

Optimization of a new infection model of murine epicutaneous dermatophytosis with *Trichophyton benhamiae*

Dermatophytoses are superficial cutaneous mycoses observed in both humans and animals caused by filamentous fungi called dermatophytes. Human dermatophytosis is widespread and most often caused by species belonging to the genus *Trichophyton*. The increasing emergence of strains resistant to currently available antifungals requires the development of new treatments. In this context, a better knowledge of the pathogenesis of dermatophytosis, including the identification of fungal virulence factors such as subtilisins, is a key step for the identification of new therapeutic targets. A method for standardizing the production of dermatophyte spores to perform experimental infections has been published by our research team (Faway *et al.*, 2021). If this method based on the use of suspensions enriched in unicellular spores allows the development of standardized infections on human epidermis reconstructed *in vitro*, it is not optimal to produce relevant *in vivo* infections. The objective of this study is to develop a new relevant mouse model of infection with the reference strain of *Trichophyton benhamiae* IHEM 20161 and derived deleted strains, using a newly standardized inoculum (spores, germ tubes and mycelium) and to evaluate the expression of genes coding for subtilisins during the infection process.

Optimization of the epicutaneous infection model



Clinical Score

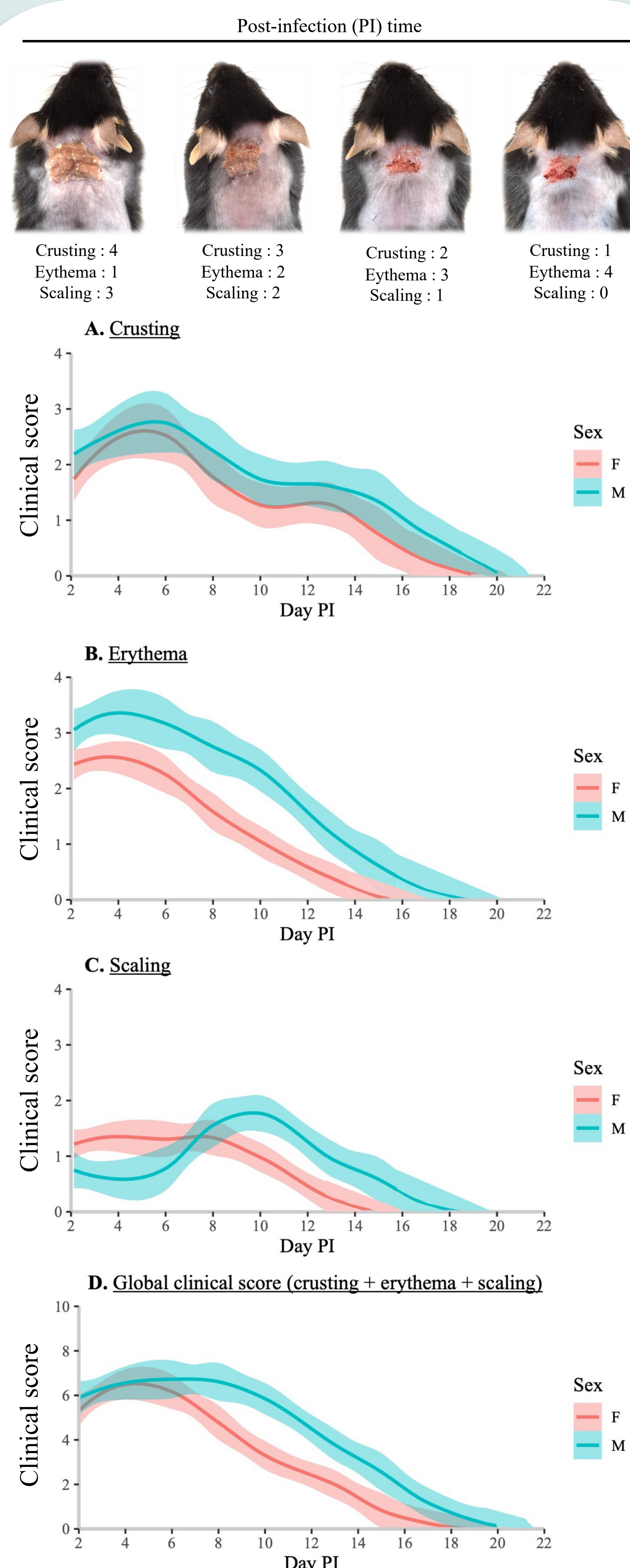


Figure 1 : Clinical scoring of crusting (A), erythema (B), scaling (C) and total cumulative scoring (D) for *T. benhamiae* wild type IHEM 20161 on murine model (n=9)
F : female, M : male, PI : post-infection

Histology

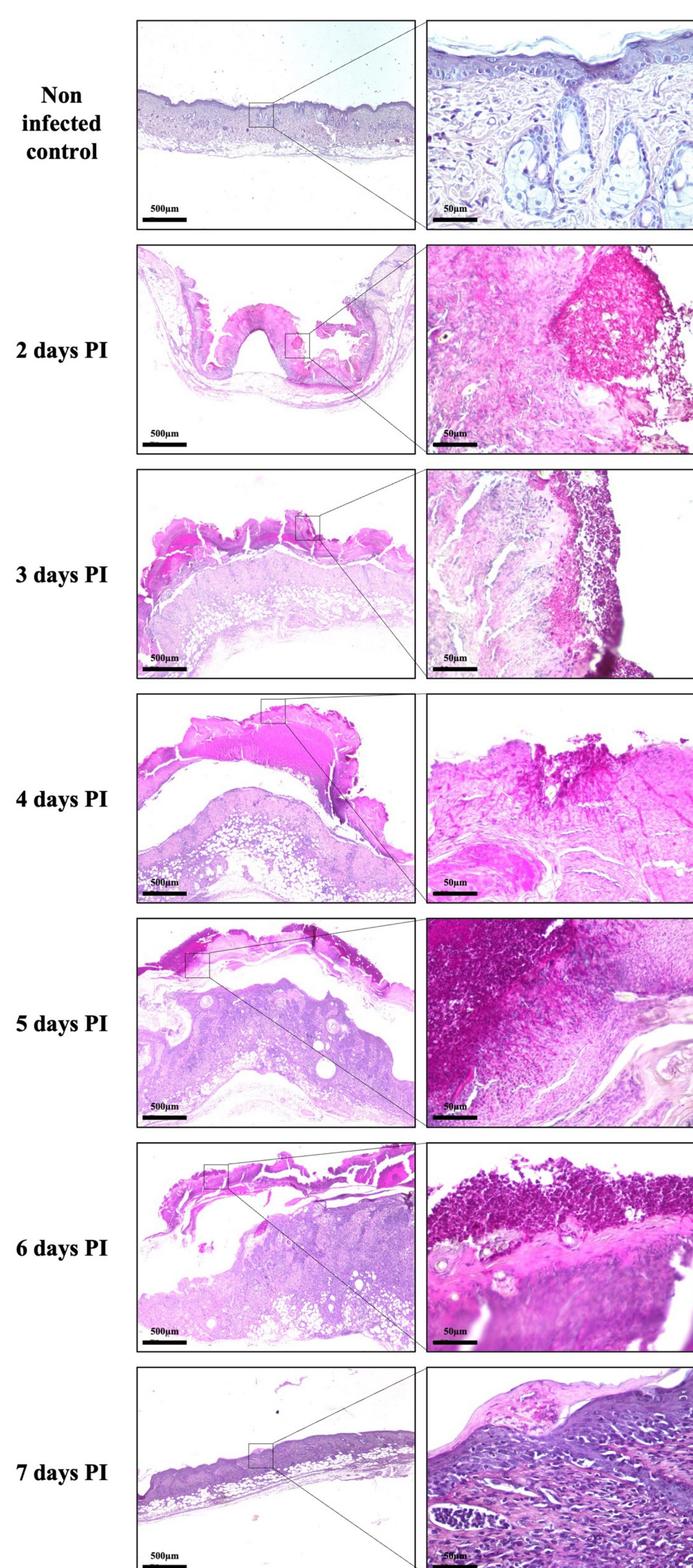


Figure 2 : Kinetics of tissue invasion by *T. benhamiae* wild type IHEM 20161 on the new murine model (PAS coloration)
PI : post-infection

Expression of markers

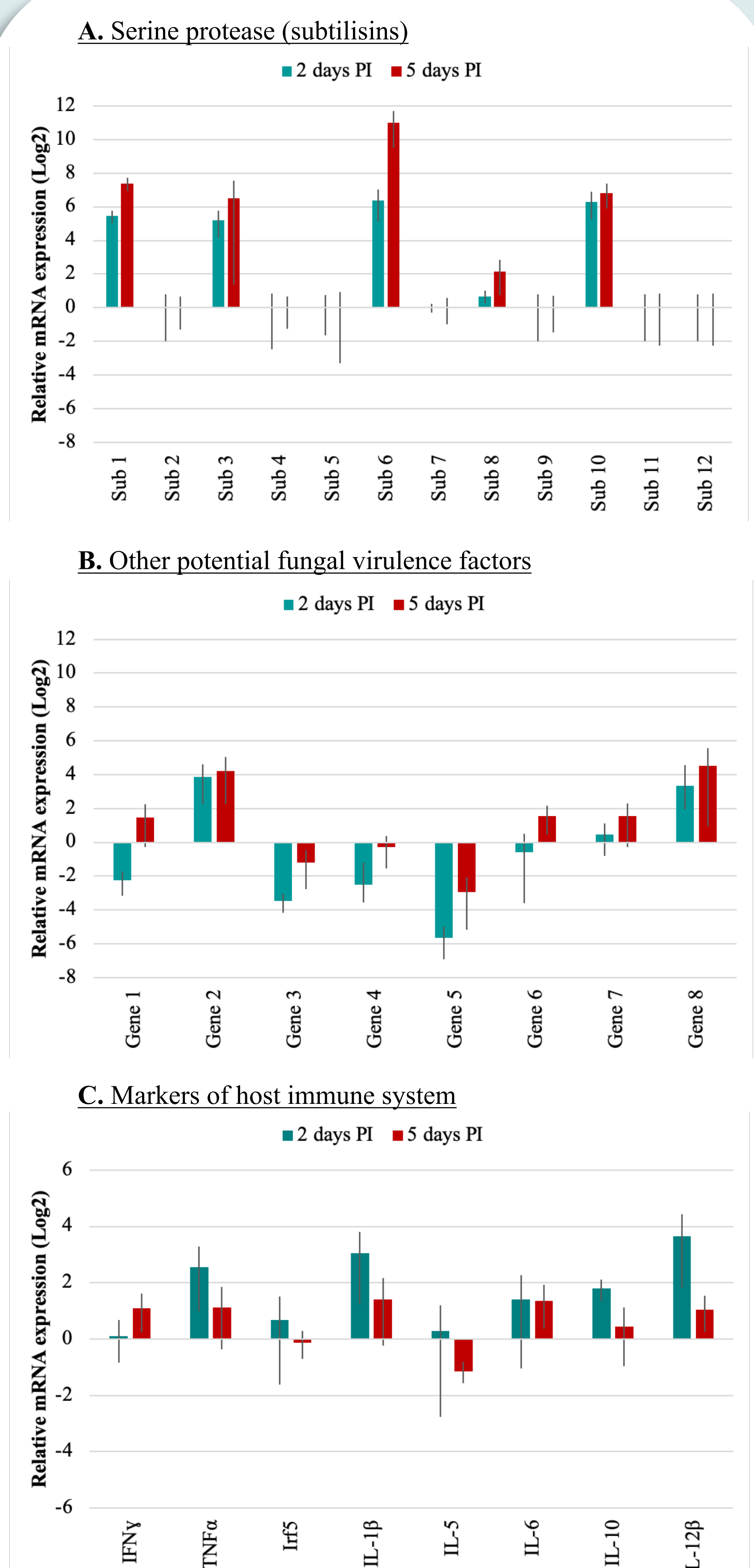


Figure 3 : Relative mRNA expression for subtilisins belonging to serine protease family (A), other potential fungal virulence factors (B) and murine immune system response (C) at 2 and 5 days PI (n=6)
(Genes 1 to 8 will be delivered in future publication)

The inoculum used in this new *in vivo* model generates clearly visible skin symptoms (Fig. 1), mimicking a self-resolving natural infection, lasting more than twice as long (min 18 days vs 7 days) as those obtained in the past using spores as inoculum only. Histopathological analyzes show an invasion of keratinized tissues by the fungus until day 6 PI, ranging from numerous fungal hyphae within epidermal crusts on day 2 PI to a progressive and intense development more deeply within the tissue on day 5 PI (Fig. 2). Concerning expression of markers of infection, Sub 1, Sub 3, Sub 6, Sub 10, gene 2 and gene 8 are overexpressed by *T. benhamiae* during the infection process, while TNF α , IL-1 β and IL-12 β are overexpressed by the host (Fig. 3). All together, we developed a new reproducible mouse model of *T. benhamiae* dermatophytosis, allowing to evaluate the expression of potential virulence factors and other important markers during the infection process.