

## Histopathological Changes in Digits of Dairy Cows Affected with Sole Ulcers

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**ABSTRACT.** Forty-eight digits of 8 dairy cows, which had been diagnosed as sole ulcers in one or two hooves, were examined histopathologically and classified into 5 grades on the basis of the severity of the circulatory disturbances and of keratogenesis. All the cows had significantly higher grades than normal cows. Although hind lateral digits were most severely damaged, there were no significant differences in the grades among claw positions except hind lateral digits. From these results, it is suggested that the cows affected with sole ulcers had some systemic factors predisposing all the digits to digital disorders. — **KEY WORDS:** cattle (dairy), digit, sole ulcer.

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Recently, the incidence of lameness in cattle has increased and causes considerable economic loss in many countries including Japan [9, 12, 13]. It is reported by several workers that claw disorders account for 70 to 90% of diagnosed cases of lameness in cattle [10, 22, 23]. There appears to be agreement that subclinical laminitis is the most important predisposing condition affecting the claws of dairy cattle [6, 11]. The subclinical laminitis is defined as a condition associated with only the hemorrhages and/or the discolouration of the solar horn without clinical lameness [5]. It is believed that these microscopic changes in the hoof corium lead to abnormal keratinization and predispose the hoof to many other lameness causing lesions, such as sole ulcers and white line disease [6, 21].

In our previous report, 239 digits of 45 dairy cows randomly collected at two slaughterhouses in the suburbs of Tokyo were examined histopathologically, and the lesions were classified into 5 grades on the basis of the severity of circulatory disturbances and of keratogenesis [18]. The lesions from Grade 1 to 5 were considered as manifestations of serial lesions indicating the advance of subclinical laminitis to other hoof lesions. Microscopic lesions classified into Grade 2, regarded as subclinical laminitis, were found in over half of all the digits. These results suggested a high prevalence of subclinical laminitis among the dairy cows in Japan.

The etiology and pathogenesis of laminitis is not fully clarified yet, but laminitis is believed to be a multifactorial disease. Many factors have been proposed as predisposing causes of laminitis, including systemic disease, nutrition, management, calving, genetics, etc. [8, 21, 27].

In the present study, the digits of the cows which were diagnosed as sole ulcers were examined histopathologically. The aims of this study are to compare degrees, incidence, and sites of the hoof lesions of the cows with sole ulcers to those of the randomly collected cows, and to discuss the predisposing factors of the hoof lesions.

Eight Holstein dairy cows (aged 3 to 8 years) from a farm in Tochigi prefecture were used in this study. In that

farm, lactating cows were raised in a free barn floored with concrete and given complete feed *ad libitum*. Individual information about physical status of each cow (for example, daily milk yield) were unknown. All of them had been clinically diagnosed as sole ulcers and culled due to severe lameness and decreasing productivity.

From their unilateral fore limbs and bilateral hind limbs, 48 digits were obtained, from which the solar regions comprising the corium and the part of the epidermis were collected. Regardless of the locations of the sole ulcers, the sampling sites were settled at the caudo-medial part in the center sole, namely "specific ulcer site". These samples were fixed in 10% formalin and embedded in paraffin. Sections 3–5  $\mu$ m thick were stained with hematoxylin and eosin.

The histopathological findings of the solar regions were classified into 5 grades on the basis of severity of the circulatory disturbances and of keratogenesis. The criteria of grade 1 to 5 were defined as a series of progressive changes of normal hoof to sole ulcers. The details of the criteria were reported previously [18]. Statistical analysis were made with Mann-Whitney test or Kruskal-Wallis test.

The distribution of the grades in all the digits were shown in Table 1. Twelve digits were clinically diagnosed as sole ulcers. All of them were located in hind limbs, 4 in medial and 8 in lateral digits. Four cows suffered from sole ulcers in two digits. Among these 12 digits diagnosed as sole ulcers clinically, 4 digits were not classified into Grade 5, although the criterion of Grade 5 indicates the disappearance of the lamellatum epidermis, namely sole ulcers. This means that different parts of the sole besides the sampling sites were affected more severely. On the other hand, 7 digits were not diagnosed as sole ulcers clinically, but were classified into Grade 5. It was likely that the ulcerative lesions were slight and missed due to the presence of double soles or attached dirt in these 7 digits, because the digits were not trimmed appropriately when diagnosed grossly.

Histopathological changes found in the present study were fundamentally similar to the results of randomly collected

Table 1. Grade distribution in individual cows

Cow #	Claw positions							
	Left Fore	Left Fore	Right Fore	Right Fore	Left Hind	Left Hind	Right Hind	Right Hind
	Lateral	Medial	Medial	Lateral	Lateral	Medial	Medial	Lateral
# 63			4	2	<u>5</u>	2	2	3
# 68			2	2	<u>5</u>	1	1	<u>4</u>
# 73	3	3			<u>5</u>	3	<u>5</u>	3
# 75			3	4	<u>5</u>	3	4	<u>5</u>
# 76			4	3	<u>5</u>	4	3	<u>5</u>
# 77			5	5	<u>5</u>	<u>5</u>	<u>5</u>	3
# 78			2	2	4	5	<u>2</u>	5
# 83			2	3	<u>4</u>	2	4	<u>4</u>
Number of Sole ulcers	0	0	0	0	4	1	3	4

Underlined letters show the digits diagnosed as sole ulcers clinically.

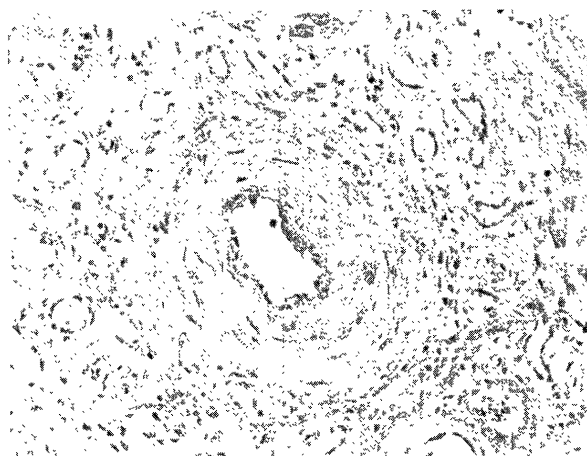


Fig. 1. Proliferation of tunica media in arterioles and the vascularization around the vessels in the solar corium of the left hind lateral digit of cow # 83 ( $\times 200$ , H. E. stain).



Fig. 2. Thrombi in the solar corium of the left hind lateral digit of cow # 75 ( $\times 100$ , H. E. stain).

digits in our previous report. In the corium, the findings were characterized by the circulatory disturbances of varying degrees. They were congestion, proliferation of tunica media in arterioles (Fig. 1), vascularization, thrombi (Fig. 2) and edema in connective tissue. The circulatory changes in the corium were found in most of all the digits. In the epidermis, dominant changes were degenerative changes, including vacuolization (Fig. 3), necrosis and loss of the cells in the stratum basale and spinosum. Various degrees of hyper-and/or parakeratosis was also found (Fig. 4). Compared with the circulatory disturbances in the corium, the degenerative changes in the epidermis were present with less frequency. Severe inflammatory changes were present only nearby the ulcerative lesions. The hypothesis about pathogenesis of laminitis, that the circulatory disturbance in the corium was the primary damage and the degeneration of the cells in the epidermis followed as a result of hypoperfusion in the corium, is widely accepted [2, 15]. In this study, it is considered to support the hypothesis that the

changes in the corium were found more frequently than in the epidermis.

Unlike the kinds of the lesions, the severity of lesions were completely different from those of our previous study. The incidence of 5 grades in all the digits was shown in Fig. 5. Grades 4 and 5 occupied over 50% in all the digits. Compared to the previous results of randomly collected samples, the digits of the cows suffering from sole ulcers had higher grades with higher incidence with significant difference ( $P < 0.001$ ) (Table 2).

The differences in the incidence of each grade among claw positions were also analyzed (Fig. 6). It was obvious that hind lateral digits in both sides were occupied with higher grades. In fact, statistical analysis showed significant difference among the distributions of the grades in 6 claw positions (all the claw positions except left fore lateral and medial) (Table 2). Furthermore, the hind digits had significantly higher grades than the fore ones, and the grades of the hind lateral digits were significantly higher than those of the hind medial ones. No significant difference was

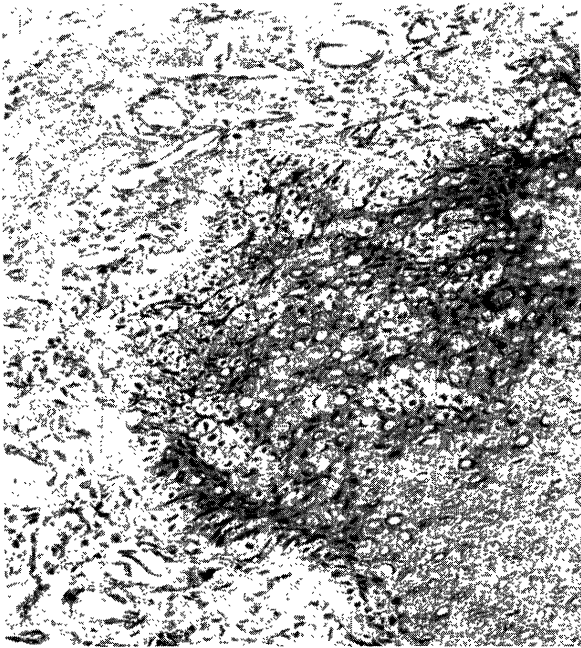


Fig. 3. Vacuolization of the cells in the stratum basale and spinosum of the right hind lateral digit of cow # 75 (× 200, H. E. stain).

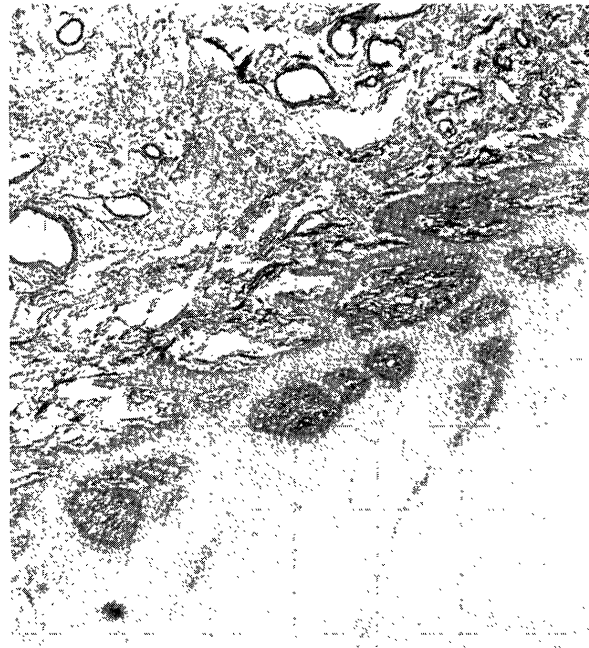


Fig. 4. Hyper-and/or parakeratosis in the epidermis of the right fore medial digit of cow # 63 (× 100, H. E. stain).

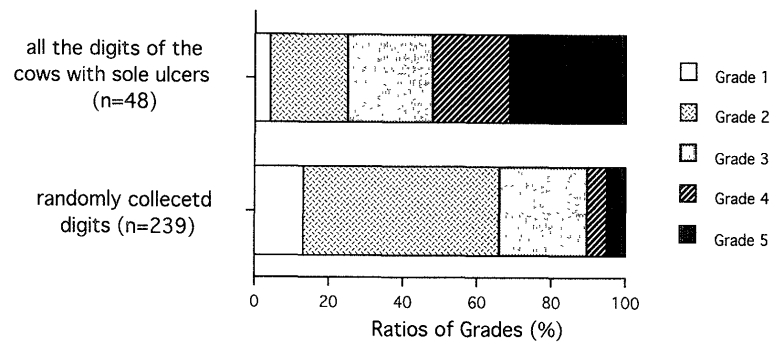


Fig. 5. Differences in ratios of grades between the digits of the cows with sole ulcers and the digits collected randomly.

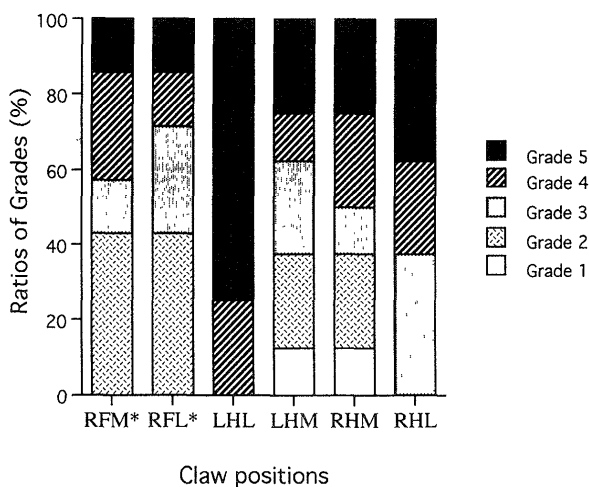


Fig. 6. Incidence of grades in each claw position. RFM: Right Fore Medial, RFL: Right Fire Lateral, LHL: Left hind Lateral, LHM: Left Hind Medial, RHM: Right Hind Medial, RHL: Right Hind Lateral. n=8 in each claw position. \*: n=7 (excluding cow # 73).

found between the right and left hind digits. Excluding hind lateral digits, there was no significant difference in the distributions of the grades among remaining 4 claw positions (right fore medial, right fore lateral, left hind medial and right hind medial). But whole 30 digits of these 4 positions had significantly higher grades than those of randomly collected samples reported previously.

These results indicated that the both sides of the hind lateral digits were affected most severely among all the claw

Table 2. Results of statistical analysis

Grouping	Analysing Method*	P Value
all digits of the cows / randomly collected samples with sole ulcers (n=48) in our previously report (n=239)	MH	< 0.0001
6 claw positions except left fore limbs fore digits (n=16) / hind digits (n=32)	KW	0.0388
hind lateral digits (n=16) / hind medial digits (n=16)	MH	0.0138
right hind digits (n=16) / left hind digits (n=16)	MH	0.2538
4 claw positions except left fore limbs and hind lateral claws	KW	0.9877
digits in 4 claw positions / randomly collected samples except hind lateral (n=30) in our previously report (n=239)	MH	0.0014

\* MH: Mann-Whitney test, KW: Kruskal-Wallis test.

positions, and that the digits in remaining 4 positions were equally affected. But it was also found that the digits in these 4 positions were much more damaged than randomly collected digits. From these results, it is speculated that all the digits of the cows suffering from sole ulcers had been damaged more severely than the normal cows. This may suggest that the cows with sole ulcers have some systemic or endogenous factors, which predispose strongly all the hooves to the circulatory disturbances.

Many endogenous factors predisposing laminitis have been proposed [8, 21, 27]. Among them, nutritional factors are believed to play an important role, because the onset of laminitis is well known to be related to the overfeeding of concentrates [14, 19]. Feeding rations high in carbohydrates can lead to alteration of bacterial flora in the rumen and decrease in the pH of ruminal fluid. Products of the deranged fermentation, such as lactic acid, histamine and endotoxin, have been considered to be causing substances of laminitis, but not been identified yet [1, 4, 24–26]. In the present study, precise quality and quantity of the rations given to the cows were unknown because they were fed *ad libitum*. But under the typical Japanese intensive husbandry management, it is easily speculated that the cows ate comparatively large amount of concentrates and suffered from metabolic stress. These systemic conditions may predispose the hoof lesions.

In many reports, as well as in this study, it was found that the lateral digits of the hind limbs in cattle were most severely affected [10, 11, 16, 17, 22, 23, 29]. In this study, it was also shown that no significant difference was found between the right and left hind digits. These results may indicate the presence of particular factors which affect the both sides of hind lateral digits to higher extent. Ossent and co-workers found overload on the lateral hoof of the hind limb in cows and they considered that the overload was one of the predisposing causes of sole ulcers [20]. In addition, other exogenous or environmental factors, such as

housing, bedding, exercise and behavior, were also considered to be involved in the onset of laminitis [3, 7, 17, 28]. Therefore, it is speculated that some systemic factors may lead the corium of all the digits to circulatory disturbances in the first place, and then the exogenous factors, such as overloading, may aggravate the lesions particularly in the hind lateral digits.

In conclusion, all the hooves of the cows suffering from sole ulcers were affected to higher extent than normal cows, and it is suggested that these circulatory disturbances in the corium were due to some systemic factors. The hind lateral digits were most severely damaged. Further study on both internal and external factors are needed to clarify the pathogenesis of hoof disease in cattle.

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