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Molecular Epidemiology of *Streptococcus uberis* Clinical Mastitis in Dairy Herds: Strain Heterogeneity and Transmission

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Multilocus sequence typing was successfully completed on 494 isolates of *Streptococcus uberis* from clinical mastitis cases in a study of 52 commercial dairy herds over a 12-month period. In total, 195 sequence types (STs) were identified. *S. uberis* mastitis cases that occurred in different cows within the same herd and were attributed to a common ST were classified as potential transmission events (PTEs). Clinical cases attributed to 35 of the 195 STs identified in this study were classified PTE. PTEs were identified in 63% of the herds. PTE-associated cases, which include the first recorded occurrence of that ST in that herd (index case) and all persistent infections with that PTE ST, represented 40% of all the clinical mastitis cases and occurred in 63% of the herds. PTE-associated cases accounted for >50% of all *S. uberis* clinical mastitis cases in 33% of the herds. Nine STs (ST-5, -6, -20, -22, -24, -35, -233, -361, and -512), eight of which were grouped within a clonal complex (sharing at least four alleles), were statistically overrepresented (OVR STs). The findings indicate that 38% of all clinical mastitis cases and 63% of the PTEs attributed to *S. uberis* in dairy herds may be caused by the nine most prevalent strains. The findings suggest that a small subset of STs is disproportionally important in the epidemiology of *S. uberis* mastitis in the United Kingdom, with cow-to-cow transmission of *S. uberis* potentially occurring in the majority of herds in the United Kingdom, and may be the most important route of infection in many herds.

Mastitis is the most significant and ubiquitous infectious disease of dairy cattle throughout the world. *Streptococcus uberis* has been repeatedly identified as the most commonly isolated pathogen from clinical and subclinical samples in several countries, including Australia, the United Kingdom, New Zealand, and Belgium (1–4). Multilocus sequence typing (MLST) is a highly discriminatory and reproducible means of investigating the molecular epidemiology of *S. uberis* (5–10). This has been undertaken primarily on isolates from clinical and subclinical mastitis, which have provided insights into the population diversity (5, 9) and enabled identification of distinct geographic variation in strain prevalence (11). Control measures for managing the risk of new *S. uberis* cases have traditionally focused on reducing the environmental infection pressure by separating the cow from sources of infection, such as pastures and certain bedding materials (12). However, several studies have hypothesized a role for contagious transmission of *S. uberis* based on MLST or pulsed-field gel electrophoresis (PGFE) strain patterns (9, 10, 13, 14). Previous studies have also suggested contagious transmission of *S. uberis* based on modeling of the temporal patterns of infection (15, 16) and response to mastitis management practices (17). However, most of these studies have been limited to small numbers of isolates and few herds; no study has estimated the incidence of potentially contagious transmission in herds within the context of the overall incidence of *S. uberis* mastitis across many herds. Transmission of infection by different routes is likely to be driven by a combination of multiple factors, including the infectious pressure and the biology of the pathogen and its host. An accurate understanding of the likely transmission route is fundamental when designing risk-based mastitis control strategies.

The aim of this study was to evaluate the heterogeneity of *S. uberis* strain types between and within herds using MLST and assess the extent of possible within-herd contagious transmission of *S. uberis*.

MATERIALS AND METHODS

Farm selection. This study utilized isolates of *S. uberis* from milk samples collected during a previous study (2). Farm selection was through the database administered by National Milk Records (NMR), Chippenham, United Kingdom, to identify 250 herds with a record of >35 cases of clinical mastitis per 100 cows during the previous 12 months (2003 to 2004). A recruitment letter was sent to the farmers inviting them to participate in the study, and 68 responded positively. Monthly recordings of somatic cell counts (SCCs) from these herds were assessed; 26 herds with an annual arithmetic mean SCC of >200,000 cells/ml (high group) and 26 with an annual arithmetic mean SCC of <200,000 cells/ml (low group) were selected. A final selection was made from farms located in one of three regions of England and Wales: a region to the north of a line from the Severn estuary to the Wash and two regions to the south of this line divided to the east and west by a line joining Oxford and Portsmouth. Within each region, herds were paired according to their annual mean SCC (high and low). Average herd size over the study period ranged from 55 to 320 cows, with a mean of 164 and a median of 163 cows per herd. The median incidence of reported clinical mastitis during the original study period in the 52 recruited herds was 66 (mean, 75) cases per 100 cows per

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365 days, with a range of 16 to 146 cases. The mean percentage of clinical cases diagnosed as *S. uberis* was 28%, with a range in individual herds of 7% to 64%. All herds were housed during the winter. Year-round and seasonal-block calving herds were represented within the sample group. For the purposes of the current study, this farm selection and categorization provided a reasonable cross section of commercial dairy herds in England and Wales.

MLST strain typing of *S uberis*. Milk samples were requested from all clinical mastitis cases that occurred in each herd during the study period. Clinical mastitis diagnosis and sample collection were performed in each herd by farm employees according to a standardized operating procedure. Pathogens were identified and classified according to the standard bacterial classification technique, as previously described (18).

More specifically, *S. uberis* was identified based on colony morphology on blood and Edwards agar after 72 h incubation at 37°C and Gram staining, catalase, and Lancefield grouping. Where necessary, further biochemical identification was performed using the API 20 Strep system (bioMérieux, Basingtoke, United Kingdom).

All isolates were stored using a bead-based micropreservation system (Protect, Technical Service Consultants; Heywood, United Kingdom) and stored at −80°C. Each isolate was subbed during the identification process onto an individual blood agar plate, and two or three colonies (from a pure plate) were subsequently selected for preservation.

All clinical cases where *S. uberis* was identified as a pure or mixed infection underwent the following recovery protocol from ultralow temperature storage at −80°C. Samples of each isolate were cultured on brain heart infusion (BHI) agar medium and incubated for 20 to 36 h at 38°C, according to the abundance of colonies identified. Initial confirmation of classification was carried out by assessment of colony morphology. Any recovered colonies subjectively judged to display atypical morphology, such as mucoid colonies, small colony size, or irregular colony margins, were subjected to further confirmatory tests with both API biochemical strips (32 Strep; bioMérieux Canada, Inc., Saint-Laurent, Quebec, Canada) and matrix-assisted laser desorption ionization–time of flight mass spectrometry (Bruker Biotyper) using an ethanol extraction protocol described previously (19).

Isolates that generated only light or nil growth under the primary recovery protocol underwent a second recovery protocol in which 10-ml aliquots of BHI broth were inoculated with a sample of each isolate and incubated for 24 h at 38°C. Samples of enriched incubated broth culture were then used to inoculate BHI agar plates, which were then incubated for 24 h at 38°C and underwent the same confirmatory tests as other isolates.

Genomic DNA was extracted from all isolates classified as *S. uberis* using the phenol chloroform extraction and purification protocol previously described (9). Genomic DNA (gDNA) concentrations and purity were assessed using the Qubit fluorimeter and NanoDrop 8000 spectrophotometer following the extraction and purification protocol. MLST was performed by the University of Oxford Department of Zoology using the internationally recognized MLST scheme (9) and the highly conserved genes *arcC*, *ddl*, *gki*, *recP*, *tdk*, *tpi*, and *yqiL* (20). Initial PCR amplifications were carried out using the standard 96-well format with a positive and negative control and the following reagents per 96-well plate: H_2O, 2,975.00 μl; Qsolution,1,000.00 μl; 10× PCR buffer, 500.00 μl; forward primer, 100.00 μl (10 μm stock); reverse primer, 100.00 μl (10 μm stock); deoxynucleoside triphosphate (dNTP), 100.00 μl; and *Taq*, 25.00 μl. This protocol was successful for loci *arcC*, *ddl*, *yqil*, *recP*, and *gki*, generating sufficient quantities of DNA, but quantities were insufficient for the *tdk* and *tpi* loci. As these two genes have longer PCR products, PCR quantities were adjusted as follows: H_2O, 2,815.00 μl; Qsolution, 1,100.00 μl; 10× PCR buffer, 500.00 μl; forward primer, 120.00 μl; reverse primer, 120.00 μl; dNTP, 120.00 μl; and *Taq*, 25.00 μl. The adjusted quantities improved the results for the *tpi* but not the *tdk* locus. Therefore, *tdk* PCR quantities were used at a reduced annealing temperature, from 72°C to 54°C. This improved the yield sufficiently for Sanger sequencing.

TABLE 1 Frequency of missing loci from 278 isolates with partial *S. uberis* MLST profiles where between 1 and 6 loci were successfully sequenced

MLST locus	Unsequenced loci from confirmed *S. uberis* isolates by MALDI-TOF MS	
	No.	%
arcC	53	7
ddl	153	20
gki	63	8
recP	47	6
tpi	218	28
yqiL	68	9

The MLST scheme was selected because of the large number of isolates already sequenced and the readily accessible online format, which facilitates analysis of these data in the broadest international context. Analysis of sequence traces was carried out using Ridom SeqSphere for automatic and manual assignment of sequence traces to alleles. Sequence profiles were catalogued for inclusion in the PubMLST database (http://pubmlst.org/). Phylogenetic and cluster analysis was performed using eBURST version 3 (11), and statistical analysis of MLST data was conducted in a MiniTab 16 using Fisher's exact chi-square tests.

Isolates from all clinical cases were assigned to one of five clinically relevant classifications, according to the observed epidemiological patterns, as follows:

Index case (I): more than one clinical case of mastitis in different cows in the same herd during the study period. These pairs or multiple cases potentially occur through contagious (cow-to-cow) transmission rather than from an environmental reservoir. The first occurrence of one of these STs in a herd was classified as the index case of that ST in that herd.

Potential transmission event (PTE): Following determination of an index case in a herd, each subsequent clinical case caused by that specific ST in another cow in that herd was classified as a PTE.

Persistent (P), using the established definition described previously (21, 22): When the same ST was identified on two or more occasions from the same mammary gland quarter, all but the first case caused by that ST were classified as persistent infections.

Unclassified (U): Clinical cases where the identified ST was isolated once in a particular herd and was also identified (as P, I, or PTE cases) in another herd.

Solitary (S): STs occurred in only one clinical case throughout the study period.

RESULTS

A total of 854 isolates (78%) were successfully recovered from the 1,099 stored *S. uberis* samples. Of these, 494 isolates were successfully sequenced, generating complete MLST profiles at all seven loci and representing 58% of the recovered samples. A further 278 isolates (25%) generated partial profiles, with 1 to 6 sequenced loci. No loci were able to be sequenced in the remaining 82 isolates. The frequency of locus absence is presented in Table 1.

Complete MLST sequence profiles were obtained from bacterial isolates cultured from clinical mastitis samples collected from 419 cows. Of these, 365 cows (87%) experienced a single case of *S. uberis* mastitis, 46 cows (11%) experienced two cases, and 8 cows (2%) experienced three or four cases.

MLST diversity and distribution. A total of 195 unique STs were identified from clinical cases of mastitis. The mean number of STs identified per farm was 3.75 and the median was 6 STs, with the range extending from 0 to 20 STs per herd (Fig. 1). The ma-

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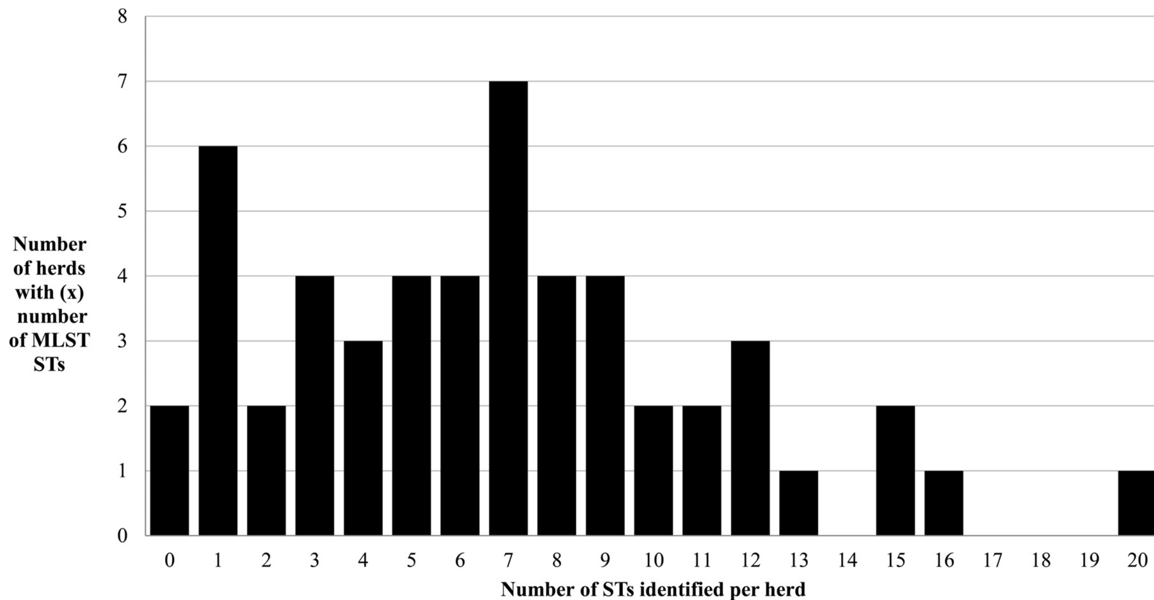

FIG 1 Number of unique MLSTs of clinical *S. uberis* isolates identified in different dairy herds.

jority of STs (148 of 195) were identified in a single herd each. The majority of these single-farm STs (127 of 148) caused one identified clinical case in the herd in which they were found. Of the remaining STs, 26 were identified in 2 herds, 16 were identified in 3 to 9 herds, and 5 were identified in 11 to 20 herds of the 52 total herds.

Cluster analysis was performed using PubMLST Web tools to establish the clonal complex structure and allow comparison with the wider PubMLST *S. uberis* database. Sequence types from three clonal complexes were identified. Clonal complex five (CC5) formed the largest group, accounting for 42.5% of all STs and 63% of clinical cases. Clonal complexes 86 and 143 accounted for 2.1% and 3.1% of STs and 1.4% and 1.2% of clinical cases, respectively, while the remaining 52.3% of STs were singletons.

Isolates from all clinical cases were assigned to one of five clinically relevant classifications according to the observed epidemiological patterns: index (I), potential transmission event (PTE), persistent (P), solitary (S), and unclassified (U) (Table 2). The solitary STs accounted for 65% of the subspecies diversity within the data set and were identified in 90% of the study herds. However, solitary STs accounted for only 25.7% of the clinical mastitis cases. The majority, 279 (56.48%) of 494 of the sequenced isolates in this data set, were attributed to one of 35 STs, all of which caused PTE cases in at least one herd (Table 3). Within this group of 279 isolates, 198 isolates (71%) occurred in pairs or multiples within a single herd or in multiple herds. Within each herd, the earliest clinical case of a given PTE-causing ST was assigned as the index case, with all subsequent isolates of that ST classified as PTE cases. If multiple isolates of the same ST were identified from the same gland, the second and subsequent cases were classified as persistent cases emanating from the earliest infection of that gland. The remaining 81 isolates attributed to these 35 STs were identified as single cases in additional herds. These 81 cases formed part of the unclassified group, along with all isolates from STs that were identified in more than one herd but only once per herd. PTE clinical cases were identified in 33 of 52 herds (63.4%).

In 17 herds (33%), index/PTE/persistent clinical cases accounted for >50% of the clinical cases illustrated in Fig. 2 (listed in descending order of *S. uberis* clinical case frequency; one outlier farm [farm 27] with very high mastitis prevalence, shown at far left in Fig. 2, is discussed in more detail below). The mean number of PTE mastitis cases following an index case was 1.6 (range, 1 to 15 clinical cases). There were no significant correlations between the proportion of isolates classified as index, PTE, or persistent and herd size, total herd mastitis prevalence, herd SCC (high/low category pairs), housing versus grazing, bedding, reported milking parlor routine, or within-herd prevalence of *S. uberis* mastitis.

Out of all 195 STs, 9 were significantly overrepresented (OVR), causing ≥10 clinical cases across all herds ($P = 0.045$, Fisher's exact chi-square test). These 9 OVR STs caused 38.25% of all clinical mastitis cases. Eight of the 9 OVR STs were found in mul-

TABLE 2 Number of *S. uberis* clinical mastitis cases grouped by case classification for all MLST sequence types across all herds

Case classification	No. (%) of clinical cases
Index case (I)	68 (14)
Potential transmission event (PTE)	108 (22)
Persistent case (P)	27 (5)
Solitary (S)	127 (26)
Unclassified (U)	164 (33)

[a] Index case (I): more than one clinical case of mastitis in different cows in the same herd during the study period. These pairs or multiple cases potentially occur through contagious (cow-to-cow) transmission rather than from an environmental reservoir. The first occurrence of one of these STs in a herd was classified as the index case of that ST in that herd. Potential transmission event (PTE): following an index case in a herd each subsequent clinical cases caused by that specific ST in another cow in that herd was classified as a potential transmission event (PTE). Persistent (P): when the same ST was identified on two or more occasions from the same mammary gland quarter, all but the first case caused by that ST were classified as persistent infections. Unclassified (U): clinical cases where the identified ST was isolated once in a particular herd but was also identified (as persistent, index, or PTE) in another herd. Solitary (S): STs occurred in only one clinical case throughout the study period.

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TABLE 3 All 35 *S. uberis* MLST sequence types from clinical mastitis isolates associated with potential transmission events (PTEs)

MLST sequence type[a]	No. (%) of all mastitis cases in all herds	No. of index case = no. of herds	No. of PTE cases	No. of persistent cases	No. of unclassified cases	MLST profile[b]	No. of nonidentical loci from founder (ST-6)
6*	49 (10.1)	9	23	5	12	1121213	0
22*	30 (6.2)	8	11		11	2121212	2
5*	23 (4.7)	6	7	2	8	1121212	1
35*	20 (4.1)	5	6	2	7	4121212	2
20*	17 (3.5)	3	8	2	4	1232116	5
233*	15 (3.1)	3	5	3	4	1122213	1
24*	14 (2.9)	1	2	1	10	2122212	3
361*	11 (2.3)	2	3	1	5	2121213	1
512*	10 (2.1)	3	3	1	3	4121213	1
67	9 (1.9)	3	3	2	1	4122212	3
343	9 (1.9)	1	2	2	4	5252333	6
10	6 (1.2)	1	2		3	1122212	2
595	6 (1.2)	1	1		4	10234373113	6
523	4 (0.8)	1	2		1	51522233	4
528	4 (0.8)	1	1	1	1	9353733	6
553	4 (0.8)	1	3			21211612	3
597	4 (0.8)	1	2	1		11222213	1
496	3 (0.6)	1	1		1	52424310	7
544	3 (0.6)	1	1		1	112172110	2
545	3 (0.6)	1	1		1	21217212	3
497	3 (0.6)	1	2			4322213	3
501	3 (0.6)	1	2			11424913	3
507	3 (0.6)	1	2			49134923	5
511	3 (0.6)	1	2			4121712	3
577	3 (0.6)	1	2			41462212	4
9	2 (0.4)	1	1			1122211	2
476	2 (0.4)	1	1			3464333	6
480	2 (0.4)	1	1			9134323	5
509	2 (0.4)	1	1			11251612	3
526	2 (0.4)	1	1			50122213	2
546	2 (0.4)	1	1			91611313	3
552	2 (0.4)	1	1			11125213	2
560	2 (0.4)	1	1			41232110	3
561	2 (0.4)	1	1			528541313	5
614	2 (0.4)	1	1			12222110	3

[a] Cases listed in descending order of their overall prevalence as distribution between classification groups. *, Statistically overrepresented (OVR) STs.
[b] MLST profiles are listed in the following locus order; *arcC*, *ddl*, *gki*, *recP*, *tdk*, *tpi*, *yqiL*.

tiple herds. Eight of the 9 STs were also identified in persistent infections (Table 3).

Twenty-seven persistent infections were identified (Table 2). The PTE STs were significantly more likely to be classified as persistent cases, accounting for 85% (23 of 27) of the persistent cases (Table 3). Within the 35 PTE STs, the OVR subgroup mastitis cases were significantly more likely to be classified as persistent, accounting for 63% (17 of 27) of the identified persistent infections in the study.

eBURST analysis of MLST groupings. A small number of STs of common lineage were identified that caused ~40% of the clinical mastitis cases attributable to *S. uberis* (Table 3). Phylogenetic analysis illustrates the relative similarity of eight of the nine OVR STs (Fig. 3): ST-5, ST-6, ST-22, ST-24, ST-35, ST-233, ST-361, ST-512, and ST-20 (separate lineage). With the exception of ST-20, the OVR STs form part of the global clonal complex previously defined as GCC5 (9). The "true singleton" STs varied by >3 alleles both from the main group and from each other, leading to the majority remaining ungrouped. In this study, ST6 is defined as the founder, four of the OVR STs are single-locus variants, and two of the OVR STs are double-locus variants (Table 3). ST20 is the fifth most commonly isolated ST and the only OVR ST found to be in a separate lineage.

DISCUSSION

S. uberis has been classified as an opportunistic environmental pathogen since the early 1970s, following its identification in straw bedding and as a commensal organism on skin and in feces (23), as well as in infected bovine mammary glands. This is in contrast to the more prevalent, obligate pathogens of that era, principally *Streptococcus agalactiae*, which appeared to reside principally or exclusively in the mammary gland, which in turn were classified as contagious pathogens. This broad classification is likely to be an oversimplification of the epidemiology (13, 14, 17). Several studies investigating *S. uberis* mastitis identified temporal patterns of clinical mastitis that were suggestive of contagious transmission (15, 16), occurring as outbreaks in individual herds. There are several published studies using MLST on relatively small numbers of samples collected from a small number of herds that have demonstrated a wide variety of STs, indicating that infections were

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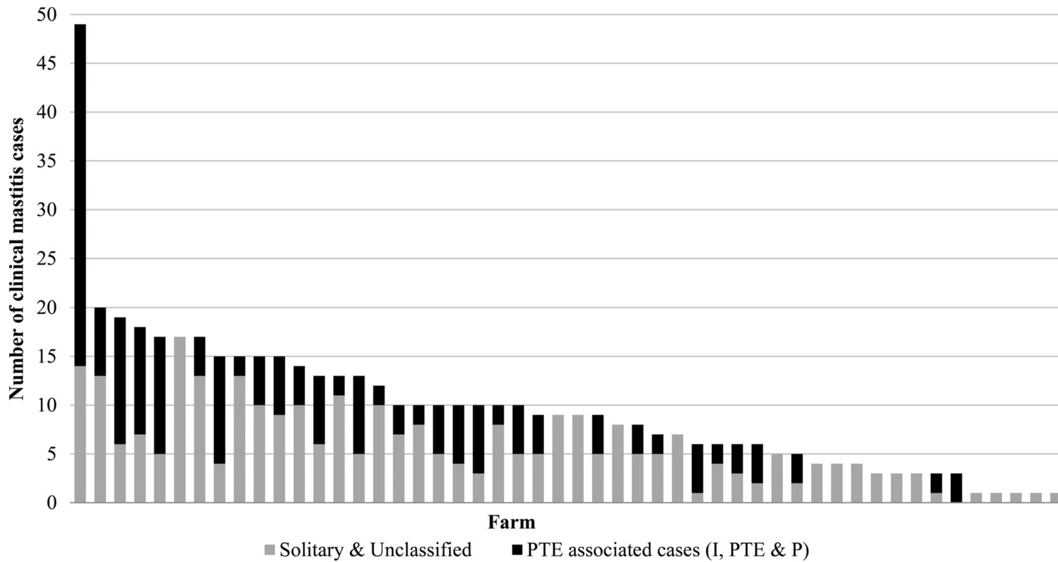

FIG 2 Mastitis clinical cases by classification groups for each farm where *S. uberis* mastitis cases were identified, with complete MLST profiles in descending number of total identified isolates. Farm 27, described in the Discussion, is on the far left.

probably acquired from diverse environmental populations (5, 9, 24). Studies have also shown that one pathogenic ST can predominate in a herd (5, 9, 13). The same STs (ST-5, -6, and -20) previously identified as predominant in different herds were identified as OVR STs in this study. The prevalence of STs in mastitis samples compared to environmental samples has been shown to be significantly different (10), indicating that a variable fitness to colonize between STs in the environment exists or some degree of

FIG 3 eBURST V3 population plot of STs identified in this study. The node size proportionate to the number of clinical mastitis isolates attributed to that ST.

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cow-to-cow transmission occurs. Given the ST diversity catalogued in the *S. uberis* MLST database (635 separate STs), the heterogeneity of environmental populations (25), and the limited number of clinical cases per herd during the study period, it seems implausible that multiple cows in the same herd would become infected with the same ST if there were no additional fitness or contagious phenotypic attributes specific to these highly prevalent strains. The potential for contagious transmission was described in several studies that demonstrated temporal patterns (15) and herd-level mastitis treatment and control protocols (16, 17) that support the hypothesis of contagious transmission.

Analysis of the relative prevalence of STs across all herds identified nine strains that were significantly more prevalent (OVR STs) than would be expected if opportunistic infection from the environment were the main transmission route. The nine OVR STs accounted for only 4.7% of the STs identified in the study. However, these STs were diagnosed in 38% of sequenced *S. uberis* clinical mastitis cases and 63% of persistent mastitis infections. While we recognize the possibility that the distribution of mastitis prevalence reflects an underlying distribution within the *S. uberis* population in the farm environment, this would seem unlikely, given that the mastitis and environment populations were previously shown to be significantly different (10).

Of the 52 herds studied, 63% had at least one pair of mastitis cases caused by the same ST in different cows. While not conclusive, this result suggests that either contagious transmission or enhanced infectivity among the environmental population may be more common and more important than previously thought. Pathogens that acquire virulence genes for intramammary infection may reside in the environment and cause new infections as environmental opportunists. However, it is logical to assume that environmental pathogens with enhanced infectivity will present an increased risk of contagion and pose the ability to maintain bacterial numbers at a high enough level for long enough to contaminate milking equipment with the requisite infectious dose. This may occur especially if their virulence mechanisms involve host immune evasion and the establishment of persistent infections, as suggested in previous studies (26). Persistent infections, by definition, are present during more milking periods than transient infections and therefore present an increased number of transmission opportunities for an infectious pathogen (22). In this study, the overwhelming dominance of PTE STs and particularly the OVR types in the persistent mastitis classification suggests that these STs would have more opportunities to be transmitted from cow to cow during milking.

Previous studies identified genetic sequences associated with important infectious processes, such as colonization (20, 27). Genomic analysis using isolates from clinical and subclinical mastitis cases show that complex multigene arrangement, rather than the simple presence or absence of virulence determinants, influences bacterial pathogenicity and the outcome of the infection (28).

The results of this study confirm the predominant clonal complex in the United Kingdom to be CC5, in contrast to those found in Australia and New Zealand, where CC86 and CC143 are more common (5, 10, 11). CC143 isolates were also identified in China (29), whereas only singleton STs were identified in India (7). While some of these studies involved only a limited number of isolates, their results do indicate geographic variation in the *S. uberis* population.

The more extensive management systems practiced in these countries and different pre- and postmilking hygiene protocols may predispose to different patterns of *S. uberis* mastitis in these countries, and it is plausible that contagious transmission may not be as likely under those conditions. This study provides the first estimate of the prevalence of these potentially contagious patterns between herds in the United Kingdom and the distribution of incidence rates of potentially contagious transmission events within herds. The finding that potential contagious transmission contributed >50% of the total clinical mastitis in 33% of the herds studied suggests that contagious transmission may be a major barrier to conventional control protocols designed to limit new *S. uberis* infections that focus on environmental factors alone.

Previous studies have discussed contagious transmission as a means of explaining dramatic clinical mastitis outbreaks in individual herds (15, 16). In this study, 1 of 52 herds (farm 27) displayed a temporal pattern of clinical cases that would have fallen within a previously proposed definition of an outbreak (15); in this single herd, 71% of the clinical cases were classified as PTEs (Fig. 2, farm 27), with high ratios of index to PTE cases for several OVR STs (e.g., 1:15 for ST-6 and 1 to 6 for ST-20). However, this study highlights the potential role of insidious, low-level cow-to-cow transmission at index-to-PTE ratios of 1:1 or 1:2 as a major component of the ongoing mastitis incidence in the majority (63%) of herds. This insidious manifestation of potentially contagious mastitis may arguably be more important and more challenging than the rare, dramatic mastitis outbreaks. It is only with the aid of molecular epidemiology that estimates of the relative importance of these two modes of transmission can be attempted.

The selection criteria used in the recruitment of herds provided a reasonable cross section of herds and management practices at the time of data collection. However, herds with a substantially higher incidence of *S. uberis* clinical mastitis or significantly different management practices may exhibit higher rates of PTEs than those identified in this study. This study has identified several specific STs, which warrant further investigation due to their higher prevalence and wider distribution. Further work is required to elucidate the underlying molecular mechanisms, host-pathogen interactions, and influence of management interventions in the colonization of mammary glands by *S. uberis*. This may allow us to understand how and why different patterns of potentially contagious or environmental mastitis appear to predominate in different herds. The variability observed between herds in this study may provide some explanation of the apparently intractable difficulties faced by the dairy industry in reducing *S. uberis* mastitis prevalence in spite of the adoption of control measures for environmental pathogens, as suggested previously (17). A more tailored risk-based approach using a combination of pathogen classification, analysis of mastitis patterns, and individual herd management practices may be more successful.

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