BRIEF NOTE

Contagious Equine Metritis: Isolation of *Haemophilus equigenitalis* from Horses with Endometritis in Japan

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Contagious equine metritis (CEM) is a highly contagious, acute venereal disease of horses which severely affects breeding operations and infertility. It is caused by a Gram negative coccobacillus classified as *Haemophilus equigenitalis* (*H. equigenitalis*) by Taylor et al. [10] and characterized by rapidly developing cervicitis, by varying amounts of exudate and by returning to estrus after covering.

Apparently recognized in Thoroughbred horses in Ireland during the 1976 breeding season [5], CEM was first reported as an outbreak of acute metritis during the 1977 breeding season in England [2] and Ireland [13]. Thereafter, the disease spread rapidly among horses in Australia [3], England [6], France, Germany [7], Ireland [11, 12] and the USA [9] during a period from 1977 to 1979. Subsequently in these or other countries, the code of practice for the control of CEM or the ban on the importation of equidae was published for the purpose of preventing the spread of CEM. As the result, there has been no report on the occurrence of CEM or the isolation of *H. equigenitalis* in other countries except these by

the present time.

This paper presents the first occurrence of CEM among horses in Japan and the isolation of *H. equigenitalis* from the cervical swabs obtained from mares with endometritis.

During a period from April 14 to May 25, 1980, cervical swab specimens were collected from 144 mares in Hidaka region, Hokkaido Prefecture, a horse breeding center in Japan. All specimens were directly put into 2 kinds of transport media supplemented with thioglycollate medium and heart infusion medium, frozen in a dry-ice box, and transported to the authors' laboratory. Initially in some instances, cervical swab specimens were collected also from mares without endometritis. Later in the investigation, when a putative pathogen had been isolated from mares, all swabs were taken only from mares with endometritis. After all swabs were adequately stirred with a micromixer (Taiyo Bussan Co., Ltd., Tokyo) and removed from the transport media, the fluids were put into a small tube and centrifuged at 2,000 rpm for 10 minutes. The sediments were suspended

in a small volume of PBS (pH 7.0) and each 0.1 ml was inoculated on blood agar, chocolate agar and MacConkey agar. The chocolate agar for isolation of *H. equigenitalis* was prepared using Eugon agar (BBL) supplemented with 10% defibrinated horse blood according to the previous report [8]. These cultures were incubated at 37°C aerobically, anaerobically (hydrogen 90 percent, carbon dioxide 10 percent v/v) and in an atmosphere of air containing 10 percent v/v carbon dioxide.

A trace of growth was occasionally observed at 24 hours on the chocolate agar incubated in an atmosphere of air containing 10 percent v/v carbon dioxide, although small colonies were usually recognisable at 48 hours. The colonies were glossy, smooth, raised, butyrous and slightly grey. These enlarged to 1–2 mm in diameter at 48 hours and to 3–5 mm in diameter at 120 hours. After prolonged incubation, beyond 72 hours, the colonies were white, raised and glistening. Some strains of the isolates showed variation in colony size at 48 hours, with many tiny colonies, 0.2–0.4 mm in diameter, intermingled with colonies of normal size. Under anaerobic conditions growth from swabs was usually obtained on chocolate agar 3 days after inoculation. These enlarged to 2–3 mm in diameter at 120 hours. Growth from these swabs was not observed after prolonged incubation either on blood agar or on MacConkey agar.

A little and pure growth of the putative pathogen was observed in 4 out of 70 mares (5.7%) during a period from April 14 to 30 (Table 1). Moreover, the organism was isolated from 16 out of 74 mares (21.6%) during a period from May 1 to 25. *Streptococcus zooepidemicus* was isolated from 38 of 144 mares and the cervical swabs from the remaining mares yield a mixed growth of staphylococci, micrococci, fecal strepto-

Table 1. Bacterial counts of *Haemophilus equigenitalis* isolated from cervical swabs of mares with endometritis

Sample No.	Date harvested	Number of microorganisms*	
		<i>H. equigenitalis</i>	others
63	April 14, 1980	++	—
2	16,	+++	—
265	19,	++	—
278	22,	++	—
84	May 1,	+++	+
89	5,	+++	+
94	10,	+++	—
101	15,	+++	—
191	16,	++	—
192	" ,	+++	+
193	" ,	+++	+
194	" ,	+++	+
195	17,	++	+++**
196	" ,	+++	—
197	" ,	+++	—
45	23,	+++	—
49	24,	+++	—
50	" ,	+++	—
118	" ,	++	—
52	25,	+++	—

* Number of colonies per swab; —: 0, +: 1–10, ++: 11–100, +++: 101–300, ++++: >300.

** *Streptococcus zooepidemicus* was isolated.

cocci, coliform bacilli and other spp.

The putative pathogens subcultured on the chocolate agar were Gram negative coccobacilli or short rods with the Gram-stain, oxidase-positive, catalase-positive, resistant to streptomycin (400 µg/ml) and unreactive in sugars. Suspensions of organisms grown on the chocolate agar were agglutinated strongly with anti-rabbit serum against the K-188 strain of *H. equigenitalis* supplied by Dr. Bryans, of the University of Kentucky. They were dependent on a carbon dioxide, but not on X or V factor. After 3rd subcultures the organisms were propagated in an incubator containing an atmosphere of 10 percent v/v carbon dioxide in air and subjected to the characterization tests as described by Taylor et al. [10] and Kilian [4]. Two strains (To-84 and

Table 2. Characterization of the isolates (To-84 and To-89) and the K-188 strain of *Haemophilus equigenitalis*

Characteristic	Strains used for the test		
	To-84	To-89	K-188
Motility	—*	—	—
Capsule	+**	+	+
Cytochrome oxidase	+	+	+
Catalase	+	+	+
CO ₂ requirement	+	+	+
X-factor requirement	—	—	—
V-factor requirement	—	—	—
Nitrate	—	—	—
Indole	—	—	—
H ₂ S	—	—	—
Urease	—	—	—
Casein	—	—	—
Gelatin	—	—	—
Arginine dehydrolase	—	—	—
Lysin decarboxylase	—	—	—
Ornithine decarboxylase	—	—	—
Carbohydrates***	—	—	—
β -galactosidase	—	—	—
Phosphatase	+	+	+
Hemolysis	—	—	—

*: Negative. **: Positive. ***: Arabinose, fructose, glucose, inulin, lactose, maltose, mannitol, mannose, raffinose, rhamnose, saccharose, salicin, sorbitol, starch, trehalose, xylose.

To-89), isolated from cervical swabs of mares with endometritis, and the K-188 strain were subjected to the tests. The characteristics summarized in Table 2 were mostly consistent with those described by Taylor et al. [10]. Namely, only biochemical tests for catalase, cytochrome oxidase and phosphatase were positive, but the remaining tests were negative. From these results, the organism was found to be very inactive biochemically.

Serological relationship between the equine isolates, strain To-84 and To-89, and the K-188 strain of *H. equigenitalis* were established in a cross tube agglutination test. Each anti-rabbit serum was prepared by five intramuscular injections of the appropriate organism (more than $10^{10.0}$ cells

Table 3. Antigenic relationship between the isolates (To-84 and To-89) and the K-188 strains of *Haemophilus equigenitalis* by the cross-agglutination test

Antigen	Rabbit antiserum		
	To-84	To-89	K-188
To-84	2,560*	2,560	1,280
To-89	2,560	2,560	1,280
K-188	1,280	1,280	640

* Expressed as reciprocals of the highest serum dilution that agglutinated by 50 percent.

/0.5 ml) inactivated with 0.2% formalin solution. The test was performed by the method of Benson et al. [1]. As shown in Table 3, the results show a close antigenic relationship of strain To-84 and To-89 with the K-188 strain. On the basis of these characteristics, the equine isolates were identified as *H. equigenitalis*. From this result, it will be necessary to immediately perform more detailed studies on the bacteriological and serological surveys of CEM among horses in the whole country.

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要 約

馬伝染性子宮炎：子宮内膜炎雌馬からの *Haemophilus equigenitalis* の分離（短報）：鎌田正信・秋山 紳（日本中央競馬会競走馬総合研究所栃木支所感染病），小田隆範・福沢慶一（日高軽種馬農業協同組合）——北海道日高地方の繁殖雌馬に発生する子宮内膜炎の原因学的調査を実施したところ，144例の子宮頸管スワブから20株の *Haemophilus equigenitalis* が我国で初めて分離され，馬伝染性子宮炎の発生が確認された．分離株と *Haemophilus equigenitalis* K-188 株（米国株）の各種性状は全く一致した．早急に，全国的な馬伝染性子宮炎の疫学調査ならびに強力な行政指導が必要であろう．