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Hyperinvasive Neonatal Group B Streptococcus Has Arisen from a Bovine Ancestor

Naiel Bisharat,[1] Derrick W. Crook,[1] James Leigh,[2] Rosalind M. Harding,[3] Phil N. Ward,[2] Tracey J. Coffey,[2] Martin C. Maiden,[3] Tim Peto,[4] and Nicola Jones[1]*

Nuffield Department of Clinical Laboratory Sciences,[1] The Peter Medawar Building for Pathogen Research,[3] and The Academic Department of Microbiology and Infectious Disease,[4] John Radcliffe Hospital, University of Oxford, Oxford, and Institute for Animal Health, Compton Laboratory, Compton, Newbury,[2] United Kingdom

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The genetic relatedness and evolutionary relationships between group B streptococcus (GBS) isolates from humans and those from bovines were investigated by phylogenetic analysis of multilocus sequence typing data. The collection of isolates consisted of 111 GBS isolates from cows with mastitis and a diverse global collection of GBS isolates from patients with invasive disease ($n = 83$) and carriers ($n = 69$). Cluster analysis showed that the majority of the bovine isolates (93%) grouped into one phylogenetic cluster. The human isolates showed greater diversity and clustered separately from the bovine population. However, the homogeneous human sequence type 17 (ST-17) complex, known to be significantly associated with invasive neonatal disease, was the only human lineage found to be clustered within the bovine population and was distinct from all the other human lineages. Split decomposition analysis revealed that the human isolate ST-17 complex, the major hyperinvasive neonatal clone, has recently arisen from a bovine lineage.

Group B streptococcus (GBS) is the paradigm of an emerging infectious disease. This bacterial pathogen, now commonplace, has a dynamic epidemiological history. *Streptococcus agalactiae*, the species designation of GBS (5), was initially described in 1887 as an animal pathogen causing bovine mastitis (28). Human infections caused by this bacterium were only reported 50 years later, in the 1930s (13, 16, 29). Neonatal disease, though, was rarely reported. However, during the 1960s numerous reports linked neonatal infections with this organism (11, 15, 18), and by the 1970s, GBS had become the leading neonatal pathogen in much of the developed world and has remained so ever since (3, 9, 17, 26). A high incidence of neonatal GBS disease has been the focus of much attention by clinicians, particularly in the United States, and measures to reduce its prevalence have been introduced (1, 2, 8). The reasons behind the rapid and sustained emergence of GBS neonatal disease have not been completely elucidated. A possible explanation has been acquisition by humans of bovine GBS, which is consistent with two previous reports showing that indistinguishable strains of GBS occur in both humans and bovines (6, 20). However, most studies have concluded that the human and the bovine GBS populations are distinct and unrelated (7, 10, 12).

Analysis of multilocus sequence typing (MLST) data collected from GBS isolate collections has provided insights into the population structure of this pathogen (22). A single homogeneous clone of capsular serotype III GBS (sequence type [ST] 17 [ST-17]) was found to be significantly associated with cases of invasive neonatal disease (22). In the 1980s, Musser and colleagues (27) had also concluded that a single virulent clone was responsible for many cases of neonatal disease. Their work, based on multilocus enzyme electrophoresis, showed that a proportion of invasive neonatal GBS strains were genetically related and possessed capsular serotype III.

The study reported here investigated the relationships of human and bovine GBS isolates using MLST data. A major objective was to determine whether human and bovine GBS strains are distinct populations or whether there is some overlap between the two populations, given the link between the two populations suggested by epidemiology.

MATERIALS AND METHODS

Bacterial strains. The bovine isolates ($n = 111$) were obtained from milk samples of cows with evidence of clinical mastitis. Twenty-five isolates were provided by the Institute of Animal Health, Compton, United Kingdom. Twelve of these had been collected by the Central Veterinary Agency at Weybridge, United Kingdom, during the mid-1950s. The remaining isolates ($n = 13$) were collected and supplied to the Institute of Animal Health by the Milk Marketing Board in 1991 and 1992. A further 86 isolates were purchased from the Veterinary Laboratories Agency, Bury St. Edmunds, United Kingdom. These had been collected between 1987 and 1996 from farms around the United Kingdom and represented a collection of diverse geographical origins. Each strain was a single isolate from an individual cow within a herd, and additional isolates were not collected from the same herd. For interest, we also included four disease-causing isolates collected from other animals (an elephant, dogs [$n = 2$], and a goat), which were supplied by the Veterinary Laboratories Agency.

The human strains comprised 83 invasive and 69 carriage isolates from the United Kingdom, the United States, Japan, New Zealand, Thailand, Singapore, and Israel. These isolates were characterized in a previous study (22) and represent a global collection. Additionally, three reference strains were included, strain ATCC 27541 (isolated from a bovine mammary gland); strain NCTC8541 (isolated from a human vaginal carrier; Public Health Laboratory, London, United Kingdom); and strain NEM316 (isolated from a patient with fatal neonatal sepsis), whose genome has been fully sequenced (14).

Identification and characterization of strains and MLST. Methods described previously for the isolation of strains, DNA extraction, and MLST were followed

* Corresponding author. Mailing address: Nuffield Department of Clinical Laboratory Sciences, Microbiology, Level 7, John Radcliffe Hospital, University of Oxford, Oxford OX3 9DU, United Kingdom. Phone: 44-1865-221226. Fax: 44-1865-764192. E-mail: nicola.jones@ndcls.ox.ac.uk.

(22). The resultant sequence data have been deposited in a database accessible on the Internet at http://sagalactiae.mlst.net. Capsular serotyping was carried out by the capillary precipitation method (24) with anti-type Ia, Ib, II, III, IV, V, VI, VII, and VIII sera (Statens Serum Institute, Copenhagen, Denmark).

Data analysis. DNA sequence-based methods of analysis were used to investigate the population structure of the collections. The nucleotide sequences of the seven alleles (459 to 519 bp each) making up the ST of each strain were pasted together to create a single larger (3,456-bp) length of DNA (concatenation) representing that strain. The relatedness of the strains was displayed by cluster analysis, using the matrix of pairwise differences in the concatenated sequences with the unweighted pair group method with arithmetic averages (UPGMA) algorithm, as implemented in MEGA (molecular evolutionary genetic analysis) software, version 2.1 (http://www.megasoftware.net) (23). The population structure of the strains was further investigated by split decomposition analysis, as implemented in SplitsTree software, version 3.2 (http://bibiserv.techfak.uni-bielefeld.de/splits) (19). Split decomposition analysis does not make the a priori assumption that sequences have a tree-like structure; and therefore, conflicting phylogenetic signals in the data, such as evidence of recombination, are presented as an interconnected network rather than as a tree.

RESULTS

Genotypes identified. Fifty STs were represented in the whole collection, of which 26 were identified only in isolates from humans, 17 were unique to the bovine isolates, and 3 were present in isolates from both humans and bovines. The remaining four STs were associated with isolates from other animals, two from dogs and one each from an elephant and a goat. The most common STs were ST-67, which accounted for 73 of the 111 (65.8%) bovine isolates, and ST-17, which was found in 44 of the 152 (29.0%) human isolates. A total of 31 STs were identified only once in this isolate collection. The characteristics of the more common genotypes identified in the data set are shown in Table 1. The remainder of the STs had a single representative only.

Capsular serotyping. Seventy-six percent of the bovine isolates were nontypeable (Table 1). Among the typeable isolates, serotype II was the most common ($n = 22$); the remaining four isolates were characterized as serotype III ($n = 3$) or serotype 1b ($n = 1$). In contrast, only 3.3% of the human isolates were nontypeable. The majority of human isolates belonged to serotype III (51%). Eight isolates (5.3%) from the human isolate collection belonged to serotype II.

Mobile genetic elements within the *glcK* gene. PCR amplification of the internal fragment of the glucose kinase (*glcK*) gene among eight isolates from the bovine collection produced a 3.0-kb band instead of the 0.5-kb band that was observed in the remaining isolates. This increase in band size was found to be due to a mobile genetic element which was inserted at the identical point in the *glcK* gene in each of the eight bovine isolates. This mobile genetic element (2,314 bp) contained one open reading frame (568 amino acids), and a search with the BLAST algorithm yielded an approximate 50% identity to the group II intron reverse transcriptase. For the purposes of the analysis with concatenated sequences, the nucleotide sequence of the mobile element was removed, leaving the intact *glcK* allele.

Relationship between bovine and human isolates. As expected, the human strains fit into the same seven lineages shown previously (22), as determined with the BURST program. The present analysis uses the UPGMA clustering algorithm of MEGA, based on the DNA sequence of the concatenated alleles, and shows that human isolate lineages 1 (ST-1 complex) (Fig. 1, cluster C) and lineages 3, 4, and 6 (Fig. 1, clusters E and D) are grouped together and separately from lineages 5 (ST-17 complex) and 7 (ST-23 complex). The bovine strains are largely clustered together (Fig. 1, cluster A). The human isolate ST-17 complex, previously identified as having increased virulence in neonates, grouped within the main bovine isolate cluster (Fig. 1, cluster A). Placement of human lineage 2 (Fig. 1, cluster B) as an outgroup of the bovine cluster rather than with the other human lineages is an apparent rearrangement compared with that in the previous analysis (22), which reflects the different method of analysis based on concatenated sequences. A minority of bovine strains ($n = 8$; 7%) represented in three STs (ST-23 [$n = 1$], ST-19 [$n = 1$], and ST-2 [$n = 6$]) are found within the main cluster of human strains. Isolates from dog and goat cluster with the human isolate STs. The elephant isolate and human ST-22 and ST-26 isolates do not appear to be closely related to any of the clusters.

The analyses so far show that the isolates of the human ST-17 complex group more closely with the bovine isolate STs than with other human isolate STs, but we lack strong support for this conclusion, given the low bootstrap values at these branches. To further assess the reliability of the structure within the tree, the data were examined by split decomposition analysis, which allows for the fact that real evolutionary data may not be best described by a branching tree format, given that recombinational events may have occurred within the population.

The split graph representation of the structure is shown in Fig. 2 and indicates that several STs are distantly related to most others. As the algorithm gives undue prominence to these distant STs, better discrimination between bovine and human isolate STs was obtained for the majority of the data set by repeating the analysis after removal of five distant human isolate STs (ST-22, ST-26, ST-23, ST-24, and ST-25) and the four STs of isolates from other species (dog [ST-81 and ST-84], goat [ST-86], and elephant [ST-82]). To allow comparison of Fig. 1 and 3, the branches have been labeled A to E.

Figure 3 shows that the bovine isolate STs are in a split separate from that of the human isolate STs. This bovine isolate split, labeled branch A, also contains the human isolate ST-17 complex (which is significantly associated with invasive neonatal disease). Other branches in Fig. 3 show the same groupings of STs apparent in Fig. 1. Note that the parallel branches for splits C and E in Fig. 3 reveal that the apparently clear phylogenetic relationships in the UPGMA dendrogram in Fig. 1 are more complicated. The human isolate ST-1 complex, grouped as split C, has affinities with those STs in splits D and E, probably reflecting ancient recombination. However, while the presence of parallel splits in Fig. 3 shows ambiguous phylogenetic relationships between clusters of mainly human isolate STs, the finding that the human isolate ST-17 complex groups within a branch of bovine strains is a clear and robust result.

DISCUSSION

We have shown that GBS populations from bovines and humans are largely discrete, which is consistent with previous work (7, 10, 12). However, the present study showed that the

TABLE 1. Characteristics of main GBS STs by country of origin, serotype, host, and disease state[a]

ST	Allelic profile[b]	No. of isolates of the ST	Country of origin (no. of isolates)	Serotype (no. of isolates)	Host (no. of isolates)	Disease state (no. of isolates)
67	13, 1, 1, 13, 1, 1, 5	73	UK (73)	II (14), Ib (1), NT (58)	B (73)	BM (73)
17	2, 1, 1, 2, 1, 1, 1	44	J (14), NZ (1), UK (13), USA (16)	III (44)	H (44)	NI (33), AC (9), AI (2)
1	1, 1, 2, 1, 1, 2, 2	21	J (10), NZ (3), UK (4), Is (3), T (1)	V (9), VIII (4), VI (4), III (2), Ib (1), NT (1)	H (21)	AC (16), AI (3), NI (2)
19	1, 1, 3, 2, 2, 2, 2	21	J (2), NZ (5), UK (9), USA (5)	III (17), II (2), V (1), NT (1)	H (20), B (1)	AC (14), NI (3), AI (2), NC (1), BM (1)
23	5, 4, 6, 3, 2, 1, 3	17	J (3), NZ (7), UK (4), Is (1), S (1), NK (1)	Ia (11), III (4), V (1), NT (1)	H (16), B (1)	AC (7), NI (5), AI (3), NC (1), BM (1)
61	13, 1, 1, 13, 1, 1, 1	9	UK (9)	III (1), II (2), NT (6)	B (9)	BM (9)
2	1, 1, 3, 1, 1, 2, 2	8	NZ (1), UK (6), Is (1)	II (7), NT (6)	B (6), H (2)	BM (6), AI (2)
8	4, 1, 4, 1, 3, 3, 2	7	J (1), NZ (2), UK (3), S (1)	Ib (6), NI (1)	H (7)	AC (4), NI (1), AI (2)
10	9, 1, 4, 1, 3, 3, 2	5	J (1), NZ (2), UK (2)	Ib (3), II (1), NT (1)	H (5)	AC (2), NI (1), AI (2)
11	9, 3, 7, 1, 3, 3, 2	5	S (5)	III (5)	H (5)	AI (5)
76	13, 1, 1, 13, 1, 14, 1	5	UK (5)	II (1), NT (4)	B (5)	BM (5)
7	10, 1, 2, 1, 3, 2, 2	3	J (3)	Ia (3)	H (3)	AC (1), NI (2)
12	10, 1, 4, 1, 3, 3, 2	3	J (2), UK (1)	Ib (3)	H (3)	NI (2), AC (1)
26	1, 1, 5, 4, 1, 4, 6	3	J (2), UK (1)	V (3)	H (3)	AC (2), NI (1)
28	1, 1, 3, 5, 2, 2, 2	3	J (2), UK (1)	II (3)	H (3)	AC (2), NI (1)
72	13, 1, 1, 13, 1, 2, 5	3	UK (3)	NT (3)	B (3)	BM (3)
4	1, 1, 4, 1, 1, 3, 4	2	J (1), UK (1)	Ia (2)	H (2)	AC (1), NI (1)
15	9, 1, 4, 1, 5, 3, 2	2	NZ (2)	Ib (2)	H (2)	AI (1), NI (1)
22	13, 3, 1, 3, 1, 1, 1	2	Is (2)	II (2)	H (2)	AC (1), AI (1)
18	3, 1, 1, 2, 1, 1, 1	1	UK (1)	III (1)	H (1)	NI (1)
29	2, 1, 1, 8, 1, 1, 1	1	J (1)	III (1)	H (1)	NI (1)

[a] Abbreviations: NT, nontypeable; A, human adult; B, bovine; M, mastitis; H, human; N, human neonatel; I, invasive disease; C, carried strain; J, Japan; NZ, New Zealand; UK, United Kingdom; USA, United States; Is, Israel; T, Thailand; S, Singapore; NK, not known.

[b] The allelic profile for each gene is presented in the order *adhP*, *pheS*, *atr*, *glnA*, *sdhA*, *glcK*, and *tkt*, respectively.

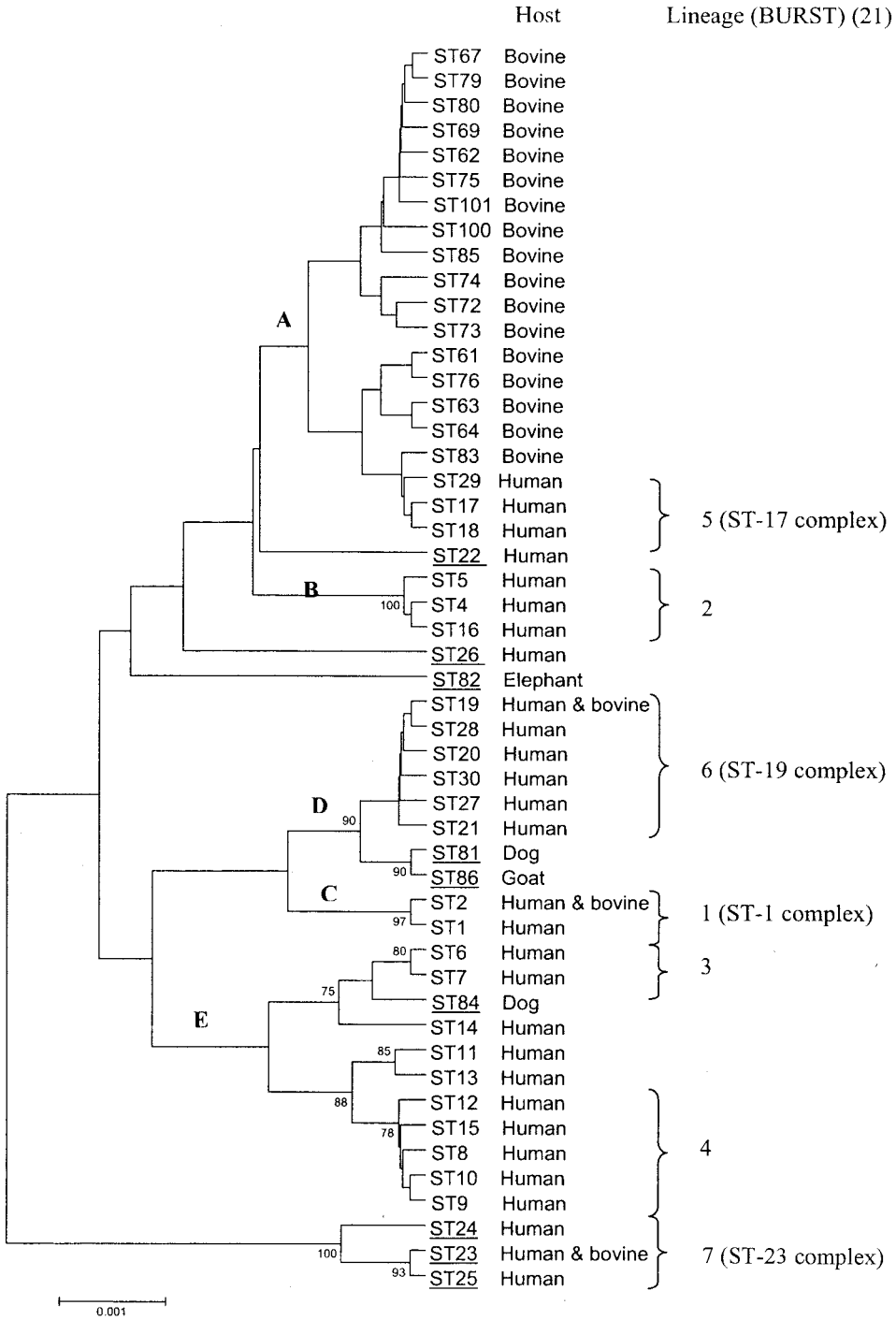

FIG. 1. UPGMA tree showing the genetic relatedness of bovine, human, and other GBS strains. Bootstrap values are the percentages of 500 computer-generated trees produced by randomly sampling the sequences and are shown at the nodes. Values of less than 70 are not shown. Underlined STs indicate those to be pruned for further analysis.

human isolate ST-17 complex is distinct from all other human isolate STs and is genetically more closely related to the main bovine isolate cluster of STs. The phylogenetic evidence indicates that the human isolate ST-17 complex, the major hyperinvasive neonatal clone (22) (which accounts for 30% of neonatal infections [N. Jones, unpublished data]), has arisen from a bovine lineage.

In order to achieve this analysis, we have studied large, carefully assembled collections of GBS isolates. The bovine strains were collected from cows with mastitis in the United Kingdom between 1950 and the present. The human strains belong to a well-characterized global collection, which has previously been described in detail (22).

Excluding the ST-23 complex, which is more distantly re-

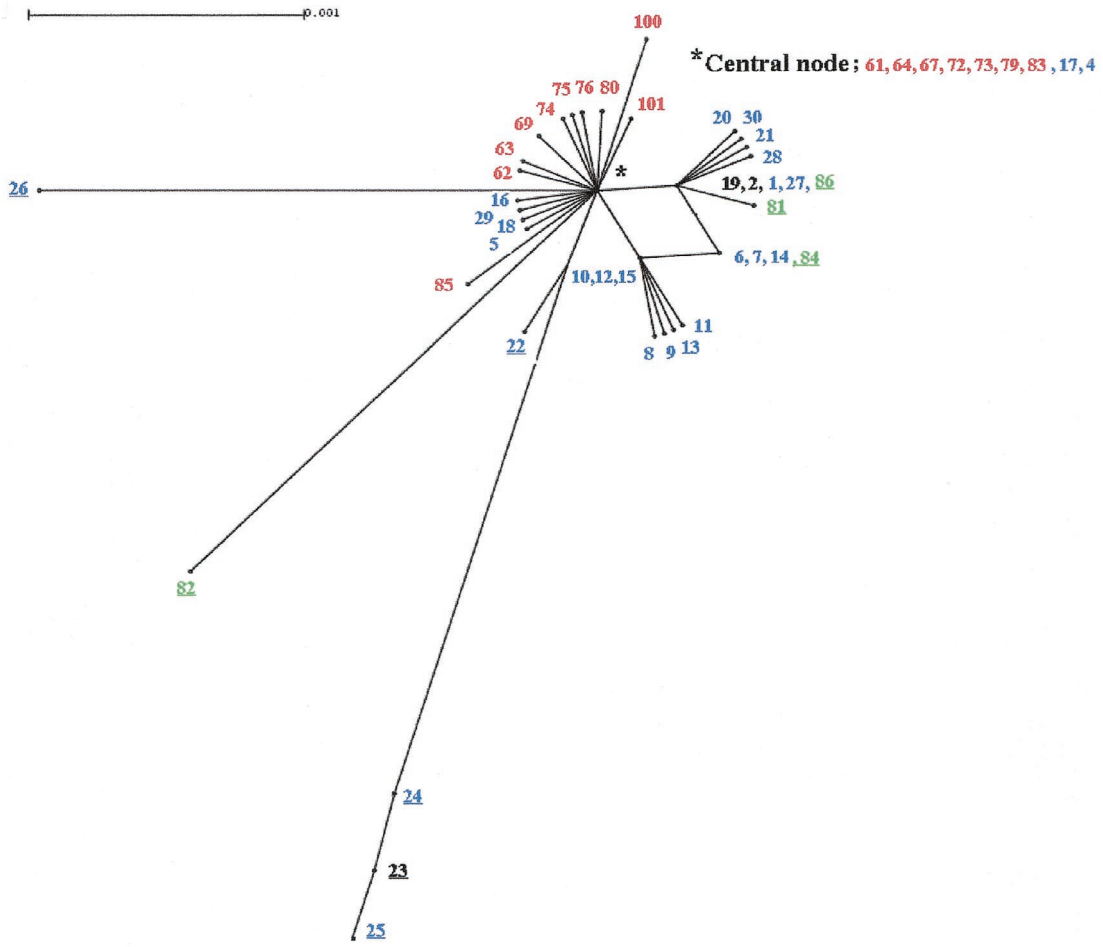

FIG. 2. Split graphs showing the relationship between bovine, human, and other GBS strains. The numbers at the nodes indicate the ST(s). Human STs are shown in blue, bovine STs are shown in red, STs found in humans and bovines are shown in black, and STs from other animals are shown in green. Underlined numbers indicate the STs to be pruned. Split graphs express conflicting phylogenies in the sequence data as net-like topologies.

lated, the remainder of the human GBS STs fall into one large group of related clusters. There is marked diversity in this group of clusters (labeled B to E in Fig. 3), demonstrated by the parallel branches of the split graphs, which reveal that the phylogenetic relationships are complicated and are indicative of recombination.

The most prevalent human isolate STs, other than ST-17, are ST-1, ST-19, and ST-23. Isolates of these STs have diverse capsular serotypes, exhibit more complicated phylogenetic relationships, and include both carried and invasive strains, suggestive of opportunistic pathogenicity. Occasional bovine strains are found within these STs. In contrast, the collection of strains from cows with mastitis are generally lacking in diversity. Almost two-thirds of bovine strains (73 [65.8%]) are ST-67. ST-67 can be found within a cluster of strains, labeled A (Fig. 3), which includes the human ST-17 complex and which has a tree-like structure indicative of clonal expansion. The variation in serotype within a single genotype and the presence of genetically diverse isolates with the same serotype (Table 1) suggest that capsular switching may have occurred through recombination. Similar observations have been made by Jones et al. (22) and Tettelin et al. (30).

Previous molecular studies have suggested that isolates of bovine origin have a high level of diversity (4, 25), which is inconsistent with the data presented here. This may reflect the fact that previous studies used typing approaches, randomly amplified polymorphic DNA analysis (25) and pulsed-field gel electrophoresis (4) fingerprinting, which may show more variability than MLST, which is based on housekeeping genes. In addition, the bovine isolates used in the present study, although all were independent and were from diverse geographical and temporal sources, perhaps reflect some geographical bias, as all were from the United Kingdom. Furthermore, the bovine GBS strains studied were collected from the 1950s onwards, when pasteurization and improved methods of hygiene on dairy farms were routine. For these reasons a more clonal population structure among bovine GBS isolates may be expected.

The greater genetic diversity of the human lineages of isolates indicates that these isolates may represent or be de-

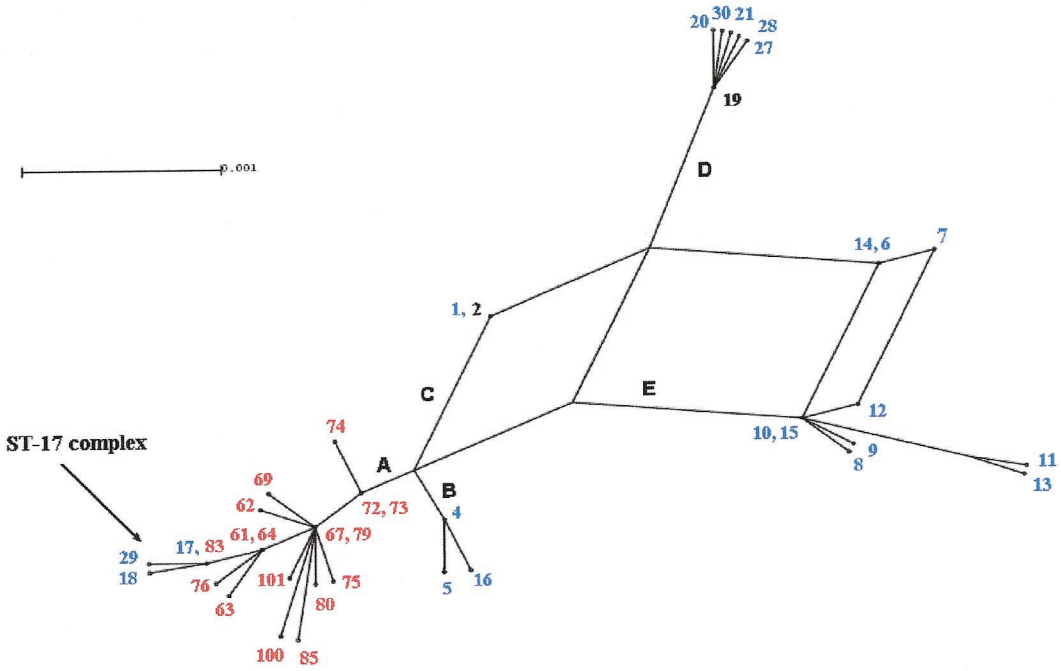

FIG. 3. Split graphs showing the relationship between bovine and human GBS strains after progressive pruning of the data. The designations A to E at the splits correspond to the letters shown in Fig. 1. The color indicators are is described in the legend to Fig. 2.

scended from a parent population of GBS isolates. The clonal expansion evident among the bovine isolate STs suggests more recent evolution from the parent population. The finding of the human neonatal hyperinvasive ST-17 complex within this cluster is a significant finding and is consistent with the concept that this lineage was acquired from the bovine subpopulation of GBS isolates relatively recently. Although no ST-17 isolates were found among the bovine isolates examined here, it is noteworthy that they have been found in a collection of North American bovine isolates (unpublished data).

In conclusion, the phylogenetic analysis indicates that the human ST-17 complex of isolates, the hyperinvasive neonatal clone, has arisen from a lineage of bovine isolates. The epidemiology of human neonatal GBS infection is that of recent and sustained emergence since the 1970s for reasons that have never been fully elucidated. It is intriguing to postulate that the increased rates of GBS infections among human neonates may in part be due to the relatively recent introduction of the GBS genetic lineage corresponding to the ST-17 complex into humans from cattle. The finding that the ST-17 complex accounts for a proportion of strains carried by adults (Table 1) suggests that it is now autonomously circulating within the human population. Further changes in animal husbandry are therefore unlikely to alter disease prevalence in neonates. Nevertheless, this represents yet another example of a pathogen that has jumped the species barrier. Further investigation of this model will require more extensive sampling of bovine and human isolates and their characterization by MLST and related techniques.

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