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Involvement of *Candida albicans* in hyperkeratosis of the Göttingen minipig

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Introduction

Hyperkeratosis is common in intensively housed pigs, and manifests as a brownish greasy scaling dorsal of the neck and back. The scales can be rubbed off, revealing normal skin underneath. Affected animals are otherwise healthy, and do not seem to have discomfort from hyperkeratosis *(Scott, 1988)*.

Lehman (1992) describes hyperkeratosis (hyperkeratinization) as a diffuse proliferative, non-elevated skin alteration on the back. In contrast to parakeratosis, which is a generalized diffuse proliferative, non-elevated skin alteration, hyperkeratosis is a local skin affection. Hyperkeratosis should not be mistaken for dermatophytosis (mycosis) or ectoparasitosis (mange).

Barrier bred Göttingen minipigs are also affected by hyperkeratosis. Young minipig boars are most frequently affected, developing signs of hyperkeratosis from the age of 6-8 weeks. Lesions may disappear spontaneuosly at a later age. Previous investigations have shown an opportunistic *Candida albicans* infection in connection with hyperkeratosis and exudative dermatitis of the peri-ocular region of microbiologically defined Göttingen minipigs *(Madsen et al. 1998)*.

The present study investigates the involvement of fungi in hyperkeratosis in the Göttingen minipig. The incidence of hyperkeratosis was recorded, and fungal investigations were performed in order to elucidate a fungal involvement.

Materials and Methods

Animals:

Animals examined were microbiologically defined Göttingen minipigs, housed at a full-barrier facility (Ellegaard Göttingen Minipigs, Dalmose, Denmark) with HEPA filtered ventilation. The animals were group housed in floor pens with solid concrete at 2/3 of the surface area and grids at the other 1/3. They were fed restrictedly from the solid concrete floor with an expanded pellet diet (SDS, Witham, England). Water was available *ad libitum*. The temperature was 20-22 °C and the relative humidity 50-70%. Health monitoring of the colony was done twice a year as described by Hansen *et al.* (1997).

Registration of hyperkeratosis:

Hyperkeratosis was registered in the animal's file when observed. A record was made if a brownish greasy scaling on the dorsal side of the neck or back was observed. The percentage of animals with hyperkeratosis was calculated on a monthly basis.

Sampling for fungal investigations:

Swabs with sterile cotton tips were taken from 25 animals, from:

-the back of 5 animals with hyperkeratosis on the dorsum,

-the head of 5 animals with hyperkeratosis on the head,

-the peri-ocular region of 5 animals with brown staining,

-the ears of 5 clinically healthy animals,
-the back of 5 clinically healthy animals.

The samples were transported to the laboratory in Stuart's transport medium (Statens Seruminstitut, Copenhagen, Denmark).
From 3 animals, swabs from the skin on the back of animals with hyperkeratosis were taken, as well as 4 mm punch biopsies of each animal. The swabs were transported to the laboratory in Stuart's Transport medium and the biopsies were fixed in 4% formaldehyde.

Microbiology:
The swabs were inoculated on Saburaud's agar, pH 4.2 (Statens Seruminstitut, Copenhagen, Denmark) at 20 °C for three to seven days. All morphologically distinct colonies were sampled and grown as pure cultures on Saburaud's agar after which they were Gram stained. Typical yeasts were identified by assimilation assays using the API ID32C kit (BioMerieux, Meyrin-Geneve, France).

Histology:
From the skin biopsies, sections were taken and stained with Giemsa and Gram as described by Bancroft & Cook (1994). Modified versions of Bancroft & Cook's (1994) methods for HE and PAS staining were performed as well.
To examine the presence of *Candida spp.* the biopsies were stained by immunohistochemistry, using a three-layer horseradish peroxidase antiperoxidase (PAP) technique with polyclonal rabbit-antibodies against mannan from *Candida albicans* (DAKO, Glostrup, Denmark) as described by Jensen et al. (1993).

Results
A total of 6.404 animals were born in the period 1995-1997. The incidence of hyperkeratosis of these animals varied from 1.4-6.9%. The averages of monthly records were 3.6%, 4.9% and 4.8% respectively for the years 1995, 1996 and 1997 (Table 1).
The results of the microbiological investigation are given in Table 2. One animal sampled on the back showed unspecific growth and infection with *Trichosporon spp.* (minipig 52081). From the animals sampled on the head, one animal was positive for *Candida spp.* (minipig 56643). From the ear, no *Candida spp.* could be cultured. Also here, one animal sampled showed unspecific growth and infection with *Trichosporon spp.* (minipig 54566). Of clinically healthy animals sampled from the back, one animal was positive for *Candida humicola* (minipig 55990) and one animal was positive for *Candida albicans* and *Candida humicola* (minipig 55653). However, animals sampled from the peri-ocular region were all positive for *Candida albicans* (minipigs 54024, 54543, 54814, 50502 and 55882) and one animal was positive for *Trichosporon spp.* (minipig 50502).
The three animals of which skin biopsies were taken were all positive for *Candida parapsilosis.* (minipigs 52032, 52081 and 54689). Histology showed the typical image of hyperkeratosis (Figure 1). PAS and Gram staining showed positive particles in the outer layer of the *Stratum corneum.*
Immunuhistochemistry demonstrated yeast - like organisms positive for *Candida* antigen in one of the tissue sections (minipig 54689). The yeast was seen in the superficial debris and did not invade the underlying epidermis. The inflammatory reaction seen adjacent to the yeast was similar to the reaction in other regions of the hyperkeratotic epidermis.

Discussion
Hyperkeratosis in Göttingen minipigs occurs at about the same frequency as in large pigs. Leman (1986) reported that 6.4% of mature boars are affected by hyperkeratosis, followed by dry sows (1.3%) and suckling sows (0.5%). In the Göttingen minipig we found that up to 6.9% of the animals were affected by hyperkeratosis. Although we did not make a division in sex and age, we observed that especially young boars at an age of 6-8 weeks were affected.
The underlying cause of hyperkeratosis remains unknown. Candida was present microscopically in low counts, and did not invade the underlying epidermis. From this it can be concluded that it was not a primary pathogen. This fits well with

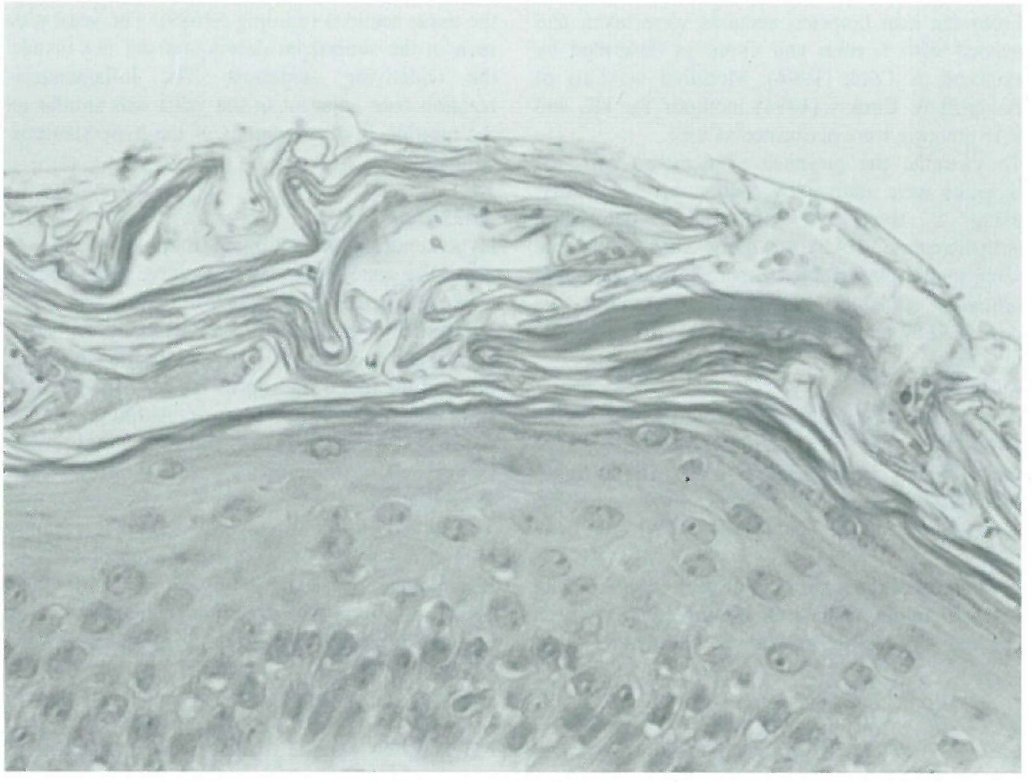
Table 1. Percentages of Göttingen minipigs with hyperkeratosis over the years 1995-1997.

	average (%)	lowest (%)	highest (%)
1995	3,6	1,4	6,5
1996	4,9	2,4	6,9
1997	4,8	2,2	6,7

Table 2. Number of Göttingen minipigs with infected hyperkeratotic or healthy skin at different sites. A total number of 5 animals per group was examined.

	C. albicans infection	Other infections	No infection
Back, hyperkeratotic	0	1	4
Head, hyperkeratotic	0	1	4
Ear, healthy	0	1	4
Back, healthy	1	2	3
Eye, brown staining	5	1	0

Figure 1. Hyperkeratotic layer (HE. 40x). The Stratum corneum is thickened. Some erythrocytes can be seen in between the keratine layers, as a result from taking the biopsy



general characteristics of *Candida* infection of the skin, which generally is of secondary grade *(Odds, 1988)*.

Whereas the brown staining of the peri-ocular region was positive for *Candida* in all cases, no clear relation between *Candida albicans* infection and hyperkeratosis could be found (table 2). Madsen et al. (1998) found that the organism was only present superficially at the peri-ocular region and did not invade the underlying epidermis, and suggested that the *Candida albicans* infection was opportunistic. The humid environment of the peri-ocular region in combination with a hyperkeratotic layer is apparently a favourable environment for *Candida* growth.

Immunuhistochemistry showed only one positive finding for yeast-like organisms in the tissue sections, whereas all animals examined for Candida growth were positive. Although Odds (1988) mentions that positive results from cultures of skin infections are relatively rare, it is even more infrequent that yeasts are included in tissues slides of mild opportunistic skin infections.

The Göttingen minipigs were housed at a full-barrier facility with floor pens with solid concrete at 2/3 of the surface area and grids at the other 1/3, and the animals were fed from the solid concrete floor. Gedek (1968) pointed out that food sources are often the cause of candidosis in pigs, and Odds (1988) mentions that yeasts generally reside in the digestive tract as commensals. Faeces are therefore an important source of infection. Since the minipigs sleep on the concrete area with remains of diet and faeces, and a local high humidity, it is very likely that this is the source of infection. However, since the colony is caesarean derived and housed at a full-barrier facility, we may presume that *Candida albicans* did not occur initially. The primary source of infection must therefore be in connection with the introduction of staff, diet or materials into the barrier facility. Since *Candida albicans* is widely spread in the environment *(Odds, 1988)*, this seems very likely.

Summary

At a breeding colony of Göttingen minipigs, 6404 animals born in 1995-1997 were examined for the occurence of hyperkeratosis, and the percentage of animals with hyperkeratosis was calculated on a monthly base. From 25 animals, skin swabs were taken, and the swabs were inoculated on Saburaud's agar, pH 4.2 at 20 °C for three to seven days. All morphologically distinct colonies were sampled and grown as pure cultures on Sabauraud's agar after which they were Gram stained. The percentage animals with hyperkeratosis varied from 1.4 to 6.9% monthly, with an average of 3.6%, 4.9% and 4.8% for the years 1995, 1996 and 1997 respectively. *Candida albicans* infection was not found on the skin of the back or head of animals with hyperkeratosis. One healthy animal was positive for *Candida albicans*. Some animals had a brown staining at the peri-ocular region. Of the sampled animals with brown staining at the peri-ocular region, all findings were positive for *Candida albicans*. Other infections found were *Candida humicola* and *Trichosporon spp.* In connection with histological examination, *Candida parapsilosis* was found. The underlying cause of hyperkeratosis remains unknown. Hyperkeratosis is not associated with *Candida albicans* infection.

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