

Histological and bacteriological evaluation of digital dermatitis in cattle, with special reference to spirochaetes and *Campylobacter faecalis*

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Tissue samples from the feet of slaughtered cattle exhibiting different stages of digital dermatitis were sectioned and stained with haematoxylin and eosin and silver staining techniques. Three morphological variations of spirochaetes were observed, whereas control samples from feet which were macroscopically negative for digital dermatitis were also negative for spirochaetes. In an immunofluorescence test, *Campylobacter faecalis* was found to be abundant on superficial wound smears from the classical ulceration of digital dermatitis.

DIGITAL dermatitis was first described by Cheli and Mortellaro (1974) as a disease of cattle which caused painful ulcerations along the coronary band. Since then it has been recognised in many countries of the world, but remains a disease of unknown aetiology, epidemiology and economic significance. The lack of a gold standard test for its diagnosis makes it difficult to consider the disease as a separate entity. Speculations about the disease's aetiology have associated it with a variety of different agents, including spirochaetes (Read and others 1992, Walker and others 1995), *Bacteroides* species (now more commonly known as *Porphyromonas* species) (Grund and others 1995), *Campylobacter* species (Cornelisse and others 1982) and viruses (Rebhun and others 1980). A lack of adequate negative controls in previous study designs have rendered these findings inconclusive. Furthermore, the disease has never been reproduced in healthy animals by the inoculation of one of these organisms.

In previous studies, histological examination revealed signs of an acute, suppurative inflammation of the epidermis with superficial necrosis and hyperkeratosis (Rebhun and others 1980). Thickening of the epidermis and numerous mitoses within the stratum basale were observed in addition to parakeratosis (Gourreau and others 1992). Microabscesses within an acanthotic and hyperkeratotic epidermis were accompanied by infiltrating neutrophils and mononuclear cells (Bassett and others 1990). Deeper layers of the epidermis showed either no signs or only slight signs of inflammatory reactions (Peterse 1982, Weaver 1993). Perivascular aggregations of lymphocytes and plasma cells indicated an inflammatory reaction in the adjacent tissues (Blowey and Sharp 1988, Blowey and others 1994).

The skin forms a crucial barrier between the animal and its environment and has essential functions in the defence and homeostasis of metabolic equilibrium. Special functions are performed

TABLE 1: Description of the four disease classes associated with the development of digital dermatitis (Döpfer 1994)

| Disease class | Description |
|---------------|---|
| 1 | Early stage of digital dermatitis with a circumscribed granulomatous area, 0.5 to 4 cm in diameter, which lies at the epithelial surface (Fig 1) or up to 2 mm underneath it (Fig 2) |
| 2 | 'Classical' ulceration of digital dermatitis, which is an area close to the coronary band affecting skin or horn, up to 7 cm in diameter, with granulomatous tissue when the lesion lies more than 2 mm underneath the epithelial level (Fig 3) |
| 3 | 'Classical' ulceration of digital dermatitis in the process of healing covered by a scab (Fig 4) |
| 4 | Alteration of the skin close to the coronary band which can be observed in the endemic situation where there is a history or suspicion of digital dermatitis. The cutaneous lesions are hyperkeratotic and can present themselves with a proliferative aspect (Fig 5) |

by the keratinocytes in terms of the production of inflammatory mediators and in the expression of autocrine receptors for these mediators; the keratinocytes influence the movement of inflammatory cells into the epidermis and their retention within it by the action of cytokines (Jubb and others 1993).

The aims of this study were to describe the histopathological changes during the course of the development of digital dermatitis and to compare skin samples, altered by inflammation caused by the disease, with control samples which were macroscopically negative for digital dermatitis.

Materials and methods

A system to classify the various stages of digital dermatitis was developed during an observational study of the evolution of the disease in two groups of 45 dairy cows on two commercial dairy farms during an outbreak of digital dermatitis. The cows were monitored for five (September 1992 to January 1993) and 11 (September 1992 to July 1993) months, respectively, during the housing (November to April) and pasture (April to November) periods. Monthly cross-sectional observations of the claw health status of all the cows and 67 follow-up periods on individual cows (16 on the first farm and 51 on the second farm) with digital dermatitis were carried out at intervals of two days during four weeks. The observations yielded a qualitative classification system with four different classes of disease (Table 1, Döpfer 1994). This classification system was used to identify disease classes in the samples taken during the present study.

The four disease classes were as follows: early lesions (Figs 1 and 2), classical ulcerations (Fig 3), healing lesions (Fig 4) and lesions of suspected digital dermatitis or with a history of the disease (Fig 5). The classes are defined in Table 1. Two types of sample were used for this study; tissue samples for haematoxylin and eosin or silver staining techniques, and superficial wound smears for immunofluorescence testing. The tissue samples were obtained from slaughterhouse material (one study animal with a history of digital dermatitis was slaughtered because of fertility problems and had one early lesion and one lesion of suspected digital dermatitis; another study animal with healthy claws was slaughtered because of fertility problems; the rest of the tissue samples originated from feet collected at the slaughterhouse). The

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FIG 1: Disease class 1 – early stage of digital dermatitis with a circumscribed granulomatous area, 0.5 to 4 cm in diameter, lying at the epithelial surface (Day 0)



FIG 2: Disease class 1 – early stage of digital dermatitis with an epithelial lesion underneath the epithelial surface (Day 30)



FIG 3: Disease class 2 – 'classical' ulceration of digital dermatitis (Day 50)



FIG 4: Disease class 3 – 'classical' ulceration of digital dermatitis after topical antibiotic treatment in the process of healing covered by a scab (Day 60)



FIG 5: Disease class 4 – endemic lesion of digital dermatitis with history of or suspicion for digital dermatitis marked by a hyperkeratotic surface (Day 71)

FIGS 1 to 5: Right foot of a dairy cow exhibiting various stages of digital dermatitis, from the early stage of the disease (disease class 1 at day 0) to suspected disease lesions (disease class 4 at day 71)

smears originated from randomly selected cows in the study group during the follow-up period.

Tissue samples from 10 early lesions (five at and five up to 2 mm below the epithelial level, see Table 1), three classical ulcerations, four lesions of suspected digital dermatitis, and six samples from control feet macroscopically negative for digital dermatitis were gathered from the hindfeet of slaughtered animals, giving a total of 78 sections originating from 23 feet (21 cows).

Disease class 3 was not present in the sample material. Haematoxylin and eosin stains (Stevens 1982), and silver staining using the technique of Bosma/Steiner (Elias and Bosma 1987), were performed on all the tissue samples, which were fixed in 15 per cent

0.001M phosphate buffer. Control samples were taken from five different locations – proximal to the interdigital cleft; proximal to the lateral claw, including the horn and skin; under the lateral accessory digit at skin level; in the interdigital cleft; and cranioproximal to the interdigital cleft – in each foot in order to standardise the physiological histology of bovine digital skin. A magnification of $\times 100$ to $\times 1000$ (with oil immersion) was used for the evaluation of the slides under a light microscope (Olympus CO11).

Eighty-three superficial wound smears from 31 animals (nine samples of disease class 1, 20 samples of disease class 2, 20 samples of disease class 3, 13 samples of disease class 4, 16 control samples macroscopically negative for digital dermatitis and five samples from a sole ulcer) were analysed for the presence of *C faecalis* using the immunofluorescence test described by Cornelisse and others (1982). The association of the microorganisms found on the smears with the disease classes was given as a risk rate, that is, the risk of finding the microorganism in a certain disease class compared to all other lesions originating from different disease classes.

Results

The histological and microbiological findings (Table 2) were used to classify the samples in terms of the four stages of disease (Table 1). Six types of microorganism were found on the superficial wound smears: *C faecalis* in its comma and spherical forms;



long thin spiral organisms presumed to be a *Fusobacterium* species or a spirochaete; paired cocci; thick branched rods; and budding irregular round microorganisms presumed to be of fungal origin.

Discussion

The histopathological changes due to digital dermatitis were compared with the normal histology of bovine digital skin. In addition to the macroscopic classification of the skin, a microscopic examination was carried out. All silver-stained control sections from all of the five sites were negative for microorganisms, except for one sample where septate hyphae, cocci and rod-like microorganisms were found in the stratum corneum.

The samples collected from diseased skin showed large numbers of spirochaetes in three morphological variations (Table 2). Type S1 supposedly had good viability, while type S2 was less viable due to the lack of twists and type S3 represented degenerative forms. Typing of these spirochaetes using DNA techniques would be required to further characterise the spirochaetes and compare them between countries.

The macroscopical classification system, based on the chronological observations of the course of disease, was helpful in separating diseased samples containing spirochaetes from normal samples without spirochaetes. These findings contradict previous studies in which spirochaetes were found in healthy and diseased bovine digital skin (Guard 1992, Weaver 1993), although a standardised comparison of healthy and diseased skin was not reported in these two studies. In the current study two feet belonged to an animal which had been followed for 11 months and was known to have had several episodes of classical ulceration due to digital dermatitis proximal to the interdigital cleft in both hindfeet during the pasture and housing periods. Although spirochaetes were found at this particular site, samples from the four other digital skin sites were negative in both feet. This finding is compatible with the idea of spirochaetes having a role in the pathogenesis of digital dermatitis. The microorganisms were found at the surface of the skin, mostly at the base of the stratum corneum, or occasionally in the stratum granulosum. Numerous spirochaetes were seen in very slight macroscopical alterations of the digital skin of disease class 4. It is possible that slight interdigital lesions of the

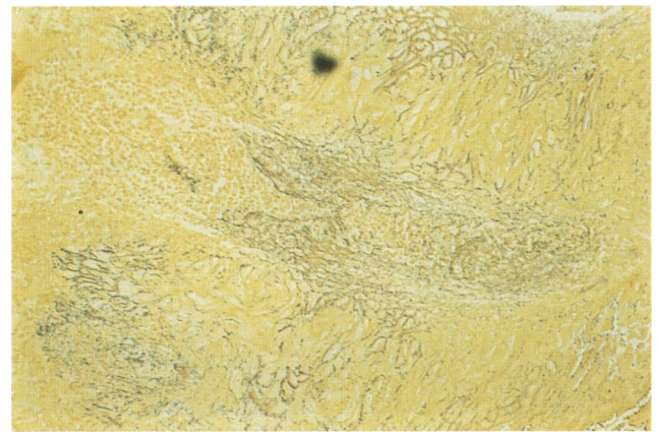


FIG 6: Silver stain using a Bosma/Steiner technique, showing spirochaetes type 2 along and perpendicular to a horny column (disease class 1); slaughterhouse material

epithelium could precede the development of digital dermatitis; Blowey and others (1994) and Walker and others (1995) have previously suggested that interdigital and digital dermatitis could be the same or related diseases.

The results of this study associate spirochaetes with cases of digital dermatitis. Two similar types of spirochaete have previously been described by Blowey and others (1994), Read and Walker (1994), and Walker and others (1995), but they did not describe the degenerative form.

The growth pattern of the spirochaetes along the horny columns supported the idea that these might be a predilection site for the presence and growth of microorganisms. The spirochaetes appeared to advance along the horny columns (Fig 6) and extremely large numbers of defence cells (mostly neutrophil granulocytes) were lined up against these sites. The microorganisms along the diskercatotic structures may enhance the defence reaction and keratinisation process in the stratum corneum. The multilayering in the stratum basale could have been due to uneven sectioning of the tissue samples, but the observations strongly suggested increased epidermal growth or decreased corneal desquamation which are both responses to chronic inflammation and altered intercellular communication.

TABLE 2: Histological and microbiological findings from 78 tissue samples and 83 superficial wound smears taken from the hind feet of slaughtered cattle

| Disease class | Histological findings in the tissue section | Microbiological findings in the tissue sections and superficial wound smears |
|---------------|--|--|
| Controls | Epithelium shows 5-15 cell layers at the minimal height and 15-35 cell layers at the maximal height; stratum corneum uniformly layered; stratum basale shows 0-4 mf/10 hpf in one layer of cells and sharply pointed tips; moderate vascularisation and infiltration of mononuclear cells in the dermis | No organisms found in the silver stains; 5x less than spiral microorganisms in the immunofluorescence test compared to all other lesion types |
| 1 | Epithelium 2-3x as high as controls; rete ridge formation; plaque with partial loss of the epithelium and parakeratosis; areas of ballooning degeneration filled with islands of fibrin; horny columns as papillary projections towards the skin surface ending in microabscesses and haemorrhages; stratum corneum hyperplastic; stratum spinosum acanthotic; stratum basale has more than one cell layer and shows 4-11 mitotic figures per 10 high power fields (mf/10 hpf) compared to the controls; perivascular infiltration in the dermis; mononuclear cells and neutrophils with occasional eosinophils present in the dermis and epidermis | Spirochaetes of the long thin type with few twists (S2) predominate and penetrate into the stratum spinosum; S2 seem to lie along the horny columns and from there turn perpendicularly into the deeper epidermal tissues (Fig 6); short thick spiral organisms coiled up into piles (S3) lie at the sites of ballooning degeneration; inconclusive pattern in the immunofluorescence test |
| 2 | Stratum corneum completely lost over the extension of the lesion; pronounced haemorrhage in its remainders at the lesion borders; horny columns, ballooning degeneration and acanthosis; stratum basale 0-11 mf/10 hpf in more than one cell layer, less mf than class 1; rete ridge formation with broad-based tips at the dermoepidermal border with microabscesses in both epidermis and dermis; pronounced perivascular infiltration in the dermis; neutrophils and eosinophils predominating in the epidermis with occasional plasma cells | Short thick spirochaetes with many twists (S1) are greater in numbers than the S2 type, penetrating very deep into the stratum spinosum; <i>C faecalis</i> in its comma form is 10x more common than other lesion types; if lesion is left untreated for five days they turn into their spherical form |
| 3 | No histological examination | Shows 3x fewer <i>C faecalis</i> in its comma form |
| 4 | Extremely proliferative epidermis three times as high as in the controls; rete ridge formation pronounced with broad-based tips at the dermoepidermal border; stratum corneum very hyperplastic; stratum spinosum very acanthotic; keratohyaline granules absent or scarce; numerous horny columns surrounded by haemorrhages and masses of cell detritus; stratum granulosum shows empty vacuoles with a sponge-like appearance; stratum basale 7-14 mf/10 hpf with 2-3 cell layers; neutrophils outnumber mononuclear cells in epidermis, many plasma cells in dermis; one sample had an intact stratum corneum w/o inflammatory cells, skin macroscopically rough | S3 type organisms in clouds and S1 and S2 type organisms reaching the stratum basale; no characteristic pattern of microorganisms on the immunofluorescence test |



The digital dermatitis lesions of disease class 4 showed pronounced ballooning degeneration of the epidermis. Intercellular communication, like mediator liberation, could be vastly altered in these areas. An inflammatory process triggered by cutaneous degeneration would enter a vicious cycle, since its progress is supposed to depend on cellular expression of mediator molecules (Jubb and others 1993). The defence cells reached the epidermis along the indentations of the stratum basale towards the exterior, creating intense contact with the modulating influences of keratinocytes, especially since rete ridge formation took place, making more interacting surface available. This might be of importance for the enhancement of inflammation in the hyperplastic epidermis. Disease class 1, the early stages, showed eosinophils. Occasional plasma cells were observed, especially in the endemic lesions (disease class 4).

It would be interesting to determine how the inflammatory membrane mediators interact with the microorganisms throughout the course of the disease. The use of monoclonal antibodies against bovine cell membrane mediators would be essential for this purpose.

Among the spirochaetes commonly found within the stratum spinosum and papillare of human skin are subspecies of *Treponema*. Yaws, an ulcerative disease of the distal human leg, is caused by *Treponema pallidum* subspecies *pertenue*, the causative agent of syphilis (Read and others 1992).

The ulcerations of digital dermatitis resemble ulcera dura or indolentia, which are painless and slow healing ulcerations associated with spirochaetes. Classical ulcerations of digital dermatitis are not necessarily painful upon digital pressure, especially in young animals.

The lesions of digital dermatitis can be compared with acutely necrotising, ulcerative gingivitis of humans. Unhygienic conditions can evoke this lesion, which is known as Plaut-Vincentstomatitis. The criteria used to diagnose this acute ulceromembranous gingivitis are necrosis and punched-out crater ulcers of the interdental papillae and gingiva. The ulcer surface is covered by a gray pseudomembranous slough, demarcated from the surrounding mucosa by a linear erythema. The exact aetiology of the disease is unknown, but an association with the fusospirochaetal complex has been suggested. These commensals of the oral mucosa proliferate during periods of host-parasite defence imbalances caused by psychological stress, debilitating disease and nutritional deficiencies. The local accumulation of these bacteria leads to an increase of the adrenergic action on capillaries due to endotoxins, resulting in ischemic necrosis of the gingiva (Macphee 1981). Histological techniques show that the epithelium is destroyed and replaced by necrotic cells and mononuclear cell infiltration. Microbes characteristic of Plaut-Vincentstomatitis are abundant. Spirochaetes, fusiforme especially *Fusobacterium* and *Bacteroides* species – are present, and *Campylobacter* and *Streptococcus* species can also be found (Carranza 1990). *C. faecalis* could be a secondary inhabitant of the eroded skin, and it is possible that its different forms could be used to describe the age of the digital lesions.

These results have shown several microorganisms present on and in the lesions of digital dermatitis. Hypotheses about the aetiology of digital dermatitis cannot be proven using this material, but the association of a spectrum of microorganisms in comparison with other ulcers, such as the Plaut-Vincentstomatitis and yaws, indicates a multiorganismic cause of the disease.

The presence of spirochaetal microorganisms in combination with *C. faecalis* remains a plausible hypothesis for the multifactorial aetiology of digital dermatitis. Classification systems based on the findings of gross lesions can be extended by microscopical data in order to standardise the description of the disease. The histology revealed progressive inflammatory patterns in the skin together with characteristic growth patterns of microorganisms. Future investigation should focus on the typing of spirochaetes, ageing of lesions, molecular inflammation modulation and more standardised data collection of digital dermatitis to compare the disease with its differential diagnoses, such as interdigital dermatitis, interdigital phlegmon and other ulcerations of the bovine skin. Future diagnosis of digital dermatitis could be based on macroscopical and microscopical aspects of digital skin lesions and the presence and typing of spirochaetes.

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Abstracts

Resynthesis of glycogen in equine skeletal muscle after exercise

SIX normal standardbred trotters in Finland underwent repeated bouts of exercise. Muscle biopsy specimens and venous blood samples were obtained at intervals, and revealed a decrease of muscle glycogen for the first four hours after exercise, and a negligible increase in the first 24 hours. Plasma concentrations of lipid metabolites, glycerol, triglycerides and non-esterified fatty acids were less than pre-exercise values for the two- to 72-hour period after exercise. The low plasma concentrations of lipid metabolites suggest that energy is produced by the oxidation of carbohydrates, resulting in less carbohydrate availability for the resynthesis of glycogen.

HYYPÄ, S., RÄSÄNEN, L. A., & PÖSÖ, A. R. (1997) *American Journal of Veterinary Research* **58**, 162

Cytogenetics of feline fibrosarcoma

SHORT-term cultures of four feline fibrosarcomas were analysed cytogenetically. There was marked genetic heterogeneity between the four cats, each showing a different clonal abnormality. The aberrations detected were one deleted B2, one marker F1 and two reciprocal translocations, t(A2q; E3q) and t(A1q; B4p).

MAYR, B., BOCKSTAHLER, B., LOUPAL, G., REIFINGER, M. & SCHLEGER, W. (1996) *Research in Veterinary Science* **61**, 268





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