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RESEARCH ARTICLE

Characterization of White Line Disease in Riding Horses and Its Therapies

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ABSTRACT

This study investigated the grades and prevalence of white line disease in 54 horses diagnosed by farriers and veterinarians among the 3,499 riding horses raised in Korea. After isolation and identification the causative agents, an antibiotic sensitivity test was performed and the effects of treatment with copper sulfate were studied. The prevalence of white line disease was 1.54% (54/3,499 horses). By region, Gangwon-do (3.27%), Gyeongsangbuk-do (1.74%), Gyeongsangnam-do (1.40%), and Gyeonggi-do (1.28%) had the highest prevalence. By location, the front of the hoof (50.0%), the back (29.6%), and the side (20.4%) were in order. The prevalence grades were grade 3 (9.2%), grade 2 (14.8%), and grade 1 (25.9%) for the front part of the hoof, but only grade 2 and grade 1 were observed for the side and back part of the hoof. As a result of identifying the causative agent from 54 WLDs, 64 bacteria of 24 species, including Acinetobacter spp., Corynebacterium glutamicum, and Escherichia coli, were identified, and 7 fungi, including Sedosporum spp., were isolated. Antibiotic susceptibility testing for these isolates revealed that they were susceptible to most of the 22 antibiotics tested, including amikacin. In addition, it was confirmed that copper sulfate is effective in treating white line disease.

INTRODUCTION

The horse's foot is largely composed of bones (second phalanx, third phalanx, and navicular bone), elastic components (plant or digital cushion), and keratin (hoof wall, sole, white line, bars, and heel bulb) (Kainer, 1989; Kim, 2018; Stashak 2002).

The hoof wall of the horse comprises three layers: the stratum externum (external layer), stratum medium (middle layer), and stratum internum (inner layer) (Redding and O'Grady, 2012; O'Grady, 2002; O'Grady and Burns, 2021). The nonpigmented zone of the middle layer (stratum medium) combined with the dermal lamellae is responsible for attaching the hoof wall to the distal phalanx. The white zone (white line) consists of intertubular horns and terminal tubules surrounded by intertubular horns, and has hard and elastic properties (Pollitt, 2010).

The white line refers to the place where the hoof-nail is inserted when the horseshoe is mounted on a horse's hoof. The white line plays a special role in the horseshoe because the hoof nails are driven in this area (Hermans, 1992; Ruthe et al., 1997). If the hoof nails are misplaced, damage to the transparent or wall dermis or bruising of the parietal dermis may be the cause. No nailing is done to the quarter area to maintain the hoof mechanism (Hermans, 1992; Ruthe et al., 1997; Dabareiner et al., 2003).

White line disease (WLD) was first described in the mid-1980s (Moyer, 2003), and the first paper was published by Redden (1990).

Shin et al. (2020) reported the prevalence of hoof disorders (thrush: 4.2%, superficial hoof wall crack [crack]: 1.2%, WLD: 1.0%, hoof wall separation [HWS]: 0.6%, hoof wall defects [HWD]: 0.5%, laminitis: 0.3%, and wounds: 0.2%) in both racing horses and riding horses in Korea.

The white line separation (WLS) is a globally recognized hoof disease and a symptom of several common horse hoof diseases. In particular, WLS is difficult to identify based on the external appearance the hoof; moreover, it is challenging to differentiate WLS from other diseases such as WLD, thereby making it difficult to determine its severity. The lesion site can only be observed by removing the horseshoe and trimming the hoof, but if early treatment is not provided, the disease can spread rapidly throughout the whole white line. In WLS, the entire statum corneum (the infected area), a cause of secondary infection, must be removed to treat the disease. If chronic laminitis develops, function can be impaired, so prevention is the best option for farriers. The exact cause of WLD is unknown, and it has been discussed in various ways, including mechanical and nutritional factors, and infections of certain fungi and bacteria (Turner, 1997; Higami, 1999; Stashak, 2002; O'Grady, 2002). It is also susceptible to WLD due to changes in hoof horns caused by chronic laminitis (founders) (Kuwano et al., 1998; O'Grady, 2002).

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In this paper, we investigated the prevalence characteristics of WLD in riding horses. After pathogens isolated from the site of occurrence, antibiotic sensitivity tests and appropriate treatment were performed.

MATERINALS AND METHODS

Ethical approval

This article does not approved by Research Ethical Committee because it is a routine hoof trimmer in horse industry. But, it also complied with the AALAC regulations (Newcomer and McGlone, 2015).

Animals

The horses used in this study were 54 horses (30 gelding, 17 mares, and 7 stallions; mean age 8–10 years; mean weight 480 kg) diagnosed with WLD in public or private equestrian clubs with relatively good breeding conditions. Figure 1 shows an example diagnosed as WLD. This study was conducted for 7 months including the general grooming period with transversal study.

Incestiagtion of WLD pattern

The WLD pattern was investigated a modified the previous method of Sato et al. (2021). The positions were classified into anterior, lateral, and posterior, whereas the grades were classified into grades 1, 2, and 3 by measuring the width and depth of the lesion using a ruler after trimming.

For lesions at the toe (front), grade 1 was defined as a lesion having a width of <2 cm and a depth of <3 cm, grade 2 was defined as a lesion having a width of 2-3 cm and a depth of 4-5 cm, and grade 3 was defined as a lesion having a width of <3 cm and a depth of <5 cm. For side lesions, grade 1 was defined as a lesion having a width of <2 cm and a depth of <2 cm, grade 2 was defined as a lesion having a width of 2-3 cm and a depth of 2 cm, and grade 3 was defined as a lesion having a width >3 cm and a depth of >2 cm. For palmar lesions, grade 1 was defined as a lesion having a width of <1 cm and a depth of <1 cm, grade 2 was defined as a lesion having width of 1-2 cm and depth of 1-2 cm, and grade 3 was defined as a lesion having a width of >2 cm and a depth of 2 cm.

Sample collection

Samples were collected from the white line using sterile swabs with a bacterial testing kit (BBL CultureSwab PLUS, Becton, USA) following the manufacturer's instructions (Figure 2). The swabs were immediately transferred to a laboratory to identify the microorganisms.

Isolation and identification of bacteria and fungus

For bacterial isolation from lesions, collected swabs were streaked onto blood agar plates and incubated at 37°C for 24 and 48 hours, respectively, under aerobic and anaerobic conditions. Isolated bacteria were identified by using Bruker Biotype 4.1 (Bruker Daltonics, Bremen, Germany). Fungal isolation was performed by streaking collected swabs onto Sabouraud dextrose agar containing chloramphenicol (SDAc) and incubating at 25°C for 7 days. Purely isolated fungi were identified by genetic analysis (Kim and Cho, 2023).

Antibiotic susceptibility tests

Antibiotic susceptibility tests were performed using the broth microdilution method. The cultured colony was inoculated into a tube containing a serial dilution of the antimicrobial agent to be tested. The tube was cultured for 24 h and then read using an automatic reader (Microflex, Bruker, Germany) (Kim and Cho, 2023).

Treatment of WLD

Treatment was performed by applying a copper sulfate solution (water: copper = 100:1, Shin-O Chemical Co., Ltd., Korea) to the lesion area, allowing sufficient copper sulfate to permeate, and then applying a cream prepared by mixing Vaseline (Unilever, Korea) and copper sulfate powder to the lesion. In the case of WLD in front of the hoof, the horseshoe was fitted with rocker toe shoes for 7 animals and quarter shoes for 20 animals to reduce the length of the front of the hoof. In the side or palmar of the hoof, the animals wore the shoes for 5 months (2 times, shoeing) to balance the hoof (Figure 3). Of 54 horses, 15 were treated with a copper sulfate solution and 5 with betadine antiseptic solution (saline: betadine = 10:1, Mundipharma Co, Ltd, Korea).

RESULTS

Pattern of WLD

The results of our investigation regarding the pattern of WLD in riding horses in Korea are shown in Table 1. Of 3,499 horses, 54 (1.54%) were diagnosed with WLD. By region, the prevalence was the highest in Gangwon-do (3.27%), followed by Gyeongsangbuk-do (1.74%), Gyeongsangnam-do (1.40%), and Gyeonggi-do (1.28%).

Table 2 shows the distribution of the WLD location and grade. WLD was most commonly detected in the front of the hoof (50.0%), followed by the palmar (29.6%) and the side (20.4%). The WLD grade distribution for front lesions was 25.9% grade 1, 14.8% grade 2, and 9.2% grade 3. For side and palmar lesions, only grade 1 and 2 lesions were observed.

Identification of bacteria and fungus

As a result of identifying the causative agent from 54 WLDs, 64 bacteria of 24 species, including *Acinetobacter* spp., *Corynebacterium glutamicum*, and *Escherichia coli*, were identified (Table 3), and 7 fungi, including Sedosporum spp., were isolated (Table 4).

Antibiotic susceptibility tests

The results of antibiotic susceptibility tests for these isolates revealed that the isolates were susceptible to most of the 22 antibiotics tested, including amikacin (Table 5). This is likely because the treatment and prevention of WLD primarily focus on stall and hoof management practices, such as hoof and stall cleaning, and antibiotics were not used routinely for treatment.

Treatment of WLD

Figure 4 shows an example of the results of WLD treatment in horses using a copper sulfate solution and a betadine disinfectant. The copper sulfate solution was more effective in completely eradicating WLD within 5 months than the betadine disinfectant.

DISCUSSION

In Korea, riding horses are usually provided with hoof care every 45-50 days (about six weeks). However, some differences in these intervals may vary depending on the equestrian park and region.

WLD was first described in the mid-1980s (Moyer, 2003), and the first paper was published by Redden (1990). The researchers have attempted to establish an infectious pathology in WLD (Redden, 1990; Kuwano et al., 1999). Most of these studies aimed to isolate and identify fungi from affected hoof tissues, thereby establishing a definite association between horses' onychomycosis and WLD (Kuwano et al., 1999).

Regarding the white line, which is a part of the horn, the ends of the dermal lamellae are surrounded by small terminal papillae. The keratinocytes (stratum germinativum) in these terminal papillae form coronary band keratin, which is similar to that in the sole. The horn of the hoof wall and the lamellae combine to form the white line. The white line is pale yellow in color and approximately 3 mm wide. As the part adjacent to the hoof wall is nonpigmented, the white line appears to be white. The outside of the white line is used as a reference point when placing nails for horseshoes. The white line is weak but acts as a shock absorber.

Diseases related to WLS are generally difficult to diagnose because they can only be examined after removing the horseshoes. The causes of WLD can be categorized as follows: 1) unfavorable hoof morphology, such as flat, broad, or low hooves, with a shallow, sloping hoof wall, poor horn quality, or weakness of the white line; 2) excessive trimming of the white line area; 3) broad or low hooves or using shoes with no slope or those with a small slope; 4) abscess of the white line area, a missing hoof, or cancer; and 5) unclean stables, lack of or delayed hoof care, continual dampness of the hoof, excessive abrasion and growth with bare feet, or excessive exercise on hard ground.

Multiple additional causes of WLD have been identified, including environmental factors, nutritional factors, mechanical factors, and infectious organisms (Turner, 1997). We examined the hooves of 3,499 riding horses and found that 54 horses (1.54%) were diagnosed with WLD.

By region, WLD prevalence was the highest in Gangwon-do (3.27%), followed by Gyeongsangbuk-do (1.74%), Gyeongsangnam-do (1.40%), and Gyeonggi-do (1.28%). The lower prevalence of WLD in Gyeonggi-do and Gyeongsangnam-do than in Gangwon-do and Gyeongsangbuk-do may have been due to better horse care and management in these regions. When we examined the location of lesions, the most common location was the front of the hoof (50.0%), followed by the palmar (29.6%) and the side (20.4%). In terms of the lesion grade, 25.9% of the lesions in the front of the hoof were grade 1, 14.8% were grade 2, and 9.2% were grade 3. Only grade 1 and 2 lesions were observed in the palmar and side of the hoof. These findings were consistent with those of Sato et al. (2021), who reported that of the 326 hooves of both forelimbs in 163 yearling Thoroughbred horses, 160 hooves had grade 0 lesions, 131 had grade 1 lesions, 31 had grade 2 lesions, and 4 had grade 3 lesions.

In the present study, the analyses of the 54 samples collected from horses diagnosed with WLD in Korea revealed that the causative agents were *Acinetobacter* spp., *Corynebacterium glutamicum*, and *Escherichia coli.* In total, 64 bacterial isolates were collected from 24 species. In addition, 7 species of fungi, including Scedosporium, were isolated. This is similar to the findings reported by Kim and Cho (2023), and Kuwano et al. (1998).

Keratinolytic bacteria and fungi have been incriminated as the cause of WLD, but given the number and variety of organisms isolated, they are probably secondary opportunists. The predominate fungus isolated also appears to vary between regions of the world, with the genus Scedosporium most common in Japan (Kuwano et al., 1998) and Scopularopsis more commonly isolated in the USA and central Europe (Tjalsma and van Maurik, 1995). The most likely scenario is that there is some predisposing factor, whether nutritional deficiency, environmental factors (excessive dry or wet conditions), or mechanical factors such as neglected overgrown feet, long toes, chronic laminitis, and hoof imbalances, that leads to damage to the hoof wall and associated white zone, with subsequent invasion and colonization by bacteria and fungus (Turner, 1997; O'Grady, 2002). In this study, Scopularopsis reported from the United States were not isolated, but more studies are needed on more samples in the future.

The results of antibiotic susceptibility tests for these isolates revealed that most of the isolates were susceptible to the 22 antibiotics tested, including amikacin. This could be attributed to the fact that the treatment and prevention of WLD primarily focused on stable and hoof management practices, such as hoof cleaning, and that antibiotics were not used routinely for treatment. Medications most

commonly used were the soluble powder forms of tetracycline or oxytetracycline (48% by veterinarians and 81% by hoof trimmers) followed by copper sulfate for veterinarians and ichthammol ointment (a sulfurous, tarry compound with mild antiseptic properties used primarily as a drawing agent) by trimmers (Shearer and van Amstel, 2017). Collaboration between the clinician and a skilled farrier is important for successful management of hoof disorders (Furst and Lischer, 2021). Good hoof care relies on close collaboration and communication between the veterinarian, farrier, and owner. Laminitis and white line disease are major clinical issues when the diet is inappropriate and underfoot conditions are too wet (Thiemann and Poore, 2019).

In 85% of the horses, at least one hoof disorder was observed during regular foot trimming (Holzhauer et al., 2017). Hoof diseases can be prevented by specific management, including appropriate feeding, providing clean stall environment, regular hoof trimming, and horseshoe application. Currently, the treatment of WLD mainly involves cutting off the necrotized frog tissue, soaking the foot in disinfectant, and/or wiping with disinfectant and drying twice a day. The most effective treatment method involves trimming of the hoof to expose the infected area to air and treating it weekly with iodine to prevent bacterial growth (Hennig et al., 2001; Johnson et al., 2015; Holzhauer et al., 2017). The results of the antibiotic sensitivity tests on bacteria isolated from horses with WLD showed that the bacteria were sensitive to most of the 22 antibiotics tested, including amikacin. This could be because most treatment and prevention methods for WLD traditionally focused on hoof management an equine management, such as trimming, and few antibiotics were used for treatment. However, the use of antibiotic ointments or injections through cooperation between farriers and veterinarians would likely be more effective in the treatment of horses with WLD. Only veterinarian can treat antibiotics. Therefore, a method in which a farrier who routine hoof management can treat using copper sulfate alone was studied. When this study compared the effects of treating WLD with copper sulfate solution and betadine antiseptic, we found that the copper sulfate solution was more effective betadine. Higami (1999) reported that the risk of WLD is high when horses are given a feed low in zinc and copper. It appears that the well-known antimicrobial effects of copper may apply to the application of copper coating of horseshoe nails in reducing the microbial damage to the horses' hoof frequently associated with horseshoe nail insertion (Hampson and Wilson, 2018). Therefore, the supply of these minerals is important for preventing WLD. Our study demonstrates that copper sulfate is effective for treating WLD; however, in the future, a larger sample of horses will need to be studied.

CONCLUSION

In this study, 54 of 3,499 riding horses raised in Korea were diagnosed with WLD by farriers and veterinarians, and the prevalence of WLD was found to be 1.54% (54 /3,499 horses). Antibiotic susceptibility testing for the causative agents of WLD revealed that they were susceptible to most of the 22 antibiotics tested, including amikacin. In addition, it was confirmed that copper sulfate is effective in treating WLD.

Author contributions: All research protocols in this study were designed and conducted by DS Kim, C Jang, YH Kim, whose also contributed to data acquisition and writing the manuscript. GJ Cho contributed to the interpretation of the experimental results and supervision of writing the manuscript. All authors have read and agreed to the published version of the manuscript.

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Conflicts of interest: The authors declare no conflict of interest.

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APPENDIX: 1

Table 1: Region and number of horses with WLD in this study

Region	No. (%) of horses with WLD
Gwangwon-do	8/245 (3.27)
Gyeonggi-do	25/1,950 (1.28)
Gyeongsangnam-do	7/500 (1.40)
Gyeongsangbok-do	14/804 (1.74)
Total	54/3,499 (1.54)

Table 2: Distribution of WLD by site and grade in this study

Site of occurrence	No. (%) of horses	Grade 3	Grade 2	Grade 1
Front	27/54 (50.0)	5 (9.2)	8 (14.8)	14 (25.9)
Side	11/54 (20.4)	0	5 (9.3)	6 (11.1)
Palmar	16/54 (29.6)	0	6 (11.1)	10 (18.5)

Table 3: Results of bacteriological examination of WLD in horses

Isolates	No. (%) of isolates	Isolates	No. (%) of isolates
Acinetobacter pseudolwoffii	4 (6.3)	Enterococcus faecium	2 (3.2)
Acinetobacter spp.	9 (14.1)	Escherichia coli	4 (6.3)
Aerococcus viridans	2 (3.2)	Glutamicibacter arilaitensis	5 (7.8)
Bacillus cereus	2 (3.2)	Microbacterium spp.	1 (1.6)
Bacillus circulans	1 (1.6)	Psychrobacter maritimus	1 (1.6)
Bacillus spp.	2 (3.2)	Proteus mirabilis	2 (3.2)
Corynebacterium glutamicum	6 (9.4)	Proteus vulgaris	2 (3.2)
Carnobacterium inhibens	5 (7.8)	Shewanella algae	1 (1.6)
Carnobacterium viridans	1 (1.6)	Streptococcus equinus	2 (3.2)
Enterococcus asini	2 (3.2)	Solibacillus silvestris	1 (1.6)
Enterococcus casseliflavus	4 (6.3)	Rhodococcus pyridinivorans	2 (3.2)
Enterobacter cloacae	1 (1.6)	Enterococcus spp.	2 (3.2)

Table 4: Results of fungal examination of WLD in horses

Isolates	No. (%) of isolates
Ascochyta meticaginicola	1 (10.0)
Candida parasilosis	4 (40.0)
Cladoporium spp.	1 (10.0)
Fusarium lichenicola	1 (10.0)
Preussia flanaganii	1 (10.0)
Scedosporum spp.	1 (10.0)
Talaromyces spp.	1 (10.0)

Table 5: Antimicrobial drug test results of bacteria isolated from horses with WLD

Isolates	Drug sensitivities
Acinetobacter pseudolwoffii	A, AC, AK, AP, C, CT, D, E, G, IM, M, O, TC
Acinetobacter spp.	A, AC, AD, AK, C, CT, CV, D, E, ER, G, IM, M, O, TS
Aerococcus viridans	A, AC, AP, C, CM, D, E, ER, IM, M, N, O, V
Bacillus cereus	A, AC, AK, C, CM, D, E, G, IM, M, N, O, TS, V
Bacillus circulans	AC, AP, AK, C, CL, CT, CV, D, E, G, IM, M, N, O, P, TS, V
Bacillus spp.	A, AC, AK, AP, C, CL, CM, CT, CZ, D, E, ER, G, IM, M, N, O, P, TS, V
Corynebacterium glutamicum	A, AC, AK, C, CT, CV, CZ, D, E, ER, G, IM, M, O, TS, V
Carnobacterium inhibens	A, AC, AP, CM, CV, D, ER, G, IM, O, P, TS, V
Carnobacterium viridans	A, AC, AK, C, CM, CZ, D, E, ER, G, IM, M, N, O, P, TC, V
Enterococcus asini	A, AC, AK, AP, C, CL, CM, CT, CV, CZ, D, E, ER, G, IM, M, N, O, P, TS, V
Enterococcus casseliflavus	AC, AP, D, E, N, O, V
Enterobacter cloacae	AK, CT, CV, E, G, IM, M, O, TS
Escherichia coli	AC, AK, AP, C, CT, CV, CZ, E, G, IM, M, N, O, TS
Enterococcus faecium	A, AC, AK, AP, C, CL, CM, CT, CV, CZ, D, E, ER, G, IM, M, N, O, P, TS, V
Enterococcus spp.	AC, AP, P, D, V, M, O, IM, N
Glutamicibacter arilaitensis	A, AC, AK, AP, C, CL, CT, CV, CZ, D, E, EP, G, IM, M, O, TS, V
Microbacterium spp.	A, AK, CM, E, ER, G, IM, M, O, V
Psychrobacter maritimus	A, AC, AK, C, CL, CV, CZ, G, IM
Proteus mirabilis	AC, AK, AP, C, CT, CV, CZ, E, G, IM, M, O, TS
Proteus vulgaris	AC, AK, CT, CV, E, G, IM, M, O, TS
Rhodococcus pyridinivorans	A, AK, C, CT, CV, D, E, ER, G, IM, N, O, V
Shewanella algae	A, AC, AK, AP, C, CV, CZ, E, G, IM, M, N, O, TS
Streptococcus equinus	A, AC, AP, C, CL, CM, CT, CV, CZ, G, IM, M, N, O, P, V
Solibacillus silvestris	A, AC, AK, AP, C, CL, CM, CT, CV, CZ, D, E, ER, G, IM, M, N, O, P, TS, V

*A, amikacin; AC, amoxicillin / clavulanic acid; AP, Ampicillin; AZ, azithromycin; CC, cefaclor; CZ, cefazolin; CT, cefotaxime; CV, cefovecin; CE, cephalexin; CL, clindamycin; D, doxycycline; EN, enrofloxacin; ER, erythromycin; G, gentamicin; I, imipenem; M, marbofloxacin; ME, metronidazole; N, nitrofurantoin; O, ofloxacin; P, penicillin; TS, trimethoprim / sulfamethoxazole; V, vancomycin.

APPENDIX: 2

Figure 1: Pattern of white line disease. A: front, B: side, C: back (palmar).

Figure 2: Samples collected from horses with WLD: A: front, B: side, and C: back of hoof (palmar).

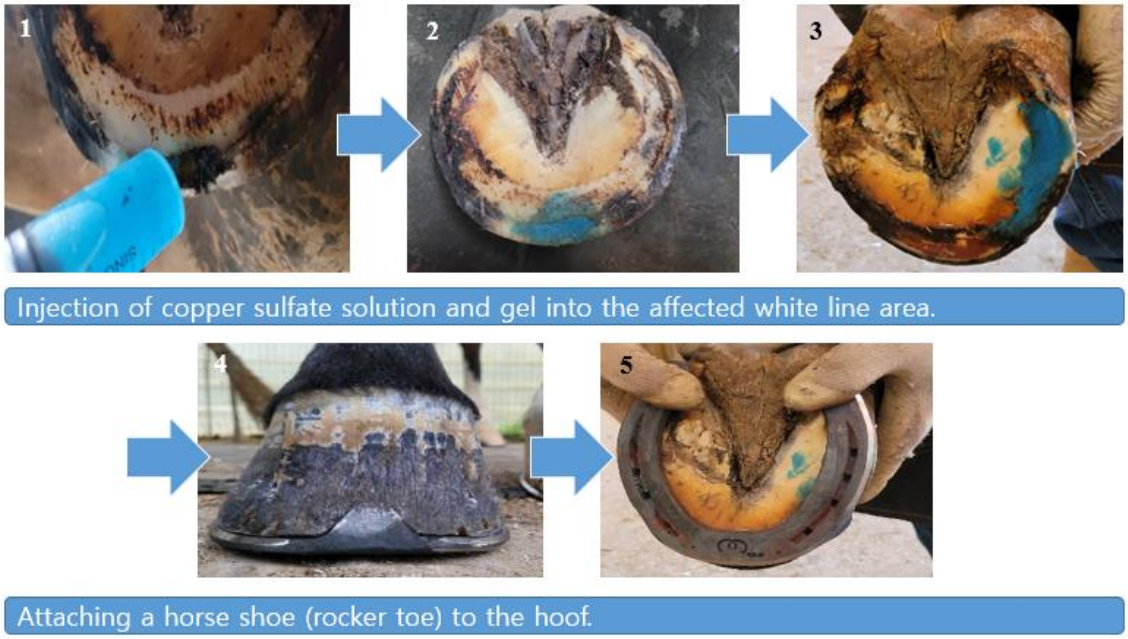

Figure 3: WLD treatment procedure using a copper sulfate solution.

Figure 4: Results of WLD treatment. A: before treatment, B: treatment using a copper sulfate solution, C: after treatment.