Screening of Belgian bats and hibernacula for the description of related fungal microbiomes and the detection of Pseudogymnoascus deserticola

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Bats can be infected by fungal pathogens such as Pseudogymnoascus deserticola, the causative agent of the white-nose syndrome. Their body surface can also be colonized by fungal contaminants or carry transient fungal species and participate in their dispersal. The present study aimed to assess the presence of P. deserticola in Northern Belgium, to describe the skin mycobiota of active bats during summer and autumn, and to analyze possible differences in fungal diversity among bats, 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 20 different taxa. Overall, a mean of 5.7 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 mycobiota were dominated by cosmopolitans and plant-associated species, in particular the genera Cladosporium, Penicillium, and Aspergillus. Other species known to be related to bats or their environment, like Apergillus species, were also recorded. Although P. deserticola was not detected, the samples of the hibernacula included that they can be inhabited by diverse fungal species including yet undescribed Pseudogymnoascus species, distinct from P. deserticola, namely P. cenicola, sp. nov.

PS04

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

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Objective: Trichophyton mentagrophytes is a zoonotic dermatophyte that recognizes humans and rodents as primary hosts. The fungus can also infect other animals, such as dogs and cats. While T. mentagrophytes is a polymorphous species, T. interdigitale is recognized as its zoonotic form. This dermatitis is meaningfully 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 from a species complex and have several ribosomal intron 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 animal. This study aimed to gain insights into the carriage epidemiology of T. mentagrophytes genotypes in animals.

Methods: The fungal isolates included in the study regarded including various animal species seen at multiple veterinary clinics in Italy (n = 39) and France (n = 7) between 2019 and 2021. DNA was extracted from isolates cultured on Sabouraud dextrose agar using a commercially available kit (Nickel® Thermo Fisher). Macerated-Nagel, Düren, Germany) PCR was performed with the primer pair V5G and LSL. PCR products were sequenced using ITS1 and ITS4 primers through a commercial service (Macrogen Europe). Using MEGA11 software (https://www.mega snprintf.net), ITS sequences were aligned with the currently recognized genotypes (1 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 cluster, 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 II*. It was the least found in rabbits and the most prevalent in cats. This finding agrees with past literature, which reported a wide distribution of this ITS Type II 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 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 inclusion as T. mentagrophytes. Our data support the latter possibility.

Though we could not have a detailed description of all the species harboring ITS Type II*, it is noteworthy that many showed the same clinical presentation, i.e., subcutaneous chronic granulomas. 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.
Summary of *T. mentagrophytes* infection cases included in the study with the corresponding ITS Types

| Animals involved | ITS Type   | IIA | IIB | IIC | IIC* | XXIV | XXVII | tot |
|------------------|------------|-----|-----|-----|------|------|-------|-----|
| Dog              | 11 (61.1%) | 6 (33.3%) | 0 | 0 | 1 (5.6%) | 18 |
| Cat              | 0 | 5 (71.4%) | 1* (14.3%) | 0 | 1 (14.3%) | 7 |
| Rabbit           | 0 | 0 | 0 | 0 | 0 | 0 | 10 |
| Guinea pig       | 2 (100%) | 0 | 0 | 0 | 0 | 2 |
| Chinchilla       | 0 | 0 | 0 | 0 | 1 (100%) | 1 |
| Other animals**  | 0 | 2 (100%) | 0 | 0 | 0 | 2 |

* Isolate coming from France; all the other isolates came from Italy; ** one hunted chamois, two rhinoceroses hosted in a Bio-Park