

Isolation of *Streptococcus equi* subsp. *equi* from Thoroughbred Horses in a Racehorse-Breeding Area of Japan

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ABSTRACT. For determination whether strangles has invaded the Hidaka district of Hokkaido, the main racehorse-breeding area of Japan, a epizootiological survey with bacterial isolation was carried out during the breeding season in 1995. *Streptococcus equi* subsp. *equi*, which is the causative agent of strangles, was isolated from two Thoroughbred horses with submandibular lymphadenitis. Isolates were identified by serological grouping, biochemical tests and analysis of cell surface proteins by Western immunoblotting. Through this survey, it revealed that *S. equi* subsp. *equi* has invaded the Hidaka district and that strangles has become prevalent in racehorse-breeding farms in this area. — **KEY WORDS:** equine, strangles, *Streptococcus equi* subsp. *equi*.

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Strangles is an enzootic disease affecting horses, especially young horses, which is characterized by regional or generalized suppurative lymphadenitis associated with upper respiratory infection [5]. *Streptococcus equi* subsp. *equi*, which is the etiologic agent of this disease, is a β -hemolytic and Lancefield's group C streptococcus which infects only the Equidae. This disease was recognized prior to the 18th century and occurs world wide [4]. In Japan, strangles was also recognized as a major disease affecting horses, it was colloquially referred to as *naira* [3]. However, outbreaks have decreased gradually after World War II, and no cases had been reported in the last 30 years before an outbreak of strangles was reported in Percheron, Belgian, Breton and their hybrid horses at horse-breeding farms in the Tokachi district of Hokkaido in 1992 [7]. And then, we isolated *S. equi* subsp. *equi* independently from two Thoroughbred horses in different area of Japan during 1993–1994 (unpublished data). The purpose of this study was to isolate *S. equi* subsp. *equi* from horses suspected as strangles bred in the Hidaka district of Hokkaido, the main racehorse-breeding area of Japan, and analyze the outbreak of strangles with epizootiological results.

From April to July in 1995, clinical samples were collected from 15 Thoroughbred horses bred in different farms who were suspected having strangles with paranasal sinusitis, inguinal lymphadenitis, submandibular lymphadenitis and abscesses. Swabs taken from their lesions were placed in a transport medium (Culturette: Becton Dickinson Microbiology Systems, Cockeysville, MD, U.S.A.) and sent to Mitsubishi Animal Clinic Center in the Hidaka district with their temperature maintained at 4°C. Colombia CNA agar (Difco Laboratories, Detroit, MI, U.S.A.) supplemented with 5% heparinized horse blood was used for isolation of the bacteria. After aerobic incubation at 37°C over night, five or more colonies of suspected β -hemolytic streptococci were picked from each isolation agar plate and were cultured on Colombia agar (Difco) supplemented with 5% heparinized horse blood.

Identification of *S. equi* subsp. *equi* was performed using STREPT LA (Denka Seiken Co., Ltd., Tokyo) for Lancefield serological grouping, API 20 STREP (bioMérieux, Marcy-l'Étoile, France) for biological test, and Western immunoblot analysis for measurement of the molecular weight of M-like protein.

Western immunoblot analysis of native M-like protein was performed according to the method described by Galan and Timoney [1]. Briefly, native M-like protein was extracted from mutanolysin (Sigma Chemical Co., St. Louis, MD, U.S.A.)-treated bacterial cells harvested from overnight cultures at 37°C in Todd-Hewitt broth (Difco) with 0.2% yeast extract. Rabbit antiserum against *S. equi* subsp. *equi* was prepared by inoculating intravenously two rabbits with heat-killed CF32, a typical strain of *S. equi* subsp. *equi* which was kindly supplied by Dr. Timoney [6]. The proteins extracted from streptococcal isolates were separated by SDS-PAGE and transferred to nitrocellulose membranes. The blots were incubated in diluted antiserum and then in peroxidase-conjugated protein G. Reactive bands were visualized with 4-chloro-1-naphthol as a substrate.

β -hemolytic streptococci were isolated from 7 horses in the 15 horses who were suspected having strangles. In the identification with STREPT LA and API 20 STREP, all of five or more isolates in a primary isolation at each sample were found to have the same characteristics in Lancefield grouping and biochemical reactions. *S. equi* subsp. *equi* was isolated from one foal and one two-year-old horse with submandibular lymphadenitis (Table 1). *S. equi* subsp. *zooepidemicus*, which was the indigenous bacterium and the opportunistic pathogen of horses, was isolated from the remaining 5 horses. These isolates of *S. equi* subsp. *equi* agglutinated latex particles sensitized with anti-group C streptococcus rabbit serum and could not produce acid from the ribose, arabinose, mannose, sorbitol, lactose, trehalose, inulin, and raffinose included in the API 20 STREP identification kit.

The two isolates of *S. equi* subsp. *equi* were also analyzed

Table 1. Isolation of β -hemolytic streptococci from clinical specimens collected from Thoroughbred horses suspected having strangles

Horse No.	Age (year)	Clinical diagnosis	Sample for isolation	Identification of the isolates
1 ^{a)}	0	Submandibular lymph. ^{b)}	Puss	<i>S. equi</i> ^{c)}
2	2	Submandibular lymph.	Puss	<i>S. equi</i>
3	0	Submandibular lymph.	Puss	None
4	0	Submandibular lymph.	Puss	None
5	1	Submandibular lymph.	Puss	None
6	2	Inguinal lymph.	Puss	<i>S. zooepidemicus</i> ^{d)}
7	2	Abcess	Puss	<i>S. zooepidemicus</i>
8	1	Abcess	Puss	None
9	3	Abcess	Puss	None
10	1	Abcess	Puss	None
11	2	Abcess	Puss	None
12	1	Paranasal sinusitis	Nasal discharge	<i>S. zooepidemicus</i>
13	1	Paranasal sinusitis	Nasal discharge	None
14	2	Paranasal sinusitis	Nasal discharge	<i>S. zooepidemicus</i>
15	9	Paranasal sinusitis	Nasal discharge	<i>S. zooepidemicus</i>

a) Each horse were bred in different farms. b) lymph.; lymphadenitis. c) *S. equi*; *Streptococcus equi* subsp. *equi* which is the causative agent of strangles. d) *S. zooepidemicus*; *Streptococcus equi* subsp. *zooepidemicus* which is the indigenous bacterium and the opportunistic pathogen of horses.

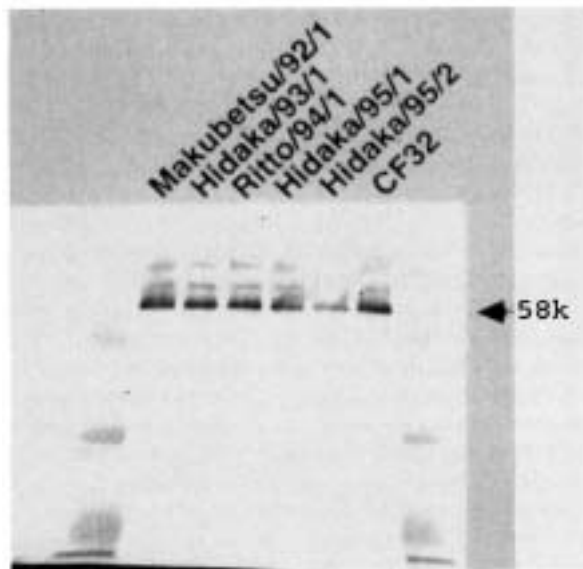


Fig. 1. SDS-PAGE and immunoblot analysis of native M-like protein from *S. equi* subsp. *equi*. The blot was developed with anti-CF32 rabbit serum. Makubetsu/92/1, Hidaka/93/1 and Ritto/94/1 were isolated from horses during the three years prior to this survey in Japan. Hidaka/95/1 and Hidaka/95/2 were isolated from clinical specimens collected from a two-year-old Thoroughbred horse and a Thoroughbred foal in this survey. CF32 was isolated from a horse with strangles in 1980 in New York [6].

by immunoblotting [1, 2]. The native M-like protein, which strongly reacts to the rabbit antiserum, in extracts from two isolates of *S. equi* subsp. *equi* (Hidaka/95/1, Hidaka/95/2), was found to have the same molecular weight of 58,000 as that of the native M-like protein in extracts of CF32 and

Table 2. An outbreak of strangles at a racehorse-breeding farm in Hidaka district at 1995

Horse No.	Age (year)	Clinical symptoms	Onset of disease	Bacterial isolation
16	?	F. ^{a)} , L.A. ^{b)} , M.N.D. ^{c)} , D. ^{d)}	May 20	ND ^{f)}
17	0	F., L.A., A.S.L. ^{e)}	May 29	ND
18	1	F., L.A., M.N.D., D.	Jun 9	ND
1	0	F., A.S.L.	Jun 22	<i>S. equi</i> ^{g)}
19	1	F., L.A., M.N.D., A.S.L., D.	Jun 25	ND
20	1	F., M.N.D., A.S.L., D.	Jun 26	ND
21	1	F., L.A., M.N.D., A.S.L.	Jun 27	ND
22	0	F., A.S.L.	Jun 30	ND

Although bacteriological examination was performed with only a horse No. 1, prevalence of strangles in this farm was suggested by clinical symptoms and continuous incidence of the disease of other 7 horses. a) F.: Fever, b) L.A.: Loss of appetite. c) M.N.D.: Mucopurulent nasal discharge. d) D.: Dysphagia. e) A.S.L.: Abscess in submandibular lymphodes. f) ND: Not done. g) *S. equi*; *Streptococcus equi* subsp. *equi*.

three Japanese strains of *S. equi* subsp. *equi* isolated from horses during 1992 to 1994 (Fig. 1).

On the other hand, according to the isolation of *S. equi* subsp. *equi* from the horse No.1 (see Table 1) and the clinical records of breeding horses in the farm, prevalence of strangles among horses had been suggested in this farm (Table 2). There have been ten mares for breeding in this farm at that year. A mare (horse No. 16) had been introduced onto the farm from other farm in same area for nursing of a foal (horse No. 17), because his dam died after partus. Clinical symptoms suspected strangles were observed in this mare immediately after the introduction. Subsequently, three foals and four yearlings at this farm developed symptoms of strangles during the ensuing 40 days

and then *S. equi* subsp. *equi* was isolated from the abscess in submandibular lymphodes of a foal (horse No. 1). It was suggested that *S. equi* subsp. *equi* was transmitted to this farm from another farm by the mare and spread in the farm.

The present study indicated that *S. equi* subsp. *equi* has been already spread in Thoroughbred breeding farms in the Hidaka district of Hokkaido in 1995. Nevertheless a lot of clinical specimens of horses has been examined bacteriologically at Mitsuishi Animal Clinic Center during last 20 years, *S. equi* subsp. *equi* had not been isolated from any horses in this area before the outbreak of strangles in the Tokachi district of Hokkaido was reported at 1992. Therefore, it can be presumed that *S. equi* subsp. *equi* had been introduced to the Hidaka district from the Tokachi district in Japan or some other country recently. On the basis of our data, we suggest that the epidemiological surveillance must be continued for the control of the disease.

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