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1 **Overview of spatio-temporal distribution inferred by multi-locus sequence typing of**
2 ***Taylorella equigenitalis* isolated worldwide from 1977 to 2018 in equidae**

3

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27

Abstract

The accurate identification of *Taylorella equigenitalis* strains is essential to improve worldwide prevention and control strategies for contagious equine metritis (CEM). This study compared 367 worldwide equine strains using multilocus sequence typing according to the geographical origin, isolation year and equine breed. The strains were divided into 49 sequence types (STs), including 10 described for the first time. Three major and three minor clonal complexes (CCs), and 11 singletons, were identified. The genetic heterogeneity was low (0.13 STs/strain) despite the wide diversity of geographical origins (n=16), isolation years (1977 to 2018) and equine breeds (n=18). It was highest outside Europe and in the 1977-1997 period; current major STs and CCs already existed before 1998. Previous data associated the major CC1 with the first CEM outbreaks in 1977-1978 in the United Kingdom, Australia and the United States, and revealed its circulation in France. Our study confirms its circulation in France over a longer period of time (1992-2018) and its distribution in Spain and Germany but not throughout Europe. In addition to CC1, relationships between non-European and European countries were observed only through ST4, ST17 and ST30. Within Europe, several STs emerged with cross-border circulation, in particular ST16 and ST46 from the major complexes CC2 and CC8. These results constitute a baseline for monitoring the spread of CEM outbreaks. A retrospective analysis of a higher number of strains isolated worldwide between 1977 and the early 2000s would be helpful to obtain an exhaustive picture of the original CEM situation.

Keywords: infectious equine disease; contagious equine metritis; *Taylorella equigenitalis*; MLST.

52 **Introduction**

53 Contagious equine metritis (CEM) is a venereal disease of equids caused by *Taylorella*
54 *equigenitalis*, a slow-growing Gram-negative, non-mobile capnophilic and pleiomorphic
55 coccobacillus. The acute form of the disease is characterised by mucopurulent vaginal
56 discharge and variable degrees of vaginitis, endometritis and cervicitis, leading to temporary
57 infertility. All infected stallions and a variable proportion of infected mares are
58 asymptomatic carriers (Sugimoto et al., 1983; Timoney, 2011). Because of its contagiousness
59 and consequential economic losses, CEM was at the origin of the Horserace Betting Levy
60 Board's Code of Practices to prevent and control specific diseases in all horse and pony
61 breeds (Allen and Wilsher, 2018). CEM must be reported to the World Organisation for
62 Animal Health (OIE) and is part of veterinary certification for international trade purposes.
63 However, the CEM disease status of many countries remains unknown due to the absence of
64 monitoring and import programmes.

65 CEM was first reported in 1977 in the United Kingdom and Ireland among Thoroughbred
66 horses (Crowhurst, 1977; Timoney et al., 1977), but is currently a worldwide concern in
67 various equine breeds (Jeoung et al., 2016; Schulman et al., 2013) with the hypothesis that
68 the episodic "source of contagion" is often mainland Europe (Schulman et al., 2013). Pulsed-
69 field gel electrophoresis (Aalsburg and Erdman, 2011; Sting et al., 2016) and several other
70 molecular typing tools including field inversion gel electrophoresis (Bleumink-Pluym et al.,
71 1990), chromosomal DNA fingerprinting (Thoresen et al., 1995), crossed-field gel
72 electrophoresis (Miyazawa et al., 1995) and more recently repetitive extragenic palindromic
73 PCR (Sting et al., 2016) have been used to genotype CEM isolates. However, these molecular
74 epidemiological tools are not very portable and inter-laboratory results are difficult to
75 compare (Maiden et al., 1998), making them ill-suited for global epidemiological studies of

76 CEM outbreaks. International equine trade stakeholders, however, need to identify and
77 track the global spread of *T. equigenitalis*. With this in mind and starting by whole genome
78 sequencing data for both *Taylorella* species (Hébert et al., 2011; Hébert et al., 2012), a
79 *Taylorella* multilocus sequence typing (MLST) scheme was developed based on the
80 nucleotide sequences of seven housekeeping genes (*glt*A, *gyr*B, *fh*, *shmt*, *tyr*B, *adk* and *txn*)
81 (Duquesne et al., 2013). This scheme provides unambiguous results directly comparable
82 between laboratories (Enright et al., 2000). The accumulation of nucleotide changes in
83 housekeeping genes is a relatively slow process and their allelic profile is sufficiently stable
84 over time to be used for global epidemiology purposes (Enright and Spratt, 1999).
85 Distinct sequence types (ST) reported by recent publications using the *Taylorella* MLST
86 scheme show that new *T. equigenitalis* strains are constantly emerging (Hwang and Cho,
87 2018; Melzer et al., 2018). Some of these strains showed no genetic links with other
88 identified STs according to the default clonal complex definition when single locus variants
89 (SLV) were considered. This is the case, for example, of the inland South Korean strain KITE-1
90 isolated in 2016 (Hwang and Cho, 2018). The aim of the present study was to assess the
91 distribution of specific *T. equigenitalis* genotypes from a large population of *T. equigenitalis*
92 strains mainly originating from several European countries using MLST. Based on the
93 resulting MLST data, the genetic diversity and phylogeny of 367 *T. equigenitalis* strains were
94 analysed and compared on the basis of different factors such as the geographical origin, year
95 of isolation and equine breed.
96
97 **Materials and methods**
98 Isolate collection

99 The study was conducted with the European Union Reference Laboratory for equine
100 diseases other than African horse sickness (ANSES, Laboratory for Animal Health, France),
101 the National Reference Laboratories for CEM from Belgium, Czech Republic, France,
102 Germany, Poland, Spain and Switzerland and two laboratories outside Europe: the Central
103 Veterinary Research Laboratory in Dubai, United Arab Emirates, and the National Veterinary
104 Services Laboratories in Ames, United States.

105 A total of 367 *T. equigenitalis* strains isolated from horses, two ponies and two donkeys,
106 were included in this study: 119 strains were obtained and characterised using MLST; the
107 MLST sequence data for 242 strains were downloaded from the online *Taylorella* MLST
108 database (https://pubmlst.org/taylorella/), and the MLST sequence data for six strains were
109 obtained from Hicks et al. (Hicks et al., 2018) (n = 4) and Delerue et al. (Delerue et al., 2019)
110 (n = 2).

111 The strains were isolated over a 42-year period between 1977 and 2018 (93.2%) or at an
112 unknown date (6.8%). Most of them were isolated in Europe (81.8%), including Austria,
113 Belgium, the Czech Republic, France, Germany, Poland, Spain, Switzerland, the Netherlands
114 and the United Kingdom. The remaining strains were isolated in Australia, Japan, South
115 Africa, South Korea, the United Arab Emirates and the United States (17.4%) or the
116 geographical location was unknown (0.8%) (Table 1). At least 18 different equine breeds
117 were represented (Table 2), which were grouped into 12 categories (Additional file 1) to
118 perform phylogenetic and goeBURST analysis. The Draught horse category grouped Comtois
119 Draught and Trait Breton breeds, while the Warmblood horse category grouped Belgian
120 Warmblood/Warmblood, Hanoverian, Oldenburg, Westfalen and Zangersheide breeds.
121 Detailed information on the geographical origin, year of isolation, clinical signs and equine

122 breed as well as the MLST sequence data relating to each strain are reported in Additional
123 file 1.
124
125 Bacterial growth and DNA extraction
126 The 119 *T. equigenitalis* strains typed in this study were isolated and identified at the
127 institutions of origin using the gold standard culture method according to OIE or national
128 culturing instructions applicable at the time of isolation. Strains were subcultured onto
129 chocolate agar at (37±2) °C with 5-10% (v/v) CO_2 in air prior to genomic DNA extraction and
130 purification using commercially available DNA extraction kits according to the
131 manufacturer's protocol.
132
133 MLST
134 The seven MLST loci (*glt*A, *gyr*B, *fh*, *shmt*, *tyr*B, *adk* and *txn*) were amplified and sequenced
135 according to previously published experimental conditions (Duquesne et al., 2013). The
136 *Taylorella* MLST database (https://pubmlst.org/taylorella/) was used to assign allele numbers
137 and STs. Novel alleles and STs were allocated by the database curator. All the sequence data
138 for the 119 strains typed in this study are available from the *Taylorella* MLST database.
139
140 Data analysis
141 The sequence data obtained from the online *Taylorella* MLST database and from the
142 literature (Delerue et al., 2019; Hicks et al., 2018) were incorporated into the dataset. The
143 sequences of every individual locus were trimmed to equivalent lengths, like in the
144 *Taylorella* MLST database.

145 Sequences were aligned and a phylogenetic tree drawn up using MEGA software version 7.0
146 (Kumar et al., 2016). For each unique ST, sequences of the seven MLST loci were
147 concatenated, giving an in-frame sequence of 3,521 bp (not including nucleotide
148 insertions/deletions). The phylogenetic tree was based on the concatenated sequences using
149 the maximum likelihood method based on the Tamura-Nei model (Tamura and Nei, 1993).
150 Support for internal nodes was estimated using the nonparametric bootstrap method with
151 1,000 replications. The tree was rooted with the ST28-concatenated sequences of the
152 *Taylorella asinigenitalis* CIP 107673^{T} reference strain as the outgroup, extracted from the
153 *Taylorella* MLST database.
154 Relatedness between STs was analysed based on allelic profiles using eBURST software
155 version 3 (Feil et al., 2004; Spratt et al., 2004). The BURST algorithm, based on a set of
156 hierarchical rules relating to the number of single-locus variants (SLV) and double-locus
157 variants (DLV) involved, identifies mutually exclusive groups named clonal complexes (CCs)
158 of related STs in the population, and attempts to identify the primary and subgroup founding
159 ST for each CC. A CC was defined as major when a primary founding ST was predicted. All
160 others were considered minor.
161 Global optimal goeBURST (Francisco et al., 2009) diagrams were drawn up using PHYLOViZ
162 software version 2 (Francisco et al., 2012) to analyse relatedness between STs and
163 geographical origins or equine breeds.
164
165 **Results**
166 The MLST analysis of the 367 *T. equigenitalis* strains included in this study divided the strains
167 into 49 genotypes or STs, i.e. 0.13 STs/strain (Additional file 1). Thirty-one STs were
168 associated with a single strain per ST, and the other 18 STs were associated with between

169 two and 67 strains per ST. ST4 (n = 67), ST33 (n = 64), ST16 (n = 49) and ST1 (n = 41) were the
170 most prevalent STs and concerned 60% of strains. MLST data for the 119 *T. equigenitalis*
171 strains typed in this study allowed us to identify three new alleles (*fh*-25, *fh*-26 and *shmt*-15)
172 and 10 new STs (ST32, ST36, ST53, ST54, ST59 and ST61 to ST65) never described before
173 (Additional file 1).
174 The eBURST analysis (Additional file 2) grouped the 49 STs into three major CCs (CC1, CC2
175 and CC8), three minor CCs (CC3, CC9 and CC10) and 11 singletons at the SLV threshold. CC1
176 was the largest CC, including 17 STs with the predicted primary founder ST1 and both
177 predicted subgroup founders ST2 and ST33. CC2 and CC8 each included seven STs with the
178 predicted primary founders ST17 and ST46 respectively. Minor complexes CC3, CC9 and
179 CC10 included between two and three STs and no primary founders were predicted. The
180 discovery of 10 new STs led to the emergence of the new CC10, and the identification of CC8
181 and CC2 as major CCs (last one following its merger with the former CC4 via ST66). At the
182 DLV threshold, all the CCs and eight singletons previously described using the SLV threshold
183 were linked; only ST9, ST31 and ST50 remained genetically independent (Additional file 2).
184 A phylogenetic tree (Fig. 1) drawn up using concatenated sequences of seven MLST loci did
185 not reveal any major clade within the 49 unique STs. However, the distribution of STs within
186 the six CCs reflects a correlation between the genetic distances and evolutionary
187 relationships among the closely-related STs. The 11 singletons were distributed throughout
188 the tree except on the upper part composed by CC1, and several of them were clustered
189 along with CC2 and CC8. The 10 new STs were distributed throughout the tree, but 50%
190 clustered in CC8.
191 Three hundred and twenty-nine *T. equigenitalis* strains out of the 367 included in this study
192 were isolated over a 42-year period, but the strain distribution over time was heterogeneous

193 (Additional file 1): 32 strains were isolated from 1977 to 1997 versus 310 strains between
194 1998 and 2018. However, the ST diversity was greater during the 1977-1997 period (0.34
195 STs/strain) than during the 1998-2018 period (0.12 STs/strain). Twelve STs, grouped into the
196 current CC1, CC2, CC8 and CC10 complexes plus three singletons, had already been
197 identified before the 1998-2018 period; they included the four most currently prevalent, i.e.
198 ST1, ST4, ST16 and ST33.
199 The 64 non-European and 300 European *T. equigenitalis* strains out of the 367 strains
200 included in this study were grouped into 12 and 40 STs respectively (Table 1, Additional file
201 1). The goeBURST analysis (Fig. 2) allowed us to connect non-European and European
202 countries through ST1, ST4, ST17 and ST30. ST19 and ST20 were only associated with non-
203 European strains but were nonetheless related to CC1, which includes European strains.
204 Singletons ST3, ST9, ST31, ST42, ST55 and ST58 were associated only with non-European
205 strains whereas CC3, CC8, CC9, CC10 and singletons ST5 and ST50 were associated only with
206 European strains. Interestingly, (Fig. 2a) Japan (ST3 and ST9) and South Korea (ST55) had no
207 connections with any other countries, whereas Australia, the United States and Europe were
208 connected through CC1, the United Arab Emirates and Europe through CC2 and ST30, and
209 South Africa and Europe through ST4. Within Europe (Fig. 2b), the Czech Republic showed
210 the least connection with other European countries (only with Germany though CC10)
211 whereas Germany and France showed the most connections with other European countries.
212 While the French strains were the most represented in this study (30.8%), unlike the German
213 strains that only represented 5.4%, they are less genetically heterogeneous (0.21 STs/strain)
214 than German strains (0.60 STs/strain). A focus on the most prevalent STs (Fig. 2) suggested
215 that ST16 (CC2) may circulate within Europe, whereas ST1 (CC1) may circulate worldwide,
216 and ST33 (France and Spain) and ST4 (Austria, France and unknown) appear to be more

217 specific to a small number of countries. With fewer strains, ST46 (CC8) showed the same
218 country distribution as ST16 without any Polish strains, and ST17 may be a European ST
219 recently linked to Emirati strains.
220 Despite unknown equine breeds representing 35.5% of the 367 strains included in this study
221 and the large number of equine breeds represented (Table 2, Additional file 1), we
222 investigated the genetic relationships between the STs and equine breeds or category of
223 equine breeds (Fig. 3). Singletons were mostly specific to an equine breed, except ST4 which
224 was associated with both South African strains from Thoroughbred and Warmblood horses,
225 and South African, Austrian and French strains from Lipizzaner horses. The largest complex,
226 CC1, was associated with up to eight different equine breeds. Details on the seven prevalent
227 equine breeds (Fig. 3) showed that except the Trotter from ST16, Draught and Trotter horses
228 were clustered within CC1 and CC3, both closely located on the phylogenetic tree (Fig. 1).
229 Thoroughbred, Warmblood and Lipizzaner horses were more dispersed through the
230 goeBURST representation, but it may be noted that STs related to Lipizzaners were close to
231 each other on the phylogenetic tree (Fig. 1). The Kladruber (ST56) and Pure Spanish (CC1)
232 horses, on the other hand, were not only specific to a ST or CC, but were also country-
233 specific (from the Czech Republic and Spain respectively).
234
235 **Discussion**
236 *T. equigenitalis* is the causative agent of CEM, a contagious equine disease leading to
237 substantial economic losses. It is essential to accurately identify the strain for both
238 epidemiological surveillance and to improve worldwide CEM prevention and control
239 strategies. CEM was first reported in the United Kingdom and Ireland among Thoroughbred
240 horses in 1977 (Crowhurst, 1977; Timoney et al., 1977). The disease spread rapidly from

241 1977 to 1978 in Europe (including Belgium, France and Germany), Australia and the United
242 States (1978) (Matsuda and Moore, 2003). CEM was then reported in Italy and Japan in 1980
243 (Matsuda and Moore, 2003) and its presence was finally described in at least 30 countries
244 worldwide in various equine breeds (Schulman et al., 2013), the most recent cases in the
245 literature being in Croatia in 2014 or 2015 (Štritof et al., 2017) and South Korea in 2015
246 (Jeoung et al., 2016). Besides being the cradle of CEM emergence, the endemic status among
247 the non-Thoroughbred horses of Europe supports the current hypothesis that the episodic
248 "source of contagion" is often mainland Europe. MLST has already been implemented to
249 examine the *T. equigenitalis* population structure. It revealed that the first CEM outbreaks in
250 the United Kingdom, Australia and United States from 1977 to 1978 were associated with
251 the founding complex, CC1 (Duquesne et al., 2013). In parallel, distinct genotypes emerged
252 over time and in different countries (Japan, the United Arab Emirates, South Korea)
253 suggesting the existence of an unidentified natural worldwide reservoir (Duquesne et al.,
254 2013; Hwang and Cho, 2018). The present study focused on a large population of 367 *T.*
255 *equigenitalis* strains to determine, through their MLST genotypes, their diversity and spatio-
256 temporal distribution according to the geographical origin, year of isolation and equine
257 breed. A hundred and nineteen strains were characterised using MLST, and the MLST
258 sequence data of 248 additional strains were accessed from the online *Taylorella* MLST
259 database and the literature (Delerue et al., 2019; Hicks et al., 2018). Despite the large
260 selection of strains in terms of geographical origin, year of isolation and equine breed, one
261 area of concern is the amount of emphasis put on the number of strains within the same ST.
262 In this study, a *T. equigenitalis* strain was usually isolated from a CEM-positive animal
263 reported in a single year. However, several strains with the same ST may be represented
264 multiple times, subjected to repeated sampling (perhaps in relation to a treatment protocol)

265 or due to animals CEM-positive over a period of several years (Additional file 1);
266 nevertheless, considering the known data on strains, this situation concerned only a limited
267 number of strains and has not been considered a significant bias.
268 The MLST analysis performed in this study suggests little genetic heterogeneity, with only 49
269 STs characterised from the 367 *T. equigenitalis* strains (0.13 STs/strain), despite marked
270 heterogeneity in terms of geographical origin (16 countries, including six outside Europe),
271 year of isolation (1977 to 2018) and equine breed (n = 18) in the analysed strain collection.
272 The genetic heterogeneity of *T. equigenitalis* was slightly higher outside Europe (0.19
273 STs/strain) than within Europe (0.13 STs/strain). More importantly, the genetic
274 heterogeneity of *T. equigenitalis* was greater in the 1977-1997 period (0.34 STs/strain) than
275 in the 1998-2018 period (0.12 STs/strain); it may be noted that the current major STs and CC
276 had already been identified during the 1977-1997 period. The STs/strain value of 113 *T.*
277 *equigenitalis* strains used to develop the *Taylorella* MLST was slightly higher (0.24 STs/strain)
278 (Duquesne et al., 2013) than for the present study but probably corresponded to genotypic
279 variability in the French strains, since 85% of the strains were from France. This is similar to
280 our study, where the French data reported 0.21 STs/strains. A comparison of whole-genome
281 sequencing reported by Hicks et al. (Hicks et al., 2018) showed diversity in genome size by up
282 to 100,000 bp however, mainly due to repeat regions and four main variable regions.
283 Moreover, the authors observed that *T. equigenitalis* was a more diverse species than the
284 whole *Mycobacterium tuberculosis complex* by an SNP comparison (Hicks et al., 2018). The
285 genomic diversity thus observed is not, however, noticeable here because of the MLST
286 principle, being based on housekeeping genes.
287 Compared to previous MLST results, the present study confirms the circulation of the
288 founding complex, CC1, in France over a long period of time (1992-2018) and confirms its

289 distribution in Spain (2015-2016) and Germany too, but not in the six other European
290 countries investigated, i.e. Austria, Belgium, the Czech Republic, the Netherlands, Poland
291 and Switzerland. In the 1970s-80s, and perhaps still today, CC1 was circulating worldwide yet
292 it does not seem to have spread throughout Europe according to the dataset analysed. This
293 observation should nevertheless be confirmed by a retrospective analysis of strains isolated
294 from the first reported outbreaks in these European countries, if they are available.
295 Apart from the epidemiological relationships described through CC1, few epidemiological
296 relationships between non-European and European countries were revealed in the present
297 study. Thus, singleton ST4 was first found in Austria (1992-2007) and France (1993-2001) and
298 then in South Africa (1996 to 2015), suggesting its spread from Europe to South Africa first
299 within the Lipizzaner population then spreading to other breeds (Warmblood and
300 Thoroughbred). Emirati strains were divided into four genotypes, three of which (ST17, ST30
301 and S42) had a DLV relationship and clustered on the phylogenetic tree, suggesting the
302 presence of a common ancestral Emirati clone. The presence of this ancestral Emirati clone
303 is, however, in contrast to the fact that ST17 (primary founder of CC2) was found in Europe
304 before Emirati strains were isolated in 2012 (ST42), 2016-2017 (ST17) and 2018 (ST30).
305 Within Europe, ST16 included in CC2 has emerged as the dominant genotype with a cross-
306 border circulation; it is composed of strains from Austria (2004), Belgium (2008-2013),
307 France (2004-2012), Germany (1989-2009), Poland and Switzerland (2005-2008). ST46, the
308 primary founder of CC8, seems to have the same profile as ST16 without any Polish strains,
309 but it was found to be mainly composed of German strains plus the strain isolated in 2018
310 from the vaginal discharge of a French mare infected by cryopreserved stallion semen
311 collected and processed in Germany in 2012 (Delerue et al., 2019). This Germanic origin
312 could be extended to CC8, which is mainly composed of German strains first isolated in

313 1981. Interestingly, the genetic heterogeneity of German strains was much higher (0.60
314 STs/strain) than that of the entire dataset despite representing only 5.4% of the strain
315 collection studied.
316
317 **Conclusion**
318 This study provides new insights into the molecular epidemiology of *T. equigenitalis* in
319 Europe and constitutes a baseline for monitoring the spread of CEM outbreaks. However, it
320 is difficult to give an exhaustive picture of the global CEM situation as there are very few *T.*
321 *equigenitalis* strains genotyped by MLST from non-European countries and in the 10-15
322 years preceding the first CEM outbreaks reported in 1977. The genotyping of *T. equigenitalis*
323 strains by MLST should be continued over an extended timeline, and a retrospective study
324 carried out on a higher number of strains isolated from 1977 to the early 2000s. In the
325 future, MLST data could be obtained using whole-genome sequencing, or a core genome
326 MLST scheme could even be developed to the maximum resolution of the genealogy within
327 the *T. equigenitalis* species.
328
329
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339

340 **Authors' contributions**

341 Conception and design of the study and writing the article: FD and SP. Acquisition of data:
342 FD, IPC, KS, FM, GO, DF, WI, MFB, UW, JK, MAG, NFS, ESMI, EP, AW, MJ, JJ, SJ and PV. Data
343 analysis and interpretation: FD, AM and SP. Participation in the drafting and critical revision
344 of the article: FD, AM, IPC, KS, WI, EP, AW, UW, DLC, AH and SP. All the authors read and
345 approved the article.
346

347 **Conflicts of interest**

348 The authors declare that they have no conflicts of interest.
349

350 **Abbreviations**

351 CC, clonal complex; CEM, contagious equine metritis; DLV, double-locus variant; MLST,
352 multilocus sequence typing; OIE, world organisation for animal health; SLV, single-locus
353 variant; ST, sequence type.
354

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435

436 **Table 1.** Countries of origin for the 367 *T. equigenitalis* strains included in this study.

Country of origin	Strain typed in this study	Strain extracted from the MLST database	Strain extracted from the literature *	Total number of strains
Australia	-	6	-	6
Austria	-	24	1	25
Belgium	1	16	-	17
Czech Republic	27	2	-	29
France	13	99	1	113
Germany	15	3	2	20
Japan	-	7	-	7
Netherlands	1	-	1	2
Poland	-	17	-	17
South Africa	-	34	-	34
South Korea	-	1	-	1
Spain	57	-	-	57
Switzerland	-	19	-	19
United Arab Emirates	5	5	-	10
United Kingdom	-	1	-	1
United States	-	5	1	6
Unknown	-	3	-	3
Total	119	242	6	367

437 *Delerue et al., 2019; Hicks et al., 2018

438 **Table 2.** Equine breeds for the 367 *T. equigenitalis* strains included in this study.

Equine breed	**Strain typed in this study**	**Strain extracted from the MLST database**	**Strain extracted from the literature ***	**Total number of strains**
Anglo-Arabian	2	-	-	2
Arabian	1	17	-	18
Baudet du Poitou	-	2	-	2
Belgian Warmblood / Warmblood	-	15	-	15
Comtois Draught Horse	-	7	-	7
French Saddlebred Horse	-	11	-	11
French Trotter / Trotter	-	44	-	44
Hanoverian	-	-	1	1
Kladruber horse	27	-	-	27
Lipizzaner	5	54	1	60
Oldenburg	-	-	1	1
Pony	-	2	-	2
Pure Spanish horse	34	-	-	34
Quarter Horse	-	1	-	1
Thoroughbred	7	7	1	15
Trait Breton	-	2	-	2
Westfalen riding horse	-	1	-	1
Zangersheide horse	-	1	-	1
Unknown	43	78	2	123
Total	119	242	6	368

439 *Delerue et al., 2019; Hicks et al., 2018

440

441 **Figure 1.** Phylogenetic tree based on 3,521-bp concatenated sequences of seven MLST
442 housekeeping genes. The tree was constructed from the MLST data of 367 *T. equigenitalis*
443 strains (49 STs) using the maximum likelihood method, and rooted with the *T. asinigenitalis*
444 CIP 107673^T reference strain as the outgroup. The bootstrap values were calculated from
445 1,000 replications. The total number of strains associated with each ST is indicated in
446 brackets. The equine breeds are indicated next to the STs using geometrical shapes, and the
447 geographical origins correspond to the colours of those geometrical shapes (see the legend).
448 The grouping of STs into CCs defined by eBURST analysis at the SLV threshold is indicated by
449 braces, while singletons are underlined. STs identified for the first time in this study are
450 identified by an asterisk. The numbers in brackets in the legend correspond to the number of
451 strains.

452

453 **Figure 2.** This population snapshot of *T. equigenitalis* displays genetic relationships between
454 the STs and countries of isolation, with a focus on non-European (**a**) and European countries
455 (**b**). It is based on a goeBURST (PHYLOViZ) analysis of country distribution among 367 *T.*
456 *equigenitalis* strains characterised by MLST. STs with an SLV relationship were linked
457 together by solid lines to form six CCs, and STs with a DLV relationship were linked together
458 by dashed lines (the length of lines are not representative of the distances between STs). Dot
459 colour fractions refer to countries of isolation associated with STs (see legend). The dot size
460 is proportional to the prevalence of STs in the analysed collection. The numbers in brackets
461 in the legend correspond to the number of strains.

462

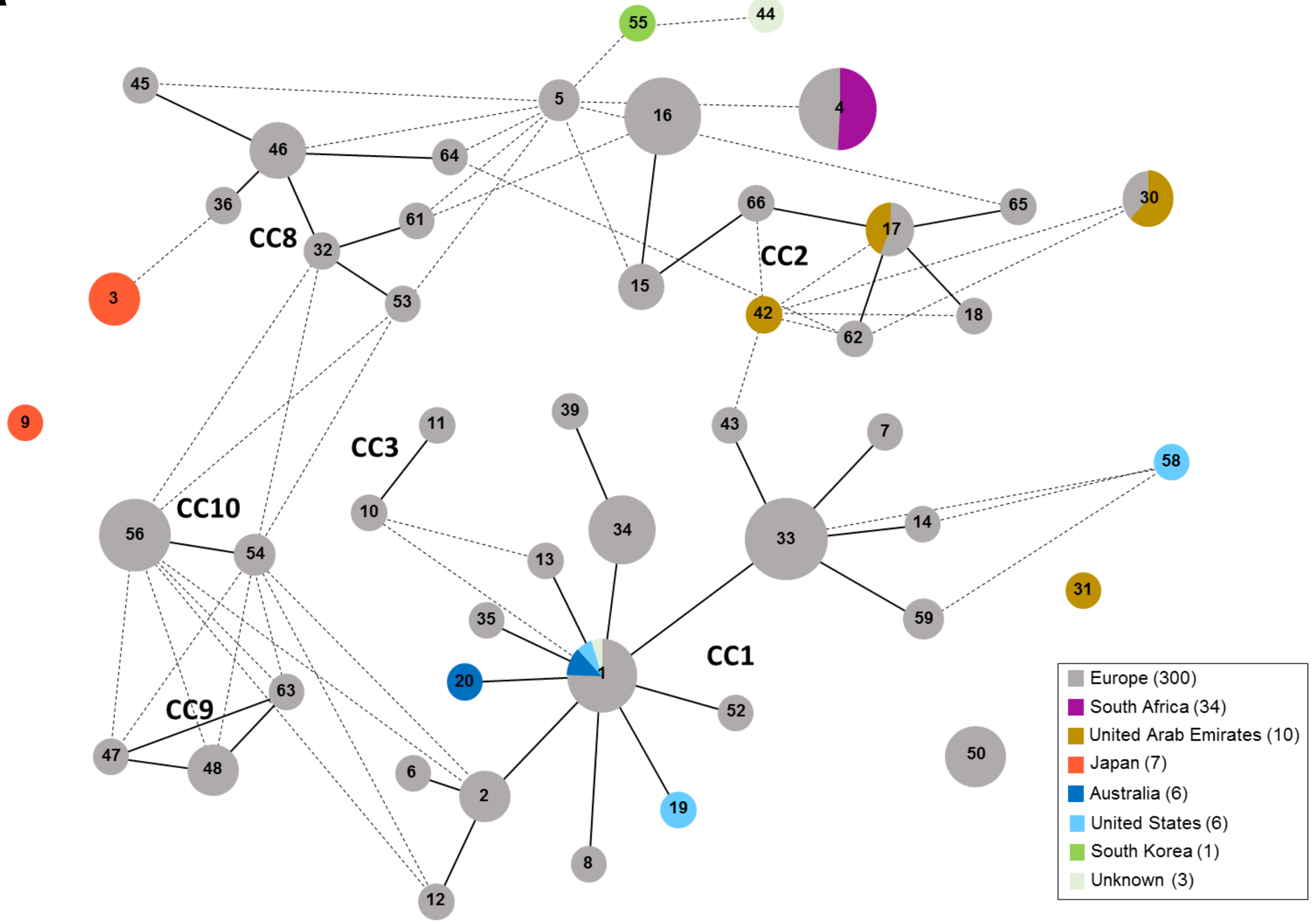
463 **Figure 3.** This population snapshot of *T. equigenitalis* displays genetic relationships between
464 the STs and equine breeds. It is based on a goeBURST (PHYLOViZ) analysis of equine breed

465 distribution among 367 *T. equigenitalis* strains characterised by MLST. STs with an SLV
466 relationship were linked together by solid lines to form six CCs, and STs with a DLV
467 relationship were linked together by dashed lines (the length of lines are not representative
468 of the distances between STs). Dot colour fractions refer to equine breeds associated with
469 STs (see legend). The dot size is proportional to the prevalence of STs in the analysed
470 collection. The numbers in brackets in the legend correspond to the number of strains.
471

Arabian & Anglo-Arabian (20)
Donkey (2)
Draught Horse (9)
Kladruber Horse (27)
Lipizzaner (60)
Pony (2)
Pure Spanish Horse (34)
Quarter Horse (1)
Thoroughbred (15)
Trotter (44)
Warmblood (30)
Unknown (123)

Australia (6)
Austria (25)
Belgium (17)
Czech Republic (29)
France (113)
Germany (20)
Japan (7)
The Netherlands (2)
Poland (17)
South Africa (34)
South Korea (1)
Spain (57)
Switzerland (19)
United Arab Emirates (10)
United Kingdom (1)
United States (6)
Unknown (3)

ST 34 (21)
ST 39 (1)
ST 52 (1)
ST 20 (1)
ST 13 (1)
ST 19* (1)
ST 01 (41)
ST 07 (1)
ST 08 (1)
ST 43 (1)
ST 33 (64)
ST 59 (2)
ST 14 (1)
ST 35 (1)
ST 02 (7)
ST 06 (1)
ST 12 (1)
CC1
ST 09 (1)
ST 58 (1)
ST 10 (1)
ST 11 (1)
CC3
ST 54* (2)
ST 56 (29)
CC10
ST 47 (1)
ST 48 (5)
ST 63* (1)
CC9
ST 50 (12)
ST 55 (1)
ST 61* (1)
ST 05 (2)
ST 64* (1)
ST 04 (67)
ST 31 (1)
ST 53* (1)
ST 32* (1)
ST 03 (6)
ST 45 (1)
ST 36* (1)
ST 46 (10)
CC8
ST 44 (1)
ST 65* (1)
ST 15 (3)
ST 16 (49)
ST 17 (7)
ST 62* (1)
ST 42 (1)
ST 66 (1)
ST 30 (8)
ST 18 (1)
CC2
Taylorella equigenitalis
Taylorella asinigenitalis
ST 28 (strain CIP 107673^{T})
0.02


a
CC8
CC2
CC3
CC10
CC9
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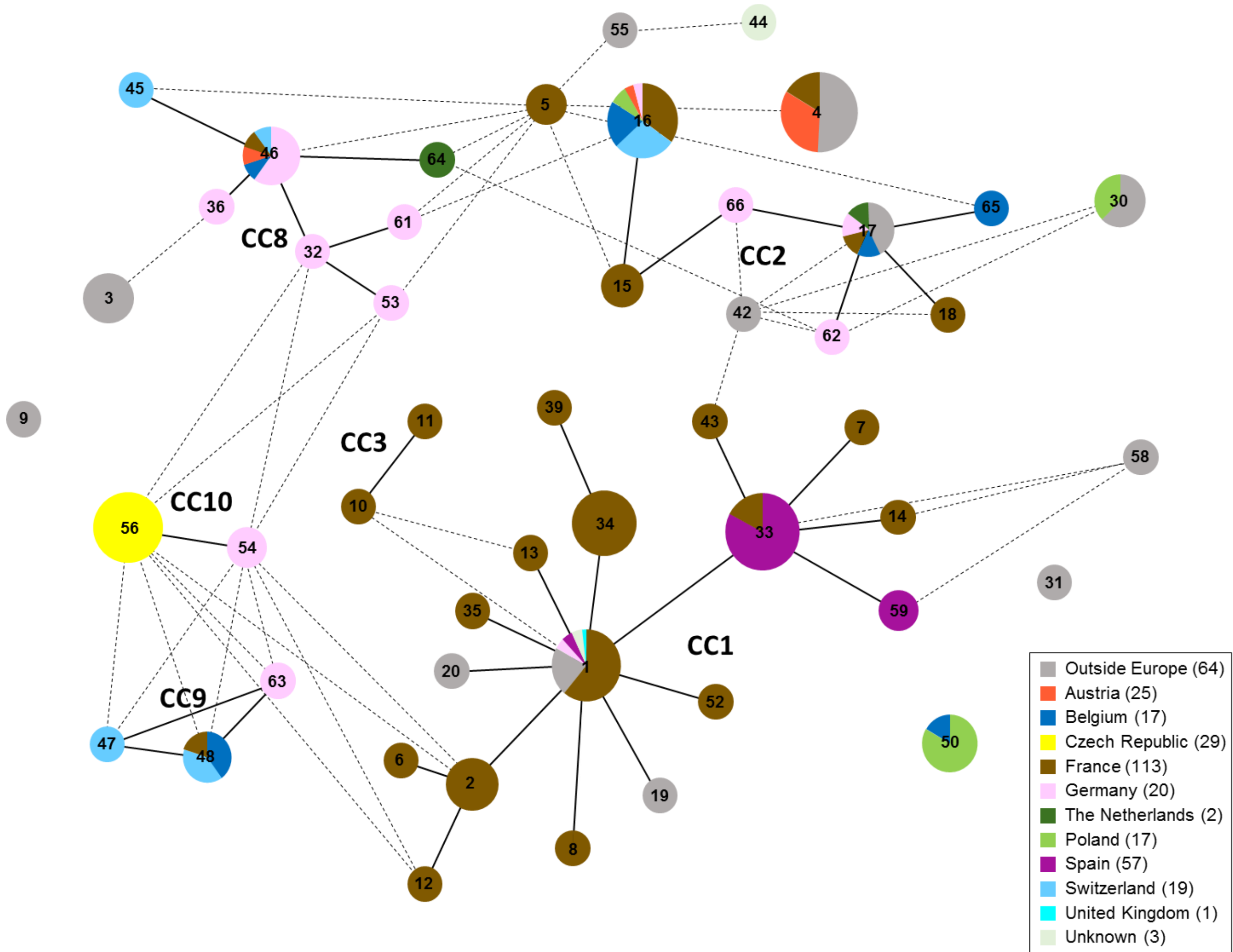
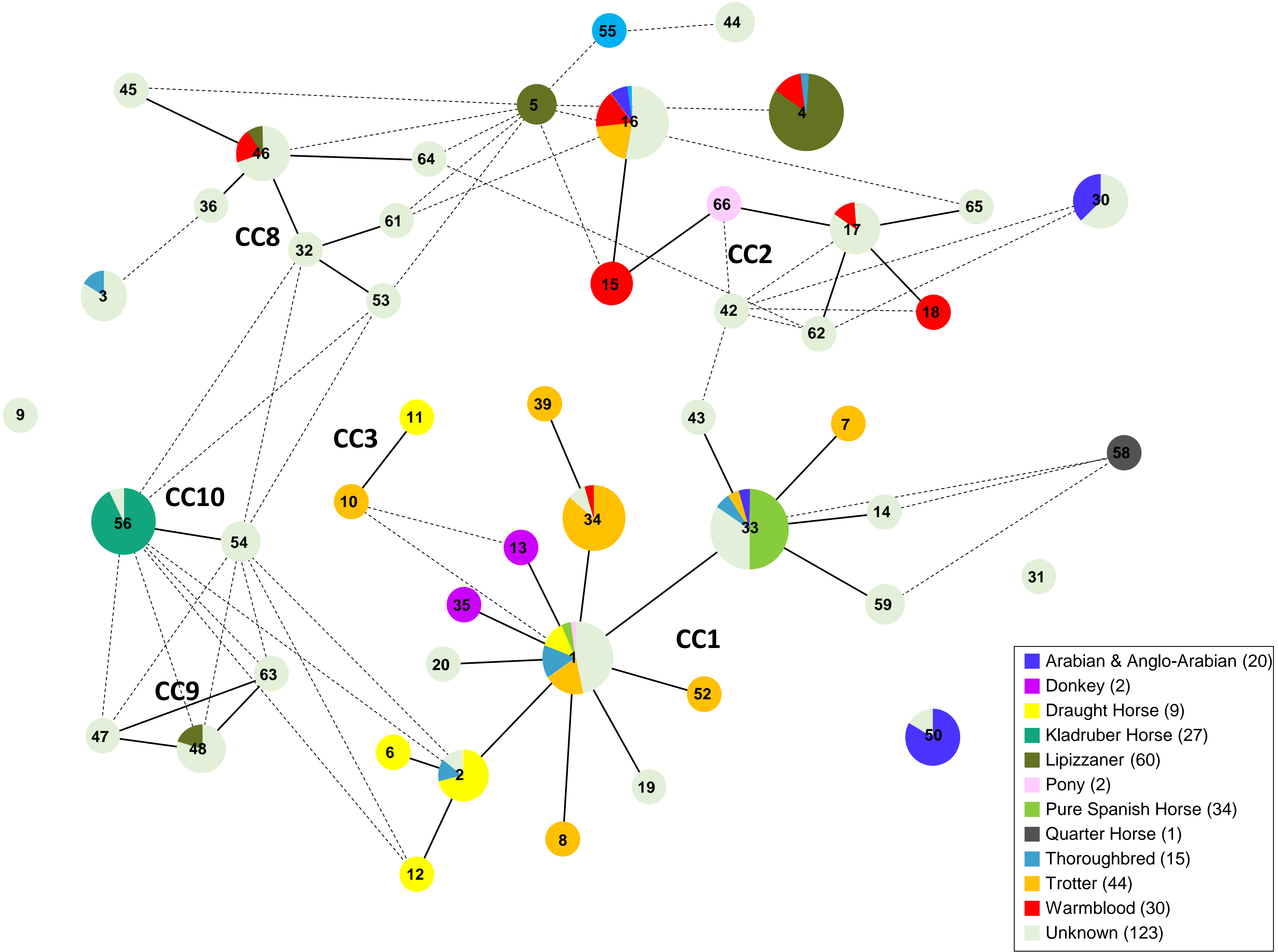
South Africa (34)
United Arab Emirates (10)
Japan (7)
Australia (6)
United States (6)
South Korea (1)
Unknown (3)


b
CC8
CC2
CC3
CC10
CC9
CC1
Outside Europe (64)
Austria (25)
Belgium (17)
Czech Republic (29)
France (113)
Germany (20)
The Netherlands (2)
Poland (17)
Spain (57)
Switzerland (19)
United Kingdom (1)
Unknown (3)


CC8
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Arabian & Anglo-Arabian (20)
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