**Emericella nidulans** Isolated from Horses with Guttural Pouch Mycosis in Japan

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*Emericella nidulans*, the teleomorph of *Aspergillus nidulans*, was isolated from the guttural pouches of 3 horses with nasal hemorrhage. All the horses had been suffering from respiratory diseases, such as rhinitis showing purulent nasal discharge, pharyngitis and mild pneumonia, since approximately 1 month before the onset of nasal hemorrhage. Fungal cultures of isolates from them showed almost the same mycological characteristics in the cultural and morphological examination. All the isolates were identified as *E. nidulans*. Guttural pouch mycosis is very rare disease in horses in Japan, while it seems to be rather prevalent in other countries. This is the first paper in Japan to report the isolation of *E. nidulans* from horses with guttural pouch mycosis.

**Key words.** horses, guttural pouch mycosis, *Emericella nidulans*

Introduction

Guttural pouch mycosis in horses is an upper respiratory tract infection. This comparatively unfamiliar fungal disease was first described by Rivolta in 1868 and appears to have been contracted by stabled horses, usually during the warmer season of the year.1,2) Some workers1,3–6) reported that *Aspergillus*, *Paecilomyces*, *Scopulariopsis* and *Penicillium* were suspected to be the causative agents of this disease. On the other hand, guttural pouch mycosis due to *Emericella nidulans*, the teleomorph of *Aspergillus nidulans*, was reported to have occurred mostly in Europe,3,7) Australia1,3) and North America,5,8,9) According to data available at present, this disease seems to be increasing in incidence on a world-wide scale.

Up to now, there has been no description on the outbreak of guttural pouch mycosis due to *E. nidulans* in Japan. This paper presents the isolation of *E. nidulans* from 3 horses with guttural pouch mycosis for the first time in Japan and the mycological characterization of this species.

**Materials and Methods**

*Isolation history.* During a period from September, 1983 to February, 1984, 2 Anglo-Arabian and 1 Thoroughbred horses 2 to 3 years of age, belonging to the Ritto or Miho Training Center, Japan Racing Association, were suddenly affected with nasal hemorrhage and subjected to autopsy and pathological ex-
amination. They had been suffering from respiratory disease, such as rhinitis showing purulent nasal discharge, pharyngitis and mild pneumonia, since approximately 1 month before the onset of nasal hemorrhage. Mucoid or mucopurulent nasal discharge from the left or bilateral nasal cavities disappeared within at least 2 weeks before the onset of nasal hemorrhage. Diphtheroid membranes or scars suggestive of guttural pouch mycosis could be observed in the 3 horses at autopsy. The horses were submitted to the author's station for bacteriological examination. Cultures were made on blood agar, MacConkey agar, Sabouraud's dextrose agar, mycobiotic agar and Candida agar plates at 37°C and room temperature. A number of fungal and bacterial colonies grew on all the agar plate after incubation at 37°C for 24 h. Alcaligenes faecalis, Streptococcus zooepidemicus and Aerococcus spp. were isolated from samples taken from Horse No. 1, Staphylococcus spp. and Aerococcus spp. from Horse No. 2, and Streptococcus faecalis was from Horse No. 3. Fungi were isolated predominantly from all the samples taken from the 3 horses. A fungal culture was selected from each sample for identification. It was subcultured on potato dextrose agar slants (Table 1) and identified by routine methods.

**Culture for identification.** All cultures were grown on potato dextrose agar and Czapek's solution agar for colonial and micromorphological observation. The microscopic morphology was also studied on slide cultures on potato dextrose agar and potato carrot agar.

**Growth examination at each temperature.** Growth examination was made on organisms (M# 784 and M# 797) at four different temperatures, or 25, 30, 37 and 42°C. These organisms on potato dextrose agar medium were incubated for 7 days.

**Results**

Three cultures isolated from the nasal lesions of the horses were subjected to a brief mycological investigation. The macro- and micro-morphological findings obtained from them are as follows.

**Culture No. M# 778** (Figs. 1–3). Colonies grew well on Czapek's solution agar at 25°C, attaining 5.5 to 6.5 cm in diameter in 10 days. They were plane or somewhat floccose. Their surface was cream, buff or pale brown in color and had cleistothecia. Their margins were thin and irregular. The colony reverse varied from purplish to colorless during the period of growth. There was neither exudate nor odor. Conidial heads were short, columnar and 50 to 65 by 25 to 35 µm in size. Conidiophores were sinuous and 50 to 110 µm in length. They had smooth walls and looked bright brown in the shade. Vesicles were usually hemispherical and 8 to 12 µm in diameter. Phialides were arranged in 2 series. Conidia were globose, rugulose and 3.0 to 3.5 µm in diameter and constituted a green mass. Cleistothecia, 100 to 200 µm in diameter, developed separately. They were in central colony areas and obscured a
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Ascospores were purple-red, lenticular and smooth-walled with 2 equatorial crests from 3.5 to 4.5 µm in length by 3.0 to 4.0 µm in breadth. The entire crests ranged from 0.5 to 1.0 µm in width. There were globose hülle cells of 15 µm in diameter which varied from hyaline to pale brown in color.

Colonies grew quite rapidly on potato dextrose agar at 25°C, reaching 6.0 to 7.0 cm in diameter in 1 week. Their reverse varied from pale purple to brown in color. There was neither exudate nor odor.

Culture No. M# 778 was identified as *E. nidulans*.

Culture No. M# 784 (Figs. 4–6). Colonies grew rapidly on Czapek's solution agar at 25°C, attaining 5.5 to 7.5 cm in diameter in 10 days. They were plane to floccose with cream buff and remarkably sectored. Their margins were thin and irregular. The colony reverse was purple during the period of growth. There was neither exudate nor odor.

Conidial heads were short and columnar, ranging from 50 to 75 µm in length. Conidiophores had smooth walls, looking bright brown in the shade and attaining 70 to 90 µm in length. Vesicles were hemispherical in shape, ranging from 8 to 12 µm in diameter. Phialides were arranged in 2 series. Conidia were globose, rugulose and 3.0 to 3.5 µm in diameter.

Cleistothecia were abundant in the central areas. They were globose, ranging from 130 to 180 µm in diameter and surrounded by a yellowish layer of mycelia bearing hülle cells. Ascospores were orange red, lenticular and had smooth walls with 2 equatorial crests each. They were 3.5
to 4.5 μm in length by 2.5 to 3.5 μm in width. The entire crests ranged from 0.5 to 1.0 μm in width. Globose or subglobose hülle cells were hyaline to pale brown in color, attaining 12 to 15 μm in diameter.

Colonies grew very rapidly on potato dextrose agar at 25°C, attaining 6.5 to 7.5 cm in diameter in 1 week. They were plane or somewhat floccose and dull green to light yellow in color. The colony reverse varied from pale brown to colorless. There was neither exudate nor odor.

Culture No. M# 784 was identified as E. nidulans.

Culture No. M# 797 (Figs. 7–9). Colonies grew well on Czapek’s solution agar at 25°C, attaining 4.5 to 5.5 cm in 10 days. They were plane to floccose and dull green to bluish green in color. Sectoring was remarkable. The margins were thin. The colony reverse varied from dull brown to pale purple during the period of growth. There was neither exudate nor odor.

Conidial heads were short and columnar, looking dull green to bluish green in the shade and ranged from 50 to 70 μm in length. Conidiophores were short, ranging from 70 to 85 μm in length. They had smooth walls with brown pigment. Vesicles were hemispherical to subglobose and 8 to 12 μm in diameter. Phialides were arranged in 2 series. Conidia were globose, rugulose and 3.0 to 3.5 μm in diameter.

Cleistothecia were globose, somewhat abundant in dull green areas, and 100 to 150 μm in diameter. Ascospores were orange red, lenticular, and had smooth walls with 2 equatorial crests. They were 3.5 to 4.5 μm in length by 2.5 to 3.5 μm in width. The entire crests ranged from 0.5 to 1.0 μm in width. Globose and sub-
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globose hüle cells were 15 µm in diameter and hyaline to pale brown in color.

Colonies grew well on potato dextrose agar at 25°C, attaining 5.5 to 6.6 cm in 1 week. They were floccose with a grayish green shade. The colony reverse was colorless. There was neither exudate nor odor.

Culture No. M# 797 was identified as *E. nidulans*.

**Discussion**

Since guttural pouch mycosis in the horses was discovered by Rivolta, some sporadic cases of it have been reported in the 1950s. During the last 2 decades the disease has been the underlying cause for the spread of equine respiratory infection in such countries as the United Kingdom, Germany, Australia, and North America. It is characterized by the formation of a diphtheritic membrane in one or both guttural pouches. It
may be manifested by one or more of the following signs: epistaxis, laryngeal hemiplegia, pharyngeal paralysis, and soft-palate paresis.\textsuperscript{3)}

The disease has been described in detail by Cook\textsuperscript{10)} and Cook et al.\textsuperscript{3)} Cook\textsuperscript{1)} also made a review of the literature of it. Numerous clinical and pathological studies have been made on it, but hardly any mycological investigation has been carried out. Some causative agents of the disease have been identified as \textit{Aspergillus} spp. in many studies, but fungal identification is still obscure.

According to Cook\textsuperscript{1,10)} and Greet,\textsuperscript{11)} a main causative agent is \textit{E. nidulans}, the teleomorph of \textit{Aspergillus nidulans}, in every country. \textit{E. nidulans} is more widely distributed in nature than any other \textit{Aspergillus} species. It mainly inhabits the soil of the ground where grasses are grown for grazing of horses. Therefore, it finds a way into the circumstances of horses and other domestic animals.

In the field of veterinary medicine, the disease caused by \textit{E. nidulans} has been reported only in birds and horses.\textsuperscript{3,12)} It is a feature of the disease to cause an epizootic among chickens. Pulmonary lesions have also been found in donkeys and horses. It is well known that \textit{E. nidulans} is an opportunistic pathogen to cause the disease and distinctly shows a pathogenic capability.

Up to the present, there have been no reports on etiological studies on guttural pouch mycosis of horses in Japan. In the present investigation three cases of this disease were first encountered and causative agents, \textit{E. nidulans} isolates, were derived from the nasal lesions of the guttural pouch in each case. All the isolates showed similar mycological characteristics, although the three cases were scattered in the country.

**Literature Cited**


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要約

真菌性喉のう炎馬から分離された *Emericella nidulans* について：高島浩介*・鶴田正信**・福永昌夫**・熊塚御堂 慶**・平澤 澄**・仁崎正之***・高田 耀****・及川正明****（*飼料食品薬品安全センター*泰野研究所 **日本中央競馬会競走馬総合研究所*新木支所 ***日本中央競馬会乗馬トレーニング・センター ****日本中央競馬会美浦トレーニング・センター）——鼻出血で死亡した3頭の馬の真菌性喉のう炎病巣部から，*Aspergillus nidulans* の完全世代である *Emericella nidulans* が分離された。死亡馬はいずれも鼻出血を発症する1カ月前に腺性鼻頭を伴う鼻炎，咽頭炎，軽度の肺炎などの呼吸器病に罹患していた。3例から分離された真菌はいずれも培養および形態学的検査において酷似しており，*E. nidulans* と同定された。真菌性喉のう炎馬は日本では極めてまれなる疾病として見出されてきたが，外国では一般的な疾病である．今回の報告は日本における真菌性喉のう炎馬から分離された *E. nidulans* 同定の初めものである。