

## Genetic Homogeneity of *Taylorella equigenitalis* from Norwegian Trotting Horses Revealed by Chromosomal DNA Fingerprinting

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**Chromosomal DNA fingerprinting indicated that Norwegian *Taylorella equigenitalis* strains are genetically homogeneous and similar to some Swedish isolates but different from other European strains. As contagious equine metritis is rarely a serious disease in Norwegian horses, we conclude that the dominant *T. equigenitalis* strain in Norway is a genetically homogeneous clone of low virulence.**

The recognition of a new venereal disease, contagious equine metritis (CEM), among thoroughbred mares in Europe and Australia during 1977 has had widespread repercussions for the thoroughbred breeding industry and veterinarians involved in equine stud practice. The causative agent of CEM is a nonmotile gram-negative coccobacillus, *Taylorella equigenitalis* (formerly *Haemophilus equigenitalis*). CEM was reported for the first time in the United Kingdom by Crowhurst in 1977 (4). An early account of CEM was presented by Powell in 1978 (12), and a review of the subject was published by Eaglesome and Garcia a year later (5).

Originally, the disease spread among thoroughbred horses to France and the United States and occurred in Australia. Since the first outbreak in 1977, the disease has also been detected in other breeds of horses in many other countries (2, 3, 14-17). In Norway, CEM was first recognized in 1985 in a thoroughbred mare. *T. equigenitalis* was then isolated from several mares. Some of the mares tested were suffering from

metritis. However, the reason for testing was not that any serious outbreaks of disease had occurred but the fact that the mares under test had experienced contact with infected Swedish stallions (6). More extensive testing revealed that *T. equigenitalis* was also widespread in another breed (Norwegian Fjord Horse) with no tradition of any mixed breeding or breeding outside Norway (13a). For this reason, we can assume that the history of *T. equigenitalis* in Norway has been long but nonserious compared with that in many other countries (7, 9, 10). From 1987 to 1990, 15 isolates of *T. equigenitalis* were obtained from 14 different horses.

The aim of the present study was to genetically compare strains of *T. equigenitalis* isolated in Norway and in other European countries.

**Bacterial strains.** *T. equigenitalis* strains from 14 Norwegian horses were from widespread localities throughout the country (N<sub>1</sub> through N<sub>14</sub>). In addition, strains from eight Swedish horses (S<sub>1</sub> through S<sub>8</sub>), three Dutch horses (D<sub>1</sub> through D<sub>3</sub>), one English horse (E<sub>1</sub>), one Swiss horse (SW), and one horse of unknown origin (X) were investigated.

Strains from the Dutch, English, and Swiss horses, plus

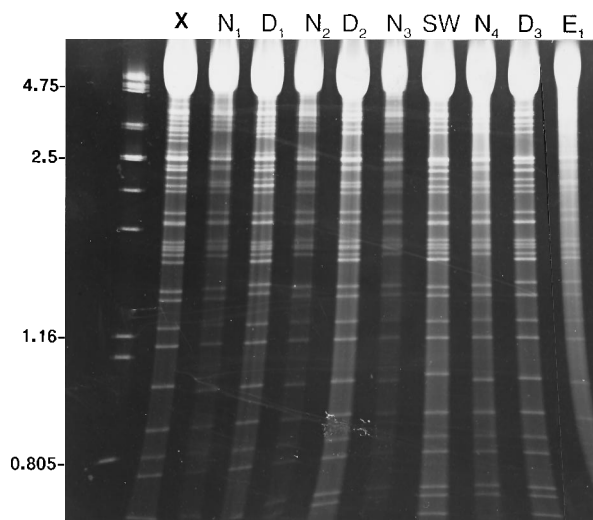


FIG. 1. Polyacrylamide gel electrophoretic analysis of *T. equigenitalis* chromosomal DNA digested with *Bam*HI. Tracks are labelled according to the strain designations given in Table 1. The unlabelled track to the left is  $\lambda$  DNA digested with *Pst*I. Sizes are indicated in kilobases.

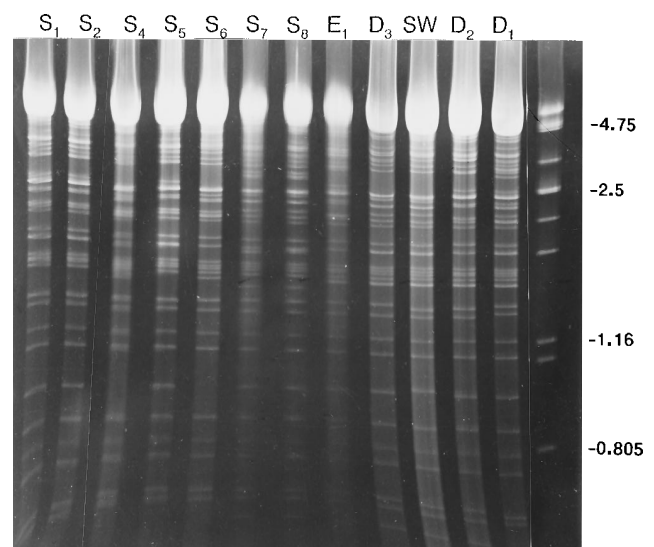


FIG. 2. Polyacrylamide gel electrophoretic analysis of *T. equigenitalis* chromosomal DNA digested with *Bam*HI. Tracks are labelled according to the strain designations given in Table 1. The unlabelled track to the right is  $\lambda$  DNA digested with *Pst*I. Sizes are indicated in kilobases.

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TABLE 1. *T. equigenitalis* strains and results of DNA typing

Horse	Country of origin	Breed <sup>a</sup>	<i>T. equigenitalis</i> type		
			DNA group	FIGE group <sup>b</sup>	Strain no.
N <sub>1</sub>	Norway	Fjord Horse	A		43461/90
N <sub>2</sub>	Norway	Norw. Coldbl. Trotter	A		38336/90
N <sub>3</sub>	Norway	Standardbred	A		19422/90
N <sub>4</sub>	Norway	Norw. Coldbl. Trotter	A		19419/90
N <sub>5</sub>	Norway	Norw. Coldbl. Trotter	A		2732/87
N <sub>6</sub>	Norway	Norw. Coldbl. Trotter	A		12719/90
N <sub>7</sub>	Norway	Norw. Coldbl. Trotter	A		13658/89
N <sub>8</sub>	Norway	Norw. Coldbl. Trotter	A		11031/89
N <sub>9</sub>	Norway	Norw. Coldbl. Trotter	A		9013/89
N <sub>10</sub>	Norway	Norw. Coldbl. Trotter	A		694/89
N <sub>11</sub>	Norway	Standardbred	A		62526/88
N <sub>12</sub>	Norway	Norw. Coldbl. Trotter	A		58505/88
N <sub>13</sub>	Norway	Norw. Coldbl. Trotter	A		16331/87
N <sub>14</sub>	Norway	Norw. Coldbl. Trotter	A		1486/87
S <sub>1</sub>	Sweden	Standardbred	A		1/82
S <sub>2</sub>	Sweden	Standardbred	A		58/84
S <sub>3</sub>	Sweden	Swed. Coldbl. Trotter	A		85/85
S <sub>4</sub>	Sweden	Fjord Horse	B		186/88
S <sub>5</sub>	Sweden	Mixed-breed pony	A		191/88
S <sub>6</sub>	Sweden	Thoroughbred	C		199/89
S <sub>7</sub>	Sweden	Ardennes	D		217/89
S <sub>8</sub>	Sweden	Fjord Horse	E		232/91
D <sub>1</sub>	The Netherlands	Dutch Saddlehorse	F	B	L10783
D <sub>2</sub>	The Netherlands	Haflinger	G	D	L68722-2
D <sub>3</sub>	The Netherlands	?	H	E	L67215
E <sub>1</sub>	England	Thoroughbred	I	A	NCTC 11184
X	?	?	J	F <sup>c</sup>	92720-2
SW	Switzerland	Nonthoroughbred	K	C	N610/88

<sup>a</sup> Norw. Coldbl. Trotter, Norwegian Coldblood Trotter; Swed. Coldbl. Trotter, Swedish Coldblood Trotter.

<sup>b</sup> Grouping based on field inversion gel electrophoresis (FIGE) analysis of Bleumink-Pluym et al. (1a). The FIGE groups of the Norwegian and Swedish strains are unknown.

<sup>c</sup> Per reference 1.

strain X, were chosen to represent the five previously determined groups based on field inversion gel electrophoresis (1a).

**DNA fingerprinting.** DNA was extracted as described by Wilson (18). *T. equigenitalis* was harvested from GC Agar Base (Mast Laboratories Ltd., Merseyside, United Kingdom) plates (with amphotericin B, lincomycin, and trimethoprim) and lysed with sodium dodecyl sulfate. Lysates were deproteinized with proteinase K, and cell wall debris was precipitated with CTAB (hexadecyltrimethyl ammonium bromide). After extraction once with chloroform-isoamyl alcohol and once with phenol-chloroform-isoamyl alcohol, DNA was precipitated from the supernatant with isopropanol, dried, and resuspended in Tris-EDTA buffer.

DNA was digested with *Bam*HI. Samples (5 µg) of DNA were run on 0.7% agarose gels overnight (16 h) at a constant voltage of 30 V as a control of DNA quality. Subsequently, 25 µg of *Bam*HI-digested DNA was run on polyacrylamide gels overnight (16 h) at 20 mA (11). Gels were stained with ethidium bromide and photographed under UV illumination according to standard protocols (13). Restriction patterns were compared visually.

There were no differences between the *Bam*HI restriction patterns of Norwegian strains; four of the Swedish strains (S<sub>1</sub>, S<sub>2</sub>, S<sub>3</sub>, and S<sub>5</sub>) were identical to the Norwegian strains. The remaining four Swedish strains (S<sub>4</sub>, S<sub>6</sub>, S<sub>7</sub>, and S<sub>8</sub>) were

different from the Norwegian strains and from all other strains investigated. All of the other six strains investigated were different from the Norwegian and Swedish strains and from each other (Fig. 1 and 2). A summary of data is given in Table 1. Analysis with *Hae*III or *Ban*II gave similar results but gave poorer resolution of strains of different field inversion gel electrophoresis groups.

We find that the degree of difference between *T. equigenitalis* strains on DNA fingerprints is quite small. For this reason, the high resolution of polyacrylamide gel electrophoresis is necessary in order to distinguish strains (Fig. 1 and 2). The low variability of DNA fingerprints implies a low degree of genetic variability among strains of *T. equigenitalis*, as is also implied by the great constancy of biochemical characteristics (8).

Outbreaks of CEM in Norway have on the whole been less serious than elsewhere in Europe. This might be explained by the presence of a single clone of low virulence in this country, and this is consistent with our data.

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