Characterization of *Streptococcus agalactiae* Isolates of Bovine and Human Origin by Randomly Amplified Polymorphic DNA Analysis

GABRIELA MARTINEZ, JOSEE HAREL, ROBERT HIGGINS, SONIA LACOUTURE,
DANIELLE DAIGNAULT, AND MARCELO GOTTSCHALK*

*Groupe de Recherche sur les Maladies Infectieuses du Porc, Faculté de médecine vétérinaire,
Université de Montréal, St-Hyacinthe, Québec J2S 7C6, Canada*

Received 21 May 1999/Returned for modification 19 July 1999/Accepted 9 September 1999

Streptococcus agalactiae is considered one of the major causes of bovine intramammary infections. It is also found in the vaginas of women without any apparent clinical symptoms, but reports of neonatal infections, causing significant morbidity, are relatively frequent. The aim of this study was to evaluate the genetic diversity of *S. agalactiae* strains isolated from bovine milk and from asymptomatic women in Québec, Canada, by randomly amplified polymorphic DNA (RAPD) analysis. A total of 185 bovine isolates and 38 human isolates were first serotyped for capsular polysaccharide by double diffusion in agarose gel (bovine isolates) and coagglutination (human isolates). Strains were then studied by RAPD using 3 primers, designated OPS11, OPB17, and OPB18, which were selected from 12 primers. Thirty-eight percent of bovine isolates and 82% of human isolates could be serotyped. Prevalent serotypes were type III (28%) for bovine isolates and types V (26%) and III (24%) for human isolates. RAPD results showed that, taken together, all isolates (of bovine and human origin) shared 58% similarity. Ninety-four percent of these isolates were clustered in four groups (I, II, III, and IV) with 70% similarity among them. Three clusters, A (48 isolates), B (14 isolates), and C (32 isolates), with 79 to 80% similarity were identified within group IV, whereas the three other groups did not present any clusters. Despite some clustering of human isolates, relatively high diversity was seen among them. Relatively high heterogeneity was observed with the RAPD profiles, not only for field strains belonging to different serotypes but also for those within a given serotype.

Mastitis remains one of the most economically important problems of the dairy cattle industry throughout the world. Milk quality and the prevalence of clinical and subclinical mastitis are major factors in determining farm profitability (16).

Streptococcus agalactiae (Lancefield group B) is a highly contagious obligate parasite of the mammary gland, where it can survive for long periods of time (19). Since this organism is susceptible to treatment with a variety of antimicrobial agents, eradication within a closed herd is possible. With increasing pressures for the reduction of antimicrobial agents in animals as well as in humans, the necessity for improved understanding of the epidemiology of this etiological agent has become apparent (1). Prevalence studies for *S. agalactiae* in cattle have been conducted in different areas of North America (19, 20, 31). Data about the epidemiology and molecular characteristics of this organism from bovine milk are not available in Canada. In the United States, only a few studies, with a limited number of strains, have been carried out on these subjects (7, 29).

S. agalactiae also causes significant morbidity and mortality in humans, both infants and adults, worldwide (3). In neonates, *S. agalactiae* is mostly acquired from the mother's vagina in early-onset disease, although nosocomial, community, and breast milk transmissions have been reported (2). In adults, *S.*

agalactiae occurs preferentially in certain individuals, such as diabetics, pregnant and postpartum women, and immunocompromised patients, emphasizing the opportunistic nature of the infection (23). Furthermore, humans act as a significant reservoir of *S. agalactiae*, since this bacterium may be carried in the vaginas of women without apparent clinical signs (14). Questions have been raised as to whether *S. agalactiae* is a zoonotic agent or whether host-specific ecovars exist. Controversial reports indicate the absence or the presence of a relationship between human and bovine *S. agalactiae* isolates (8, 18). Some epidemiological studies on *S. agalactiae* infections have been based on serotyping techniques, but these traditional procedures are limited in that their discriminatory potential is too low. DNA-based subtyping techniques, such as pulsed-field gel electrophoresis (PFGE) (10), ribotyping (5), restriction enzyme analysis (REA) (9), multilocus enzyme electrophoresis (28), and random amplification of polymorphic DNA (RAPD) (6) have been used efficaciously to subtype *S. agalactiae* isolates of human origin. Methodologies such as ribotyping and PFGE usually involve time-consuming steps and/or sophisticated equipment. REA has the advantages of simplicity and high discriminatory power but is sometimes difficult to interpret because of the large number of restriction fragments generated. RAPD is an accessible and sensitive method based on the use of arbitrary primers to amplify polymorphic segments of DNA. This technique has been widely used in recent years for detection of diversity among isolates (25, 34, 36).

The objective of this work was to study, by RAPD, the genetic diversity of a collection of *S. agalactiae* isolates originating from dairy cattle in different parts of Québec, Canada. Data were used to standardize the technique and evaluate the discriminatory power of the primers used. In addition, some

* Corresponding author. Mailing address: Groupe de Recherche sur les Maladies Infectieuses du Porc, Département de Pathologie et Microbiologie, Faculté de médecine vétérinaire, Université de Montréal, C.P. 5000, St-Hyacinthe, Québec J2S 7C6, Canada. Phone: (450) 773-8521, ext. 8374. Fax: (450) 778-8108. E-mail: gottschm@medvet.umontreal.ca.

FIG. 1. Illustration of the RAPD patterns generated with primers OPS11, OPB17, and OPB18. Lanes 1, reference strain SS615 (serotype I_a); lanes 2 nontypeable bovine isolate from region 3; lanes 3, serotype III bovine isolate from region 2; lanes 4, serotype III bovine isolate from region 7; lanes 5, serotype IV human isolate from region 4; lanes 6, serotype II bovine isolate from region 6; lanes 7, serotype V human isolate from region 4; lanes 8, serotype I_b bovine isolate from region 2; lanes M, 1-kb DNA ladder (DNA molecular size marker).

isolates from asymptomatic healthy women were also analyzed and compared to bovine isolates.

MATERIALS AND METHODS

Bacteria. Reference strains of *S. agalactiae* serotypes I_a (SS 615), I_b (SS 618), II (SS 619), III (SS620), IV (3139), and V (SS 1169 [NT1]) were used as positive controls in serotyping and RAPD experiments. All reference strains originated from the Centers for Disease Control and Prevention, Atlanta, Ga., except for *S. agalactiae* serotype IV (3139), which was kindly sent by J. Henrichsen, Statens Serum Institut, Copenhagen, Denmark.

A total of 297 bovine isolates were collected in cases of bovine mastitis or from a bulk tank of unrelated herds by the seven provincial laboratories of Québec, Canada, during 1996 and 1997. All agricultural regions of Québec were represented. In addition, 38 *S. agalactiae* isolates were collected from vaginal or rectal swabs of asymptomatic pregnant women. These isolates originated from two different geographical regions (representing 29 and 9 isolates, respectively). *S.*

agalactiae was isolated by using Trypticase soy agar supplemented with 5% sheep blood. All isolates were identified as *S. agalactiae* based on a positive Christie, Atkins, and Munch-Peterson (CAMP) reaction, lack of esculin hydrolysis, and a positive latex agglutination test for Lancefield group B (22). The latter test was conducted with a commercial kit (Patho D_x; Diagnostic Products Corporation) according to the manufacturer's recommendations.

Serotyping. Human isolates were serotyped on the basis of capsular polysaccharides by the coagglutination method (21). Since most bovine isolates were autoagglutinable, they were serotyped by double diffusion in agarose gels (17). Anti-type I_a, I_b, II, III, IV, and V sera were purchased from Oxoid (Basingstoke, England).

RAPD fingerprinting. All human isolates and 185 representative bovine isolates were analyzed by RAPD. This analysis was performed as described by Williams et al. (36) with some modifications. The PCR mixture consisted of buffer (10 mM Tris-HCl [pH 8.3], 50 mM KCl; 2.5 mM MgCl₂), 100 μM each of the four deoxynucleoside triphosphates (Pharmacia Biotech Inc., Baie d'Urfé, Québec, Canada), 0.4 μM primer, 50 ng of DNA extracted and purified as

TABLE 1. List of primers tested by RAPD for study of *S. agalactiae* field isolates

Primer ^a	Sequence, 5' → 3'	% CG	Bands (n)	Patterns (n)
A4	GCATCAATCT	40	3–5	3
AP42	AACGCGCAAC	60	2–5	3
OPS11*	AGTCGGGTGG	70	4–7	6
OPS16	AGGGGGTTCC	70	3	2
OPB04	GGACTGGAGT	60	1–4	4
OPB05	TGCGCCCTTC	70	≤2	2
OPB06	TGCTCTGCCC	70	1–5	5
OPB07	GGTGACGCAG	70	2–5	7
OPB08	GTCCACACGG	70	1	1
OPB10	CTGCTGGGAC	70	2–4	4
OPB17*	AGGGAACGAG	60	5–8	6
OPB18*	CCACAGCAGT	60	5–8	8

^a Asterisks indicate primers selected for the present study.

previously described (27), and 2.5 U of *Taq* DNA polymerase (Pharmacia) in a total volume of 25 µl. The primers used are shown in Table 1 and were synthesized by Gibco BRL Custom Primers (Burlington, Ontario, Canada). Each sample was subjected to the first cycle of denaturation (5 min at 94°C) in a DNA Thermal Cycler 480 (Perkin-Elmer Applied Biosystems, Foster City, Calif.). Each of the 35 subsequent cycles consisted of denaturing at 94°C for 30 s, annealing at 35°C for 30 s, and extension at 72°C for 1 min. The last cycle included an extension at 72°C for 5 min. Amplified products were separated by electrophoresis in a 1.4% agarose gel (Sigma) and visualized as white bands on a black background by UV transillumination following ethidium bromide staining. A 1-kb DNA ladder (Gibco) was used in each gel as molecular size standards. A negative control, consisting of the same reaction mixture but with water instead of template DNA, was included in each run. In addition, a positive control, containing the same reaction mixture with a template of DNA from a well-characterized reference strain (*S. agalactiae* SS 615), was also included. Each isolate was tested under the same conditions at least three times with the selected primers.

Pattern analysis. Photographs of each gel were digitalized with a video camera connected to a microcomputer (Alpha ease; Alpha Innotech Corp., San Leandro, Calif.). After conversion, the data were normalized and analyzed. Degrees of homology were determined by Dice comparisons, and clustering correlation coefficients were calculated by the unweighted pair group method with arithmetic averages. When the calculations were completed, a dendrogram showing the hierarchic representation of linkage level between isolates was drawn. All these processes were carried out with Molecular Analyst Software, Fingerprinting, version 1.12 (Bio-Rad Laboratories, Mississauga, Ontario, Canada).

Discriminatory analysis. The probability that two unrelated isolates sampled from the test population will be placed into different typing groups or clusters was assessed according to the Hunter-Gaston formula (15). This probability is calculated as

$$D = 1 - \frac{1}{N(N-1)} \sum_{j=1}^s n_j(n_j - 1)$$

where *N* is the total number of isolates in the sample population, *s* is the total number of Rapid's patterns described, and *n_j* is the number of isolates belonging to the *j*th type.

RESULTS

Identification of informative primers. To identify primers that generate informative arrays of PCR products, eight unrelated *S. agalactiae* isolates were selected from the entire panel of isolates. They had been isolated from different geographic sites and belonged to different serotypes or were nontypeable (Fig. 1).

Twelve oligonucleotides, each 10 nucleotides long, with a G+C content of 40 to 70%, and containing no palindromic sequences, were tested (Table 1). The choice of selected primers was based on the number of bands generated (with as few low-intensity bands as possible) as well as the quantity of different and reproducible patterns yielded. Three primers (OPS11, OPB17, and OPB18) were selected because they satisfied the characteristics described above (Fig. 1). A set of

reproducible bands produced for a particular primer was defined as a "pattern".

Serotyping. The double diffusion method was used to serotype bovine isolates because most of them were autoagglutinable. However, nonagglutinable bovine isolates were analyzed by both methods with identical results (data not shown). Results of serotyping for bovine and human isolates can be observed in Table 2. Sixty-two percent of bovine isolates were nontypeable. The remaining bovine isolates were found to belong to four different serotypes. Serotype III was the most prevalent, representing 28% of all bovine isolates. All six serotypes tested were identified among the 38 human isolates (Table 2). Only five human isolates were nontypeable and two autoagglutinated. Serotypes V and III were the most frequently identified serotypes, with prevalences of 26 and 24%, respectively.

Genetic diversity as defined by RAPD fingerprinting. The genetic relationship among all RAPD patterns of *S. agalactiae* based on the combination of data obtained with the three primers is represented in the dendrogram shown in Fig. 2. Overall, *S. agalactiae* isolates presented 58% similarity. A total of 94% of the isolates were clustered in four groups (I, II, III, and IV) with 70% similarity among them. Three clusters, A (48 isolates), B (14 isolates), and C (32 isolates), with 79 to 80% similarity, were identified within group IV. The other three groups did not present any clustering. The percent similarity of each group oscillated between 70 and 77%. The heterogeneity of the population was significantly increased by 14 nongrouped isolates.

Genetic variation in relation to serotype. The serotype distribution for each RAPD pattern is indicated in Fig. 2. In addition, the relationship between serotype and RAPD group and/or cluster is also observed in Tables 3 and 4 for isolates of bovine and human origin, respectively. Half of serotype III and II isolates of bovine origin were in group II. Nontypeable bovine isolates were proportionally distributed in all groups (Table 3). All serotype I_a isolates and most serotype III isolates recovered from humans were in cluster C. Most human isolates of serotype V were included in group I (Table 4). Clustering was not observed for other serotypes.

Genetic variation of isolates in relation to geographical distribution. In general, clustering was not observed for bovine isolates originating from the same region, except for those from regions 1 and 2 (Fig. 2). In spite of the existing diversity, it was possible to find at least one pair of isolates sharing the same RAPD pattern in most of the regions.

Genetic variation of isolates in relation to host origin. In general, clustering was observed in most *S. agalactiae* isolates

TABLE 2. Distribution of *S. agalactiae* isolates of bovine and human origins according to capsular serotype

Serotype	No. (%) of:	
	Bovine isolates ^a	Human isolates ^b
I _a	6 (2)	6 (16)
I _b	2 (1)	2 (5)
II	22 (7)	3 (8)
III	82 (28)	9 (24)
IV		1 (3)
V		10 (26)
NT ^c	183 (62)	7 ^d (18)

^a As tested by double diffusion in an agarose gel test.

^b As tested by the coagglutination test.

^c NT, nontypeable.

^d Two strains were autoagglutinable.

FIG. 2. Genetic relationship among 223 *S. agalactiae* isolates (of bovine and human origins) as estimated by clustering analysis of RAPD patterns obtained with three primers. The dendrogram was generated by the unweighted pair group method with arithmetic means. H, carrier woman; B, bovine milk; UT, untypeable; AA, autoagglutinable.

FIG. 2—Continued.

TABLE 3. Distribution of *S. agalactiae* isolates of bovine origin in different RAPD groups and/or clusters according to capsular serotype^a

Serotype	Total no. of isolates	No. of isolates in the following group and/or cluster:						No. of nongrouped isolates
		I	II	III	IV			
					A	B	C	
I _a	6		2		1		3	
I _b	2	1						1
II	22	1	11	6		1	1	2
III	82	1	40	4	28	2	4	3
NT ^b	73	4	16	15	17	11	5	5

^a Groups and clusters are derived from a dendrogram generated with *S. agalactiae* isolates of human and bovine origins (Fig. 2).

^b NT, nontypeable.

of human origin (Fig. 2). Fifty percent of human isolates were placed in cluster C of group IV, and 37% belonged to group I. The human isolates were then analyzed separately to verify this apparently high homology. Figure 3 shows relatively high variability among human isolates, since only 65% similarity was found. A principal group (group ii), in which approximately 79% of isolates clustered, and one minor group (group i) can be observed (Fig. 3). Two clusters, "a" (9 isolates) and "b" (21 isolates), with 80 to 82% similarity, were identified within group ii. The other group did not present any clear cluster. The percent similarity of each group oscillated between 76 and 77%. None of the human isolates shared identical RAPD patterns with the three primers. In addition, in the dendrogram illustrating the cluster analysis of *S. agalactiae* isolated from asymptomatic women, most isolates of serotype V appeared in cluster "a" (Fig. 3). This confirmed results obtained with the general dendrogram that included the analysis of bovine and human isolates (Fig. 2).

Identical RAPD patterns (for the combination of the three primers) between human and bovine isolates were observed only in one case (Fig. 2). The isolate of human origin belonged to serotype V, whereas the isolate of bovine origin was nontypeable; both of them were placed in group I of the dendrogram.

RAPD typing as an epidemiological tool. The discriminatory capacity of the RAPD typing was determined in order to eval-

TABLE 4. Distribution of *S. agalactiae* isolates of human origin in different RAPD groups and/or clusters according to capsular serotype^a

Serotype ^b	Total no. of isolates	No. of isolates in the following group and/or cluster:						No. of nongrouped isolates
		I	II	III	IV			
					A	B	C	
I _a	6							6
I _b	2	1						1
II	3	2					1	
III	9				1		8	
IV	1	1						
V	10	7					2	1
NT	5	2			1		1	1
AA	2	1					1	

^a Groups and clusters are derived from a dendrogram generated with *S. agalactiae* isolates of human and bovine origins (Fig. 2).

^b NT, nontypeable; AA, autoagglutinable.

FIG. 3. Genetic relationship among 38 *S. agalactiae* isolates from asymptomatic pregnant women as estimated by cluster analysis of RAPD patterns obtained with three primers. The dendrogram was generated by the unweighted pair group method with arithmetic means. UT, untypeable; AA, autoagglutinable.

uate the suitability of the primers chosen in this study for the epidemiological analysis of *S. agalactiae* isolated from milk. It was possible to define 215 RAPD types for the 223 isolates (index of discrimination [D] = 0.9996) by combining data obtained with the three primers, whereas only seven serotypes for the 223 isolates (D = 0.6908) were identified by serotyping.

DISCUSSION

Limited information was available on the epidemiology of Canadian *S. agalactiae* isolates recovered from bovine milk. To our knowledge, few studies using DNA-based techniques have been carried out with a large collection of field isolates of bovine origin in North America. Previous studies on *S. agalactiae* isolates of human origin have suggested that RAPD is superior to serotyping for epidemiological evaluations of this pathogen (6, 24). In the present work, RAPD was used to study a large collection of bovine isolates from Canada. In general, high genetic diversity was found. A possible explanation for this diversity is that different isolates originated from different herds. Similar results were obtained by using other molecular techniques in Australia, Denmark, and the United States, even

though these studies were based on lower numbers of herds and field strains (1, 18, 29).

S. agalactiae can be differentiated on the basis of distinct polysaccharide surface antigens. In this study, as in others (4, 11), most bovine isolates were nontypeable by polysaccharide antigens. Only 38% of isolates of bovine origin were typeable; serotype III was the most important. Previous studies showed a relative heterogeneity in the distribution of different serotypes of bovine isolates (4, 18, 26, 35). The importance of invasive serotype III strains is well known among human isolates (28), but the significance for bovine isolates is unknown yet.

In general, no evident correlation could be established between serotyping and RAPD patterns. Before this study, data which combine genomic diversity and antigenic typing were not available for bovine isolates. Results showed genetic heterogeneity not only among different serotypes but also among isolates belonging to same serotype. This suggests that the RAPD technique may be more accurate than capsular serotyping in differentiating *S. agalactiae* isolates from a bovine population. RAPD of *S. agalactiae* of bovine origin therefore appears to be of great value for epidemiological studies.

Clustering was not observed for bovine isolates originating from the same region, except for those from regions 1 and 2. This result is consistent with a previous report of Rivas et al. (29), who analyzed *S. agalactiae* of bovine origin by automated ribotyping. They could not find one ribotype in all three regions delineated in New York State. In the present study, at least two isolates with an identical RAPD pattern were found in each region. This fact might suggest that, in some instances, there may be a common source of *S. agalactiae* in different herds from the same region.

The serotype distribution of *S. agalactiae* of human origin appears to have changed over time. Until recently, the predominant serotypes that were detected among clinical isolates by the Centers for Disease Control and Prevention and other laboratories were I_a and III (3, 13, 14). A striking change, however, occurred in the 1990s, when the percentage of serotype V climbed from 2.6% in 1992 to 14% in 1993 and then to 20% in 1994 (12). The reasons for this increase are still unclear. Interestingly, serotypes V and III were identified in the present study as the most frequent serotypes among isolates from carrier women, with prevalences of 26 and 24%, respectively.

Reports on the genetic diversity of *S. agalactiae* isolated from healthy women are controversial. Huet et al. concluded that the genetic polymorphism of isolates from carrier women, as evaluated by ribotyping, is relatively limited (14). However, this technique appears to have low discriminatory power when it is used alone for epidemiological studies of *S. agalactiae* (14). On the other hand, Helmig et al. observed considerable heterogeneity in a population of *S. agalactiae* isolates from asymptomatic women (13). In agreement with other studies (7, 33), data presented here indicate that isolates from asymptomatic women have a slightly closer relationship than isolates of bovine origin. In spite of some clustering of human isolates, relatively high diversity was seen among them.

In this study, only one pair of human (serotype V) and bovine (nontypeable) isolates showing the same RAPD pattern was found. This suggests the possibility of a common origin for both isolates. This is in agreement with the results of Jensen and Aarestrup, who detected identical ribotypes for isolates from milk and dairy workers (18). Despite the fact that a common source of human and bovine isolates is possible (18), results obtained in this work do not allow confirmation of this hypothesis. Isolates belonging to different serotypes but indis-

tinguishable by genetic analysis have already been described (2, 18). One possible explanation is the ability of *S. agalactiae* to regulate capsule expression in a phase shift-like manner (32). The ability to phase shift may be of particular interest in *S. agalactiae* mastitis, since bacterial adherence is an important factor in the pathogenesis of bovine mastitis, and the adhesion of *S. agalactiae* to epithelial cells seems to be inversely proportional to the degree of encapsulation (30).

The selection of primers is critical in maximizing the discriminatory power of RAPD typing. An index of discrimination (*D*) greater than 0.90 is necessary for interpreting typing results with confidence (15). Two previous studies have reported genetic analysis of *S. agalactiae* isolates of human origin by RAPD (6, 24). In one of those studies, a partially degenerated oligonucleotide with a *D* of 0.98 was used (24), whereas in the other, a combination of four primers with a *D* of 0.90 was obtained (6). Our data suggest that the RAPD typing generated by the combination of OPS11, OPB17, and OPB18 primers (*D* = 0.9996) has increased the ability of the methodology to detect variability between isolates. Potential applications include identification of isolates that appear to have broad geographic distribution, suggesting interfarm transfer, and discrimination among recurrent versus new intramammary infections. Such information may allow the establishment of control and eradication programs at the herd level. Furthermore, RAPD typing may be used to study the relationship between human and bovine infection.

ACKNOWLEDGMENTS

We thank the different provincial laboratories of Québec for providing the isolates of bovine origin. We are also indebted to Philippe Jutras (Centre Hospitalier de Rimouski) and Monique Goyette (Hôpital Saint-Joseph, Trois-Rivières) for the group B *Streptococcus* isolates of human origin.

This work was supported by a grant from NSERC-RII (195831-96) and the Dairy Farmers of Canada.

REFERENCES

1. Baseggio, N., P. D. Mansell, J. W. Browning, and G. F. Browning. 1997. Strain differentiation of isolates of streptococci from bovine mastitis by pulsed-field gel electrophoresis. *Mol. Cell. Probes* 11:349-354.
2. Bingen, E., E. Denamur, N. Lambert-Zechovsky, Y. Aujard, N. Brahimi, P. Geslin, and J. Elion. 1992. Analysis of DNA restriction fragment length polymorphism extends the evidence for breast milk transmission in *Streptococcus agalactiae* late-onset neonatal infection. *J. Infect. Dis.* 165:569-573.
3. Blumberg, H. M., D. S. Stephens, C. Licitra, R. Pigott, R. Facklam, B. Swaminathan, and I. K. Wachsmuth. 1992. Molecular epidemiology of group B streptococcal infections: use of restriction endonuclease analysis of chromosomal DNA and DNA restriction fragment length polymorphisms of ribosomal RNA genes (ribotyping). *J. Infect. Dis.* 166:574-579.
4. Bopp, V., and C. Lammler. 1995. Comparative studies on group-B streptococci isolated from bovine milk samples in Thuringia and Hesse. *Zentbl. Veterinarmed.* B 42:427-433.
5. Chatellier, S., H. Huet, S. Kenzi, A. Rosenau, P. Geslin, and R. Quentin. 1996. Genetic diversity of rRNA operons of unrelated *Streptococcus agalactiae* strains isolated from cerebrospinal fluid of neonates suffering from meningitis. *J. Clin. Microbiol.* 34:2741-2747.
6. Chatellier, S., C. Ramanantsoa, P. Harriau, K. Rolland, A. Rosenau, and R. Quentin. 1997. Characterization of *Streptococcus agalactiae* strains by randomly amplified polymorphic DNA analysis. *J. Clin. Microbiol.* 35:2573-2579.
7. Denning, D. W., C. J. Baker, N. J. Troup, and L. S. Tompkins. 1989. Restriction endonuclease analysis of human and bovine group B streptococci for epidemiologic study. *J. Clin. Microbiol.* 27:1352-1356.
8. Devriese, L. A. 1991. Streptococcal ecovars associated with different animal species: epidemiological significance of serogroups and biotypes. *J. Appl. Bacteriol.* 71:478-483.
9. Ellis, S., M. Kotiw, and S. M. Garland. 1996. Restriction endonuclease analysis of group B streptococcal isolates from two distinct geographical regions. *J. Hosp. Infect.* 33:279-287.
10. Fasola, E., C. Livdahl, and P. Ferrieri. 1993. Molecular analysis of multiple isolates of the major serotypes of group B streptococci. *J. Clin. Microbiol.* 31:2616-2620.

11. Finch, L. A., and D. R. Martin. 1984. Human and bovine group B streptococci: two distinct populations. *J. Appl. Bacteriol.* **57**:273–278.
12. Harrison, L. H., D. M. Dwyer, and J. A. Johnson. 1995. Emergence of serotype V group B streptococcal infection among infants and adults. *J. Infect. Dis.* **171**:513.
13. Helmig, R., N. Uldbjerg, J. Boris, and M. Kilian. 1993. Clonal analysis of *Streptococcus agalactiae* isolated from infants with neonatal sepsis or meningitis and their mothers and from healthy pregnant women. *J. Infect. Dis.* **168**:904–909.
14. Huet, H., C. Martin, P. Geslin, F. Grimont, and R. Quentin. 1993. Ribotyping of *Streptococcus agalactiae* strains isolated from vaginas of asymptomatic women. *Res. Microbiol.* **144**:457–465.
15. Hunter, P. R., and M. A. Gaston. 1988. Numerical index of the discriminatory ability of typing systems: an application of Simpson's index of diversity. *J. Clin. Microbiol.* **26**:2465–2466.
16. Janzen, J. J. 1970. Economic losses resulting from mastitis. A review. *J. Dairy Sci.* **53**:1151–1161.
17. Jensen, N. E. 1979. Production and evaluation of antisera for serological type determination of group B streptococci by double diffusion in agarose gel. *Acta Pathol. Microbiol. Scand. Sect. B.* **87**:77–83.
18. Jensen, N. E., and F. M. Aarestrup. 1996. Epidemiological aspects of group B streptococci of bovine and human origin. *Epidemiol. Infect.* **117**:417–422.
19. Keefe, G. P. 1997. *Streptococcus agalactiae* mastitis: a review. *Can. Vet. J.* **38**:429–437.
20. Keefe, G. P., I. R. Dohoo, and E. Spangler. 1997. Herd prevalence and incidence of *Streptococcus agalactiae* in the dairy industry of Prince Edward Island. *J. Dairy Sci.* **80**:464–470.
21. Kirkegaard, M. K., and C. R. Field. 1977. Rapid slide coagglutination test for identifying and typing group B streptococci. *J. Clin. Microbiol.* **6**:266–270.
22. Lancefield, R. C. 1934. Serologic differentiation of specific types of bovine hemolytic streptococci (group B). *J. Exp. Med.* **61**:335–349.
23. Lerner, P., K. Gopalakrishna, E. Wolinsky, M. D. McHenry, J. S. Tan, and M. Rosenthal. 1977. Group B streptococcus (*S. agalactiae*) bacteremia in adults: analysis of 32 cases and review of the literature. *Medicine* **56**:457–473.
24. Limansky, A. S., E. G. Sutich, M. C. Guardati, I. E. Toresani, and A. M. Viale. 1998. Genomic diversity among *Streptococcus agalactiae* isolates detected by a degenerate oligonucleotide-primed amplification assay. *J. Infect. Dis.* **177**:1308–1313.
25. Maslow, J. N., M. E. Mulligan, and R. D. Arbeit. 1993. Molecular epidemiology: application of contemporary techniques to the typing of microorganisms. *Clin. Infect. Dis.* **17**:153–164.
26. Mosabi, J. M., S. M. Arimi, and E. K. Kang'ethe. 1997. Isolation and characterization of group B streptococci from human and bovine sources within and around Nairobi. *Epidemiol. Infect.* **118**:215–220.
27. Pitcher, D. G., N. A. Saunders, and R. J. Owen. 1989. Rapid extraction of bacterial genomic DNA with guanidium thiocyanate. *Lett. Appl. Microbiol.* **8**:151–156.
28. Quentin, R., H. Huet, F.-S. Wang, P. Geslin, A. Goudeau, and R. K. Selander. 1995. Characterization of *Streptococcus agalactiae* strains by multilocus enzyme genotype and serotype: identification of multiple virulent clone families that cause invasive neonatal disease. *J. Clin. Microbiol.* **33**:2576–2581.
29. Rivas, A. L., R. N. Gonzalez, M. Wiedmann, J. L. Bruce, E. M. Cole, G. J. Bennett, H. F. Schulte III, D. J. Wilson, H. O. Mohammed, and C. A. Batt. 1997. Diversity of *Streptococcus agalactiae* and *Staphylococcus aureus* ribotypes recovered from New York dairy herds. *Am. J. Vet. Res.* **58**:482–487.
30. Salasia, S. I. O., I. W. T. Wibawan, C. Lammler, and M. Sellin. 1994. Phase variation in streptococci of serological group B. *APMIS* **102**:925–930.
31. Sargeant, J. M., H. Morgan Scott, K. E. Leslie, M. J. Ireland, and A. Bashiri. 1998. Clinical mastitis in dairy cattle in Ontario: frequency of occurrence and bacteriological isolates. *Can. Vet. J.* **39**:33–38.
32. Sellin, M., M. Linderholm, M. Norgren, and S. Hakansson. 1992. Endocarditis caused by a group B streptococcus strain, type III, in a nonencapsulated phase. *J. Clin. Microbiol.* **30**:2471–2473.
33. Wanger, A. R., and G. M. Dunny. 1985. Development of a system for genetic and molecular analysis of *Streptococcus agalactiae*. *Res. Vet. Sci.* **38**:202–208.
34. Welsh, J., and M. McClelland. 1990. Fingerprinting genomes using PCR with arbitrary primers. *Nucleic Acids Res.* **18**:7213–7218.
35. Wibawan, I. W. T., and C. Lammler. 1990. Properties of group B streptococci with protein surface antigens X and R. *J. Clin. Microbiol.* **28**:2834–2836.
36. Williams, J. G. K., A. R. Kubelik, K. J. Livak, J. A. Rafalski, and S. V. Tingey. 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Res.* **18**:6531–6535.