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

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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.
Introduction

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

CEM was first reported in 1977 in the United Kingdom and Ireland among Thoroughbred horses (Crowhurst, 1977; Timoney et al., 1977), but is currently a worldwide concern in various equine breeds (Jeoung et al., 2016; Schulman et al., 2013) with the hypothesis that the episodic “source of contagion” is often mainland Europe (Schulman et al., 2013). Pulsed-field gel electrophoresis (Aalsburg and Erdman, 2011; Sting et al., 2016) and several other molecular typing tools including field inversion gel electrophoresis (Bleumink-Pluym et al., 1990), chromosomal DNA fingerprinting (Thoresen et al., 1995), crossed-field gel electrophoresis (Miyazawa et al., 1995) and more recently repetitive extragenic palindromic PCR (Sting et al., 2016) have been used to genotype CEM isolates. However, these molecular epidemiological tools are not very portable and inter-laboratory results are difficult to compare (Maiden et al., 1998), making them ill-suited for global epidemiological studies of
CEM outbreaks. International equine trade stakeholders, however, need to identify and track the global spread of *T. equigenitalis*. With this in mind and starting by whole genome sequencing data for both *Taylorella* species (Hébert et al., 2011; Hébert et al., 2012), a *Taylorella* multilocus sequence typing (MLST) scheme was developed based on the nucleotide sequences of seven housekeeping genes (*glt*A, *gyr*B, *fh*, *shmt*, *tyr*B, *adk* and *txn*) (Duquesne et al., 2013). This scheme provides unambiguous results directly comparable between laboratories (Enright et al., 2000). The accumulation of nucleotide changes in housekeeping genes is a relatively slow process and their allelic profile is sufficiently stable over time to be used for global epidemiology purposes (Enright and Spratt, 1999).

Distinct sequence types (ST) reported by recent publications using the *Taylorella* MLST scheme show that new *T. equigenitalis* strains are constantly emerging (Hwang and Cho, 2018; Melzer et al., 2018). Some of these strains showed no genetic links with other identified STs according to the default clonal complex definition when single locus variants (SLV) were considered. This is the case, for example, of the inland South Korean strain KITE-1 isolated in 2016 (Hwang and Cho, 2018). The aim of the present study was to assess the distribution of specific *T. equigenitalis* genotypes from a large population of *T. equigenitalis* strains mainly originating from several European countries using MLST. Based on the resulting MLST data, the genetic diversity and phylogeny of 367 *T. equigenitalis* strains were analysed and compared on the basis of different factors such as the geographical origin, year of isolation and equine breed.

**Materials and methods**

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

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

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

Bacterial growth and DNA extraction

The 119 T. equigenitalis strains typed in this study were isolated and identified at the institutions of origin using the gold standard culture method according to OIE or national culturing instructions applicable at the time of isolation. Strains were subcultured onto chocolate agar at (37±2) °C with 5-10% (v/v) CO₂ in air prior to genomic DNA extraction and purification using commercially available DNA extraction kits according to the manufacturer’s protocol.

MLST

The seven MLST loci (gltA, gyrB, fh, shmt, tyrB, adk and txn) were amplified and sequenced according to previously published experimental conditions (Duquesne et al., 2013). The Taylorella MLST database (https://pubmlst.org/taylorella/) was used to assign allele numbers and STs. Novel alleles and STs were allocated by the database curator. All the sequence data for the 119 strains typed in this study are available from the Taylorella MLST database.

Data analysis

The sequence data obtained from the online Taylorella MLST database and from the literature (Delerue et al., 2019; Hicks et al., 2018) were incorporated into the dataset. The sequences of every individual locus were trimmed to equivalent lengths, like in the Taylorella MLST database.
Sequences were aligned and a phylogenetic tree drawn up using MEGA software version 7.0 (Kumar et al., 2016). For each unique ST, sequences of the seven MLST loci were concatenated, giving an in-frame sequence of 3,521 bp (not including nucleotide insertions/deletions). The phylogenetic tree was based on the concatenated sequences using the maximum likelihood method based on the Tamura-Nei model (Tamura and Nei, 1993). Support for internal nodes was estimated using the nonparametric bootstrap method with 1,000 replications. The tree was rooted with the ST28-concatenated sequences of the *Taylorella asinigenitalis* CIP 107673\(^T\) reference strain as the outgroup, extracted from the *Taylorella* MLST database.

Relatedness between STs was analysed based on allelic profiles using eBURST software version 3 (Feil et al., 2004; Spratt et al., 2004). The BURST algorithm, based on a set of hierarchical rules relating to the number of single-locus variants (SLV) and double-locus variants (DLV) involved, identifies mutually exclusive groups named clonal complexes (CCs) of related STs in the population, and attempts to identify the primary and subgroup founding ST for each CC. A CC was defined as major when a primary founding ST was predicted. All others were considered minor.

Global optimal goeBURST (Francisco et al., 2009) diagrams were drawn up using PHYLOViZ software version 2 (Francisco et al., 2012) to analyse relatedness between STs and geographical origins or equine breeds.

**Results**

The MLST analysis of the 367 *T. equigenitalis* strains included in this study divided the strains into 49 genotypes or STs, i.e. 0.13 STs/strain (Additional file 1). Thirty-one STs were associated with a single strain per ST, and the other 18 STs were associated with between
two and 67 strains per ST. ST4 (n = 67), ST33 (n = 64), ST16 (n = 49) and ST1 (n = 41) were the most prevalent STs and concerned 60% of strains. MLST data for the 119 *T. equigenitalis* strains typed in this study allowed us to identify three new alleles (*fh*-25, *fh*-26 and *shmt*-15) and 10 new STs (ST32, ST36, ST53, ST54, ST59 and ST61 to ST65) never described before (Additional file 1).

The eBURST analysis (Additional file 2) grouped the 49 STs into three major CCs (CC1, CC2 and CC8), three minor CCs (CC3, CC9 and CC10) and 11 singletons at the SLV threshold. CC1 was the largest CC, including 17 STs with the predicted primary founder ST1 and both predicted subgroup founders ST2 and ST33. CC2 and CC8 each included seven STs with the predicted primary founders ST17 and ST46 respectively. Minor complexes CC3, CC9 and CC10 included between two and three STs and no primary founders were predicted. The discovery of 10 new STs led to the emergence of the new CC10, and the identification of CC8 and CC2 as major CCs (last one following its merger with the former CC4 via ST66). At the DLV threshold, all the CCs and eight singletons previously described using the SLV threshold were linked; only ST9, ST31 and ST50 remained genetically independent (Additional file 2).

A phylogenetic tree (Fig. 1) drawn up using concatenated sequences of seven MLST loci did not reveal any major clade within the 49 unique STs. However, the distribution of STs within the six CCs reflects a correlation between the genetic distances and evolutionary relationships among the closely-related STs. The 11 singletons were distributed throughout the tree except on the upper part composed by CC1, and several of them were clustered along with CC2 and CC8. The 10 new STs were distributed throughout the tree, but 50% clustered in CC8.

Three hundred and twenty-nine *T. equigenitalis* strains out of the 367 included in this study were isolated over a 42-year period, but the strain distribution over time was heterogeneous.
(Additional file 1): 32 strains were isolated from 1977 to 1997 versus 310 strains between 1998 and 2018. However, the ST diversity was greater during the 1977-1997 period (0.34 STs/strain) than during the 1998-2018 period (0.12 STs/strain). Twelve STs, grouped into the current CC1, CC2, CC8 and CC10 complexes plus three singletons, had already been identified before the 1998-2018 period; they included the four most currently prevalent, i.e. ST1, ST4, ST16 and ST33.

The 64 non-European and 300 European *T. equigenitalis* strains out of the 367 strains included in this study were grouped into 12 and 40 STs respectively (Table 1, Additional file 1). The goeBURST analysis (Fig. 2) allowed us to connect non-European and European countries through ST1, ST4, ST17 and ST30. ST19 and ST20 were only associated with non-European strains but were nonetheless related to CC1, which includes European strains. Singletons ST3, ST9, ST31, ST42, ST55 and ST58 were associated only with non-European strains whereas CC3, CC8, CC9, CC10 and singletons ST5 and ST50 were associated only with European strains. Interestingly, (Fig. 2a) Japan (ST3 and ST9) and South Korea (ST55) had no connections with any other countries, whereas Australia, the United States and Europe were connected through CC1, the United Arab Emirates and Europe through CC2 and ST30, and South Africa and Europe through ST4. Within Europe (Fig. 2b), the Czech Republic showed the least connection with other European countries (only with Germany though CC10) whereas Germany and France showed the most connections with other European countries. While the French strains were the most represented in this study (30.8%), unlike the German strains that only represented 5.4%, they are less genetically heterogeneous (0.21 STs/strain) than German strains (0.60 STs/strain). A focus on the most prevalent STs (Fig. 2) suggested that ST16 (CC2) may circulate within Europe, whereas ST1 (CC1) may circulate worldwide, and ST33 (France and Spain) and ST4 (Austria, France and unknown) appear to be more
specific to a small number of countries. With fewer strains, ST46 (CC8) showed the same country distribution as ST16 without any Polish strains, and ST17 may be a European ST recently linked to Emirati strains.

Despite unknown equine breeds representing 35.5% of the 367 strains included in this study and the large number of equine breeds represented (Table 2, Additional file 1), we investigated the genetic relationships between the STs and equine breeds or category of equine breeds (Fig. 3). Singletons were mostly specific to an equine breed, except ST4 which was associated with both South African strains from Thoroughbred and Warmblood horses, and South African, Austrian and French strains from Lipizzaner horses. The largest complex, CC1, was associated with up to eight different equine breeds. Details on the seven prevalent equine breeds (Fig. 3) showed that except the Trotter from ST16, Draught and Trotter horses were clustered within CC1 and CC3, both closely located on the phylogenetic tree (Fig. 1). Thoroughbred, Warmblood and Lipizzaner horses were more dispersed through the goeBURST representation, but it may be noted that STs related to Lipizzaners were close to each other on the phylogenetic tree (Fig. 1). The Kladruber (ST56) and Pure Spanish (CC1) horses, on the other hand, were not only specific to a ST or CC, but were also country-specific (from the Czech Republic and Spain respectively).

Discussion

*T. equigenitalis* is the causative agent of CEM, a contagious equine disease leading to substantial economic losses. It is essential to accurately identify the strain for both epidemiological surveillance and to improve worldwide CEM prevention and control strategies. CEM was first reported in the United Kingdom and Ireland among Thoroughbred horses in 1977 (Crowhurst, 1977; Timoney et al., 1977). The disease spread rapidly from
1977 to 1978 in Europe (including Belgium, France and Germany), Australia and the United States (1978) (Matsuda and Moore, 2003). CEM was then reported in Italy and Japan in 1980 (Matsuda and Moore, 2003) and its presence was finally described in at least 30 countries worldwide in various equine breeds (Schulman et al., 2013), the most recent cases in the literature being in Croatia in 2014 or 2015 (Šritof et al., 2017) and South Korea in 2015 (Jeoung et al., 2016). Besides being the cradle of CEM emergence, the endemic status among the non-Thoroughbred horses of Europe supports the current hypothesis that the episodic “source of contagion” is often mainland Europe. MLST has already been implemented to examine the *T. equigenitalis* population structure. It revealed that the first CEM outbreaks in the United Kingdom, Australia and United States from 1977 to 1978 were associated with the founding complex, CC1 (Duquesne et al., 2013). In parallel, distinct genotypes emerged over time and in different countries (Japan, the United Arab Emirates, South Korea) suggesting the existence of an unidentified natural worldwide reservoir (Duquesne et al., 2013; Hwang and Cho, 2018). The present study focused on a large population of 367 *T. equigenitalis* strains to determine, through their MLST genotypes, their diversity and spatio-temporal distribution according to the geographical origin, year of isolation and equine breed. A hundred and nineteen strains were characterised using MLST, and the MLST sequence data of 248 additional strains were accessed from the online *Taylorella* MLST database and the literature (Delerue et al., 2019; Hicks et al., 2018). Despite the large selection of strains in terms of geographical origin, year of isolation and equine breed, one area of concern is the amount of emphasis put on the number of strains within the same ST. In this study, a *T. equigenitalis* strain was usually isolated from a CEM-positive animal reported in a single year. However, several strains with the same ST may be represented multiple times, subjected to repeated sampling (perhaps in relation to a treatment protocol).
or due to animals CEM-positive over a period of several years (Additional file 1); nevertheless, considering the known data on strains, this situation concerned only a limited number of strains and has not been considered a significant bias.

The MLST analysis performed in this study suggests little genetic heterogeneity, with only 49 STs characterised from the 367 *T. equigenitalis* strains (0.13 STs/strain), despite marked heterogeneity in terms of geographical origin (16 countries, including six outside Europe), year of isolation (1977 to 2018) and equine breed (*n* = 18) in the analysed strain collection.

The genetic heterogeneity of *T. equigenitalis* was slightly higher outside Europe (0.19 STs/strain) than within Europe (0.13 STs/strain). More importantly, the genetic heterogeneity of *T. equigenitalis* was greater in the 1977-1997 period (0.34 STs/strain) than in the 1998-2018 period (0.12 STs/strain); it may be noted that the current major STs and CC had already been identified during the 1977-1997 period. The STs/strain value of 113 *T. equigenitalis* strains used to develop the *Taylorella* MLST was slightly higher (0.24 STs/strain) (Duquesne et al., 2013) than for the present study but probably corresponded to genotypic variability in the French strains, since 85% of the strains were from France. This is similar to our study, where the French data reported 0.21 STs/strains. A comparison of whole-genome sequencing reported by Hicks et al. (Hicks et al., 2018) showed diversity in genome size by up to 100,000 bp however, mainly due to repeat regions and four main variable regions. Moreover, the authors observed that *T. equigenitalis* was a more diverse species than the whole *Mycobacterium tuberculosis* complex by an SNP comparison (Hicks et al., 2018). The genomic diversity thus observed is not, however, noticeable here because of the MLST principle, being based on housekeeping genes.

Compared to previous MLST results, the present study confirms the circulation of the founding complex, CC1, in France over a long period of time (1992-2018) and confirms its
distribution in Spain (2015-2016) and Germany too, but not in the six other European
countries investigated, i.e. Austria, Belgium, the Czech Republic, the Netherlands, Poland
and Switzerland. In the 1970s-80s, and perhaps still today, CC1 was circulating worldwide yet
it does not seem to have spread throughout Europe according to the dataset analysed. This
observation should nevertheless be confirmed by a retrospective analysis of strains isolated
from the first reported outbreaks in these European countries, if they are available.

Apart from the epidemiological relationships described through CC1, few epidemiological
relationships between non-European and European countries were revealed in the present
study. Thus, singleton ST4 was first found in Austria (1992-2007) and France (1993-2001) and
then in South Africa (1996 to 2015), suggesting its spread from Europe to South Africa first
within the Lipizzaner population then spreading to other breeds (Warmblood and
Thoroughbred). Emirati strains were divided into four genotypes, three of which (ST17, ST30
and S42) had a DLV relationship and clustered on the phylogenetic tree, suggesting the
presence of a common ancestral Emirati clone. The presence of this ancestral Emirati clone
is, however, in contrast to the fact that ST17 (primary founder of CC2) was found in Europe
before Emirati strains were isolated in 2012 (ST42), 2016-2017 (ST17) and 2018 (ST30).

Within Europe, ST16 included in CC2 has emerged as the dominant genotype with a cross-
border circulation; it is composed of strains from Austria (2004), Belgium (2008-2013),
France (2004-2012), Germany (1989-2009), Poland and Switzerland (2005-2008). ST46, the
primary founder of CC8, seems to have the same profile as ST16 without any Polish strains,
but it was found to be mainly composed of German strains plus the strain isolated in 2018
from the vaginal discharge of a French mare infected by cryopreserved stallion semen
collected and processed in Germany in 2012 (Delerue et al., 2019). This Germanic origin
could be extended to CC8, which is mainly composed of German strains first isolated in
1981. Interestingly, the genetic heterogeneity of German strains was much higher (0.60 STs/strain) than that of the entire dataset despite representing only 5.4% of the strain collection studied.

**Conclusion**

This study provides new insights into the molecular epidemiology of *T. equigenitalis* in Europe and constitutes a baseline for monitoring the spread of CEM outbreaks. However, it is difficult to give an exhaustive picture of the global CEM situation as there are very few *T. equigenitalis* strains genotyped by MLST from non-European countries and in the 10-15 years preceding the first CEM outbreaks reported in 1977. The genotyping of *T. equigenitalis* strains by MLST should be continued over an extended timeline, and a retrospective study carried out on a higher number of strains isolated from 1977 to the early 2000s. In the future, MLST data could be obtained using whole-genome sequencing, or a core genome MLST scheme could even be developed to the maximum resolution of the genealogy within the *T. equigenitalis* species.

**Acknowledgements**

We are very grateful to Isabelle Brodard from the Institute of Veterinary Bacteriology, University of Bern, Switzerland and to David Craig from the Emirates Racing Authority. We would also like to thank Delphine Libby-Claybrough, professional translator/editor and native English speaker, for her editorial assistance.

**Financial support**
This work was mostly supported by the European Commission (via the budget of the EURL for equine diseases other than African horse sickness).

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

Conflicts of interest
The authors declare that they have no conflicts of interest.

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


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

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<th>Country of origin</th>
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<th>Strain extracted from the MLST database</th>
<th>Strain extracted from the literature *</th>
<th>Total number of strains</th>
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*Delerue et al., 2019; Hicks et al., 2018*
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<td>54</td>
<td>1</td>
<td>60</td>
</tr>
<tr>
<td>Oldenburg</td>
<td>-</td>
<td>-</td>
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<td>1</td>
</tr>
<tr>
<td>Pony</td>
<td>-</td>
<td>2</td>
<td>-</td>
<td>2</td>
</tr>
<tr>
<td>Pure Spanish horse</td>
<td>34</td>
<td>-</td>
<td>-</td>
<td>34</td>
</tr>
<tr>
<td>Quarter Horse</td>
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<td>-</td>
<td>1</td>
</tr>
<tr>
<td>Thoroughbred</td>
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<td>7</td>
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<td>15</td>
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<tr>
<td>Trait Breton</td>
<td>-</td>
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<td>-</td>
<td>2</td>
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<tr>
<td>Westfalen riding horse</td>
<td>-</td>
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<td>-</td>
<td>1</td>
</tr>
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<td>Zangersheide horse</td>
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<td>-</td>
<td>1</td>
</tr>
<tr>
<td>Unknown</td>
<td>43</td>
<td>78</td>
<td>2</td>
<td>123</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>119</strong></td>
<td><strong>242</strong></td>
<td><strong>6</strong></td>
<td><strong>368</strong></td>
</tr>
</tbody>
</table>

*Delereue et al., 2019; Hicks et al., 2018
Figure 1. Phylogenetic tree based on 3,521-bp concatenated sequences of seven MLST housekeeping genes. The tree was constructed from the MLST data of 367 *T. equigenitalis* strains (49 STs) using the maximum likelihood method, and rooted with the *T. asinigenitalis* CIP 107673^T^ reference strain as the outgroup. The bootstrap values were calculated from 1,000 replications. The total number of strains associated with each ST is indicated in brackets. The equine breeds are indicated next to the STs using geometrical shapes, and the geographical origins correspond to the colours of those geometrical shapes (see the legend). The grouping of STs into CCs defined by eBURST analysis at the SLV threshold is indicated by braces, while singletons are underlined. STs identified for the first time in this study are identified by an asterisk. The numbers in brackets in the legend correspond to the number of strains.

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

Figure 3. This population snapshot of *T. equigenitalis* displays genetic relationships between the STs and equine breeds. It is based on a goeBURST (PHYLOViZ) analysis of equine breed
distribution among 367 *T. equigenitalis* strains characterised by MLST. STs with an SLV relationship were linked together by solid lines to form six CCs, and STs with a DLV relationship were linked together by dashed lines (the length of lines are not representative of the distances between STs). Dot colour fractions refer to equine breeds associated with STs (see legend). The dot size is proportional to the prevalence of STs in the analysed collection. The numbers in brackets in the legend correspond to the number of strains.