

managing the pandemic as, wave after wave, swept across our nation. Moreover, our indigenous drug makers jumped into the fray and excelled on par with our western counterparts in the timely development of effective vaccines.

In a country like India, we are faced with additional challenges in the form of certain infectious diseases whose burden is borne mainly by the socio-economically disadvantaged, mainly in the tropics. These diseases and strategies for their mitigation have been taken up by the World Health Organization (WHO) in a big way by means of the Initiative for Control of Neglected Tropical Diseases (NTDs). Besides bacterial and viral diseases, Eukaryotic organisms are also important entities as etiological agents of NTDs.

Eukaryotic medical microbiology (EMM) comprising medical mycology, medical protistology, medical helminthology, and medical entomology needs greater attention in India. Except malaria which has been at the forefront owing to the huge disease burden and associated mortality, several other parasitic infestations have indeed been neglected—an issue which the WHO rightly highlighted as part of its NTD elimination initiative.

Though Mycetozoa, chromoblastomycosis, and other deep mycoses are the only fungal diseases to find a place on the list of NTDs, many other fungal diseases continue to plague our communities. Mucocutaneous candidiasis and dermatophytosis may neither be commoner in the tropics nor are they neglected (rather, dermatophytosis tends to be overtreated in many cases!) but they merit attention simply because of the discomfort and disruption to daily life that they cause.

We propose that a network of Medical eukaryotic microbiology (MEM) Laboratories be set up with special emphasis on the NTDs on a regional and national scale in our country to effectively deal with these infectious diseases prevalent in our communities.

The diagnostic services offered by these 'MEM-NTDLs' can comprise of:

Microscopy, including fluorescent microscopy and special stains

Microarrays

MALDI-TOF

Multiplex PCR

NGS, including handy DNA sequencing technologies exemplified by the Nanopore MinION

For dealing with these eukaryotic diseases, we believe that we need to adopt advanced techniques like MALDI and NGS in a big way.

Culture, which continues to be the gold standard for bacteriological diagnoses, may now be termed to have historical significance at best in the case of fungal diseases because of the slow turnaround time and by the ubiquitous presence of fungal spores behaving as lab contaminants. Similarly, serological techniques may have a very limited role in the workup of eukaryotic infections owing to the complex antigenic profile of these organisms and the chronicity of the disease conditions. Microscopy can be retained for the relative procedural simplicity and rapidity of results.

The 'advanced' diagnostic techniques mentioned above have been around for quite some time now and have been widely applied in the microbiology diagnostic laboratory as well. With requisite training and provision of equipment and appropriately trained technical staff, a MEM-NTDL can be operated successfully at the district level in a planned manner. This would greatly enhance the quality of health services available to our population and enable our country to reach the goal of Health for All in the near future.

Following identification, comes the very important aspect of antimicrobial chemotherapy susceptibility testing. Even here, alternatives to the growth-based, culture-dependent systems should be sought—the role of Biomarkers related to the growth and metabolism of eukaryotic organisms should be explored and incorporated in practical diagnostics as an aid to infectious diseases therapy and patient management. This can be part of the scope of the MEM-NTDL of the future.

#### P503

##### Molecular epidemiology of *Trichophyton* mediated infections among canines from Northern India

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Objectives: Dermatophytes are *keratinophilic* fungi that cause skin infections among animals and humans. Recently, the incidence rates of fungal infections especially due to the *Trichophyton* spp. are being considered as endemic in many geographical locations. The cause of recent surge of dermatophytosis due to this agent in humans is not known. It is assumed that pets may be one of the sources which are not established till now. The present study was conducted to understand the molecular heterogeneity of *Trichophyton* spp. of canines and felines, and their phylogenetic relationship with human isolates.

Methods: The samples (skin scrapings) were collected from 386 canines and 56 felines exhibiting clinical signs of ringworm during the period of 2020-2021 from the veterinary hospitals and farms in the states of Uttar Pradesh and Kerala, India. All the samples were attempted for isolation on Sabouraud's dextrose agar (with chloramphenicol and cycloheximide at 0.05 and 0.5 g/L, respectively). The antifungal susceptibility assay was performed by following the Clinical and Laboratory Standards Institute (CLSI) guidelines, document M38-Ed3 for filamentous fungi (CLSI: Wayne, PA, USA, 2017). The isolates presumptively identified as *Trichophyton* spp. were characterized further based on PCR and sequencing of three genetic markers such as ITS, Tef1- $\alpha$ , and beta-tubulin genes. Phylogenetic analysis and taxonomical determination of the *Trichophyton* isolates were performed. Three human isolates of *T. mentagrophytes* were used for comparative study.

Results: A total of 67 (15.16%, 67/442) samples revealed the presence of fungal hyphae on direct microscopic examination. On culturing, 52 samples were found to be positive for dermatophytes. Among these, 10 isolates were presumptively identified as *T. mentagrophytes* spp. based on morphological and microscopic examinations. Most of the strains were sensitive to all drugs tested except fluconazole, which showed a resistance pattern for most strains. Based on sequence homology and phylogenetic inferences, the *Trichophyton* isolates belonged to four different species/genotypes, such as *T. mentagrophytes* genotype VIII (5), *T. interdigitale* (2), *T. simmi* (2), and *T. quinckeianum* (1). Human isolates were represented as *T. mentagrophytes* genotype VIII (2) and *T. benhamiae* (1).

Conclusion: To conclude, the study reports for the first time the prevalence, species diversity, and antifungal resistance among *Trichophyton* spp. from canines in India. Even though the *Trichophyton* prevalence was lower in canines, the presence of *T. mentagrophytes* genotype VIII/*T. indotimae* is of great public health significance. This indicates the zoonotic sharing of strains especially *T. mentagrophytes* genotype VIII in both hosts that are also considered as the recently endemic pathogenic clone in India.

#### P504

##### Screening of Belgian bats and hibernacula for the description of related fungal microbiomes and the detection of *Pseudogymnoascus destructans*

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Bats can be affected by fungal pathogens such as *Pseudogymnoascus destructans*, the causative agent of the white-nose syndrome. Their body surface can also be colonized by fungal commensals or carry transient fungal species and participate in their dispersal. The present study aimed to assess the presence of *P. destructans* in Northern Belgium, to describe the skin mycobiome of active bats during summer and autumn, and to analyze possible differences in fungal diversity among bat species, sampling sites, and seasons. In total, 114 bat specimens belonging to seven species were sampled from various localities. Culture-based methods revealed an important mycological diversity with 209 different taxa. Overall, a mean of 3.7 taxa per bat was recorded but significant differences were observed between sampling sites and seasons with a higher diversity in autumn as compared to summer. The mycobiomes were dominated by cosmopolitan and plant-associated species, in particular from the genera *Cladosporium*, *Penicillium*, and *Aspergillus*. Other species known to be related to bats or their environment, like *Apiotrichum otae*, were also retrieved. Although *P. destructans* was not detected, the sampling of the hibernacula indicated that they can be inhabited by diverse fungal species including a yet undescribed *Pseudogymnoascus* species, distinct from *P. destructans*, namely *P. cavicola*, *sp. nov.*

#### P505

##### Genotyping of *Trichophyton mentagrophytes* infections in animals in Italy through sequencing of the ITS region

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Objectives: *Trichophyton mentagrophytes* is a zoophilic dermatophyte that recognizes lagomorphs and rodents as primary hosts. The fungus can also infect other animals, such as dogs and cats. While *T. mentagrophytes* is a polymorphic sexual species, *T. interdigitale* is recognized as its clonal offshoot. This delineation is meaningful from a clinical point of view in human patients. *Trichophyton interdigitale* is exclusively anthropophilic and mainly causes non-inflammatory chronic *tinea pedis* or onychomycosis. *Trichophyton mentagrophytes* is predominantly of animal origin and often leads to the development of inflammatory lesions. These two dermatophytes form a species complex and have several ribosomal internal transcribed spacer (ITS) region genotypes. Identifying the ITS type allows species attribution and simultaneously strain typing. Many studies have been dedicated to this argument concerning human infections, while scarce information is available regarding animals. This study aimed to gain insights into the current epidemiology of *T. mentagrophytes* genotypes in animals.

Methods: The fungal isolates included in the study regarded cases involving various animal species seen at multiple veterinary clinics in Italy ( $n = 39$ ) and France ( $n = 1$ ) between 2005 and 2021. DNA was extracted from isolates cultured on Sabouraud dextrose agar using a commercially available kit (NucleoSpin® Tissue, Macherey-Nagel, Düren, Germany). PCR was performed with the primer pair V9G and LR3. PCR products were sequenced using ITS5 and ITS4 primers through a commercial service (Macrogen Europe). Using MEGA11 software (<https://www.megasoftware.net/>), ITS sequences were aligned with the currently recognized genotypes (6 and 22 for *T. interdigitale* and *T. mentagrophytes*, respectively).

Results: Figure 1 shows the ITS Type attribution for our isolates within a phylogenetic tree that includes the currently recognized genotypes. A new genotype (that, following the nomenclature, we called XXVII) was found in two isolates coming from a dog and a cat living in the same city. Figure 2 shows the distribution of the genotypes according to the animal host.

A total of 23 samples out of 40 (57.5%) belonged to the ITS Type III\*. It was the lone found in rabbits and the most prevalent in cats. This finding agrees with past literature, which reported a wide distribution of this ITS type in European animals. Of note is the high number of isolates with ITS Type II\* found in dogs. ITS Type II\* differs only by one nucleotide substitution from *T. interdigitale* and is considered an 'intermediate' entity between it and *T. mentagrophytes*. Clinical pictures, as well as molecular data, would suggest attributing this genotype to *T. interdigitale*. On the other hand, it has been detected from animal sources (chinchilla, guinea pig, and brown rat) which would justify its interpretation as *T. mentagrophytes*. Our data support the latter possibility.

Though we could not have a detailed description of all the dogs harboring ITS Type II\*, it is noteworthy that many showed the same clinical presentation, i.e., exfoliative chronic disseminated alopecia. Moreover, in most cases, despite the extensive lesions, the infection was not transmitted to the owners.

Conclusions: This study adds information on the molecular epidemiology of *T. mentagrophytes* infections in animals.