An animal free mycetoma grain model to study the therapeutic efficacy of various antifungal agents against the clinical entity of this infection

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Eumycetoma is a mutilating, chronic, granulomatous, progressive disease of mainly the foot which is most commonly caused by *Madurella mycetomatis*. Treatment of this infection is difficult and success rates are poor, even although the fungus itself is extremely susceptible towards the azole class of antifungal agents, in vitro. These in vitro results were generated against fungal hyphae, but in vivo, mycetoma causative agents organize themselves in granules called grains. These grains are composed of the causative agent and a protective cement-material. Therefore, in developing a new therapy for mycetoma, the efficacy of the antimicrobial agents needs to be determined against grains. Until now, grains could only be generated in animal models. It is not possible to generate grains in vitro. Several animal models have been developed for mycetoma over the years. These models have been established in different hosts, ranging from mice and guinea pigs to goats and monkeys. But there is a large drawback to these animal models. From our own experience we noted that, already minutes after infection, mice become discomforted. They stay in this miserable state till 3 days after inoculation when they either tend to recover or die. The mortality rate for this infection model is relatively high (20-90% for the high load infections). Therefore there is a need for a model in which grains can be formed without having to use animals. At the moment, the most studied alternative for mammal infection models is the wax moth larvae Galleria mellonella. These larvae have a cellular and humoral immune system and can be maintained at 37^0C, conditions beneficial for mimicking the attacks mycetoma causative agents will encounter when entering the human host. Furthermore, these larvae have been used as alternative model systems to study the fungal infections candidiasis, aspergillosis and cryptococcosis. For some of these infections, therapeutic efficacy studies have been performed in these models, resembling the therapeutic response found in animal models and in the clinical situation, indicating that Galleria mellonella would indeed be a good model system for the fungal mycetoma infection. In this presentation, I will highlight the development of a *M. mycetomatis* grain model in larvae of the wax moth Galleria mellonella, the infection itself but also the responses towards commonly used antifungal agents will be shown.