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Rapid fungal ID in the veterinary sector

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Rise of fungal infections and their impact

Prudently estimated, there are approximately 1.5 million species of fungi.[1] Many of them coexist with animals and humans without causing any harm (commensalism), but under certain circumstances, they can lead to diseases. The number of fungal species identified as significant pathogens is not to be underestimated, and they typically have the ability to infect not only animals but humans and plants as well.[2] Pathogenic fungi predominantly encompass yeasts and filamentous fungi including molds.

Given the vast number of species that can cause fungal infections, known as mycoses, they are generally classified based on the infection's location. This classification includes superficial, cutaneous, subcutaneous, and deep/systemic mycoses. Additional distinctions must be made between infections affecting healthy individuals and opportunistic mycoses, which develop exclusively in those with weakened immune defenses.

Fungal infections can have various origins. We categorize endogenous infections as those originating from fungi naturally present in the upper respiratory tract, skin, or mucous membranes. The transition from harmless coexistence (commensalism) to disease can be triggered by various predisposing factors. It's important to note that these types of infections constitute only a small percentage of the fungal infections affecting animals and humans. The majority of mycoses are typically transmitted from the external environment, either through direct contact with spores, between infected individuals, or indirectly through contact with contaminated materials.

Despite the inevitability of contact due to the large number of potentially pathogenic fungal spores in the environment, fungal infections have historically been relatively rare compared to infectious diseases caused by bacteria and viruses.[3,4] The thermal barrier of mammalian bodies has, in fact, limited the pathogenic potential of fungi.[5]

In recent decades, a notable surge in refractory and recurrent fungal infectious diseases has been observed globally, attributed primarily to climate changes. This trend is particularly pronounced among companion and livestock animals.[6,7,8,9]

Climate change has played a significant role in transforming formerly benign fungal species into infectious agents.[10,11] The consistently elevated global average temperatures, coupled with increased moisture in certain regions, have facilitated the continuous expansion of the geographic ranges of known fungal pathogens.[12]

The rise in fungal infections among animals has resulted in both economic consequences, including escalating costs associated with prevention and treatment, and heightened concerns for human health. Animal fungal infections are of concern due to their potential transmission to humans. Indeed, zoonoses are reported as the primary cause of fungal infections in humans.[13]

This scenario has prompted research into novel methodologies within the veterinary domain for quickly identifying the responsible fungal species. Timely and accurate detection of fungal pathogens at the species or strain level is often pivotal for disease surveillance and for organizations dedicated to animal welfare, facilitating the implementation of effective management strategies.

Advancements in the identification of fungal pathogens: New perspectives and methodologies

Conventional methods for identifying fungal species include isolation and culture, reinoculation, microscopic techniques, and biochemical tests.[14] However, these approaches have notable drawbacks.[15,16]

Direct microscopy remains a crucial tool for rapid and cost-effective "interception" of a fungal infection, allowing to observe the cellular immune response and the detection of pathogens simultaneously. However, the sensitivity of microscopic examinations varies based on the individual agent, the source and quality of the sample, as well as the skills and experience of the laboratory technician. Moreover, diagnosing invasive fungal infections in animals via direct microscopy and histopathology may not always lead to fungal identification.[17]

Biochemical methods, particularly serum immunological tests seeking fungal wall components like Beta-Glucan, have limitations such as low sensitivity, affinity, and potential interference from contaminants.[18]

Developing new and effective identification methods is imperative yet challenging for several reasons. Fungal pathogens can form complexes of multiple species or exist in low concentrations, and within species, different molecular genotypes/variants may exhibit diverse pathogenic profiles and virulence levels.

Identifying filamentous fungi remains difficult due to their growth within solid substrates and challenges in collection, leading to prolonged and labor-intensive workflows due to agar contamination.

To address these challenges, increasingly advanced molecular techniques like fluorescent in situ hybridization (FISH), DNA array technology, multiplex tandem PCR, and Padlock probe technology with rolling-circle amplification and loop-mediated isothermal amplification (LAMP) are employed. However, these methods demand significant effort and resources for individual analyses, limiting accessibility.

Amid these emerging technologies, Mass Spectrometry, in particular MALDI-TOF MS, stands out as a promising solution to address the challenge.

MBT HT Filamentous Fungi Module: Improved identification of challenging organisms

MALDI-TOF MS is a cornerstone technology for spectrum analysis of proteomic fingerprints, crucial in discriminating and identifying various species. Our MALDI Biotyper has played a pioneering role in introducing this methodology to microbiological labs and pushing the capabilities in recent years.

Its application has garnered widespread recognition, particularly in identifying bacteria and yeasts. With the introduction of the MBT HT Filamentous Fungi Module, it has become easier to identify filamentous fungi as well. This module integrates an extensive library of reference spectra for filamentous fungi with optimized software and acquisition parameters, ensuring a high identification success rate.

Filamentous fungi growth on agar plates can result in spore formulation and heterogenous biomass, resulting in varying proteomic spectra. Bruker has developed a liquid-based cultivation method to guarantee the reproducibility of reference spectra for filamentous fungi. This standardized approach minimizes the impact of cultural conditions on mass spectra, thus creating highly reproducible library entries. By preventing germination and spore formation, this method enables rapid and reliable species identification directly from the mycelium, bypassing the need for intricate cultures. Although this approach delivers spectra of the highest quality, it might not be the easiest workflow to implement in routine laboratories. Therefore, we recently introduced a new sample preparation workflow called the Mycelium transfer (MyT) method, it provides increased identification rates while keeping the workflow simple and not requiring liquid cultivation.

The ongoing evolution of this technology promises to reshape the landscape of microbiological identification, offering increasingly efficient solutions to tackle the evolving challenges in public health and clinical research.

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