SS.4d Population biology of hagfishes genus *Eptatretus* in Iceland

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SS.4c Animal model systems, September 23, 2022, 3:00 PM - 4:30 PM

*Eptatretus* is a main component of hagfishes in hagfishes and is increasingly reported from human infections worldwide. It is found in wild European hagfishes (*Eptatretus stouti*) but also in the African four-eyed hagfish (*Eptatretus allisoni*), a species of similar size to the animal worldwide. Little is known about the taxonomy and population genetics of this hagfish despite its increasing importance in clinical practice. Notably, whether there are different populations or even species associated with different hosts or geographic regions is not known. To answer these questions, we collected 161 individuals, per formed genotyping and phylogenetic analyses, determined the haplogroups and morphology. Multiple phylogenetics and microbiome analysis supported *E. stouti* as a monophyletic species, in contrast to highly incon gruent tree topologies from predominant morphological and phylogenetic analyses. The data from wild hagfishes indicated that unusual reproduction in *E. stouti* and the course of infection from hagfishes are probably contemporary and that clonal horizontal spread is strong. The molecular typology applied in this study should be employed for future studies on hagfishes, a species with high ecological and medical importance.

The results of this study also highlighted the need to use a multiple phylogeny ideally in combination with other determinants in molecular markers to understand the species boundaries of *Eptatretus*.}

SS.5c Genotyping and phylogenetics of AD-hybrids

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SS.5c Genotyping of Cryptococcus neoformans and C. gattii, September 25, 2022, 3:00 PM - 4:30 PM

Objectives: In a previous study a set of new molecular-type specific primers were designed to apply the standard DSHAM consumes multi-locus sequence typing (MLST) scheme for Cryptococcus neoformans AD-hybrids. In the present study, we report the preliminary results of the investigations by MLST of a large number of AD hybrids with the aim to identify the circulating genotypes, their phylogenetic relationships and population genetics.

Methods: A total of 50 AD hybrid isolates from different parts of the world and from different sources were genotyped by MLST. Minimum spanning tree and Coalescent algorithms were generated by comparing hybrid genotypes and by computing separately either allele-A and allele-D portions of the hybrid genotypes to the haplotypes recorded in the MLST global database. Results: Analysis identified 32 hybrids genotypes grouped in these distinct main clusters (CC1, CC2, CC11) including 12 isolates each. Both CC1 and CC11 clusters included isolates from different countries and continents but the former grouped only isolates with mating type AdA and the latter those with mating type aAa. Cluster CC2 included isolates only from India. Heterozygous allele combinations in some of the MLST profiles had presented two or three combinations more frequent than the others. In these isolates, one or more alleles were not amplified after multiple attempts, and therefore, were considered as missing. A total of 22 MLST profiles were identified by analyzing separately the allele-A or allele-D combinations of the hybrids. Comparison with all MLST profiles of VNI, VNN and VN1 included in the MLST global database showed that the allele-A portion of the hybrid genotypes was grouped in few VNN or VN1 clusters. None of the investigated hybrids were closely related to any of the VNI genotypes from VN1 genotypes. Similarly, when the MLST profiles of allele-D portion of hybrids was compared to all VN1 genotypes present in the global MLST Database, few clusters were identified but, in this case, mostly originated from US isolates not part of any well-defined MLST clades. Conclusions: These preliminary results suggest that the AD hybrids have intrinsic origin from the mating of A haploids very common in both clinical and environmental isolates and D haploids that are not circulating at present or very rare. Therefore, it is likely that AD hybrids originated in the environment where VN1 genotypes diversity is higher and specific AD combinations can occur. Sequencing of further AD hybrids is in progress to confirm these results.

SS.5d Cryptococcus neoformans and Cryptococcus gattii clinical isolates from Colombia develop heteroresistance to fluconazole at high concentrations

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3SS.5d Genotyping of Cryptococcus neoformans and C. gattii, September 25, 2022, 3:00 PM - 4:30 PM

Introduction: Cryptococcus is a worldwide mycosis caused by Cryptococcus neoformans and Cryptococcus gattii. Although resistance to antifungals is infrequent, isolates with decreased susceptibility to fluconazole have been reported globally, including Colombia, which may be due to 1) heteroresistance, defined as the ability to adapt to increasing concentrations of this antifungal in the laboratory, and 2) point mutations in the ERG11 gene encoding the fluconazole target enzyme, lanosterol 14α-demethylase.

Objective: To determine the development of heteroresistance to fluconazole in C. neoformans and C. gattii clinical isolates from Colombia and to analyze and sequence the ERG11 gene of the isolates to seek for mutations that might characterize resistant or heteroresistant phenotypes.

Methods: The minimum inhibitory concentration (MIC) to fluconazole was determined in 25 and 24 isolates of C. neoformans and C. gattii, respectively, using broth microdilution. Heteroresistance was evaluated by plating each isolate on YPD agar that contained fluconazole at concentrations equal to the MIC of each isolate. Heteroresistant colonies were then replated at increasing concentrations of fluconazole. MIC of each isolate was determined in each step.

Results: All isolates were susceptible to fluconazole with MICs of 1 µg/ml (n = 2), 2 µg/ml (n = 4), 4 µg/ml (n = 17), 8 µg/ml (n = 23), 16 µg/ml (n = 5), and 32 µg/ml (n = 1). However, all isolates developed heteroresistance colonies, with increasing MIC of 0.5 µg/ml (2×) to 2 µg/ml (8×) to 4 µg/ml (16×) for C. neoformans and 8.5 (3×) of C. gattii, respectively.

Conclusion: Clinical isolates of C. neoformans and C. gattii that develop heteroresistance to fluconazole in clinical isolates from Colombia, which is important since this characteristic contribute to the development of cryptococcosis drug therapy with this triazole.