Pathomorphological Findings in a Case of Onychomycosis of a Racehorse

Atsutoshi KUWANO, Masaaki OKAWA, and Kousuke TAKATORI

Pathology Division, Equine Research Institute, Japan Racing Association, 27–7 Tsurumaki 5-chome, Setagaya-ku, Tokyo 154, Division of Microbiology, National Institute of Health Sciences, 18-1, Kamiyoga 1-chome, Setagaya-ku, Tokyo 158, Japan
(Received 16 April 1996/Accepted 2 July 1996)

ABSTRACT. The hooves of a racehorse which were affected with white line disease and hoof wall disorders on both forelimbs were histopathologically investigated using thin ground section and standard paraffin section techniques. On both hooves, large quantities of fungus were found to have invaded the white line tissues, especially in the terminal horn which were markedly damaged. The fungus was also present among the cellular debris in the fissures of horny tissues. The morphological characteristics of the fungus were brown (its natural color), PAS-positive, mold-like shape with septa inside the tissues, and unicellular spores inside the tissues. These findings suggest that onychomycosis was a primary and/or secondary cause of white line disease in this subject. — KEY WORDS: equine, onychomycosis, white line disease.


White line disease is a hoof wall defect in which the keratinized tissues of the white line on the hoof experience brittle fractures [8]. Causes of white line disease include a worse environment, nutritional problems, poisoning by excessive intake of trace elements, and infection [3]. In addition to bacteria, fungi such as Candida albicans [4], Pseudoallescheria spp., and Scopulariopsis spp. [2] have been isolated from the white lines of hooves suffering from white line disease. However, the pathogenic significance of these fungi to the occurrence of white line disease has not yet been elucidated. Using thin ground section technique [5] enabling observation of the hoof capsule over a wide range, we conducted a pathomorphological study of hooves affected with white line disease where fungus was observed in the terminal horn of the white line. Our findings contributed to an improved understanding of the infectious characteristics and pathological nature of the fungus.

A 6-year-old male thoroughbred racehorse was returned to the Rittsu Training Center of Japan Racing Association after pasturing. It was diagnosed with serious white line disease, including wide spread horn separation, and a slight rotation of the coffin bone in the right forelimb. During the preliminary examination, the horse did not show any lameness, and a hoof-tester examination found no pain, so the horse was not clinically treated by the veterinary staff. However, the dorsal hoof wall was extensively trimmed (Fig. 1-a) and then a filling material (Equilox Adhesive System: Gauthier Medical, Inc.) was applied to the resected area by the farrier staff. About one month later, sole bulging developed in conjunction with a protrusion of the distal phalanx, which was followed by supporting limb lameness. At the same time, the right dorsal hoof wall underwent a concave deformation and a light divergent growth, causing the filling material to separate from the hoof wall. The surface of the resected area on the wall was thus left exposed for about three months. As the right hoof lesions progressed, one month after separation of the filling material, serious support laminitis, graded grade IV as described by Obel [7], occurred in the left forelimb as well, along with mild white line disease and a longitudinal fissure in the apex of the hoof wall. Accordingly, the horse was humanly destroyed at the owner’s request.

After a postmortem examination, the hoof was cut longitudinally, using an electric band saw (Makita Co., Ltd., Japan) and sections of five-mm thickness were prepared. After these sections were observed macroscopically, they
were fixed in a 10% buffered neutral formalin solution for at least three days. After dehydration by rinsing through graded alcohol, thin ground sections were prepared according to method reported above [5] and standard paraffin sections were made. Both kinds of tissue sections were then stained by hematoxylin-eosin stain (H.E. stain), and the paraffin sections only were stained by Periodic Acid Schiff reaction (PAS reaction).

At the time of the postmortem examination, the surface of the resected area on the right hoof wall was extremely irregular and brown-colored. In the sagittal section, yellowish clots of white line-like tissues proliferated between the hoof wall and the dorsal cortex of the coffin bone (Fig. 2). Fissures had formed in these white line-like tissues, and were filled with brown-colored, destroyed horn tissues.

In the sagittal section of the left hoof, a longitudinal fissure of 2-3 cm length formed, which rose linearly from the bearing surface, in the boundary region between the hoof wall and the white line at the toe. The region in a fissure was filled with destroyed horn tissues which had turned brown.

On the right foot, in the proliferating white line-like tissues, there were interdigitating formations of eosin-amblychromatic primary epidermal lamellae and terminal horns with horn tubules which were well stained by the eosin; these took on a convoluted, undulating appearance (Fig. 2-a). These white line-like tissues were subsequently confirmed to be proliferating white line tissues.

PAS-positive fungus was also confirmed on the white line tissues near the fissures. On the surface of the proliferating white line tissues and among the horny debris in the fissures, the spores were globose, subglobose, or racquet shaped, with the shape of yeast-like fungus (Fig. 2-b).

In keratinized tissues, especially in the terminal horns of the white line, fungus manifested the shape of mold-like fungus, and was contiguous with the yeast-like fungus outside the tissues (Fig. 3). Mold-like fungus was septated and some showed fragmentation (Fig. 4). Also, racquet-shaped conidia developed from some hypha. In unstained thin ground sections, brown fungal bodies were observed to be the natural color of the fungus (Fig. 3-a).

Meanwhile, on the horny tissues of the left hoof, dense formations of fungus having the same shape as in the right hoof were present among the exfoliated horn tissues in the fissure, and in the terminal horns adjacent to this crack. Passing the terminal horn, fungus had invaded the inner part of the stratum medium of the wall, and proliferated in the marrow of the horn tubules (Fig. 5).

The most noteworthy of our findings was the fungal invasion of the white line tissues accompanied by fissure formation. Fungus took on a yeast-like appearance under the aerobic environment of the hoof fissures; under the
anaerobic environment present inside the horny tissues, however, they took on a mold-like appearance. The shape of the fungus inside the tissues was different from that outside. However, fungus on the horn surface was contiguous with that inside the horn, and was the same color. Accordingly, these formations were thought to be homologous. The fungus had the following morphological characteristics: a) brown color; b) yeast-like and mold-like shapes outside and inside the tissues; c) mold types had septa, and some were fragmented; d) spores were unicellular, including globose, subglobose, or racquet-shaped. Two representative fungi exhibiting these morphological characteristics and which invade keratinized tissues are *Myceliophthora* spp. [9] and *Pseudallescheria* spp. [6].

Since this fungus was observed in the fissured region, and was present in large quantities in the terminal horn where keratinized tissues suffered extensive destruction, the following conclusions are suggested:

Even if fungus was the primary factor in the fissuring

---

Fig. 3. Mold-like fungus inside the horny tissues (H). (a) Mold-like fungus is contiguous with the yeast-like fungus outside the horny tissues. Unstained thin ground section viewed under high contrast. (b) The yeast-like fungus has grown into the horny tissues (arrow). Paraffin section. PAS reaction. (a) × 300. (b) × 850.

Fig. 4. Fungus develops in the terminal horn (Th) specifically, but less fungus is observed in the primary epidermal lamella (Pel). Mold-like fungus is septated, and some shows fragmentation irregularly (inset). Paraffin section. PAS reaction. × 300. Inset, × 600.

Fig. 5. (a) Fungus also develops in the marrow of horn tubules (M) near the tips of the terminal horns (Th) which have been destroyed on the left foot. (b) High magnification of a horn tubule in a square of (a). Paraffin section. PAS reaction. (a) × 102. (b) × 600.
seen in the white-line tissue, or was responsible for a secondary invasion after fissure formation, the fungus digested or hydrolized the keratinized tissues of the terminal horns while also producing protease [1]; this caused the horn to become brittle and induced disruption of the white line.

This study supports the possibility that the presence of onychomycosis leads to a pathological state of white-line disease [8, 10], and suggests that pathomorphologically, the terminal horn is the most susceptible region for fungal invasion.

REFERENCES