity of different nanoparticle preparations against *C. albicans* biofilms, as well as against mixed fungal/bacterial biofilms that are particularly difficult to treat with conventional antimicrobial therapy. Lastly, our group has developed a novel technique consisting of nano-scale culture of microbial biofilms on a microarray platform. Using this technique, hundreds to thousands of microbial biofilms, each with a volume of approximately 30–50 nanolitres, can be simultaneously formed on a standard microscope slide. This new technology platform allows for the implementation of true throughput screening techniques that can speed up discovery of new drugs with anti-biofilm activity. Overall, successful development of these anti-biofilm strategies and therapeutics should have a profound impact on the management of patients suffering from these difficult to treat infections.

S7.1c
Fungal-bacterial biofilms: consequences in an intra-abdominal infection model

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The pleiomorphic fungus *Candida albicans* (*Ca*) and the ubiquitous bacterial pathogen *Staphylococcus aureus* (*Sa*) are responsible for a myriad of biofilm-mediated human diseases, often co-infecting critically ill or immunocompromised patients. Although many *Candida*-bacterial interactions are antagonistic, the relationship between *C. albicans* and *S. aureus* is mutually beneficial, enabling them to act as co-pathogens. *S. aureus* preferentially adheres to *C. albicans* hyphae, and polymicrobial biofilms of both species display enhanced growth, antimicrobial drug resistance, and virulence. To study this polymicrobial interaction further, we established a murine model of intra-abdominal infection (IAI) where co-infection with both species results in 80–100% mortality within 96 h post-inoculation compared to no mortality from infections with either *Ca* or *Sa* alone. Furthermore, co-infection led to formation of biofilm-like polymicrobial structures on the surface of target organs. The polymicrobial-specific host response is characterized by dramatic up-regulation of local and systemic pro-inflammatory cytokines, prostaglandins, and polymorphonuclear infiltrate, all hallmarks of lethal sepsis. We tested several other non-albicans *Candida* species (NAC) and found that co-infection with the closely related *C. dubliniensis* (*Cd*) resulted in <10% mortality. Of particular interest, survivors of *Cd*/*Sa* or *Cd* alone re-challenged with a lethal co-infection (*Ca*/*Sa*) resulted in >90% survival. The protective response is sustained long term (up to at least 60 days prior to re-challenge) and is broad-spectrum providing protection against similarly lethal *C. tropicalis*/*Sa* and *C. krusei*/*Sa* IAI. Surprisingly, the *Cd*-induced protection against lethal IAI is NOT mediated by adaptive immunity, but instead appears to be through a mechanism of trained innate immunity (non-specific memory mediated by innate cells). Preliminary data indicate that the *Cd*-mediated protective trained innate response is mediated by Gr-1+ myeloid derived suppressor cells (MDSC) that have been reported in human sepsis. These results suggest that low virulence *Candida* species can induce protection against lethal fungal/bacterial IAI with *C. albicans* and *S. aureus* that is mediated by MDSCs as a unique form of trained innate immunity. This provides a unique mechanistic pathway for protective immunity and potentially new avenues for vaccine strategies to protect against intra-abdominal polymicrobial infections resulting in lethal sepsis.