



## Characterization of the Microbial Flora Isolated from Horses with Hoof Thrush and Their Sensitivity for Selected Antibiotics

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Article History: 22-771

Received: 15-Dec-22

Revised: 06-Jan-23

Accepted: 12-Jan-23

### ABSTRACT

The horse industry is a large industry with a high scope for growth and employment. It encompasses production and nurturing of horses, as well as other activities involving horses. The domestic horse industry in South Korea has shown remarkable growth since the Horse Industry Promotion Act was enacted in 2012. However, research on horse health-care, especially hoof diseases, is insufficient. The most frequent hoof diseases in Korea are thrush, cracks, and white line disease. In this study, we aimed to identify the causative agents of thrush, the most frequent hoof disease among horses in Korea, and perform antibiotic sensitivity tests on the isolates. In 55 samples collected from 47 horses diagnosed with thrush during grooming among 2,973 horses raised in Korea, and the causative agents, *Bacillus* spp., *Streptococcus* spp., *Staphylococcus* spp., *Pseudomonas* spp., *Enterococcus* spp., *Aerococcus* spp., *Corynebacterium* spp., *Clostridium* spp., and *Aspergillus flavus*. However, *Fusobacterium necrophorum* was not isolated from horses with thrush in Korea. Moreover, antibiotic susceptibility tests for these isolates showed that they were sensitive to most of the 22 antibiotics tested, including amikacin. This is attributable to the fact that the treatment and prevention of thrush is primarily focused on stall and hoof management procedures, such as grooming, and that antibiotics have not been used for treatment. These results are expected to greatly contribute to the treatment of equine hoof diseases, especially thrush, in Korea. Research with more samples can improve our understanding of the relationship between thrush and occurrence of other hoof diseases.

**Key words:** Bacterial Flora, Horse, Thrush, Antibiotic Susceptibility, Health-Care.

### INTRODUCTION

The horse industry is a complex industry extending from the primary to the tertiary industry and has gained the reputation of being an eco-friendly industry with a high scope for growth and industrial employment compared to industries involving other animals (Kim 2015; Abdulkarim et al. 2018; Seo et al. 2021).

In addition to the production and nurturing of horses, the horse industry encompasses a various activity, mainly horse racing, horseback riding (including rehabilitation horseback riding), tourism (including horse-cart, trekking), meat, pet food, horse equipment production, feed production and processing (Kim 2015).

The breeds of horses raised in South Korea can be primarily divided into racing horses (Thoroughbred and Jeju horses), riding horses (such as Halla, and Warmblood horses, and Shetland ponies), and animals for tourist rides (donkeys). Currently, in Korea, approximately 1,000 donkeys and 27,000 domestic horses exist, including

12,000 Thoroughbreds, 1,000 horses from other breeds (e.g., Warmblood and Quarter horses), and 7,000-10,000 native horses (Jeju and Halla horses), of which approximately 7,000 Jeju horses have been designated as natural monument No. 347 by the government (Kim et al. 2021). The Korean domestic horses industry has achieved remarkable growth since the Horse Industry Promotion Act was enacted for the first time in 2012 (Kim 2015). However, research on horse healthcare, especially hoof diseases, is insufficient. The hoof disease most frequently observed in Korea, in the order of their incidence rates, include thrush, cracks, and white line disease (WLD) (Shin et al. 2020).

Horses are herbivores, belonging to the order Perissodactyla and show a remarkably developed third toe (third metacarpal bone) with only traces of the second and fourth metacarpal bones, all the rest have odd, degenerated hooves. Unlike other animals, horses are not susceptible to foot-and-mouth disease (Oikawa et al. 1991).

**Cite This Article as:** Kim BJ and Cho GJ, 2023. Characterization of the microbial flora isolated from horses with hoof thrush and their sensitivity for selected antibiotics. International Journal of Veterinary Science x(x): xxxx. <https://doi.org/10.47278/journal.ijvs/2023.005>

Horse hooves are largely composed of the bone (the second and third phalanx and navicular bone), elastic components (the plantar or digital cushion, which is a flexible elastic body on the upper part of the frog in the latter half of the hoof, and the lateral cartilages, which are wing-like structures attached to the sides (wings) of the coffin bone), and keratin (the hoof wall, sole, white line, bars, and heel bulb). The hoof bone is responsible for the solidity of the foot and serves to attach and protect blood vessels and nerves that affect the sensory structure of the foot. The elastic components reduce the pressure on the hoof when the leg touches the ground through the action of elastomers and plays a role in lightly lifting the leg. The keratin-based components help to protect the bone and preserve the elasticity of the elastic components. The moisture content in both fore and hind hooves is in the order of the hoof wall (wall), sole, and frog. Among these components, the frog contains more than 40% moisture and is a flexible and tough, reaction buffer area; therefore, it is recommended to always maintain an appropriate moisture content in this area. The degree of hardness of the hoof varies depending on the age, area, moisture content, season, and soil conditions. In general, the outer surface of the wall is the hardest, and hardness decreases from the top to bottom. The hardness of the wall is similar to that of an acrylic plate. The sole, bars, frog, bulb heel, and hoof varnish (the shiny thin film on the outer surface of the hoof to prevent excessive drying and wetting of the hoof) are quite flexible (Stashak 2002; Kim 2018).

The hoof component is essentially non-conductive to heat; thus, even if the horseshoe is heated to a high temperature (approximately 350°C) during loading, the dermis will not be burned if the duration of exposure to high temperatures is relatively short. In addition, the hard shell protects the dermis from frostbite even with prolonged exposure to ice or snow in extreme areas. While hoof temperature rises during disease or after exercise, healthy hooves generally show a low temperature to touch during rest. In addition, the hoof temperature tends to rise and fall in relation to the daily temperature differences and changes in the four seasons and is 25-32°C in summer, 20-28°C in spring and autumn, and 5-10°C in winter. The hoof temperatures of the four legs are similar, with no constant association between the body and hoof temperatures. The hoof temperature in young horses is usually slightly higher than that in adult horses. In hoof surface temperature measurements obtained with an electronic warmer (average temperature of the three hooves, 22°C), the temperatures of the bulb heel, sole, and frog were 32.2, 27, and 26.4°C, respectively. When the hoof keratin comes into contact with acid, it turns yellow and weakens and breaks; however, it is not affected by weak acids, and is decomposed by alkaline substances (Kim 2018). It can also be weakened by ammonia in urine. The shape of the horse's hoof is circular in the forefeet and egg-shaped in the hind-feet, and it grows by an average of around 0.19-0.28mm/day and 5.7-8.4mm/month (Glade and Salzman 1985; Pollitt 1990) or 8-9 mm per month with gelatin (Balch et al. 1991). Hoof growth is influenced by various factors, including trimming and shoeing and nutrition (Glade and Salzman 1985; Reilly et al. 1998; Halsberghe 2018).

A brief introduction to horse hoof diseases is as follows. The term "thrush" refers to the decay of dead skin cells. The healthy frog is corroded and gradually decomposed by ammonia gas, and long-term exposure to unclean environments result in bacterial infections. Equine hoof thrush is an infection of the frog adjacent to the sulci (Whitton et al. 2000). In severe cases, the thrush may spread to the white line, sole, and sensitive layers of the foot, potentially resulting in permanent lameness. Although hoof thrush is a common problem in horses, limited information is available on the microorganisms associated with this condition (Petrov and Dicks 2013). Without prompt and appropriate treatment, equine hoof thrush can worsen into equine canker, a proliferative pododermatitis that manifests as chronic, hyperproliferative, suppurative, or pyogranulomatous dermatitis in the frog, bars, and sole as well as the adjacent hoof wall in severe cases (Pascoe and Knottenbelt 1999; Whitton et al. 2000). Grossly, the lesion appears as a soft, whitish, cauliflower-like proliferation associated with a foul-smelling caseous exudate. It frequently occurs in horses raised in stalls, where pollution or humidity is too high due to feces and urine. This disease is generally more common on the hind feet than on the front feet and is caused by humid conditions in tropical climates (Kim 2018). Inflammation of the bulb, which forms the basis of the hoof, is an inflammation of the dermis. The disease occurs as a result of prolonged exercise on hard ground or under pressure in horses with a bulb wound caused by a collision or another horse's hoof (Kim 2018).

A crack is a disease that occurs when a portion of the hoof wall is split horizontally or vertically. Prolonged dry conditions in winter can substantially reduce the moisture content of the hoof wall, and cracks may occur when vigorous exercise is performed by horses with a hardened hoof wall. Thus, the occurrence of cracks is greatly influenced by climate, exercise, and ground conditions (Stashak 1998; Whitton et al. 2000). In contrast, WLD refers to the separation of the wall due to external shock and bacterial growth in the white line. WLD is caused by filthy stall environments, lack of hoof grooming, persistent lack of moisture in the hoof, excessive abrasion, and excessive growth (Kim 2018). Laminitis occurs in the sole and mainly presents in the front of the fore-hoof. It is characterized by inflammation in the follicle layer inside the hoof, which separates the hoof wall and crust, and necrosis of the inflammatory part in all legs. Laminitis is attributable to various causes but is often caused by the accumulation of intestinal toxins in the hooves of horses that are prone to digestive disorders and fed a large amount of concentrated feed, especially those that lack exercise. The symptoms of laminitis include hoof wall separation, palm rotation, and displacement of the coffin bone on radiographs. Palmar rotation and displacement of the coffin bone can be associated with laminitis (Little and Schramme 2007; Yang and Lopez 2019), in which the laminae suspending the coffin bone within the hoof capsule fail in an inflammatory process (Baxter et al. 2011). This disease also causes a disturbance in the laminar tissue due to the progression of WLD and hoof wall defect, contributing to palmar rotation and

displacement of the coffin bone in addition to inflammatory laminitis. The displacement of the coffin bone results in marked lameness in cases of laminitis (Baxter et al. 2011). Deterioration with wedge shoes, restriction to small and dry paddocks, and administration of phenylbutazone are recommended for treatment (Redden 1997).

According to a survey on hoof diseases in domestic horses, the most prevalent hoof disorder was thrush (4.2%). Other identified disorders included superficial hoof wall crack (SHWC, 1.2%), WLD (1.0%), hoof wall separation (HWS, 0.6%), defect of the hoof wall (DHW, 0.5%), laminitis (0.3%), wounds (0.2%), quittor (0.1%), and inflammation of the bulb (IB, 0.0%). Based on a pain and inspection test, the lesions were classified as severe (SHWC, HWS, laminitis), moderate (thrush, HWS, DHW), and mild (wounds, quittor) (Shin et al. 2020).

In this study, the causative agents of the most frequent hoof diseases in horses were isolated and the antibiotic sensitivity of these isolates was studied.

## MATERIALS AND METHODS

### Ethical Approval

This study was carried out on the care and use of experimental animals according to the guidelines of the Animal Ethics Committee (KNU2019-0091) of Kyungpook National University in Korea.

### Sample Collection

After screening 2,973 horses raised in Korea for primary thrush during grooming by a farrier, 55 samples were collected from 47 horses diagnosed as showing thrush by a horse veterinarian. The farriers and veterinarians who participated in sampling diagnosed thrush using standardized methods. Samples were collected from each hoof diagnosed as showing thrush and were obtained from the lesions in the clefts of the hooves with sterile swabs using the manufacturer's instructions for a bacterial test kit (BBL CultureSwab PLUS, Becton, USA) before and after grooming. The swabs were immediately transferred to the laboratory to separate microorganisms.

### Isolation and Identification of Bacteria

For bacterial separation, the collected swabs were streaked on a Blood Agar Plate (BAP) and cultured at 37°C under anaerobic and 5% CO<sub>2</sub> conditions for 24 and 48h, respectively. Isolated bacteria were identified using Bruker Biotyper 4.1 (Bruker Daltonics, Bremen, Germany) to obtain pure cultured bacterial colonies. Approximately 1µL was obtained from a bacterial colony, applied to a Maldi plate (MSP 96; Bruker Daltonics, Bremen, Germany), and then using 1µL of 70% formic acid (Sigma-Aldrich, St. Louis, MO, USA). Then, 1µL of an α-cyano-4-hydroxycinnamic acid matrix solution in acetonitrile: water: TFA (50:47.5:2.5, v/v) was applied and dried. Finally, the bacteria were identified using Bruker Biotyper 4.1 (Bruker Daltonics, Bremen, Germany).

### Isolation and Identification of Fungi

For fungal separation, the collected swabs were streaked on Sabouraud dextrose agar containing chloramphenicol (SDAc) and incubated at 25°C for 7 days. Purely separated fungi were identified by genetic analysis. PCR and sequencing were performed using the primers [ITS1 (TCC GTA GGT GAA CCT GCG G) / ITS4 (TCC TCC GCT TAT TGA TAT GC)] as the target region of 5.8 S rRNA (ITS).

### 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-hs and then read using an automatic reader (Microflex, Bruker, Germany).

## RESULTS

### Identification of Bacteria

In analyses of the 55 samples collected from 47 horses diagnosed with thrush during the grooming of 2,973 horses raised in Korea, the causative agents were, *Bacillus* spp., *Streptococcus* spp., *Staphylococcus* spp., *Pseudomonas* spp., *Enterococcus* spp., *Aerococcus* spp., *Corynebacterium* spp., *Clostridium* spp., and *Aspergillus flavus*. However, *Fusobacterium necrophorum* was not isolated from the cases of thrush in Korea (Table 1).

**Table 1:** The results of bacteriological examinations from hoof thrush in horse

Isolates	No. (%) of Isolates	Isolates	No. (%) of Isolates
<i>Acinetobacter gandensis</i>	1 (1.2)	<i>Corynebacterium</i> spp.	1 (1.2)
<i>Acinetobacter towneri</i>	2 (2.5)	<i>Enterococcus asini</i>	1 (1.2)
<i>Aerococcus viridans</i>	4 (4.9)	<i>Enterobacter cloacae</i>	1 (1.2)
<i>Bacillus cereus</i>	8 (9.8)	<i>Enterococcus casseliflavus</i>	1 (1.2)
<i>Bacillus circulans</i>	2 (2.5)	<i>Enterococcus hirae</i>	1 (2.5)
<i>Bacillus endophyticus</i>	2 (2.5)	<i>Lysinibacillus fusiformis</i>	1 (1.2)
<i>Bacillus flexus</i>	1 (1.2)	<i>Lysinibacillus sphaericus</i>	1 (1.2)
<i>Bacillus indicus</i>	1 (1.2)	<i>Metabacillus indicus</i>	2 (2.5)
<i>Bacillus licheniformis</i>	10 (12.0)	<i>Paenibacillus odorifer</i>	1 (1.2)
<i>Bacillus megaterium</i>	8 (9.8)	<i>Paenibacillus phoenicis</i>	2 (2.5)
<i>Bacillus mycoides</i>	1 (1.2)	<i>Pseudomonas putida</i>	2 (2.5)
<i>Bacillus pumilus</i>	5 (6.1)	<i>Solibacillus silvestris</i>	2 (2.5)
<i>Bacillus sonorensis</i>	1 (1.2)	<i>Staphylococcus hominis</i>	1 (1.2)
<i>Bacillus subtilis</i>	2 (2.5)	<i>Staphylococcus sciuri</i>	1 (1.2)
<i>Clostridium perfringens</i>	2 (2.5)	<i>Staphylococcus simulans</i>	1 (1.2)
<i>Streptococcus equinus</i>	4 (4.9)	<i>Streptococcus lutetiensis</i>	1 (1.2)
<i>Streptococcus infantarius</i>	1 (1.2)	<i>Fusobacterium necrophorum</i>	0 (0.0)

### Antibiotic Susceptibility Tests

Antibiotic susceptibility tests for these isolates revealed that most of the 22 antibiotics tested (amikacin, amoxicillin/clavulanic acid, ampicillin, azithromycin, cefaclor, cefazolin, cefotaxime, cefovecin, cephalixin, clindamycin, doxycycline, enrofloxacin, erythromycin, gentamicin, imipenem, marbofloxacin, metronidazole, nitrofurantoin, ofloxacin, penicillin, trimethoprim/sulphamethoxazole, and vancomycin) were susceptible (Table 3). This may be attributed to the fact that the treatment and prevention of thrush primarily focused on stall and hoof management practices, such as grooming, and that antibiotics were not used for treatment.

### DISCUSSION

In Korea, unlike Thoroughbred racehorses which undergo grooming at 3-week intervals and receive aluminum shoes, riding horses undergo grooming every six weeks on average and usually receive iron horseshoes. Hoof diseases are known to occur more frequently with longer grooming intervals. In the present study, the average age of the 47 horses diagnosed with thrush among the 2,973 horses breeding at 273 horse riding grounds in Korea was 6.5 years (range, 3-10 years).

Equine hooves are exposed to a variety of microorganisms, which can be subdivided into gram-positive, skin inhabitants, such as *Staphylococcus (S.) epidermidis*, *S. aureus*,  $\alpha$ -*Streptococcus viridans*, *Micrococcus* spp., *Bacillus* spp., and *Corynebacterium* spp., gram-negative bacteria of fecal origin such as *Escherichia coli*, *Klebsiella pneumoniae*, and *Klebsiella* spp., and hoof-capsule residents such as *Acinetobacter* spp. (Pichinoty et al. 2013). This transient microflora is influenced by the presence of exudate, hair, dirt, and high moisture levels in the environment (Vela et al. 2013). In this study, *Bacillus* spp., *Streptococcus* spp., *Staphylococcus* spp., *Pseudomonas* spp., *Enterococcus* spp., *Aerococcus* spp., *Corynebacterium* spp., *Clostridium* spp., etc. were isolated, consistent with the results of previous studies (Janna 2013; Pichinoty et al. 2013). The causative agent of thrush in horses have only been described in a few reports. However, although *Fusobacterium (F.) neuroporum* and *Dichelobacter nodosus* have been suggested to be major causes of

**Table 2:** The results of fungus examinations from hoof thrush in horse

Isolates	No. (%) of isolates
<i>Aspergillus flavus</i>	2 (2.5)
<i>Nectriaceae</i> spp.	1 (1.2)
<i>Trichoderma atroviride</i>	1 (1.2)

**Table 3:** Antimicrobial drug test of bacteria isolated from hoof thrush in horse

Isolates	Type of sensitivity drugs
<i>Acinetobacter gandensis</i>	A, AC, AP, AZ, CC, CT, D, EN, G, I, M, O, P
<i>Acinetobacter townneri</i>	A, AC, AP, AZ, CC, CE, CT, CV, CZ, D, EN, G, I, M, N, O, P, TS
<i>Aerococcus viridans</i>	AC, AP, AZ, CC, CE, CL, CT, CZ, D, EN, ER, G, I, M, N, O, P, TS, V
<i>Bacillus cereus</i>	A, AC, AZ, CC, CL, EN, ER, G, I, M, N, O, TS, V
<i>Bacillus circulans</i>	A, AC, AP, AZ, CC, CE, CL, CT, CV, CZ, D, EN, ER, G, I, M, N, O, P, TS, V
<i>Bacillus endophyticus</i>	A, AC, AP, AZ, CC, CL, CT, CV, CZ, D, EN, ER, G, I, M, N, O, P, TS, V
<i>Bacillus flexus</i>	A, AC, AP, AZ, CC, CE, CL, CT, CV, CZ, D, EN, ER, G, I, M, N, O, P, TS, V
<i>Bacillus indicus</i>	A, AC, AP, AZ, CC, CE, CL, CT, CV, CZ, D, EN, ER, I, M, N, O, P, TS, V
<i>Bacillus licheniformis</i>	A, AC, AP, CC, CE, CT, CZ, D, EN, G, I, M, N, O, P, TS, V
<i>Bacillus megaterium</i>	A, AC, AP, AZ, CC, CE, CT, CV, CZ, D, EN, ER, G, I, M, N, O, P, TS, V
<i>Bacillus mycoides</i>	A, AC, AP, AZ, CC, CE, CT, CV, CZ, D, EN, ER, G, I, M, N, O, P, V
<i>Bacillus pumilus</i>	A, AC, AP, AZ, CC, CE, CZ, D, EN, ER, G, I, M, N, O, P, V
<i>Bacillus sonorensis</i>	A, AC, AP, CC, CE, CZ, D, EN, G, I, M, N, O, TS, V
<i>Bacillus subtilis</i>	A, AC, AP, AZ, CC, CE, CL, CT, CV, CZ, D, EN, ER, G, I, M, N, O, P, TS, V
<i>Clostridium perfringens</i>	AC, AP, AZ, CC, CL, CT, CZ, EN, I, M, N, O, P, TS, V
<i>Corynebacterium</i> spp.	AC, AP, AZ, CC, CL, CZ, EN, I, M, N, O, P, TS, V
<i>Enterobacter cloacae</i>	A, CT, EN, G, I, M, O, P, TS
<i>Enterococcus asini</i>	A, AC, AP, AZ, CC, CE, CT, CV, CZ, D, EN, ER, G, I, M, N, O, P, TS, V
<i>Enterococcus casseliflavus</i>	A, AC, AP, AZ, CC, CE, CT, CZ, D, EN, ER, G, I, M, N, O, P, TS, V
<i>Enterococcus hirae</i>	A, AC, AP, AZ, CC, CE, CT, CZ, D, EN, ER, G, I, M, N, O, P, TS, V
<i>Lysinibacillus fusiformis</i>	A, AC, AP, AZ, CC, CE, CL, CT, CZ, D, EN, ER, G, I, M, N, O, P, TS, V
<i>Lysinibacillus sphaericus</i>	A, AC, AZ, D, EN, G, I, M, O, TS
<i>Metabacillus indicus</i>	A, AC, AP, AZ, CC, CE, CT, CV, CZ, D, EN, ER, G, I, M, N, O, P, TS, V
<i>Paenibacillus odorifer</i>	A, AC, AZ, CC, CE, CT, CZ, D, EN, ER, G, I, M, N, O, TS, V
<i>Paenibacillus phoenicis</i>	A, AC, AZ, CC, CZ, D, EN, ER, G, I, M, N, O, TS, V
<i>Pseudomonas putida</i>	A, CT, EN, G, I, M, O, P, TS
<i>Solibacillus silvestris</i>	A, AC, AP, AZ, CC, CE, CL, CT, CV, CZ, D, EN, ER, G, I, M, N, O, P, TS, V
<i>Staphylococcus hominis</i>	A, AC, AP, AZ, CC, CL, CT, CV, CZ, EN, ER, G, I, M, N, O, P, TS, V
<i>Staphylococcus sciuri</i>	A, AC, AP, AZ, CC, CL, CT, CZ, EN, ER, G, I, M, N, O, P, TS, V
<i>Staphylococcus simulans</i>	A, AC, AP, AZ, CL, CT, CV, CZ, EN, ER, G, I, M, N, O, P, TS, V
<i>Streptococcus equinus</i>	AC, AP, CC, CE, CT, CV, CZ, ER, I, P, V
<i>Streptococcus infantarius</i>	AC, AP, AZ, CC, CE, CL, CT, CV, CZ, ER, G, I, N, P, TS, V
<i>Streptococcus lutetiensis</i>	AC, AP, AZ, CC, CE, CT, CV, CZ, ER, G, I, N, P, TS, V

\*Amikacin (A), Amoxicillin / Clavulanic Acid (AC), Ampicillin (AP), Azithromycin (AZ), Cefaclor (CC), Cefazolin (CZ), Cefotaxime (CT), Cefovecin (CV), Cephalixin (CE), Clindamycin (CL), Doxycycline (D), Enrofloxacin (EN), Erythromycin (ER), Gentamicin (G), Imipenem (I), Marbofloxacin (M), Metronidazole (ME), Nitrofurantoin (N), Ofloxacin (O), Penicillin (P), Trimethoprim / Sulphamethoxazole (TS), Vancomycin (V).

thrush, their relevance in horses is unclear, since studies of *Fusobacterium* spp. have been reported in other animals. Much of the present understanding of this infection has been gained from research published on *Fusobacterium* spp. isolated from sheep (Egerton and Roberts 1971), goats (Bennett et al. 2009), cattle (Sun et al. 2011), and pigs (Zhou et al. 2010) with foot rot. Dorsch et al. (2001) showed that *Fusobacterium* spp. isolated from horses with oral-associated diseases are genetically different from *F. necrophorum* and classified the strains as *F. equinum*. *F. equinum* is a normal inhabitant of the gastrointestinal, respiratory, and genitourinary tracts of horses and is generally associated with abscesses and necrotic infections (Trevillian et al. 1998; Racklyeft and Love 2000). However, unlike the findings reported by Petrov and Dicks (2013), *F. necrophorum*, which is known as the causative agent of thrush (Petrov and Dicks 2013), has not been isolated from domestic horses.

Our analyses of the causative agents of thrush in horses raised in Korea identified 98% aerobic bacteria, 3.1% anaerobic bacteria, and 0.01% fungi. Although anaerobic bacteria are known to be involved in thrush due to the high moisture content, this study also confirmed that aerobic bacteria exist in all thrush lesions. However, more samples should be studied in future research to determine whether these bacteria are the primary cause of thrush. In addition, *Aspergillus flavus*, *Nectriaceae* spp., and *Trichoderma atroviride* have been isolated from thrush which may be attributable to the presence of WLD rather than a direct cause of thrush. Thus, future studies should aim to evaluate the interrelationships between thrush and WLD.

Hoof diseases can be sufficiently prevented by good specification management, including appropriate feed benefits, provision of a clean stall environment, regular trimming, and horseshoe application. Currently, the treatment of thrush mainly involves cutting off the necrotized frog tissue, soaking feet in disinfectant, or wiping and drying with disinfectant twice a day. In addition, treatment is mainly dependent on the application of sterilizing ointment. The best treatment method is trimming of the hoof to expose the infected area to the air and treating it weekly with iodine to prevent bacterial growth (Hennig et al. 2001; Johnson et al. 2015). Antibiotic sensitivity tests on bacteria separated from the thrush showed sensitivity for most of the 22 antibiotics tested, including amikacin. This is believed to be due to the fact that most of the treatment and prevention of thrush was focused on hoof and horse management practices, such as trimming, and that antibiotics were not used as therapeutic agents. In addition, frog tissue with thrush should be removed as much as possible and the frog and stall should be kept dry when thrush is diagnosed. However, the use of antibiotic ointments or injection through cooperation between farriers and veterinarians can be expected to be more effective in the treatment of horses with thrush.

## Conclusion

The causative agents of thrush included, *Bacillus* spp., *Streptococcus* spp., *Staphylococcus* spp., *Pseudomonas* spp., *Enterococcus* spp., *Aerococcus* spp.,

*Corynebacterium* spp., *Clostridium* spp., and *Aspergillus flavus*. However, *Fusobacterium necrophorum* was not isolated from cases of thrush in Korea. In addition, antibiotic susceptibility tests for these isolates showed that they were sensitive to most of the 22 antibiotics tested, including amikacin. The current research results are expected to greatly contribute to the treatment of horse hoof diseases, especially thrush, in Korea.

## Author's Contribution

All research protocols and animal experiments in this study were designed, and conducted by BJ Kim, who also contributed to data acquisition. GJ Cho contributed to the interpretation of the experimental results and writing the manuscript.

## Funding

This research was supported by the National Research Foundation of Korea, funded by the Ministry of Education, Science and Technology (NRF-2020R111A3067905) and Kyungpook National University.

## Conflicts of Interest

The authors declare no conflict of interest.

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