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Investigation of the involvement of Candida albicans in hyperkeratosis of the Göttingen minipig

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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 and grids at 1/3 of the surface. To prevent competition, they were fed resrictedly from the solid concrete floor with an expanded pellet (SDS, Witham, England). Water was available ad libitum. The temperature was 20-22° C and the relative humidity 50-70%. The colony was health monitored twice a year as described by Hansen et al. (1997).

Registration of hyperkeratosis:

Hyperkeratosis was registered in the animal's electronic file when observed. A registration 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 base.

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 at that place,
-the ears of 5 clinically healthy animals,
-the back of 5 clinically healthy animals.

Stuart's transport medium (Statens Seruminstitut, Copenhagen, Denmark).

The samples were transported to the laboratory in 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 Sabauraud'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 Sabauraud'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 6404 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 registrations were 3.6%, 4.9% and 4.8% respectively for the years 1995, 1996 and 1997 (Table 1).

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

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).

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

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 animals 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 infected with 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, with PAS and Gram positive particles in the outer layer of the Stratum corneum (Figure 1).

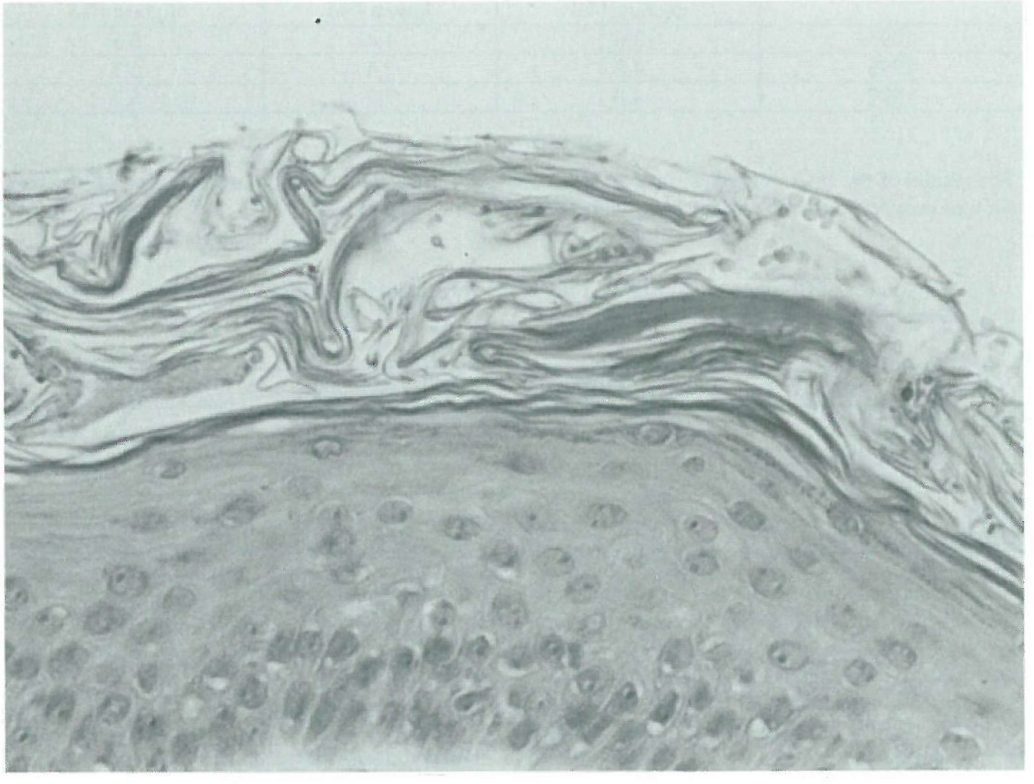
Immunuhistochemistry demonstrated yeast-like organisms positive for candida antigen in one of the tissue sections (minipig 54689). The yeast was seen in the superficial debris and did not invade the underlying epidermis. The inflammatory reaction seen adjecent to the yeast was similar to the reaction 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 general characteristics of candida infection of the skin, which generally is of secondary grade (*Odds*, 1988).

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.



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 culturs of skin affections 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 and grids at 1/3 of the surface, 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 a 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 the source of infection origins here. However, since the colony is caesrean 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 connected to the introduction of staff, diet or materials into the barrier facility. Since Candida albicans is widely spread in the environment and also occurs atmospheric (*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. The percentage affected animals 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. Of the animals with brown staining at the periocular 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.

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. This in contradiction to parakeratosis, which is described as a generalized diffuse proliferative, non-elevated skin alteration. 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 recover spontaneuosly at a later age. Previous investigations have shown an opportunistic Candida albicans infection in connection with hyperkeratosis and exudative dermatitis of the periocular region of microbiologically defined Göttingen minipigs (*Madsen et al.* 1998).

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

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