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Abstracts of the International Workshop "ONYGENALES 2020: Basic and Clinical Research Advances in Dermatophytes and Dimorphic Fungi"

Prague, Czech Republic, September 10–11, 2020

The ONYGENALES workshop is a bi-annual meeting organised by ISHAM Working Group ONYGENALES (onygenales.org). It brings together researchers, students, clinicians, laboratorians and public health professionals across biomedical disciplines, who are interested in current developments in dermatophyte, dimorphic and keratinophilic fungi research.

The abstracts are arranged according to the thematic sessions as they appeared in the programme:
Session 1: Antifungal resistance and susceptibility testing
Session 2: Taxonomy of keratinophilic and dimorphic fungi
Session 3: Taxonomy of dermatophytes
Session 4: Population genetics and genomics
Session 5: Emerging and zoonotic pathogens
Session 6: Epidemiology
Session 7: Diagnostics and treatment approaches
Session 8: Virulence factors and pathogenesis

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SESSION 1: ANTIFUNGAL RESISTANCE AND SUSCEPTIBILITY TESTING

EUCAST susceptibility testing of microconidia-forming dermatophytes

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Antifungal drug resistance in dermatophytes was first reported shortly after the turn of the millennium and has today been reported in *Trichophyton* and occasionally in *Microsporum*, but not in *Epidermophyton* species. Although drug resistance in dermatophytes is not investigated routinely, resistance in *Trichophyton* spp. is reported increasingly worldwide. The highest rates are observed in India [36% and 68% for terbinafine (MIC ≥4 mg/l) and fluconazole (MICs ≥16 mg/l), respectively], and apparently involve the spread of a unique clade related to the *T. mentagrophytes* / *T. interdigitale* complex.

The EUCAST-AFST (European Committee on Antimicrobial Susceptibility Testing Subcommittee on Antifungal Susceptibility Testing) has developed a new method (E.Def 11.0) for antifungal susceptibility testing against microconidia-forming dermatophytes, including tentative MIC ranges for QC control strains and tentative breakpoints against *T. rubrum* and *T. interdigitale*. The method is based on the EUCAST microdilution method for moulds but significant differences include: (a) an altered test medium selective for dermatophytes, (b) an altered incubation time and temperature, and (c) a different endpoint criterion (spectrophotometric determination) of fungal growth. The method can easily be implemented in laboratories already performing EUCAST microdilution methods. The method was recently validated for terbinafine, voriconazole, itraconazole and amorolfine against the wildtype as well as molecularly characterised terbinafine-resistant mutants of *T. rubrum* and *T. interdigitale* in a multicentre study. Modes for the wildtype and mutant populations were ≥7 two-fold-dilutions apart in all cases. Excluding one I121M/V237I *T. rubrum* mutant and two mixed wildtype/mutant *T. interdigitale* specimens, the number of VMEs were: *T. rubrum* CC-visual, 1/67 (1.5%); CC-spec-90%, 3/59 (5.1%); CC-spec-50%, 1/67 (1.5%) and *T. interdigitale* none. Voriconazole and amorolfine MICs were quite uniform but trailing growth complicated visual and spec-90% MIC determination of itraconazole.

Although none of the laboratories were experienced in dermatophyte testing, error rates were low. We recommend the CC-spec-50% method and provide QC-ranges and WT-ULs for wildtype/non-wildtype classification. This standardised procedure with automated endpoint reading will allow broader implementation of susceptibility testing of dermatophytes and thus facilitate earlier appropriate

therapy. This is important, as resistance is rapidly emerging and largely underdiagnosed.

In vitro antifungal susceptibility testing and point mutations in the squalene epoxidase gene of *Trichophyton mentagrophytes / T. interdigitale* complex

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During the past decade, a severe outbreak of terbinafine-resistant strains in the *Trichophyton mentagrophytes / T. interdigitale* complex has taken place in India. In this study, a total of 145 isolates of the complex were collected from India, China, Australia, Germany, and The Netherlands. Antifungal susceptibility testing of the isolates was carried out for itraconazole, fluconazole, ketoconazole, sertaconazole, miconazole, terbinafine, naftifine and griseofulvin, according to modified EUCAST 9.3.1 guidelines. Based on the latest taxonomic insights, the strains are classified into three species: *T. mentagrophytes* s. str. (n = 39), *Trichophyton indotineae* (n = 72 representing the Indian clone) and *Trichophyton interdigitale* s. str. (n = 34). High (>16 mg/l, cut-off) minimum inhibitory concentrations (MICs) to terbinafine were noted in 34 (47.2%) *T. indotineae* isolates. C to A and T to C transversions were responsible for amino acid substitution in the 397th position of the SE gene in high terbinafine-resistant isolates. Moderate (0.5 mg/l, cut-off) MICs to terbinafine were noted in 2 (2.78%) *T. indotineae* isolates. C to A and T to C transversions were responsible for amino acid substitution in the 415th and 393th positions of the SE gene in moderate terbinafine resistant isolates. Low (0.125 mg/l, cut-off) MICs to terbinafine were noted in 8 (11.1%) *T. mentagrophytes* and *T. indotineae* strains; no mutation of the SE gene was found. Antifungal susceptibility testing was performed again in these high terbinafine-resistant isolates after replication for 10 times in drug-free PDA medium; no difference was found compared to the previous results.

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Terbinafine resistance of dermatophytes in India and Germany

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An incredible increase in chronic recalcitrant dermatophytoses has been noted in India over the past few years. Due to travelling and migration, spread of the mostly terbinafine-resistant strains of *T. mentagrophytes* Type VIII from India to other parts of the world, e.g. Germany, is ongoing. In India, three epidemiologic studies were undertaken. In Germany, from September 2016 to March 2020, altogether 29 strains of *T. mentagrophytes* Type VIII India were isolated. Out of 402 Indian samples, 289 (71.9%) revealed growth of *T. mentagrophytes*. *Trichophyton rubrum* was cultivated from 19 (4.7%) samples. 71% of isolates were resistant to terbinafine. The amino acid substitution Phe^{397}Leu in the squalene epoxidase of resistant *T. mentagrophytes* was highly prevalent (91%). Two novel substitutions in resistant *Trichophyton* strains, Ser^{395}Pro and Ser^{443}Pro, were discovered. In vitro resistance testing revealed that 13 strains out of 29 (45%) were terbinafine-resistant. Point mutation analysis showed that among 13 resistant strains, 10 exhibited Phe^{397}Leu amino acid substitution of squalene epoxidase (SQLE). Two resistant strains showed combined Phe^{397}Leu and Ala^{448}Thr, and one strain single Leu^{393}Phe substitution. The dramatic increase in terbinafine-resistant *T. mentagrophytes* ITS VIII from all over India within such a short period of time underscores the issue of development of resistance in patients with chronic dermatophytoses. Transmission of the Indian *T. mentagrophytes* VIII to other countries due to travel, migration and globalisation in general appears to be a serious issue from a public health perspective. The 29 Indian strains of *T. mentagrophytes* ITS VIII isolated from patients with dermatophytoses residing in Germany, presented here, are probably 'The tip of the iceberg'.

Screening for terbinafine resistance in Australian dermatophyte isolates from toenails

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Onychomycosis is frequently recalcitrant to treatment. The issue of terbinafine resistance has been raised by the current epidemic of dermatophytoses in India.

Laboratory identification and microdilution susceptibility testing of the causative agent is costly and time consuming. It may be more efficient to screen cultured isolates for resistance. A screening assay, covering a range of terbinafine concentrations, was developed to indicate which clinical strains might be resistant. Four 6 cm Petri dishes were used per isolate. All plates contained solid PDA with 0, 0.05 µg/ml, 0.28 µg/ml or 0.5 µg/ml terbinafine concentrations. From each isolate, 4 mm^2 of conidia/hyphae was scraped from the colony surface, suspended in 5 ml of sterile water and vortexed. From this suspension, 0.15 µl was spread onto each plate, incubated for up to 7 days and checked for growth. Growth on any of the three terbinafine-containing plates was considered to indicate a degree of resistance. In this way seventy-three isolates were tested: 59 isolates of *Trichophyton interdigitale*, 2 of *T. rubrum*, 10 of *Arthroderma quadrifidum*, 1 of *A. melbournense* and 1 *of Paraphyton cookei*, along with known MIC reference strains of *T. rubrum* and *T. interdigitale.* Four strains (6.8%) of *T. interdigitale* showed good growth on all terbinafine-containing plates. Two strains (20%) of *A. quadrifidum* grew well on the 0.05 µg/ml plates. One *A. quadrifidum* isolate did not grow on the stronger concentrations and the other showed a reduction in colony count and size. These results indicate that this method may be useful in screening for resistance, giving quick information to clinicians who could guide treatment. It may also enable cost-effective testing of a large number of isolates, facilitating the identification of resistant strains for further study. Routine screening would also establish data regarding resistance in regions under study. This screening assay could further be adapted for the testing of other antifungals.

Multiple resistances of Indian *Trichophyton mentagrophytes* squalene epoxidase double mutants

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The Indian population of *T. mentagrophytes* ITS genotype VIII shows a high amount of different *erg1* (ergosterol) mutants encoding for squalene epoxidase, which catalyses the first step of ergosterol biosynthesis. The phenotype of point mutations at position $Ala^{448}Thr$ in single and double *erg1 T. mentagrophytes* mutants was analysed in detail because mutants of this type were abundantly found within the Indian fungal population. Growth in fluconazole- or terbinafine-containing medium was analysed using a growth assay based on microplate-laser-nephelometry (MLN). $Ala^{448}Thr$ *erg1* single mutants were sensitive to terbinafine

but about 50% of isolates showed an increased resistance to fluconazole, whereas 95% of the double mutants (Phe[397]Leu, Ala[448]Thr) demonstrated combined resistance to terbinafine and fluconazole. The new Indian *T. mentagrophytes* populations show several point mutations in *erg1*. Point mutations at position 397 were previously described to cause resistance to terbinafine. A large part of the double mutants exhibit resistance to terbinafine and fluconazole, demonstrating a selective advantage of the combination of both mutations.

First case of *tinea corporis* due to *Trichophyton mentagrophytes* genotype VIII in South-East Europe (Greece)

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Changes in dermatophyte epidemiology in Europe should be continuously monitored and reported in view of the increased human mobility from and to endemic areas and the increased cohabitation of humans with animal carriers. Recently, zoophilic *Trichophyton mentagrophytes* genotype VIII, presenting increased resistance to antifungals, has been reported from Asian countries such as India. The present report is the first case of *tinea corporis* caused by this genotype in Greece.

It concerns a 32-year-old male patient with a multiple skin lesion of 6 months duration, discovered in 2019. The annular or arch-like with central clearing lesions were located in the nuchal area, axillae and left calf. The patient had received oral terbinafine 250 mg o.d. for 2 months with only partial response. He reported no contact with animals, close contact with potentially infected people, visit of athletic spaces, and he had travelled in Egypt and UAE in 2018 as a mariner on a commercial ship, living in exclusive premises. Direct microscopy was positive for hyphae and the culture produced a mould with granular surface producing spherical to elongated microconidia. Internal transcribed Spacer (ITS) sequencing of the strain showed it to belong to *T. mentagrophytes* genotype VIII. Antifungal susceptibilities were determined by means of microdilution standard CLSI M38-A2 and the strain had high MICs to clotrimazole, fluconazole and terbinafine, low ones to itraconazole and ketoconazole, and MICs of ≤1 µg/ml to amorolfine, ciclopirox and griseofulvin. The patient was treated with local ketoconazole b.i.d. for 3 months and itraconazole 200 mg o.d. for 1.5 months and subsequently 400 mg o.d. for one month, with complete recovery.

This is the first report of zoophilic resistant *T. mentagrophytes* genotype VIII from Greece. It has also been reported from India and countries such as Iran,

Oman, Australia and European countries such as the Netherlands. Although our patient had travelled in the Middle East, the source of infection remains unclear. It is also possible that the fungus has entered Greece and the transmission happened inside the country in an anthropophilic manner. Further surveillance of dermatophytes from *tinea corporis* is necessary and will clarify the actual epidemiology of the genotype.

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Epidemiological survey of dermatophyte resistance in Europe

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Dermatophytosis, *tinea*, is a common infection distributed world-wide. It has until recently been considered a mild infection and easy to treat. Unfortunately, an epidemic of atypical widespread recalcitrant and terbinafine-resistant dermatophytosis is currently spreading in India. For some years it was considered an Indian problem only, but sporadic reports of resistance have been noted in Germany, Switzerland and Denmark, both as imported cases and among European residents. Therefore, a pilot study under the auspices of the European Academy of Dermatology and Venereology (EADV) Task Force of Mycology was initiated in order to explore possible European clinically and mycologically proven antifungal drug resistance to dermatophytes.

A standardised questionnaire was sent through the EADV Task Force of Mycology network to dermatologists in Europe as a pilot study to explore the extent of the problem. The study is ongoing, but to date, results from 20 countries have been obtained, of which 15 (79 %) have observed clinical and/or mycological antifungal resistance. Dermatophyte species with suspected resistance include *Microsporum canis*, *Trichophyton mentagrophytes* [6 specified as genotype VIII (Indian strain)], *T. rubrum*, *T. interdigitale*, *T. tonsurans*, *T. verrucosum*, *T. violaceum*, *M. audouinii*, *Epidermophyton floccosum* and *Nannizzia gypsea*. The most prevalent drug with antifungal treatment failure was terbinafine (approx. 50% of the cases) followed by itraconazole, fluconazole and griseofulvin.

This pilot study confirms that both clinically and mycologically, anti-fungal resistance exists in Europe.

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SESSION 2: TAXONOMY OF KERATINOPHILIC AND DIMORPHIC FUNGI

An overview of molecular phylogeny of *Onygenales*

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Members of the order *Onygenales* can be found in diverse sources. Before the molecular techniques were introduced in mycology, identification and classification of the species were mainly based on morphological features. Molecular techniques have provided new aspects and species have been described in the *Ajellomycetaceae*, *Arachnomycetaceae*, *Arthrodermataceae*, *Ascospheraceae*, *Eramascaceae*, *Gymnoascaceae*, *Myxotrichaceae*, *Nannizziopsidaceae*, *Onygenaceae*, *Spiromastigaceae*, and as *incertae cedis* in the order. However, using molecular techniques or morphological characteristics alone is not adequate to classify and describe species, and causes nomenclatural and taxonomic problems. Additionally, while the number of newly described species is increasing, the relationship between ecological behaviour and taxonomy of the species is underestimated. In the current study, multilocus phylogeny using TUB, RPB, TEF3, ITS, and LSU loci was compared with the ecology of the species. ITS and LSU loci

with Bayesian inference analyses provided better resolution than the other loci at the family level. Taxonomic resolution and habitat choice of the families *Ajellomycetaceae*, *Arthrodermataceae*, *Ascospheraceae*, and *Nannizziopsidaceae* were well defined. Members of *Gymnoascaceae*, *Onygenaceae* and *Spiromastigaceae* did not reveal consistent habitat preference, but a certain tendency was found among the clades in the families. A phylogeny based on LSU loci could be useful to establish a phylogenetic relationship between the families in the *Onygenales*. Number and diversity of the species and methods used in the analyses affect the results. The strict differentiation between phylogenetically close but ecologically very different families might be due to inadequate sampling or unknown species which could be member of a new family intermediate between two known families.

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New genera of *Onygenales*: their morphology, ecology and molecular phylogeny

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The *Onygenales* order is unique in the sense that most of its members have affinity to keratin. This feature of keratin degradation is rarely found in any other order in the whole fungal kingdom. There are around 55 genera currently known in the *Onygenales* and it seems that there are several other ones which are still undescribed. Recently, we found two of these genera in the soil of Maharashtra State, India, in keratin-rich habitats. These two genera, *Currahmyces* and *Canomyces*, are phylogenetically placed in the *Onygenaceae* family and both are monotypic. *Currahmyces* has fragile ascomata which could not be picked up intact with a needle at maturity. It was isolated from only one soil sample (hen-resting area) out of more than 500 analysed. Its peridial hyphae possess spiny crystals, which makes it unique in the *Onygenales*. The other genus, *Canomyces*, was also isolated from only one soil sample (collected under a tree) out of >500 analysed. Both are phylogenetically close to *Neogymnomyces* and *Renispora*, but are distinct morphologically. Our study indicates that there is a need to explore a broader geographical area to unearth rare Onygenalean taxa from keratin-rich habitats. It is reiterated that, had we missed to collect the two soil samples in our study, we would not have isolated and reported these new genera of *Onygenales*.

Intraspecific diversity and taxonomy of *Emmonsia crescens*

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Emmonsia crescens is known as an environmental pathogen causing adiaspiromycosis in small rodents. As the generic name *Emmonsia* is no longer available for this species, its taxonomic position is reevaluated. The intraspecific variation of *Emmonsia crescens* was analysed using molecular, morphological and physiological data, and the relationship between frequency of adiaspiromycosis and body temperature of host animals was explored. A North American and a pan-global lineage could be discerned, each with subclusters at low genetic distance. European strains produced the classical type of very large adiaspores, while in the North American lineage adiaspores were relatively small, resembling the broad-based budding cells of *Blastomyces*. Members of the closely related genus *Emergomyces* may exhibit large, broad-based cells in addition to small, narrow-based budding cells. We conclude that the morphology of the pathogenic phase in these fungi differs gradationally between species and even populations, and is therefore less suitable as a diagnostic criterion for generic delimitation. Two *Emmonsia* species are reclassified in *Emergomyces*.

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New *Keratinophyton* species (*Onygenaceae*) from Europe

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Four new *Keratinophyton* species (Ascomycota: Pezizomycotina, *Onygenales*), isolated from soil samples originating from Europe (Austria, Italy and Slovakia), are described, illustrated and presented here. The new taxa are well supported by phylogenetic analysis of the internal transcribed spacer (ITS) region, the nuclear large subunit (LSU) rDNA, and their phenotype. Within the *Keratinophyton* clade, *Keratinophyton* sp. 1 is clustered with *K. durum*, *K. hubeiense*, *K. submersum* and *K. siglerae*, while *Keratinophyton* sp. 2, *Keratinophyton* sp. 3 and *Keratinophyton* sp. 4 are resolved in a separate terminal cluster. All four new species can be well distinguished from other asexual taxa in the genus *Keratinophyton* based on phenotypical characteristics alone. Ten new combinations are proposed for all other *Chrysosporium* asexual morphs which are resolved in the monophyletic *Keratinophyton* clade.

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SESSION 3: TAXONOMY OF DERMATOPHYTES

Revised taxonomy of zoonotic pathogens in the *Trichophyton benhamiae* complex

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Species of the *Trichophyton benhamiae* complex are predominantly zoophilic pathogens with a worldwide distribution. These pathogens have recently become important due to their epidemic spread in pets and pet owners. Considerable genetic and phenotypic variability has been revealed in these emerging pathogens, but the species limits and host spectra have not been clearly elucidated. In this study, we used an approach combining phylogenetic analysis based on four loci, population-genetic data, phenotypic and physiological analysis, mating type gene characterisation and ecological data to resolve the taxonomy of these pathogens. This approach supported the distinction of nine taxa in the complex, including three new species and one new variety. *Trichophyton benhamiae* var. *luteum* var. nov. ('yellow phenotype' strains) is currently a major cause of zoonotic *tinea corporis* and *capitis* in Europe (mostly transmitted from guinea pigs). This variety exhibits unique phenotypic and ecological characteristics compared to *T. benhamiae* var. *benhamiae* and is distinguishable by using microsatellite markers but not with the conventional DNA sequence markers used here. We demonstrated that isolates of the 'white phenotype' do not form a monophyletic group and are divided into *T. benhamiae* var. *benhamiae* (mostly from North America; dogs), *T. europaeum* sp. nov. (mostly from Europe; guinea pigs), and *T. japonicum* sp. nov. (the major cause of zoonotic infections in Japan, but also found in Europe; rabbits and guinea pigs). The new species *T. africanum* sp. nov. is proposed for the 'African race' of *T. benhamiae*. The introduction to new geographic areas and host jump followed by extinction of one mating type gene have played important roles in the evolution of these pathogens. A microsatellite typing scheme consisting of ten markers was developed for the purpose of epidemiological surveillance of these emerging pathogens. Our preliminary data showed that the MALDI-TOF mass spectrometry method is able to discriminate between the newly proposed species and varieties, suggesting that this method is useful for identification in clinical practice.

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Molecular and physiological analysis of the *Trichophyton mentagrophytes / T. interdigitale* species complex using a global set of strains

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A severe outbreak of highly virulent and multi-resistant dermatophytosis is ongoing in India. There is a debate about the correct identification of the etiologic agent: *Trichophyton mentagrophytes* or *T. interdigitale*. In order to distinguish the species limits, a taxonomic study was undertaken combining molecular, morphological, and physiological characteristics as classification evidence. The molecular characteristics show that *T. mentagrophytes* s. str., *Trichophyton indotineae* and *T. interdigitale* s. str. can be distinguished by sequences of the HMG gene. Besides, the entities can be separated in a multilocus analysis using tanglegrams. Concerning morphological characteristics, the colony pigmentation of *T. mentagrophytes* and *T. interdigitale* is similar, but *Trichophyton indotineae* is different. Physiological studies showed that *T. interdigitale* and *T. mentagrophytes* strains had high urease, keratinase and lipophilic activities. Conversely, *Trichophyton indotineae* strains tended to have lower urease, keratinase and lipophilic activity. From these preliminary data it may be concluded that *Trichophyton indotineae* represents a separate species. Since we are dealing with a serious public health problem, it will be important to establish the origin of the disease, either zoonotic or widespread misuse of antifungal creams.

Correlation between genotype, clinical picture and morphology of *Trichophyton interdigitale / T. mentagrophytes* isolates

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Trichophyton interdigitale is one of the most common agents of *tinea pedis* and onychomycosis in humans. The closely related species *T. mentagrophytes* is primarily the agent of superficial infections in animals (e.g. rabbits, cats and dogs), but it also commonly causes zoonotic infections in humans, especially *tinea corporis*. Due to the differences in the source of infections and the clinical manifestation, the differentiation of these species is clinically and epidemiologically significant. Based on the original concept, these species should be distinguishable by characteristic phenotypic features, including macromorphology of colonies, presence/absence of macroconidia and spiral hyphae, etc. However, other recent studies have indicated that the correlation between the clinical picture of infection and phenotype and genotype of the pathogen is not so clear as expected. Due to this, the differentiation of these taxa mostly relies on the several substitutions in the internal transcribed spacer region (ITS).

The aim of this study was to examine the correlation between multilocus genotype, clinical picture and morphology of *T. interdigitale* / *T. mentagrophytes*. For this purpose, a total of 120 isolates were obtained from Czech patients with various clinical manifestations of dermatophytosis (*tinea pedis*, *corporis* and *unguium*). An analysis of micro- and macromorphology, and physiology was performed together with molecular characterisation of the strains by DNA sequences from three loci: ITS, β-tubulin and translation elongation factor 1-α (TEF). The mating-type genes were also characterised. A recombination analysis was performed to identify recombining lineages. Statistical analysis of the association of the mentioned features with recombining lineages was performed.

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Deciphering the composition of dermatophyte complexes

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In recent years many advances have been made in clearing up the phylogenetic relationships within the family *Arthrodermataceae*. However, certain closely related taxa still contain poorly resolved species boundaries. Here we try to elucidate the species composition of the *Trichophyton rubrum* and *T. benhamiae* species complexes using a combined approach consisting of multi-gene phylogenetic analysis, morphological analysis and spectral comparison. Within the species *T. benhamiae* we demonstrate the existence of at least 3 species which

are distinguishable using common phylogenetic markers (ITS and β-tubulin 2), maldi-tof spectrometry and morphological differences. We also confirm that the distinct "yellow" and "white" phenotypes of *T. benhamiae* do not have a clear genetic basis and should thus be considered varieties. Some lineages within the *T. benhamiae* complex show minimal but distinct genetic differences from one another while being phenotypically indistinguishable, raising the question where the species boundaries should be drawn in the genomic era. In the *T. rubrum* complex we show the existence of 4 genetic lineages, each displaying its own distinct morphological and spectral characteristics. ITS and Bt2 genetic markers are, however, not sufficient to acknowledge these lineages as monophyletic species, so perhaps other biomolecular techniques should be explored.

SESSION 4: POPULATION GENETICS AND GENOMICS

Unravelling the genetic variability of *Microsporum canis*

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Microsporum canis is the most common dermatophyte in domestic and wild carnivores, with cats considered to be the most important reservoir hosts. The species is distributed worldwide and plays an important zoonotic role. Our research group has been working for years on the characterisation of this dermatophyte at strain level. The method employed is based on a panel of 8 microsatellite markers. Over time we have built a database which includes information regarding fungal isolates coming from different hosts (animals and humans) and geographical origins. Most of the isolates were collected from Europe (Italy, France, Germany, Austria, Belgium) but we also had the opportunity to analyse isolates from Egypt, Turkey, China, Korea and Brasil. The microsatellite typing technique allowed us to assign a multi-locus genotype (MS type) associated with a serial number to each isolate. Sampling sites are reported on a freely accessible Google Map document to allow an overview of the MS-type distribution and a 'geographically based' access to the data. We have obtained a total of 91 different MS types from 264 samples (with a 'clone-corrected' approach) which corresponds to a genetic diversity of 96%. Some MS types are overrepresented, suggesting the presence of clonal lines of 'major success' due to a stronger parasitic aptitude. Furthermore, some MS types appear related to

specific geographical origins (e.g. MS types 5 and 90, largely distributed in Europe and Asia, respectively). However, the presence of genetic lineages with higher zoonotic potential is poorly supported. Indeed, considering the isolates from Europe (where we collected a similar number of samples coming from humans and animals), it can be noted that many MS types were responsible for human infection (45 MS types out of 124 samples, genetic diversity 94%), with a similarly high genetic diversity also found in feline and canine populations.

This database and the related Google map represent a useful tool for researchers with interest in the genetic diversity of dermatophytes. Database and map may indeed make it possible to compare results obtained for isolates in other parts of the World.

Genotyping of Russian isolates of fungal pathogen *Trichophyton rubrum*, based on simple sequence repeat and single nucleotide polymorphism

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The *Trichophyton rubrum* species group consists of prevalent causative agents of human skin, nail and hair infections, including *T. rubrum* sensu stricto and *T. violaceum*, as well as other less well established or debatable taxa like *T. soudanense*, *T. kuryangei* and *T. megninii*. Our previous study provided limited evidence in favour of the existence of two genetic lineages in the Russian *T. rubrum* sensu stricto population. We aimed to study the genetic structure of the Russian population of *T. rubrum*, and to identify factors shaping this structure. We analysed the polymorphism of 12 simple sequence repeat (SSR, or microsatellite) markers and single-nucleotide polymorphism in the TERG_02941 protein-coding gene in 70 *T. rubrum* isolates and performed a phylogenomic reconstruction. All three types of data provided evidence that the population consists of two genetic lineages. Clustering, performed by means of microsatellite length polymorphism analysis, was strongly dependent on the number of nucleotide repeats in the 5'-area of the fructose-1,6-bisphosphate aldolase gene. Analysis of molecular variance (AMOVA) on the basis of SSR typing data indicated that 22–48% of the variability was among groups within *T. rubrum*. There was no clear connection of population structure with types of infection, places of geographic origin, aldolase gene expression or urease activity. Our results suggest that the Russian population of *T. rubrum* consists of two cosmopolitan genetic lineages.

Population biology of *Trichophyton erinacei*, an emerging cause of dermatophytosis

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Trichophyton erinacei is increasingly reported as a cause of dermatophytosis in wild and pet hedgehogs, their breeders, and owners worldwide. The pathogen was originally described in the European hedgehog (*Erinaceus europaeus*), occurring naturally in the UK and Northern and Western Europe; but it has also been imported to New Zealand and Japan. Moreover, *T. erinacei* has been reported from the African wild-living four-toed hedgehog (*Atelerix albiventris*), which has become a popular pet animal worldwide. Little is known about the taxonomy and population genetics of this pathogen despite its increasing importance in clinical practice. Especially it is not known if there are different populations or even cryptic species associated with different animal hosts or geographic regions. To answer these questions, we assembled more than 170 strains from animals and humans isolated in different European countries, Africa, New Zealand, and Japan. We conducted DNA sequencing of four genetic loci, microsatellite analysis (7 loci), analysis of morphology, and physiological testing. Three populations were found among the examined isolates. One population was associated with free-living African hedgehogs, a second with pet African hedgehogs from the households of different countries, and the last population was isolated exclusively from wild-living European hedgehogs (*Erinaceus europaeus*). Based on the close genetic relatedness of strains from the first two populations, we assume that the population of *T. erinacei* occurring in pet African hedgehogs originated from Africa and was introduced into many countries by animal trade. The low genetic diversity of the pathogen in pet hedgehogs may indicate a founder effect and clonal spread of the pathogen. Preliminary phenotypic data and relatively low genetic divergences between populations do not support recognition of these population as separate species despite significant ecological differences.

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Onygenalean fungi detected in *Martes zibellina* by culturomics and NGS approaches

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The aim of the study was to detect potentially pathogenic fungi in skin lesions of wild sables (*Martes zibellina*) from the Siberia region of Russia. Outbreaks of mass skin lesions in Siberian sables are known for decades, but their true etiology is still unknown. To reveal the possible etiological role of fungi, 15 samples from affected skins were submitted to conventional mycological investigation, and three of these samples were subjected to next-generation sequencing (NGS), targeting the internal transcribed spacer (ITS) region on an Illumina MiSeq instrument. Of the three samples, only one yielded a sufficient quantity of fungal DNA to perform NGS analysis. A total of 34,810 assigned sequences and 249 operational taxonomic units (OTU) were yielded from the sample with NGS. Of these, 9% OTU belonged to the *Onygenales* order. In *Onygenales*, the *Arthrodermataceae* fraction was 18%, *incertae sedis* families 81%, *Ajellomycetaceae* 0.5%, and an unidentified family 0.5%. In *Arthrodermataceae*, the genus *Arthroderma* was represented by the species *A. insingulare*. The genus *Trichophyton* included *T. onychocola* and *Trichophyton* sp. Interestingly, the *Trichophyton* sp. was closely related to *Arthroderma cuniculi* (93.5% of ITS homology revealed by BLAST). In *incertae sedis* families, the genus *Chrysosporium* dominated and included *C. carmichaelii*, *C. undulatum* and *Chrysosporium* spp. (unidentified). The genus *Malbranchea* was represented by *M. cinnamomea* only. The *Ajellomycetaceae* family was represented by the genus *Emmonsia*, which consisted of *E. helica* only, the possible agent of adiaspiromycosis. From the cultural study, 31 fungal isolates were obtained, three of them belonging to *Onygenales* (9.6%). Two of them were preliminarily identified as *Arthroderma cuniculi* (GenBank accessions MN534766.1 and MN653980.1) and the third as *Chrysosporium carmichaelii* (GenBank accession MT556012.1). Each of the Onygenalean fungi was isolated from different skin samples, and no *Onygenales* representatives were isolated from the NGS-processed sample. In conclusion, a range of

keratinophilic fungi belonging to *Onygenales* were detected in affected wild sables, thus showing a probable fungal etiology in the mentioned mass skin disease. NGS revealed that up to six keratinophilic species can be found in the same animal. Of these, only two were detected by means of conventional cultural analysis. Pathogenic properties of the detected Onygenalian fungi should be established in successive studies.

Detection and monitoring of *Coccidioides* spp. in the environment

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Valley fever or coccidioidomycosis is a life-threatening fungal infection caused by inhalation of arthroconidia (airborne particles) of the two closely related species, *Coccidioides immitis* and *C. posadasii*, present in soil and dust of desert areas in South, Central and North America. Unlike most other human pathogenic fungi, *Coccidioides* spp. can infect immunocompetent individuals and cause a broad spectrum of diseases ranging from asymptomatic infection to mild respiratory illness to severe life-threatening infections. The total number of infections remains unknown: in the United States, over 10,000 of human cases are reported each year and the actual burden of the disease is estimated to be close to 100,000 cases per year. The disease is also prevalent in Mexico and has been reported in Argentina, Bolivia, Brazil, Guatemala, Panama and Paraguay. Recent data suggest that the incidence of valley fever is increasing and the pathogen's endemic range is expanding. For example, in Arizona, the reported incidence of valley fever has risen by 900% in the past decade. Furthermore, in the United States, clusters of valley fever and viable arthroconidia of *C. immitis* in the environment have been reported in the southeast of Washington State, well outside of the currently accepted endemic range. Although the exact causes of the increased incidence and the expanding geographic range of coccidioidomycosis are unknown, climate and population changes which have occurred in recent decades have likely contributed to these trends.

To better understand the distribution and spread of *Coccidioides* spp. in the environment and to develop measures to prevent human exposure, we have developed several methods for detection and monitoring of these pathogens in soil and air samples. This presentation discusses our recent findings, describing the distribution of *Coccidioides* spp. in the US.

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Genomic diversity and population differentiation of *Histoplasma* spp. in the American continent

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Histoplasmosis is one of the most prevalent fungal infections (500,000 infections estimated per year) and is one of the most common pulmonary diseases in the world. The prevalence of histoplasmosis varies greatly by continent, being rare in Europe and Oceania, moderately common in Africa and Southeast Asia, and frequent in the Americas. In recent studies, Teixeira et al. (2016) proposed at least 17 cryptic phylogenetic species causing histoplasmosis worldwide with strong geographical bias. Based on phylogenomic concordance, Sepúlveda et al. (2017) contributed to a further refinement of the clustering of *Histoplasma* isolates, reclassifying 4 *Histoplasma* phylogenetic lineages into 4 species as follows: NAm 1 (*H. mississippiense*), NAm2 (*H. ohiense*), LAm A (*H. suramericanum*), Panama (*H. capsulatum* sensu stricto). However, only a low number of taxa were genotyped using fully sequenced genomes. As part of the global consortium for *Histoplasma* whole-genome typing project, we used Illumina short-read sequencing to evaluate DNA polymorphisms, phylogenomic and population distribution of over 200 strains from the American continent. We investigated the effects of the Ohio and Mississippi Valleys on the *Histoplasma* species distribution and admixture in North America. Moreover, a high genomic variation and complex population structure was observed in isolates retrieved from countries nested within the Guyana Shield and Amazon basin.

SESSION 5: EMERGING AND ZOONOTIC PATHOGENS

Emergomycosis – the global rise of a new dimorphic and systemic mycosis: current trends and future perspectives

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Emergomycosis is a systemic fungal disease caused by thermally dimorphic fungi of the genus *Emergomyces*, named because of its recent global emergence. It was formerly classified under the genus *Emmonsia* but a taxonomic revision based on ribosomal DNA sequences, using concatenated sequence data of the loci LSU, ITS, TUB2, TEF3 and RPB2, has placed all *Emmonsia*-like fungi in a separate genus, *Emergomyces*. The genus contains *Emergomyces pasteurianus* (most widespread) as the type species and the species *Emergomyces africanus*, *E. canadensis*, *E. orientalis* and *E. europaeus*. Whole-genome sequencing data show that both *E. africanus* and *E. pasteurianus* contain the alpha mating-type locus (MAT1-1).

The disease classically manifests itself in disseminated form with extensive cutaneous involvement, usually in immunodeficient patients. So far, only a few sporadic cases have been reported from Asia, Europe, Africa and North America. However, considering the overwhelming population of immunocompromised patients, it is presumed that the disease has a worldwide distribution with many cases going undetected. Emergomycosis should be considered in the differential diagnosis of histoplasmosis, as there is considerable clinical and histopathological overlap between the two diseases. Internal transcribed spacer (ITS) sequencing of ribosomal DNA is the gold standard for identification but its application is jeopardised in resource-limited settings. Therefore, the development of an affordable, accessible and feasible diagnostic test should be prioritised to enable the diagnosis in endemic regions and also for epidemiological surveillance. There are limited studies on the antifungal susceptibility profile of *Emergomyces*, and clinical breakpoints and consensus treatment guidelines are missing. This presentation addresses taxonomic, clinical, diagnostic and therapeutic aspects of emergomycosis worldwide. It also highlights the potential areas of future studies which may open up new therapeutic approaches for better patient management and improved outcomes. More research is wanted to understand the geographic range, ecology, epidemiology and immunopathogenesis of this mycosis, to understand the full clinical spectrum of the disease and to optimise clinical diagnostics, therapeutic efficacy and overall patient management.

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Emerging fungal pathogens of captive reptiles in the Russian Federation

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Exotic reptiles have taken in a niche of popular companion animals in recent decades. However, the importance of fungal infections in reptiles is still underestimated in many cases. Mycoses in reptiles can be caused by a variety of fungal species. However, the leading role belongs to representatives of the genera *Nannizziopsis*, *Paranannizziopsis*, *Ophidiomyces* (*Onygenales*, *Onygenaceae*), which were previously treated as members of the anamorphic polyphyletic genus *Chrysosporium*. Here we present our data on the etiology of fungal infections in reptiles in Russia. Ninety-two samples from pet reptiles demonstrating skin lesions were examined mycologically, most samples (47%) from green iguanas (*Iguana iguana*). Fungal etiology was confirmed in a total of 79% of reptiles. The prevalent pathogen was identified with conventional methods as *Chrysosporium* anamorph of *Nannizziopsis vriesii* (37%). The ITS sequencing of typical isolates from green iguana revealed 100% homology with *Nannizziopsis guarroi* (GenBank accessions MN443762, MN443763). Based on observations in the Moscow Zoo, the predisposed reptile species were green iguana (*Iguana iguana*) and bearded dragons (*Pogona vitticeps*). Moreover, mycoses caused by *Chrysosporium*-related fungi were diagnosed in reptile species *Tiliqua scincoides*, *Lacerta lepida*, *Lacerta rudis*, *Sceloporus occidentalis*, *Homopus areolatus*, *Pelodiscus sinensis* and *Varanus exanthematicus*. *Chrysosporium*-related pathogens in these species had previously not been reported. In snakes, fungal infections have suddenly emerged in recent years. *Ophidiomyces ophiodiicola* is currently treated as the major pathogen of snake fungal disease (SFD) in wild and captive snakes. Recently we diagnosed three cases of *O. ophiodiicola* in file snakes *Acrochordus granulatus* imported into Russia from Indonesia. The cultures were isolated on DTM-type media and the identification was performed by ITS sequencing (GenBank accession MT271736). Snakes showed multiple bullous white vesicles filled with exudate. All three snakes died within a month after arrival. In conclusion, Onygenalean fungi have recently emerged as cardinal pathogens in captive reptiles. Pathogenic species *N. guarroi* and *O. ophiodiicola* were detected in Russia for the first time.

Ringworm in wild European hedgehogs: clinical and therapeutic approach in a French wildlife rehabilitation centre

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The increasing number of European hedgehogs (*Erinaceus europaeus*) admitted every year to wildlife rehabilitation centres in Western Europe might be a source of concern to animal and public health, since transmissible diseases such as dermatophytosis can be easily spread after direct contact with affected animals. The objective of the present study was to determine the presence of dermatophytes in hedgehogs admitted to a wildlife rehabilitation centre in the Ile-de-France region, France, and to assess the risk of contamination in order to adapt control measures. A longitudinal cohort study was performed in 412 hedgehogs hosted at the Wildlife Animal Hospital of the Veterinary College of Alfort (Chuv-FS) from January to December 2016. Animals were sampled once a month for fungal culture tests. Dermatophyte colonies were obtained from 177 out of 726 skin surface samples (29.5%). *Trichophyton erinacei* was the predominant species, detected in 23.3% of sampled animals, and its distribution does not seem to be associated with age, sex, season or geographical origin. Among *T. erinacei* positive animals, 79.2% were asymptomatic carriers. Although healing required several months of treatment with topical and systemic azoles, dermatophytosis did not seem to reduce the probability of release. This study provides some suggestions to be adopted by animal care workers at rescue centres.

Nation-wide analysis of prevalence and proliferation factors of the zoonotic dermatophyte *Trichophyton benhamiae* in Germany

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For about 10 years, a new variant of the pathogen *Trichophyton benhamiae* has been appearing in Germany, characterised by a previously unobserved culture phenotype with a strong yellow reverse. A few studies suggest that this new variety is now the most common zoophilic dermatophyte in Germany. Guinea pigs are the main carrier. Exact prevalence measurements are not yet available.

Thus, the aim of the present study was to collect data on the frequency and geographic distribution of the pathogen and its phenotypes (white and yellow) in humans and guinea pigs throughout Germany. Studies have already shown that animals from large breeding farms are affected particularly heavily. In contrast to this, 21 small private breeds were sampled and husbandry conditions recorded. This enabled us to identify propagation factors and to give recommendations for containment. For animals from private breeds, we detected *T. benhamiae* with a prevalence of 55.4%, which is less than half compared to animals from large breeding farms. As risk factors, we identified the type of husbandry and the contact to other breeds. Furthermore, certain breeds, like Rex guinea pigs and breeds with long curly hair were predestined for colonisation by *T. benhamiae* due to their phenotypic coat characteristics. A prevalence of 36.2% was determined for symptomatic pet guinea pigs suspected of having dermatophytosis, which is comparable to the study by Kraemer et al., showing a prevalence of 34.9% in 2009 in Germany. The prevalence in humans is stable with about 2–3%, comparing data of 2010–2013 and 2018 in Thuringia. The new type of *T. benhamiae* was by far the most frequent cause in all settings.

SESSION 6: EPIDEMIOLOGY

Belgian national survey on *tinea capitis*: epidemiological considerations

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Tinea capitis (TC) is a superficial infection of the scalp caused by dermatophyte fungi which affects mainly prepubescent children. In the past decade, a huge increase of African anthropophilic strains causing *tinea capitis* has been observed in Europe, probably due to immigration waves from African countries. The Belgian National Reference Center for Mycosis (NRC) conducted a surveillance study of TC in 2018.

Belgian laboratories were invited to send all dermatophyte strains isolated from scalps from January to December 2018. Dermatologists were involved and were asked to fill in a form containing various epidemiological information about

the patient. Strain identification was confirmed by means of ITS sequencing. A multiplex pan-dermatophyte real time PCR assay (DermaGenius®, PathoNostics) was applied if necessary.

A total of 337 strains were collected from 337 patients. The main population affected by TC was children from 5–9 years. Males were more affected than females. The majority of the strains was collected in the area of Brussels followed by Liege. Other Belgian towns were less affected by TC. Among people of known ethnical origin, African natives were more affected by TC than Europeans. The main transmission mode of TC was the familial way. The major etiological agent was *Microsporum audouinii*, followed by *Trichophyton soudanense*.

African anthropophilic dermatophytes are mainly responsible for *tinea capitis* in Belgium. Large cosmopolitan cities like Brussels and Liege are the most affected. People of African origin are the most affected by TC.

Eight-year molecular epidemiology of dermatophytosis in the Czech Republic and antifungal susceptibility patterns of selected species

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A large-scale molecular epidemiology project has been conducted by a collaborative network of 8 institutions in the Czech Republic. Isolates and associated data obtained between 2012 and 2019 were analysed. This huge collection comprising almost nine thousand isolates has been set up and identified using ITS rDNA sequencing (with exception of morphologically typical *Trichophyton rubrum* strains). In total, 30 dermatophyte species were identified, including several hitherto undescribed species. In the Czech Republic, the most common clinical entity is onychomycosis, predominantly caused by *T. rubrum* and *T. interdigitale*

(together almost 99% of all cases). *Tinea pedis* has a similar etiology. *Tinea corporis* is mostly caused by anthropophilic species (~54%), especially by *T. rubrum* (~51%), and zoophilic species (~42%), especially by members of the *T. benhamiae* complex (~24%), *Microsporum canis* (~10%) and *T. interdigitale / T. mentagrophytes* (~8%). The remaining 4% of *tinea corporis* cases are caused by species-rich geophilic dermatophytes. *Tinea capitis* is a relatively uncommon clinical unit in the Czech Republic, which is predominantly of zoonotic origin (*M. canis* and species of the *T. benhamiae* complex cause >70% of cases).

In 2019, we established standardised antifungal susceptibility testing methods for filamentous fungi (EUCAST-AFST methodology), including dermatophytes, which had not been available in the Czech Republic. Antifungal susceptibilities to the eight antifungals (fluconazole, terbinafine, itraconazole, ketoconazole, clotrimazole, amorolfine, ciclopirox olamine and efinaconazole) were determined in sixty-nine isolates of zoophilic species of the *T. benhamiae* complex. A similar approach was used in 70 geophilic *Arthroderma* species occurring in the clinical samples. This set comprised nine different species (e.g. *A. quadrifidum*, *A. insingulare* and *A. onychocola*). Considering the increasing incidence of terbinafine-resistant *T. interdigitale* and *T. rubrum* strains worldwide, we have started to screen for terbinafine resistance in these species since January 2020 and preliminary data are presented. The large collection of dermatophyte isolates and available genomic DNAs offer a great potential for future epidemiological, population-genetic and taxonomic investigations, and also collaboration with other laboratories worldwide.

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Trends in the epidemiology of dermatophytes and related infections in Iran

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Changes in the epidemiology of dermatophyte fungi and related infections (dermatophytosis or *tinea*) in Iran are presented. A comprehensive literature search was carried out. We found that mycological and clinical features of dermatophyte infections in Iran have been altered significantly during time. *Tinea capitis* has remained as the main dermatophytosis in preschool and school children all over the country, although its occurrence has notably decreased. In higher age groups, *tinea pedis* followed by *tinea corporis* and *tinea cruris* were the more abundant infections, and currently an increase in their occurrence is

observed. Some infections were correlated with some age groups and special species. The anthropophilic species *Trichophyton schoenleinii* and *T. violaceum* were the main species causing infection in the past, whereas currently *Epidermophyton floccosum*, *T. rubrum*, *T. tonsurans* and the *T. mentagrophytes* / *T. interdigitale* species group (TMTISG) are the most prevalent causative agents. The incidence of dermatophytoses, caused by zoophilic species *Microsporum canis* and *T. verrucosum* has decreased. *Nannizzia fulva* is the most abundant species found in the soil, but *N. gypsea* is the dominant geophilic species isolated from clinical specimens. In Iran, currently an emergence of infections with less-frequent and non-indigenous species is seen. These include e.g. *T. benhamiae*, *T. simii*, *M. ferrugineum* and also the long-forgotten species *T. eriotrephon*, all of which were detected by DNA sequencing. Some recently isolated clinical *Trichophyton* strains, phylogenetically close to *T. benhamiae*, *T. simii* and *T. quinckeanum*, potentially represent new species.

Epidemiology of dermatophyte infections in Slovenia

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Several countries have reported differences in dermatophyte epidemiology associated with human migration from endemic areas. We analysed notified dermatophytosis/*tinea* infections (N = 52,600) in Slovenia in 2006–2019. *Tinea* most frequently affected persons aged 40–55 years (19.3%) and ≥65 years of age (20.2%). Skin changes appeared most frequently on feet (17.5%), followed by trunk (11.3%), face/scalp (8.9%) and arms (7.2%). Pathogens were laboratory-confirmed in 4.5% of cases on average. In children, *Microsporum canis* (84.1%) was the most frequently diagnosed dermatophyte, and *Trichophyton rubrum* (42.8%) was the most prevalent among adults. Elderly persons and school children seem to contract the infection more likely ($p < 0.05$). To better understand and trace the source of infection, a higher proportion of laboratory-identified infections is essential. In the future, it is essential to collect precise epidemiological, clinical and microbiological/isolation data to identify potential risk factors and transmission pathways to be able to implement targeted measures, especially in more susceptible populations of older people and in schools. It is important to conduct multisectoral research on fungal infections by dermatophytes with a focus on outbreaks of virulent and resistant species in human and veterinary medicine and the environment applying the "One Health" approach.

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Molecular epidemiology of dermatophytes in Iraq and Cambodia

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From April to December 2019, skin scrapings were taken from 92 patients with superficial dermatophytoses in Baghdad, Iraq. In Phnom Penh, Cambodia, from June 2017 to July 2018, 67 patients were included in the study. Iraq: The following dermatophytes (out of 63 positive samples, 43 by culture, 20 by PCR) were found: *Trichophyton (T.) mentagrophytes* / *T. interdigitale* (TM/TI) 40 (63%, 26 by culture, 14 by PCR), *Microsporum (M.) canis* 7 (11%, 6 by culture, 1 by PCR), *Epidermophyton floccosum* 6 (10%, 3 by culture, 3 by PCR), *T. rubrum* 5 (8%, 3 by culture, 2 by PCR), *T. violaceum* 2 (3%, 2 by culture). One strain each of *Nannizzia incurvata* (2%), *T. benhamiae* (2%), and *M. ferrugineum* (2%) was isolated. Based on the results of sequencing, we were able to demonstrate that among 26 cultural isolated *T. mentagrophytes* strains, 18 were surprisingly *T. mentagrophytes* ITS Type VIII India, 5 were *T. mentagrophytes* ITS Type V Iran, and 2 belonged to the anthropophilic *T. interdigitale* ITS Type II*. Seven (39%) out of 18 Indian *T. mentagrophytes* ITS Type VIII samples were terbinafine-resistant. The resistant strains exhibited the amino acid substitution Phe^{397}Leu of the squalene epoxidase gene. Cambodia: out of 52 strains, *Trichophyton rubrum* 36 (69%, 29 by culture, 7 by PCR), TM/TI 9 (17%, 6 by culture, 3 by PCR), and *M. canis* 5 (10%, all by culture). One strain each of *N. incurvata* and *N. nana* was isolated. Two new *T. mentagrophytes* ITS Type XXV Cambodia strains were detected. One *T. mentagrophytes* strain Type VIII India was terbinafine-resistant (amino acid substitution Phe^{397}Leu of the squalene epoxidase gene). Three strains of *T. interdigitale* Type II* were isolated. In Iraq, this is the first survey on the epidemiology of dermatophytes in 20 years. Surprisingly, *T. mentagrophytes* represented the most frequent dermatophyte in Baghdad, and has replaced *T. rubrum*. The most frequent dermatophyte was *T. mentagrophytes* VIII. Currently, *T. rubrum* represents the most frequent species in Cambodia. A highlight was the first description of *T. mentagrophytes* XXV Cambodia.

SESSION 7: DIAGNOSTICS AND TREATMENT APPROACHES

Evaluation of the new Id-Fungi plates medium from Conidia® for MALDI-TOF MS identification of filamentous fungi

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The first purpose of this study was to compare the efficiency of mould/dermatophyte identification by matrix-assisted laser desorption ionisation time of flight mass spectrometry (MALDI-TOF MS) using a new medium called Id-Fungi plates from Conidia® and two different databases. For the second purpose, we evaluated the Id-Fungi plates for the direct inoculation of nail, hair and skin samples and compared the efficiency of MALDI-TOF MS identification of dermatophytes to classical methods based on culture and microscopy.

A total of 71 strains including 13 genera of filamentous fungi and 9 species of dermatophytes were cultured on Id-Fungi plates from Conidia® and Sabouraud gentamicin plates (bioMérieux, SGC2) and then identified with MALDI-TOF MS respecting several conditions and using two different databases. For an evaluation of the combination of Id-fungi plates and MALDI-TOF MS as an identification method for dermatophytes, 428 samples of hair, nail and skin were cultivated in parallel on Id-fungi plates and Sabouraud +actidione medium (bioMérieux, SAB-ACTI).

For *Aspergillus* sp. and non-*Aspergillus* moulds, the best performances were obtained on Id-Fungi plates medium after 48h growth, following protein extraction. For dermatophytes, the best condition was obtained using the Id-Fungi plates medium after 72h, after extended direct deposit. After extraction, no better result was observed for dermatophytes even after 96h of incubation. Regarding the direct inoculation of nails, hair and skin on Id-Fungi plates, 129/428 (30.1%) showed a positive culture against 150/428 (35%) on SAB-ACTI medium. Of the 129 positive strains, the identification with MALDI-TOF MS was correct for 92/129 (71.4%). This comprised dermatophytes but also non-dermatophyte species.

The Id-Fungi plates allows the generation of better spectra using MALDI-TOF MS compared to the SGC2 medium. It accelerates the growth (especially in dermatophytes) and facilitates sampling and deposit on the target plate even with fluffy fungi. Regarding the use of this medium directly on nail, hair and skin samples, this medium seems less sensitive than the SAB-ACTI medium but for positive strains, the rate of correct identification with MALDI-TOF MS is satisfactory.

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Possibilities of dermatophyte identification with the MALDI Biotyper – new and old approaches

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Moulds and dermatophytes keep appearing more frequently in the microbiological routine. The diagnosis of mould infections is often based on morphological criteria. These may encounter time-consuming sporulation as a prerequisite for identification. Based on this, molecular methods like DNA sequencing are more frequently used for identification to species and/or genus level.

MALDI-TOF MS has improved microbial identification, especially of yeasts and bacteria, dramatically in recent years. The identification of filamentous fungi and dermatophytes by MALDI has not increased as fast due to e.g. difficulties in handling and databases with limited entries. However, MALDI shows a high species resolution similar to sequencing and is faster. Here we give an overview of the current state and possibilities of identifying filamentous fungi and dermatophytes with the MALDI Biotyper, MBT (Bruker Daltonik, Bremen). Samples can either be identified directly from an agar plate, applying a protein extraction step using material from the agar plate or applying a protein extraction step from liquid cultivation. Depending on the growth behaviour of the fungus, the most suitable identification approach can be chosen. Identification success with the MALDI Biotyper also depends on the quality of the acquired spectra. Hence using a new spectrum acquisition method enables the performance of more direct identification approach and efficient measurements of samples, which will be also presented here.

As mentioned, besides the preparation and acquisition method used, the MALDI library plays an important role. In the past years, the commercially available MBT RUO filamentous fungus library has been expanded, starting from 364 database entries (44 dermatophytes, comprising 13 different dermatophyte species) to the current 786 entries (124 dermatophytes with 33 different dermatophytes). The current dermatophyte species can be divided into about 25 separate species, with the genus *Trichophyton* as the largest coherent group.

Since the taxonomy in filamentous fungi and dermatophytes is rapidly evolving, cooperation with various groups interested in the identification of fungi is highly appreciated. To provide an up-to-date and wide-ranging database, impact from the scientific community is necessary.

Inactivation of dermatophytes and treatment of onychomycosis using non-thermal plasma

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Onychomycosis is the most common nail disorder. Current treatment of this infection does not achieve ideal results in efficacy and has the potential for adverse effects. The aim of this study was to determine the optimal conditions of Non-Thermal Plasma (NTP) exposure required to inactivate the most common dermatophytes in vitro and to apply it for healing patients. NTP was applied to a group of 40 patients with onychomycosis, partially in combination with Nail Plate Abrasion and Refreshment (NPAR) or antimycotics. The cohort included 17 patients of NPAR in combination with NTP, 11 with antimycotics and NTP, and 12 with NPAR only. The exposure of dermatophytes to NTP produced by negative DC corona discharge caused efficient inactivation of *Trichophyton* spp. in its early growth phases. NTP exposure directly after inoculation led to full inactivation of *Trichophyton* and decreased to a negligible effect to exposure after 6 days after inoculation. For patients, the combination of NPAR and NTP leads to a positive effect in more than 70% of patients and the synergistic effect of NPAR and NTP shows a 85.7% improvement in mycological cure of the affected nail plate. We conclude that NTP provides many benefits to the current therapy of nail onychomycosis.

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Dealing with *Histoplasma* and *Paracoccidioides* in non-endemic areas

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Histoplasma and *Paracoccidioides* are both thermodimorphic fungi endemic in particular geographic areas. Paracoccidiomycosis is a systemic fungal disease occurring in Latin America and it is more prevalent in South America. *Histoplasma* is the infectious agent of histoplasmosis, a disease which is endemic mainly in the American continent but is now being discovered globally, but with hyperendemic areas. The epidemiology of these infections seems to be shifting. Factors such as human migration and tourism, use of immunosuppressive drugs, and organ transplantation contribute to the increase of this infectious disease.

In non-endemic areas, the medical community is less aware of this type of infections, which may delay the diagnosis or even lead to a failure in its detection. These infections spread rarely, however they can be fatal if not treated. Since they are not notifiable diseases, the true burden outside of endemic regions is not known. The diagnosis can be difficult: conventional laboratory tests include culture and histological methods which are the gold standard to diagnosis, but we deal with slow growing organisms, obtaining culture results can thus require a long time, and sensitivity is low. On the other hand, histology can be very sensitive if targeted fungal stains are used (Periodic acid–Schiff, Grocott methenamine silver) but requires practice with identification of typical structures. Despite the development of several molecular methods, these are not included as diagnostic tools for proven infections.

This presentation aims to discuss new trends in the epidemiology of these endemic infections, to describe our experience in the methods used for laboratory diagnosis of histoplasmosis and paracoccidiomycosis, and to discuss how molecular methods have contributed to the diagnosis of the cases detected. Two clinical cases already published on the subject will be presented to illustrate the decisive role of the laboratory concerning the diagnosis of these infections.

The new Atlas of Clinical Fungi

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This autumn, the Atlas of Clinical Fungi will appear in print. With several thousands of pages in full colour covering more than 700 fungi, the book provides a wealth of information on medical mycology. The several volumes are organised in analogy to the Fungal Kingdom, and because the Atlas includes numerous opportunists it is also useful for workers outside the medical area. Complementary to the search tools of the website, the book provides an overview of the rich data collected over 30 years of medical mycology, covering diagnostics, taxonomy, phylogeny, nomenclature, antifungals, and histopathology, validated by almost 8000 references. The layout makes it pleasant to read, and the photo plates are stunning, often showing unique features of the fungi. The Atlas of Clinical Fungi is a non-profit project and at € 250 (excl. postage) the book is reasonably priced. Please note that pre-ordering of the book is compulsory.

SESSION 8: VIRULENCE FACTORS AND PATHOGENESIS

In vitro evaluation of enzymatic virulence activities and antifungal susceptibility profile of *Microsporum canis* strains from various sources

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Microsporum canis is one of the major pathogenic and most prevalent dermatophytes of domestic animals causing *tinea capitis* which differs from one country to another worldwide. To have an effective therapy and to direct future research, it is essential to understand the virulence factors and antifungal susceptibility profiles of *M. canis* infection. The present study assesses the capability of *M. canis* strains from different hosts in producing virulence factors, evaluates the in vitro antifungal profile and assesses the relationship between virulence, antifungal profile and occurrence of lesions from different hosts. A total of 100 *M. canis* strains grouped (according to origin and presence of lesions) into humans (n = 10), animals with skin lesions (n = 64) and animals without skin lesions (n = 26) was used to evaluate the production of virulence enzymes and thermotolerance activity. Additionally, the in vitro activity of 7 antifungal drugs was evaluated according to the CLSI M38-A2 methodology.

Microsporum canis strains showed phospholipase Pz (94.1%), hemolytic Hz (92.2%), lipase Lz (100%) and catalase Ca activities (100%). The Lz and Ca values were lower in strains without lesions ($p < 0.05$). The growth of colonies at 28 °C was better than at 35 °C ($p < 0.05$). The number of strains with low thermotolerance

(76.5%) were higher than those with high thermotolerance (23.5%) ($p < 0.05$). VOR, TER and PSZ were the most active drugs against *M. canis* strains, followed by KTZ GRI, ITZ and FLZ in order of activity in the different hosts. Significant positive correlation of low and high VOR/FLZ MICs with Hz and Ca production was observed, suggesting that these enzymes can play a significant role in the increased probable azole resistance. Our results confirmed that reliable determination of the relationship between virulence factors and probable antifungal resistance may highlight new therapeutic strategies based on involvement of the virulence mechanism in the effectiveness of treatment.

Virulence factors of epidemically spreading population of the *Trichophyton benhamiae* clade

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White and yellow strains of *Trichophyton benhamiae* are well known pathogens of pet animals with frequent transmission to humans. Based on the recent data, white phenotype strains form three subgroups which are closely related but separate from yellow phenotype strains. While the incidence of white phenotype strains has remained roughly constant for several decades, yellow strains are currently spreading epidemically through Europe between guinea pigs and their breeders. To answer the question what stands behind its this successful spread, we compared gene expression and spectra of volatile organic compounds (VOCs) of epidemic and non-epidemic strains. Gene expression of strains grown in a liquid medium and on ex vivo murine skin explants was studied by sequencing of RNA and by RT-qPCR. Spectra of the produced VOCs were analysed with the GC-MS method. Although taxa in the white phenotype group did not differ significantly in the examined factors, yellow phenotype strains are significantly separated from the others. Expression of genes connected to production of secondary metabolites and stress management increases in yellow epidemical strains at the expense of genes involved in nutrient intake and primary metabolism in general. This supports the initial assumption that epidemical strains developed a different and probably more successful strategy of spreading in hosts. Finally, we propose catalase EasC, protein with fasciclin domain, and VOCs with possible bioactive potential as new putative virulence factors in this study.

Spherule remodelling and endospore development in the fungal pathogen *Coccidioides posadasii*

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Coccidioides immitis and *C. posadasii* are soil-dwelling dimorphic fungi found in North and South America. Inhalation of environmentally produced conidia results in infection. *Coccidioides* spp. make specialised parasitic spherules, which contain endospores that are released into the host upon spherule rupture. The molecular determinants involved in this key developmental step of infection remain largely elusive. In this study, we utilised an attenuated mutant strain, Δcts2/Δard1/Δcts3, in which chitinase genes 2 and 3 were deleted, previously created for vaccine development. This strain does not complete endospore development, which prevents completion of the parasitic lifecycle. We sought to identify pathways active in the wild-type strain during spherule remodelling and endospore formation which had been affected by gene deletion. We compared the transcriptome and volatile metabolome of the mutant Δcts2/Δard1/Δcts3 to the wild-type C735 strain. First, the global transcriptome was compared for both isolates using RNA sequencing. The raw reads were aligned to the reference genome and transcript expression analysed. Expression of genes of interest was assessed in vivo using NanoString technology. Using solid phase microextraction (SPME) and comprehensive two-dimensional gas chromatography – time-of-flight mass spectrometry (GC×GC-TOFMS), volatile organic compounds (VOCs) were collected and analysed. Our RNA-seq analyses reveal approximately 280 significantly differentially regulated transcripts that are either absent or improperly up- or down-regulated in the mutant compared to the parent strain. This suggests that these genes are tied to networks impacted by deletion and may be critical for proper development. Of these genes, 14 were specific to the *Coccidioides* spp. Finally, the wild-type and mutant strains differed significantly in their production versus consumption of metabolites, with the mutant displaying increased nutrient scavenging. Overall, our results provide a set of key genes that are active during endospore formation, and demonstrate that this bioinformatics approach can define logical targets for future functional studies.

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Histoplasma relies on gluconeogenic substrates to circumvent nutritional limitations within macrophages

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Microbial pathogens rely on exploiting host nutrients to proliferate during infection. Intracellular pathogens, particularly those exclusively living in phagosomes, such as the primary pathogen *Histoplasma capsulatum*, must be able to assimilate available carbon sources within the phagosome to meet their nutritional needs. In this study, we investigated which host nutrients could be utilised by *Histoplasma* as the major carbon source to proliferate within macrophages. *Histoplasma* yeasts can grow on hexoses and amino acids but not fatty acids as the sole carbon source in vitro. Transcriptional analysis and metabolism profiling showed that *Histoplasma* yeasts down-regulate glycolysis and fatty acid utilisation but up-regulate gluconeogenesis within macrophages. Neither depletion of glycolysis nor fatty acid utilisation pathways prevent *Histoplasma* growth within macrophages nor impair virulence in vivo. However, loss of function in Pck1, the enzyme catalysing the first committed step of gluconeogenesis, impairs *Histoplasma* growth within macrophages and severely attenuates virulence in vivo. Summarising, our data indicate that *Histoplasma* yeast catabolises gluconeogenic substrates (e.g. amino acids) to proliferate within macrophages.