Histopathological Findings of the Digits in Dairy Cows in Japan

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ABSTRACT. Two hundred and thirty-nine digits of 45 Holstein dairy cows, which were raised in typical Japanese dairy farms and received poor hoof management, were randomly obtained in the slaughterhouses and examined histopathologically. The findings were classified into 5 grades on the basis of the severity of circulatory disturbances and of keratogenesis. The lesions from Grade 1 to 5 were considered as manifestations of serial lesions indicating that subclinical laminitis advanced to other hoof lesions. The incidence of Grade 2, regarded as subclinical laminitis, reached approximately 50% of digits examined. The lesions classified as Grades 3 (23.9%) and 4 (5.4%) were mainly characterized by circulatory disturbances, which were similar to those of chronic laminitis in the previous reports. The incidence of Grade 5, characterized by sole ulcer, was 5.4%. It is suggested that a considerable number of dairy cows in Japan suffered from subclinical laminitis, which may be the cause of recent high incidence of hoof diseases in dairy cows.—**KEY WORDS:** dairy cattle, digit, histopathology, incidence, laminitis.

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A recent increase in the incidence of digital disorders in cattle has been alarming in many countries including Japan, and its economic importance is comparable with other production diseases such as mastitis, metabolic diseases and reproductive disorders [2, 7, 9, 11, 17, 19–21, 23, 24]. Among various kinds of digital disorders, laminitis (pododermatitis aseptica diffusa) is believed to be the most important cause of other hoof lesions, such as sole ulcer and white zone diseases. In dairy cows there are 4 types of laminitis: acute, subacute, chronic and subclinical [4]. Subclinical laminitis is defined as a condition with slight microscopic lesions without clinical lameness [4], which may lead to the lesions such as hemorrhage in the sole, soft waxy horn and deformation of the hooves several months later [5].

The etiology and pathogenesis of laminitis are still not fully understood. Environmental factors, such as poor cubicle housing, lack of exercise and lack of hoof trimming, seem to be very important causes of this disease [2, 7]. At the same time, in dairy cows, nutritional factors are thought to play an important role in the etiology of subclinical laminitis [19]. David [8] reported that the rise in the incidence of lameness in dairy herds had paralleled the increased use of concentrates. Rowland [22] and other workers reported that about a half of the lameness cases occurred during the first four months of lactation. These facts may be related to metabolic stress from maximum milk yield after calving, which may exacerbate subclinical problems such as abnormally soft horn.

Most of the dairy farms in Japan are in a small scale without pasture, and dairy cows are kept in small stalls all the year round without correct hoof management and supplied with comparatively high concentrates/low roughage ration. Therefore, there may be some differences in the incidence of the digital disorders between in

Japan and in other countries. However, only a few epidemiological and histopathological studies concerning the digital disorders of dairy cows have been reported in Japan [12, 13].

In the present study, the digits of dairy cows in Japan were examined histopathologically to investigate the prevalence of the subclinical laminitis.

MATERIALS AND METHODS

Two hundred and thirty-nine digits of 45 Holstein dairy cows, including 28 digits of 5 cows that were slaughtered due to severe hoof lesions, were used in this study. The digits from the right fore limb and both hind limbs were randomly obtained from two slaughterhouses in Ibaraki and Tochigi prefectures. The husbandry forms of these cows were unknown, however, it was considered that these animals were managed in the typical Japanese intensive methods, i.e. supplied with rather high concentrates, kept in a small stall without exercise at a paddock or a pasture and received poor claw management. Immediately after slaughtered, the hooves were cleaned and observed macroscopically. If necessary, the sole horn was trimmed to expose and examine the fresh surface. Then, each hoof was sewn along the sagittal direction at the center of the hoof, and the caudo-medial part of the solar regions, namely 'specific ulcer site', comprising the corium and the part of the epidermis were collected for histopathological examination [1, 3]. These samples were fixed in 10% formalin and embedded in paraffin. Sections of 3–5 μ m in thickness were stained with hematoxylin and eosin and phosphotungstic acid hematoxylin (PTAH).

RESULTS

Macroscopic findings: The incidences of macroscopic abnormalities in 211 digits of 40 cows without sole ulcer were shown in Table 1. Most of the digits (72.3%) were overgrown claws [10]. Severe solar horn hemorrhages were found in only 12.1% of the digits examined. However, after paring the solar horns, petechial hemorrhages on the fresh surfaces were seen in most of the digits. These hemorrhages were mainly located in the central sole and the white line and could be pared out to reveal apparently normal horn.

All five cows slaughtered due to severe hoof lesions had sole ulcers and some of the cows had the infectious lesions extended to the third phalanx.

Histopathological findings: Various degrees of circulatory disturbance and keratogenesis were observed in most of the digits. Thus, the digits were histopathologically classified according to the following criteria.

Grade 1 was considered to be almost normal. The border between the stratum internum and medium was distinct and regular in shape (Fig. 1). The papillae of the stratum internum also formed a regular pattern (Fig. 2). Circulatory disturbances, such as distention of the vessels, congestion and vacuolization of the vessel wall, were rarely found.

In the tissues classified as Grade 2, the border between the stratum internum and medium was irregular in shape (Fig. 3). Occasionally, keratin bodies and eosinophilic degenerative cells were found in the stratum spinosum. Some of the cells in the stratum basale were vacuolized and pyknotic. In the corium, fibrosis and mild inflammation associated with accumulations of a few neutrophils and round cells were locally observed. Disturbances of circulation in the corium, which comprised mild vacuolization of the vessel wall, diapedesis, congestion, thrombosis or dilation of the vessels, were found.

In the tissues classified as Grade 3, the border between the stratum internum and medium was irregular, and keratin bodies in the stratum spinosum increased in number (Fig. 4), suggesting parakeratosis and hyperkeratosis. In the stratum basale, vacuolization was obvious and irregular proliferations were locally recognized. In the adjacent stratum spinosum, degenerative and necrotic changes were present (Fig. 5). The pathological changes in the corium were characterized by the moderate disturbances of circulation, consisting of congestion, dilation of blood vessels, vascularization and thrombi. There was edematous degeneration in the vessel walls, and especially in arterioles, proliferation of tunica media (Fig. 6) was dominant.

In Grade 4, the changes were characterized by cornification overall the stratum internum (Fig. 7). In some cases, the stratum internum was locally degenerated and/or necrotic. The obvious signs indicating the disturbances of circulation were frequently present. In the corium, dilation of the vessels and vascularization were found in most digits classified into this group, and thrombosis was seen in

Table 1. The incidences of macroscopic hoof lesions in the cows without sole ulcer (211 digits of 40 cows)

Overgrown claw	72.7%
Traumas or cracks in the solar horn	49.4%
Heel erosion	14.8%
White zone disease	27.5%
Severe hemorrhage in the solar horn	12.1%

more than half of the digits (Fig. 8). In addition, fibrosis of the capillary wall, edematous changes in collagenous fibers and perineurial fibrosis were observed.

In Grade 5, sole ulcer was the characteristic sign. The stratum corneum was so friable that the lamellatum epidermis was partly disappeared and as a result, sole ulcer was formed (Fig. 9). The corium was infected around the ulcerated region. In severely damaged digits, the infection extended to the third phalanx. In the corium, there was severe inflammation, associated with hemorrhage and infiltration of a large number of neutrophils and round cells. At the same time, thrombosis and the abnormalities of the vessels described above were observed with a high incidence.

Incidence of each grade: The incidence of each grade is shown in Table 2. In all the samples, the ratio of Grade 1 was only 11.7%. Grade 2 was the largest group, which occupied over 50% of all the digits. Twenty-three point nine percent of the digits belonged to Grade 3 and 5.4% belonged to Grade 4. These two groups occupied over a quarter of all the samples. The incidences of Grade 5 was 5.4%.

The differences in the incidences of each histopathological grade among each claw position were also examined. Except the right fore medial claws, Grade 2 accounted for over 50% in all the digit (Table 2). There was no evident difference in the ratio between the fore and hind digits and between the right and left hind digits (Fig. 10). Grades 3 and over tended to exist with high frequency in the fore medial and hind lateral digits (Fig. 11).

DISCUSSION

In this study, the individual physical conditions including the presence of lameness and the management they had received were unknown. However, in these districts, most of the dairy cows are raised in a tie-stall, stanchion or small concrete-floored barn with lack of exercise, and fed rather high concentrates. In 211 digits without obvious hoof lesions, 72.3% were overgrown claws and about 50% had traumas or cracks in the soles. These high incidences suggested that most of the cows had undergone poor hoof management such as lack of hoof trimming, poor exercise spaces and unsuitable floor surfaces of the stall.

The details of pathology of bovine laminitis were reported by several workers [1, 3, 15, 18]. In acute laminitis, hyperemia, edema, thrombi and hemorrhage are observed in the corium. In the epidermis, cells of the

Fig. 1. Histologic findings of Grade 1. Note the regular shapes of the border between the stratum internum and medium. ($\times 100$, H. E. stain).

Fig. 2. Normal appearance of the stratum basal and stratum spinosum in Grade 1 ($\times 200$, H. E. stain).

Fig. 3. Histologic findings of Grade 2. Keratin bodies in the stratum spinosum (arrows) ($\times 100$, H. E. stain).

Fig. 4. Histologic findings of Grade 3. Progressive keratinization in the stratum spinosum ($\times 100$, H. E. stain).

stratum basale are enlarged and vacuolized locally, and eosinophilic keratin bodies appear in the stratum spinosum. In chronic laminitis, the lesions in the corium are characterized by the vascular changes like arteriosclerosis and arteriolosclerosis including intimal proliferation, hypertrophy of the tunica media and fibrosis of the tunica

adventitia. In addition, dilation of the vessels, vascularization, chronic thrombi and fibrosis around nerves are observed. In the epidermis, hyper- and parakeratosis are present.

Recently, the term "subclinical laminitis" was introduced for the disease peculiar to cattle [5]. This condition

Fig. 5. Vacuolization of the stratum basale and the degenerative changes of the stratum spinosum in Grade 3 ($\times 200$, H. E. stain).

Fig. 6. Edematous degeneration of the arteriole in Grade 3. Note the proliferation of the tunica media ($\times 200$, PTAH stain).

Fig. 7. Histologic findings of Grade 4. Cornification of all area of the stratum internum ($\times 100$, H. E. stain).

Fig. 8. Multiple thrombi in the corium in Grade 4 ($\times 200$, H. E. stain).

is defined as the hoof problem with mild pathologic changes including hemorrhages in the sole, yellowish discoloration of the horn and other sole lesions, but without any noticeable clinical signs. Hypothesis that bovine subclinical laminitis is a major predisposing cause

of other hoof problems has been proposed by several workers [5, 6, 11]. In their reports, it is suggested that microscopic lesions in the corium, characterized by circulatory disturbances, may cause poorer horn quality and lead to sole ulcer and white zone disease after several

Fig. 9. Histologic findings of Grade 5. Partial loss of the stratum corneum ($\times 100$, H. E. stain).

Table 2. The incidence of grades in each claw position

Claw Position	Grade					total
	1	2	3	4	5	
Right fore medial	6 <i>14.6</i>	17 <i>41.5</i>	13 <i>31.7</i>	3 <i>7.3</i>	2 <i>4.9</i>	41
Right fore lateral	6 <i>15.0</i>	13 <i>57.5</i>	8 <i>20.0</i>	1 <i>2.5</i>	2 <i>5.0</i>	40
Left hind medial	2 <i>5.1</i>	23 <i>59.0</i>	9 <i>23.1</i>	2 <i>5.1</i>	3 <i>7.7</i>	39
Left hind lateral	4 <i>10.5</i>	23 <i>60.5</i>	7 <i>18.4</i>	2 <i>5.3</i>	2 <i>5.3</i>	38
Right hind medial	5 <i>12.8</i>	21 <i>53.9</i>	10 <i>25.6</i>	1 <i>2.6</i>	2 <i>5.1</i>	39
Right hind lateral	5 <i>11.9</i>	21 <i>50.0</i>	10 <i>23.8</i>	4 <i>9.5</i>	2 <i>4.8</i>	42
Total	28 <i>11.7</i>	128 <i>53.6</i>	57 <i>23.9</i>	13 <i>5.4</i>	13 <i>5.4</i>	239

(The italic numbers show the percentages in each grade)

months.

The lesions classified into five grades in this study are considered as manifestations of serial lesions, which may indicate that subclinical laminitis advances to other hoof lesions. The lesions classified as Grades 3 and 4 are quite similar to those reported as chronic laminitis, such as arteriolosclerosis, vascularization, chronic thrombi and perineurial fibrosis [1, 15, 18]. These lesions are mainly characterized by circulatory disturbances. Since any in-

Fig. 10. Differences in ratios of grades between the fore and hind, and right and left limbs.

Fig. 11. Differences in ratios of grades between the medical and lateral digits.

flammatory findings are hardly seen, it is considered that the degenerative changes and para- or hyperkeratosis in lamellae epidermales are not primary but secondary changes due to the circulatory disturbances in the corium. Andersson and Bergman [1] hypothesized that the circulatory change in the hooves caused an insufficient nutrient supply to the keratin producing cells with a synthesis of structurally incompetent keratin. Our findings in this study may support this hypothesis.

The histopathologic findings of Grade 2 were essentially the same as those of Grades 3 and 4, though milder than them. This may suggest that Grade 2 is the prior stage of Grade 3 and that Grade 2 may be regarded as subclinical laminitis. The incidence of this grade reaches over 50%. At the same time, although severe solar horn hemorrhages were found in only 12.1% of the digits, petechial hemorrhages in the central sole and the white line were seen in most of all the digits. Hemorrhage of the sole in cows of various ages have been reported by several workers, and it is suggested that the solar hemorrhage is an indication of subclinical laminitis [5, 6, 11]. These histopathological and macroscopical findings in this re-

search are highly suggestive of the presence of subclinical laminitis in the majority of the cows in Japan.

It is widely accepted that laminitis is a local manifestation of a generalized condition. For example, the practice feeding large amounts of concentrate in the absence of roughage could cause a reduction in rumen pH, which is believed to predispose the animal to laminitis. Histamine, lactic acid and endotoxins have been believed to be involving factors, most of which are not clearly understood [2, 14, 20]. In the present study, the incidence of Grade 2 is about 50% in any position of claws examined. This similarity in the incidence of Grade 2 among all claws is considered to be due to a certain systemic factor. It is suggested that Japanese dairy cows suffer from metabolic stress through the typical Japanese intensive husbandry management and that these systemic conditions predispose to the hoof lesions.

It has been reported that lesions causing lameness occur in the lateral claws in more than 80% of cows with hind limb lameness [9, 21, 23]. Maclean [16] reported that the lesions in the hind claws are three times as common as in the fore claws. Greenough and Vermunt [11] reported that the severity of sole hemorrhages is three times greater in the lateral hind than medial hind claws. In the present study, although there was no evident difference, Grades 3–5 with comparatively severe changes tended to exist with a higher frequency in the fore medial and the hind lateral digits. Therefore, the following hypothesis could be proposed: the dairy cows supplied with large amounts of concentrates are under metabolic stress. In the corium of all claws, they have slight microscopical lesions like Grade 2 as the local manifestation of the systemic condition. In addition to this internal factors, the external factors, such as trauma, overloading and dirty environment, predispose the claws in the specific positions (*i.e.* the lateral hind claws) to the horny lesions, which lead to lameness. Further investigations on many other factors causing laminitis in the cows are needed.

In conclusion, it is suggested that the dairy cows under common Japanese husbandry system are affected with subclinical laminitis in high incidence and that these conditions are related to the recent increase in the incidence of the lameness in cows.

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