NOTE *Bacteriology*

Contagious Equine Metritis Eradicated from Japan

Toru ANZAI[1)]*, Masanobu KAMADA[2)], Hidekazu NIWA[2)], Masashi EGUCHI[3)] and Hideki NISHI[4)]

[1)]*Equine Research Institute, Japan Racing Association, 321–4 Tokami, Utsunomiya, Tochigi 320–0856, Japan*
[2)]*Epizootic Research Center, Equine Research Institute, Japan Racing Association, 1400–4 Shiba, Shimotsuke, Tochigi 329–0412, Japan*
[3)]*Research Institute for Animal Science in Biochemistry and Toxicology, 3–7–1 Hashimotodai, Sagamihara, Kanagawa 229–1132, Japan*
[4)]*Livestock Farming Promotion Division, Bureau of Food Safety Promotion, Department of Agriculture, Hokkaido Government, Nishi-6, Kita-3, Chuhoh-ku, Sapporo, Hokkaido 060–0003, Japan*

(Received 22 July 2011/Accepted 13 November 2011/Published online in J-STAGE 25 November 2011)

ABSTRACT. Contagious equine metritis (CEM), a contagious venereal disease of horses, invaded Japan in 1980 and spread in the Thoroughbred population of the Hidaka-Iburi district of Hokkaido. To eradicate CEM, we ran a program aimed at detecting *Taylorella equigenitalis,* the causal agent, in carrier horses by using the PCR test, followed by culling or treatment. In 2001, the first year of the program, 12,356 Thoroughbred racing stallions and mares were tested and 11 carriers were found. Four, two, one, and one carrier mares were detected in 2002, 2003, 2004, and 2005, respectively, by application of the program at the same scale as in 2001. No PCR-positive horses were found from 2006 to 2010. These results strongly suggest that CEM was eradicated from Japan by 2010.
KEY WORDS: CEM, eradication, PCR, *Taylorella equigenitalis*.

doi: 10.1292/jvms.11-0347; *J. Vet. Med. Sci.* 74(4): 519–522, 2012

Contagious equine metritis (CEM) is a highly contagious venereal disease of horses that is caused by *Taylorella equigenitalis*. The causative bacterium is a persistent inhabitant of the smegma on the penis or clitoris in horses, and it invades the uterus at the time of copulation. Mares infected with this bacterium develop endometritis and short-term infertility, and the disease is spread rapidly by mating. CEM therefore severely affects horse-breeding operations. Since the first outbreaks were reported in England and Ireland in 1977 [5, 15], CEM has spread throughout the world, including to some European countries, Australia, and the United States of America [12, 14].

In May 1980, the first outbreak of CEM was confirmed in the Hidaka-Iburi district of Hokkaido, which is a major Japanese area for the breeding of Thoroughbreds for racing [7, 8, 13]. *Taylorella equigenitalis* (formerly called *Haemophilus equigenitalis*) was isolated from cervical swab specimens collected from mares with clinical signs of endometritis. By the end of the same year, 321 mares and stallions had been diagnosed with CEM by bacterial isolation testing.

Since then, bacterial isolation tests have been conducted on all breeding Thoroughbreds before the breeding season every year, and surveillance by isolation testing combined with serological testing using a passive hemagglutination test [6] is conducted during the breeding season. On the basis of these tests, the movement and mating of positive horses have been limited.

*CORRESPONDENCE TO: ANZAI, T., Equine Research Institute, Japan Racing Association, 321–4 Tokami, Utsunomiya, Tochigi 320–0856, Japan.
e-mail: Toru_Anzai@jra.go.jp

By 1997, as a result of these preventive measures, the number of horses from which *T. equigenitalis* had been isolated had decreased. Although eradication had not yet been achieved, CEM had become virtually confined to the Thoroughbred population of the Hidaka-Iburi district. The addition of CEM in 1998 to the list of diseases notifiable by law clearly demonstrated the continuing lack of spread of CEM beyond the Hidaka-Iburi district, and it enabled the number of infected horses in Japan to be determined officially (Table 1).

The results of molecular epidemiologic studies suggest that although *T. equigenitalis* invaded Japan on one occasion in or before 1980, the bacterium has not invaded since then. From 1980 to 1996, Matsuda and Miyazawa performed crossed-field gel electrophoresis of restricted genomic DNAs, after separate digestion with *Apa*I and *Not*I, against 130 isolates of *T. equigenitalis* from Thoroughbred mares and stallions in Japan. They reported that only a single genotype was present among all of the isolates [9, 10].

The above findings suggest that eradication of CEM would be achievable if thorough testing and selection were performed, using a highly sensitive method that could detect asymptomatic carriers, in Thoroughbreds destined for breeding.

In 1999, we developed a PCR method for direct rapid detection of *T. equigenitalis* in clinical specimens and reported its specificity and sensitivity as a diagnostic test for CEM [1, 2]. PCR testing has five times the sensitivity of bacterial isolation testing in detecting carrier horses. We proved that this PCR test was effective in revealing carriers in field; this suggested that eradication of CEM would be achievable by continued field application of the test [4].

The purpose of this paper is to report the results of a program aimed at eradicating CEM from Japan by using this diagnostic PCR test.

Table 1. Numbers of horses in Japan confirmed from 1980 through 2010 to have CEM

Area	Year													
	1980	1981	1982	1983	1984	1985	1986	1987	1988	1989	1990	1991	1992	1993
Hidaka-Iburi district of Hokkaido	321	57	35	25	33	212	100	96	96	70	24	33	19	23
Other areas of Japan	0	1	0	0	0	0	0	0	0	0	0	3	0	0
Total	321	58	35	25	33	212	100	96	96	70	24	36	19	23

Year																
1994	1995	1996	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010
11	0	23	7	5	0	1	11	4	2	1	1	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
11	0	23	7	5	0	1	11	4	2	1	1	0	0	0	0	0

After 1998, the numbers of CEM cases were reported officially because the disease became notifiable.

The eradication program was designed to operate by detecting *T. equigenitalis* in carrier mares and stallions by using the PCR test, followed by culling or treatment. In accordance with epidemiological data obtained up until the year the program started, we applied the program at all Thoroughbred racing breeding farms and studs in Japan. All horses, including teasers and other horses, bred on these farms and studs were tested by PCR before the breeding season every year, and a health certificate was issued if the result was negative. When the result was positive, the horse owner had to choose between culling and treatment.

Treatment was performed according to a proven method described previously [3]. Briefly, in stallions the penis was coated with gentamicin ointment after the removal of smegma by using a disinfectant solution. In mares, the uterus was treated first by using gentamicin solution, except if the mares were pregnant. In both pregnant and non-pregnant mares the clitoris was treated with disinfectant solution and gentamicin ointment. Additionally, clitoral sinusectomy was performed whenever possible in mares after treatment with disinfectant and antibiotics. After treatment for 5 consecutive days, each horse was checked by PCR testing three times.

Horses were issued with a health certificate and allowed to mate only if all results were negative. Treated horses were regarded as high-risk horses for 3 years, during which time all high-risk horses had to be checked 3 times by PCR testing before mating. Additionally, surveillance PCR tests were performed during the breeding season on horses that were suspected clinically of having genital infections despite having negative PCR tests before the breeding season; these surveillance tests were also performed on horses with low conception rates and horses that had been mated with PCR-positive horses.

For PCR testing before mating, the clitoral fossa and clitoral sinuses of mares were swabbed together, whereas the urethral sinus and penile sheath of stallions were swabbed separately, using a commercial swabbing and transportation kit without charcoal (CULTURETTE or MINI-TIP CULTURETTE; Becton Dickinson Microbiology Systems, Sparks, MD, U.S.A.). Swabs were also collected from multiple genital sites (e.g., the uterine cervix of mares and the urethral orifice of stallions) for surveillance.

Although PCR testing was performed by a direct, two-step, semi-nested method, its protocol was changed partly from that used previously in order to treat multiple samples by using microplates [4]. Swabs taken from genital sites were sent at 5°C to a laboratory, and the materials were suspended in 100 μl of distilled water in deep well microplates. DNA was extracted by boiling for 10 min, and the supernatant solution was used as a DNA template for initial PCR amplification after centrifugation at 15,000 *g* for 10 min. Initial PCR was performed in a 50 μl volume containing 5 μl of supernatant, 20 mM Tris-HCl (pH 8.0), 100 mM KCl, 3.0 mM $MgCl_2$, 0.2 mM (each) deoxynucleoside triphosphate, 0.4 μM (each) of P1 [5'-CCATTAGAGGCTGTTATCAATCGGGAAACC-3'] and N2 [5'-GTGTCATTAAGGTGTGTATTTGGTCTGGTG-3'] PCR primers and 1.25 U of Z-Taq (TAKARA BIO INC., Otsu, Shiga, Japan). Amplification was performed by one denaturation step at 95°C for 3 min, followed by 30 amplification cycles consisting of denaturation at 98°C for 1 sec, annealing at 68°C for 5 sec, and elongation at 72°C for 3 min in a Gold 96-Well GeneAmp PCR System 9700 (Applied Biosystems, Foster City, CA, U.S.A.). Secondary PCR was performed in a 50 μl volume also containing 0.5 μl of the initial PCR amplicons for the DNA template and P2 [5'-CCATACCGAACCCAATACCAAGCACACAAG-3'] and N2 PCR primers. Secondary PCR amplification was performed under the same conditions as used for the initial amplification. Amplicons of secondary PCR were analyzed by 2% agarose gel electrophoresis together with a reaction control constructed with recombinant plasmid from a strain of *T. equigenitalis* [11].

The results of the CEM eradication program from 2001 to 2010 are shown in Table 2. In 2001, the first year of the program, 12,356 stallions and mares were tested by PCR, and one stallion and 10 mares were found to be CEM. Of these PCR-positive horses, two mares were culled and the other horses were treated. In 2002, PCR testing was conducted on the same scale as in 2001 and four mares were found to be PCR positive before mating. One mare was culled and the others were treated that year. Two positive mares were detected and treated before mating in 2003.

Table 2. Results of the program to eradicate CEM, 2001 to 2010

Category	Year									
	2001*	2002	2003	2004	2005	2006	2007	2008	2009	2010
Registered stallions	411	412	389	351	331	305	281	282	311	269
Registered mares	12,411	12,276	11,499	11,130	10,670	10,297	10,253	10,263	9,872	10,765
PCR-tested	12,356	12,762	12,124	12,152	11,769	12,650	12,738	12,261	12,305	11,796
PCR-positive stallions	1	0	0	0	0	0	0	0	0	0
PCR-positive mares	10	4	2	1	1	0	0	0	0	0

Numbers of registered stallions and mares are total true head counts of Thoroughbreds registered for breeding for racing in Japan. 'PCR-tested' is the total head count of stallions and mares tested by PCR. Numbers of PCR-positive stallions and mares are total true head counts of PCR-positive horses. Five PCR-positive mares (two in 2001, one in 2002, 2004, and 2005) were culled, and the other mares and the stallion were treated. *The total number of horses in Japan in 2001 was 104,561, including 59,833 light horses. Of the light horses, 411 were registered as stallions and 12,411 were registered as breeding mares.

One positive mare was detected and culled in 2004, and one in 2005. Among the total of 19 horses found to be PCR positive in the period 2001 to 2010, one stallion was treated, five mares were culled, and 11 of 13 treated mares underwent clitoral sinus removal. No samples taken from the 14 treated high-risk horses in a 3-year period after treatment were PCR positive. In the 5 years from 2006 to 2010, no PCR positive horses were found, even though the PCR tests were performed on the same scale as in previous years.

An epidemiological survey of the 10 mares and one stallion that tested PCR positive in 2001 revealed that four of the mares had been mated with the PCR-positive stallion and five of the mares had been mated with two stallions standing at the same stud. These epidemiological data suggest that the small outbreaks occurring that year were associated with these stallions. Furthermore, three of the four mares found to be PCR positive in 2002 and both mares found to be PCR positive in 2003 were epidemiologically related to the horses that were PCR positive in 2001. In contrast, each of the mares found to be PCR positive in 2004 and 2005 had no epidemiological relationship to any of the horses diagnosed as PCR positive before, and no PCR-positive horses were found in the following 5 years. These findings suggested that the CEM was confined effectively and that the presence of carriers gradually but definitely disappeared as a result of the program.

With two exceptions, CEM infection of Thoroughbreds has been recognized in the Hidaka-Iburi district only. In Chiba Prefecture in 1981, bacteria were isolated from a Thoroughbred mare that had been moved from a farm in Hokkaido in 1980 when the first outbreak of CEM was found [16]. From November 1991 through to March 1992, *T. equigenitalis* was also isolated from three Thoroughbred mares that had been moved to Aomori from Hokkaido after being mated with a PCR-positive stallion. In these two instances there was no further infection, and on further testing both areas have since remained free from CEM.

Cases of infection in non-Thoroughbreds have also been limited. Only one instance, in 17 Japanese native ponies, has been confirmed, at Hidaka in Hokkaido, in 1987. Because these horses had no direct contact with Thoroughbreds, indirect transmission via a person or equipment was suspected. Since these ponies were treated, there have been no other positive cases in non-Thoroughbreds.

This epidemiological background suggests that expansion of the distribution of CEM in Japan was virtually limited to the Thoroughbred population of the Hidaka-Iburi district in Hokkaido, and that the program effectively eradicated CEM from Japan.

In conclusion, these findings strongly suggest that eradication of CEM was finally achieved by 2010. CEM carriers were removed year by year as the program progressed, and no PCR-positive horses were found in the last 5 years.

ACKNOWLEDGMENTS. We thank Kohei Yoshida, Hiroshi Nomura, Michio Komano, Shingo Nakanishi, Sadao Eguchi, Yoshizumi Kimura, Katsutomo Kashiwagi, Takashi Hatazoe, Shinichi Terai, Shigeru Nakajima, Hiroyoshi Komazawa, Ryohei Takahashi, Noboru Seno, and Ichiro Igari for conducting the program. We are also grateful to Takashi Tamura, Mariko Eto, Takehisa Yamamoto, Takeshi Nishida, Takuo Sawada, and Shinji Takai for evaluating the results of the program.

REFERENCES

1. Anzai, T., Kamada, M., Eguchi, M., Sekizaki, T., Yamamoto, K. and Okuda, T. 1999. Development of a PCR test for rapid diagnosis of contagious equine metritis. *J. Vet. Med. Sci.* **61**: 1287–1292. [Medline] [CrossRef]
2. Anzai, T., Eguchi, M., Eto, M., Okuda, T., Aoki, T., Sawada, T. and Matsuda, M. 2001. Establishment and evaluation of the PCR test for diagnosing contagious equine metritis. *J. Jpn. Vet. Med. Assoc.* **54**: 345–348 (In Japanese with English summary).
3. Anzai, T. 2001. Treatment of contagious equine metritis. *J. Jpn. Vet. Med. Assoc.* **54**: 18–20 (In Japanese).
4. Anzai, T., Wada, R., Okuda, K. and Aoki, H. 2002. Evaluation of the field application of PCR in the eradication of contagious equine metritis from Japan. *J. Vet. Med. Sci.* **64**: 999–1002. [Medline] [CrossRef]
5. Crowhurst, R. C. 1977. Genital infection in mares. *Vet. Rec.* **100**: 476. [Medline] [CrossRef]
6. Eguchi, M., Kamada, M., Tsukuba, S., Nishimori, K., Anzai, T. and Kuniyasu, C. 1994. Use of the modified passive haemagglutination test for detection of Taylorella equigenitalis-infected mares. pp. 207–210. *In*: Proceedings of Seventh International Conference on Equine Infectious Diseases

(Plowright, W. and Nakajima, H. eds.), R and W Publications, Newmarket, U.K.
7. Kamada, M., Akiyama, Y., Oda, T. and Fukunaga, Y. 1981. Contagious equine metritis: isolation of *Haemophilus equigenitalis* from horses with endometritis in Japan. *Jpn. J. Vet. Sci.* **43**: 565–568. [Medline] [CrossRef]
8. Kikuchi, N., Tsunoda, N., Kawakami, Y., Murase, N. and Kawata, K. 1982. An outbreak of contagious equine metritis in Japan: isolation of *Haemophilus equigenitalis* from thoroughbred mares with genital infection in Hokkaido. *Jpn. J. Vet. Sci.* **44**: 107–114. [Medline] [CrossRef]
9. Matsuda, M., Miyazawa, T. and Anzai, T. 1999. Detection of a common genotype among strains of *Taylorella equigenitalis* isolated from Thoroughbred horses in Japan between 1994 and 1996. *J. Basic Microbiol.* **39**: 127–130. [Medline] [CrossRef]
10. Miyazawa, T., Matsuda, M., Isayama, Y., Samata, T., Ishida, Y., Ogawa, S., Takei, K., Honda, M. and Kamada, M. 1995. Genotyping of isolates of *Taylorella equigenitalis* from Thoroughbred brood mares in Japan. *Vet. Res. Commun.* **19**: 265–271. [Medline] [CrossRef]
11. Niwa, H., Anzai, T. and Hobo, S. 2007. Construction of a recombinant plasmid as reaction control in routine PCR for detection of contagious equine metritis (CEM-PCR). *J. Vet. Med. Sci.* **69**: 1199–1201. [Medline] [CrossRef]
12. Powell, D. G. 1978. Contagious equine metritis. 1978. *Equine Vet. J.* **10**: 1–4. [Medline] [CrossRef]
13. Sugimoto, C., Isayama, Y., Kashiwazaki, M., Fujikura, T. and Mitani, K. 1980. Detection of *Haemophilus equigenitalis*, the causal agent of contagious equine metritis, in Japan. *Natl. Inst. Anim. Health Q. (Tokyo)* **20**: 118–119. [Medline]
14. Swerczek, T. W. 1978. Contagious equine metritis in the USA. *Vet. Rec.* **102**: 512–513. [Medline] [CrossRef]
15. Timoney, P. J., Ward, J. and Kelly, P. 1977. A contagious genital infection of mares. *Vet. Rec.* **101**: 103. [Medline] [CrossRef]
16. Uchimura, K., Miwa, R., Ide, Y., Nagata, T., Sugimoto, T. and Isayama, Y. 1981. Detection of *Haemophilus equigenitalis* from a pregnant mare in Chiba prefecture. *Bull. Nat. Inst. Anim. Health* **82**: 47–49 (In Japanese).