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cgMLST of Finnish *Taylorella equigenitalis* isolates



Finnhorse



Fjord horse

Photo: BiancaGrueneberg Canva.com

Kirsti Pelkola DVM, Senior Researcher,
Specialist in Infectious Animal Diseases
Finnish Food Authority Helsinki

History of CEM in Finland



- Testing started in 1980's
 - Import, export, clinical suspicions
 - From 1995 on, studs used for AI have been tested before each breeding season
 - According to Finnish veterinary legislation (AI) and Finnish horse breeding association (Suomen Hippos Ry) guidance
 - No clear rules for natural service especially in the early 2000's (awareness of infectious diseases...)
 - Sampling and testing according to WOAH (culture, ID, PCR, qPCR)
 - 300-500 studs tested annually
- First detected in 1992
 - Clinical CEM in three Standardbred (trotter) mares serviced by a visiting German stallion
- *T. equigenitalis* detected from 28 horses by 2021
 - 34 isolates from 24 horses available for the cgMLST study
 - Rare Finnhorse and Fjord horse isolates
 - One Russian Warmblood and one Swedish Warmblood isolate sampled upon import examination

8/2023 Icelandic horse stallion qPCR-pos.,
and a contact mare with CEM was positive
also in culture



Our study

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Core genome multilocus sequence typing analysis of Finnish *Taylorella equigenitalis* isolates

Kirsti Pelkola^{a,*}, Sirpa Heinikainen^b, Tarja Pohjanvirta^b

^a Animal Health Diagnostics Unit, Finnish Food Authority, Mustialankatu 3, Helsinki FI-00790, Finland

^b Animal Health Diagnostics Unit, Finnish Food Authority, Neulaniementie 4, Kuopio FI-70210, Finland

The *T. equigenitalis* isolates analysed in this study.

Horse ID	Isolate ID	Isolation year	Horse breed	Horse sex	Reason for sampling
1	FIXT-839	1992	Standardbred	Mare	Clinical signs
2	FIXT-840	1992	Standardbred	Mare	Clinical signs
3	FIXT-841	1992	Standardbred	Mare	Clinical signs
4	FIXT-842, FIXT-843, FIXT-844, FIXT-845 ¹⁾	1993-1994	Warmblood	Mare	Import
5	FIXT-846	1994	Warmblood	Stallion	Import
6	FIXT-853	2002	Standardbred	Stallion	Breeding
7	FIXT-856, FIXT-860, FIXT-861 ¹⁾	2003-2004	Standardbred	Stallion	Breeding
8	FIXT-857, FIXT-858, FIXT-859 ¹⁾	2003	Arabian horse	Stallion	Breeding
9	FIXT-854	2003	Standardbred	Stallion	Breeding
10	FIXT-855	2003	Standardbred	Stallion	Breeding
11	FIXT-868	2005	Standardbred	Stallion	Breeding
12	FIXT-863	2005	Finnhorse	Stallion	Breeding
13	FIXT-766	2005	Finnhorse	Mare	Contact to ID 19
14	FIXT-864	2005	Fjord horse	Stallion	Breeding
15	FIXT-865	2005	Fjord horse	Mare	Contact to ID 14
16	FIXT-866	2005	Fjord horse	Gelding	Contact to ID 14
17	FIXT-867, FIXT-769, FIXT-768 ¹⁾	2005-2006	Fjord horse	Gelding	Contact to ID 14
18	FIXT-862	2005	Standardbred	Stallion	Breeding
19	FIXT-767	2005	Finnhorse	Stallion	Breeding
20	FIXT-770	2006	Finnhorse	Stallion	Breeding
21	FIXT-761	2014	Shetland pony	Stallion	Breeding
22	FIXT-762	2014	Fjord horse	Stallion	Breeding
23	FIXT-763, FIXT-764 ¹⁾	2017, 2019	Fjord horse	Stallion	Breeding
24	FIXT-765	2021	Finnhorse	Stallion	Breeding

The 34 isolates from 24 horses

- **9 Standardbreds (trotters):**
11 isolates (1992 and 4 years period 2002-2005)
- **6 Fjord horses:**
9 isolates (2005-2006, 2014, 2017-2019)
 - 2 geldings
- **5 Finnhorses:**
5 isolates (2005-2006, 2021)
- **2 Warmbloods (riding horses):**
5 isolates (1993-1994, from Swedish ♀ and Russian ♂)
- **1 Arabian horse:**
3 isolates (2003)
- **1 Shetland pony:**
1 isolate (2014)
- **15/24 horses during 5 years period 2002-2006**

2002-2006

1) All isolates originate from different sampling time points.

WGS procedure



- The DNA was extracted using DNeasy Blood & Tissue kit in QIAcube classic instrument (gram-positive bacteria protocol)
- The integrity of the DNA was controlled with agarose gel electrophoresis
- The DNA concentrations were quantified using the Cubit dsDNA BR Assay Kit (Thermo Fisher Scientific, Waltham, USA)
- The library for WGS was prepared using Illumina DNA Prep kit
- Sequencing: using Illumina chemistry and MiSeq benchtop sequencer (Illumina, San Diego, CA, USA).



- **ad hoc cgMLST scheme**

- The scheme was defined using a target definer tool within the Ridom SeqSphere+ software (Ridom Münster, Germany) to identify **1259 target loci** from the reference strain CP002456.1 (*T. equigenitalis* MCE9) and seven complete query genomes obtained from GenBank (NC_018108.1, NZ_CP021201.1, NZ_CP021200.1, NZ_CP021199.1, NZ_CP021060.1, NZ_CP021246.1 (12-OCT-2021) and NZ_LR134346.1).
- The cgMLST scheme targets covered 76,5 % of the reference genome
- Sequences were subjected to an ad hoc cgMLST analysis using Ridom SeqSphere+ software
- Results visualized as minimum spanning tree MST: revealing allelic differences. Comparison of genes: pairwise, ignoring missing values, cluster distance threshold value 12 (0,9% of the targets)

- **Classical MLST and clonal complexes CC**

- The **seven housekeeping gene MLST** profiles (Duquesne et al. 2013) and **CCs** were extracted from the WGS data using *Taylorella* MLST Database scheme (Jolley et al. 2018) in Ridom SeqSphere+ software.
- Duquesne F, Hébert L, Breuil MF, Matsuda M, Laugier C, Petry S. Development of a single multi-locus sequence typing scheme for *Taylorella equigenitalis* and *Taylorella asinigenitalis*. *Vet Microbiol.* 2013 Dec 27;167(3-4):609-18. doi: 10.1016/j.vetmic.2013.09.016. Epub 2013 Sep 24. PMID: 24139720.
- Jolley KA, Bray JE, Maiden MCJ. Open-access bacterial population genomics: BIGSdb software, the PubMLST.org website and their applications. *Wellcome Open Res.* 2018 Sep 24;3:124. doi: 10.12688/wellcomeopenres.14826.1. PMID: 30345391; PMCID: PMC6192448.
- > The cgMLST and MLST results were combined with the known epidemiological data of the horses

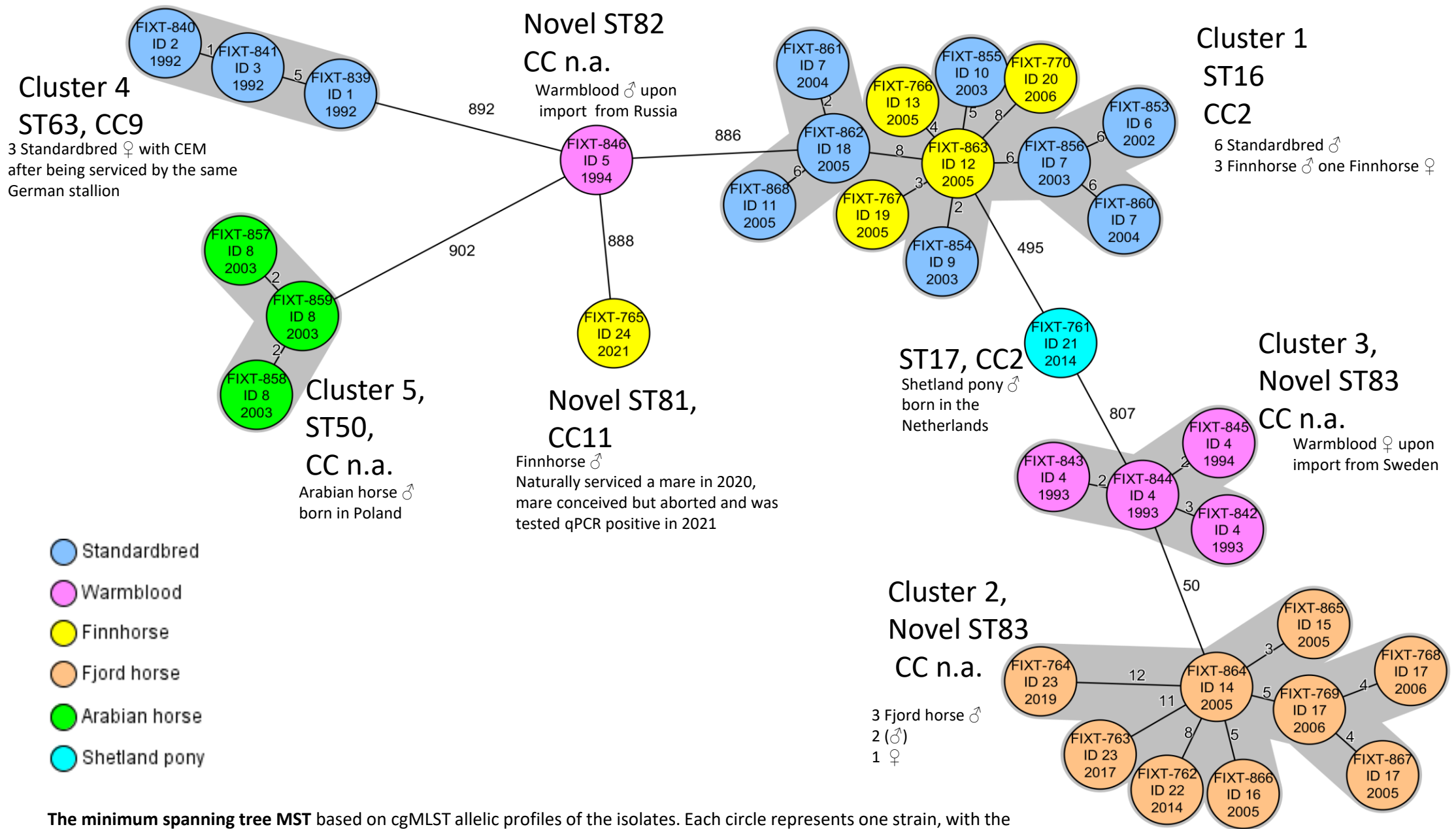
The clonal complexes, core genome MLST clusters and MLST sequence types of the *T. equigenitalis* isolates.

Clonal complex	cgMLST cluster	ST	Horse ID	Breed and sex	Isolate ID	Year	Epidemiological data
CC 2	Cluster 1	16	6	Standardbred ♂	FIXT-853	2002	Born in the USA. Imported from Sweden. At stud farm 1 in 2000-2005. Naturally serviced an imported mare in 2000 and another mare in 2002.
			7	Standardbred ♂	FIXT-856, FIXT-860, FIXT-861	2003-2004	Born in the USA. Imported from Sweden. Owned by A. At stud farm 1 in 2000-2001. At stud farm 2 in 2002.
			9	Standardbred ♂	FIXT-854	2003	Born in the USA. Owned by A. At stud farm 1 in 2000-2002.
			10	Standardbred ♂	FIXT-855	2003	Born in the USA. Imported from Sweden. At stud farm 1 in 2002.
			11	Standardbred ♂	FIXT-868	2005	Born in the USA. Owned by A. At stud farm 1 in 2000-2002.
			12	Finnhorse ♂	FIXT-863	2005	At stud farm 2 in 2002.
			13	Finnhorse ♀	FIXT-766	2005	Naturally serviced by ID 19 in 2003. Conceived but aborted.
			18	Standardbred ♂	FIXT-862	2005	Born in Germany. Owned by A. At stud farm 1 in 2000-2002.
			19	Finnhorse ♂	FIXT-767	2005	Naturally serviced ID 13 in 2003.
			20	Finnhorse ♂	FIXT-770	2006	A foal with ID 13 in 2005 (contact to ID 13 in 2004).
NA ¹⁾	Cluster 2	83 ²⁾	14	Fjord horse ♂	FIXT-864	2005	While still a colt shared a paddock with IDs 16 and 17. Naturally serviced ID 15. At stable 1.
			15	Fjord horse ♀	FIXT-865	2005	Born in Norway. Imported from Norway in 2005. Was naturally serviced by ID 14, did not conceive. At stable 1.
			16	Fjord horse (♂)	FIXT-866	2005	Born in Norway. Imported from Norway in 2003. Shared a paddock with ID 14. At stable 1.
			17	Fjord horse (♂)	FIXT-867, FIXT-769, FIXT-768	2005-2006	Born in Denmark. Imported from Denmark in 1997. Shared a paddock with ID 14. At stable 1.
			22	Fjord horse ♂	FIXT-762	2014	Offspring of ID 14. At stable 1.
			23	Fjord horse ♂	FIXT-763, FIXT-764	2017, 2019	Contact with stable 1 horses in 2012.
NA	Cluster 3	83 ²⁾	4	Warmblood ♀	FIXT-842, FIXT-843, FIXT-844, FIXT-845	1993-1994	Born in Sweden. Imported from Sweden.
CC 9	Cluster 4	63	1	Standardbred ♀	FIXT-839	1992	Naturally serviced by a German stallion.
			2	Standardbred ♀	FIXT-840	1992	Naturally serviced by a German stallion.
			3	Standardbred ♀	FIXT-841	1992	Naturally serviced by a German stallion.
NA	Cluster 5	50	8	Arabian horse ♂	FIXT-857, FIXT-858, FIXT-859	2003	Born in Poland. Imported from Denmark.
NA	single	82 ²⁾	5	Warmblood ♂	FIXT-846	1994	Born in Russia. Imported from Russia.
CC 2	single	17	21	Shetland pony ♂	FIXT-761	2014	Born in the Netherlands.
CC 11	single	81 ²⁾	24	Finnhorse ♂	FIXT-765	2021	Naturally serviced a mare in 2020, which was tested positive in 2021.

¹⁾ NA: not applicable. ²⁾ Novel ST.

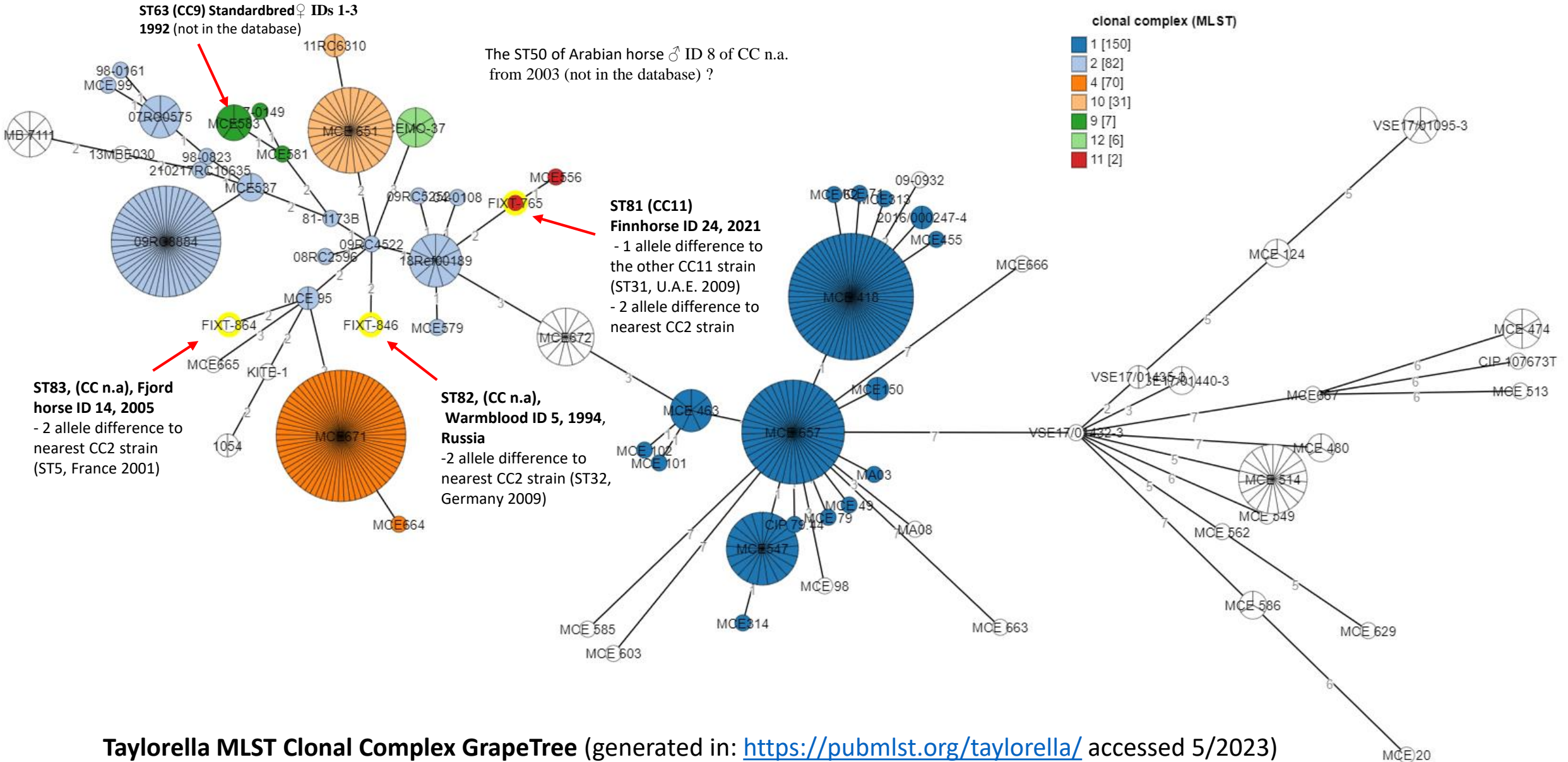
Results

- 5 clusters (1-5) congruent with MLST STs, 3 singletons
- 34 isolates > 34 cgSTs
- 7 different MLST STs
 - **3 novel STs:** ST81 (minor CC11), ST82 (CC n.a.) and ST83 (CC n.a.)
 - 2 separate clusters of novel ST83
 - **4 previously described STs:** ST16 (major CC2), ST17 (CC2), ST50 (CC n.a.) and ST63 (minor CC9)
 - ! No isolates belonging to the founding major CC1
- All isolates of horses with known epidemiological links clustered together:
 - **Links of Cluster 1 horses (6 Standardbreds, 4 Finnhorses, ST16):**
 - all 6 Standardbreds were imported, 4 of them had the same owner, visits at same stud farms and artificial insemination (biosecurity!), also some natural services (5 year period 2002-2006)
 - **Links of cluster 2 (6 Fjordhorses, ST83):**
 - 5/6 horses (incl. geldings!) at the same stable with close physical contacts, natural service, the sixth horse had close contact with the other stable horses in 2012. Role of the two imported geldings (introduced or harboured the infection in the herd?)
 - **Links of cluster 4 horses (3 Standardbreds, ST63):**
 - All mares were serviced by the same German stallion
- All isolates of a same horse clustered together
- Several of the horses - other than Finnhorses - were **imported**



The minimum spanning tree MST based on cgMLST allelic profiles of the isolates. Each circle represents one strain, with the isolate ID number, horse ID number and the isolation year indicated. The numbers between the circles denote the allelic difference between the isolates. CC: clonal complex, n.a.: not applicable.

Genomic relationship of the novel STs 81, 82 and 83 with the *Taylorella* pubMLST database strains



Discussion and conclusions



- We developed a local ad hoc gcMLST scheme (1259 targets) for 34 Finnish *T. equigenitalis* isolates from 1992-2021
 - Rare isolates from Finnhorses, Fjord horses, Russian horse, Swedish horse
- MLST STs extracted from WGS data (7 genes): 3 novel STs (added to Taylorella MLST database) and 4 previously described STs
- The seven MLST STs were divided into 34 cgSTs, 5 clusters (congruent with STs) and three singeltons
- No isolates belonging to founding CC1
- All isolates of horses with epidemiological link and isolates from a same horse clustered together
- cgMLST could separate the two ST83 clusters with no epidemiological link (50 allele difference)
- ! Since 2006: no ST16 in Finland nor *T. equigenitalis* in Standardbreds or Warmbloods (practically only AI) very likely due to:
 - Successful treatment of positive horses, improved biosecurity at stud farms after 2006 and limiting natural service of studs accepted for AI after negative test result
 - Isolates after 2006 were not connected to AI



- cgMLST: good discriminatory power compared to classical MLST
- Results contribute the worldwide phylogenetic and spatio-temporal epidemiological analyses of *T. equigenitalis* (sequences are published in European Nucleotide Archive ENA, project PRJEB64295 <https://www.ebi.ac.uk/ena/browser/home>)
- Generally in bacteriology: the highly discriminatory cgMLST is replacing classical MLST:
- Developement of standardised curated cgMLST scheme for *T. equigenitalis* would:
 - > Improve understanding the epidemiology and global transmission routes of the bacterium
 - > Assist control measures of the infection



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Thank you!



Finnhorse. Photo: Sinikka Pelkonen