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Epidemiological aspects of group B streptococci of bovine and human origin

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SUMMARY

Restriction fragment length polymorphism of the gene encoding rRNA (ribotyping) was used in combination with conventional epidemiological markers to study phenotypic variations among *Streptococcus agalactiae* of bovine origin and the possible epidemiological interrelationship between the bovine and human reservoirs of *Streptococcus agalactiae*.

The bovine material constituted 53 strains (9 antigen combinations) isolated from 11 herds. Herds with a uniform as well as heterogenic antigenic pattern were included. Furthermore, strains isolated in the course of time from the same persistently infected quarters were examined. The human material constituted 16 strains, 4 each of 4 serotypes, isolated from healthy carriers. Finally, nine serotype- and the group reference strains were examined. All strains were serotyped by double diffusion in agarose gel, biotyped (lactose ±), and ribotyped using two restriction enzymes, *Hin*d III and *Hha* I.

All isolates could be typed by ribotyping and seven ribotypes were identified among the reference strains. The restriction enzymes used alone or in combination gave typing results that allowed discrimination between and within serotype. Combined use of serotype, *Hin*d III and *Hha* I ribotypes produced 11 types among the 16 human strains. Ribotype analysis discriminated between herds infected with the same serotype. Strains of varying antigenic patterns from the same herd had the same ribotype. Phenotypic variations in serotype observed in persistent intramammary infection were not related to genetic changes as monitored by ribotype. Two ribotypes were represented among both bovine and human strains. The discriminating capability of lactose fermentation was of limited value.

INTRODUCTION

Streptococcus agalactiae, the only recognized group B streptococcus (GBS) species, is a cause of neonatal septicaemia and meningitis and is implicated in a variety of invasive diseases in humans [1, 2]. In many countries GBS occur as a significant agent causing bovine mastitis. Questions have been raised whether GBS is a zoonotic agent or whether host-specific ecovars exist [3, 4]. In Denmark eradication programmes have cleared this infectious agent from most herds, but new infections, apparently from non-bovine sources, are detected at a rate of 0·5–1 % of the herds per year [4]. The human is a significant reservoir [5] and the epidemiological interrelationship between GBS isolated from cattle and people in Denmark has been studied by traditional phenotypic subtyping methods such as serotyping and biotyping [4]. Approximately a quarter of Danish bovine GBS strains are deficient in polysaccharide antigens and 5 % are non-typable (NT). The discriminating power of serotyping is limited by the fact that 58 % of Danish bovine and 39 % of human strains belong to serotype III [9, 10]. As to the biochemical types, 95 % of Danish human strains do not ferment lactose whereas nearly 90 % of bovine strains are lactose fermenters. GBS of bovine

Table 1. *Serotype, biotype and ribotype distribution among 45 bovine strains of group-B streptococci isolated from five herds with uniform and three with varying antigenic patterns*

Herd no.	Number of strains	Serotype	Lactose fermentation	Ribotype	
				Hind III	*Hha* I
1	5	III	+	9	2
2	5	III	+	9	2
3	5	III	+	10	2
4	2	Ia/c	+	2	2
	4	Ia/c	−	2	2
5	6	NT/c	+	9	3
6	1	II/c	+	8	3
	2	NT/oX	+	8	3
	2	NT/c	+	8	3
7	4	III/X	+	10	4
	2	III	+	10	4
8	5	Ib/c*	+	8	3
	2	NT/c†	+	8	3

* Two quarter milk strains and three strains from the tonsils of milkers.
† Two bulk milk strains.

and human origin have been divided into different ecovars based on this property [3]. However, the interpretation of results of serological typing and biotyping of bovine GBS strains is difficult and may often lead to erroneous conclusions since the polysaccharide and the protein antigens are not always present in different isolates recovered during the course of intramammary infection [6–8]. Phenotypic variations also occur during the course of human infections [9, 10].

Evidently, the epidemiological unit of concern in bovine infections is the herd, not the single quarter (infected gland) or cow. Since neither the polysaccharide nor the protein antigens are always demonstrable on an infecting strain, it is probably preferable to classify bovine GBS strains as 'herd types'. By this approach phenotypic variation was identified and a similarity was demonstrated among GBS strains isolated from cattle and humans in Denmark [4].

DNA-based subtyping techniques have been used successfully to subtype strain collections of human origin [11–14], but to our knowledge this technique has had only limited use with GBS of bovine origin [15].

This study describes the use of newer DNA-based typing techniques on selected human and bovine GBS in an attempt to confirm the hypothesis of a direct epidemiologic relationship between the bovine and the human reservoir and to define the extent of phenotypic variations.

MATERIALS AND METHODS

Bacterial strains

In selecting the strains for the study emphasis was placed on testing further the following assumptions: (1) In general, dairy herds in Denmark are infected from one source only and (2) variations in serotype within a herd/cow/quarter are phenotypic and not due to the introduction of a new infecting strain. The study was based on a limited number of epidemiologically characterized bovine and human isolates and the type reference strains. All field strains were isolated originally during 1980, serologically typed and then kept lyophilized until used in this study.

The bovine material constituted 53 strains isolated from quarter milk samples (48), bulk milk samples (2) and tonsils of dairy workers (3 strains) from 11 different herds. Twenty-seven strains originated from five herds, each with a uniform GBS serotype pattern. Serotype III was present in 3 herds and Ia/c and NT/c in 1 herd each (Table 1). Eighteen strains were isolated from 3 herds in which a varying antigenic pattern was present among the GBS-strains. These included 2 strains isolated from bulk milk and 3 isolated from the tonsils of 2 dairy workers (Table 1). Finally, 12 strains displaying variation in serological type in the course

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Table 2. *Serotype and ribotype distribution among 12 bovine strains of group-B streptococci isolated on repeated examinations of five persistently infected quarters with varying antigenic pattern*

Quarter no.	Strain no.	Serotype	Ribotype	
			Hind III	*Hha* I
1	1	III	10	4
	2	III/X	10	4
2	1	III	10	4
	2	III/X	10	4
3	1	NT/c	8	3
	2	NT/	8	3
4	1	III/X	9	2
	2	III	9	2
	3	III/X	9	2
5	1	NT	8	3
	2	NT/cx	8	3
	3	Ib/c	8	3

Table 3. *Serotype and ribotype distribution among 16 human urogenital strains of group-B streptococci*

Serotype	*Hind* III ribotype	*Hha* I ribotype
II/R	1	6
	4	7
	4	7
	4	7
III/R	4	7
	5	8
	6	2
	2	2
Ia/c	2	2
	2	2
	2	2
	2	2
Ib/c	3	1
	2	2
	7	2
	8	3

of an intramammary infection were included (Table 2). The strains were isolated from quarter milk samples from 5 cows from 4 different herds. Reisolation took place at intervals of approximately 3 months. Four of these isolates are included in Table 1.

The human material constituted 16 epidemiologically unrelated GBS strains isolated from the urethra or cervix of 16 healthy carriers. Four strains each of serotype II/R, III/R, Ia/c and Ib/c were selected (Table 3).

Finally, the 9 type- and the group B-reference strain were included in the study (Ia(090), Ib(H36B), Ia/C(A909), II(18RS21), III(D136C), IV(3139), V(SS1169), R (R Compton), X (X Compton), and group B (090R)).

Serotyping

Serological typing was performed by double diffusion in agarose gel [5]. This method will reveal the following antigens: Ia, Ib, II, III, IV, V, c, R, X, and the group B-antigen. When these strains were retyped during 1994 antisera against the type IV and V polysaccharide antigens were included. None reacted to these. Serological types are given in Tables 1 and 2.

Ribotyping

Ribotyping was performed as described by Aarestrup and colleagues [16], with the modification that bacterial cells were lysed using 25 μl of lysozyme (10 mg/ml) instead of 15 μl of lysostaphin. All isolates were digested using *Hind* III and *Hha* I restriction enzymes (Amersham, Arlington Heights, IL, USA).

Serotyping

The capability to ferment lactose was assessed in test tubes using a basal medium of peptone and beef extract to which 0·5% lactose was added using phenol red as indicator. Reactions were recorded daily up to 5 days.

RESULTS

Digestion with *Hind* III and *Hha* I, and subsequent blotting and hybridization, resulted in 10–12 and 5–8 bands, respectively. Both restriction patterns were easy to interpret (Figs 1 and 2). *Hind* III and *Hha* I ribotyping yielded 11 and 10 types, respectively. When used in combination a total of 16 types were identified.

The 10 reference strains comprised 7 *Hind* III and 7 *Hha* I types. *Hind* III types 11 and *Hha* I types 5, 9 and 10 were not recorded among either the bovine or the human strains. Two *Hha* I types (2 and 3) and one *Hind* III type (9) could be subtyped further by *Hind* III and *Hha* I respectively giving a total of 9 ribotypes when the 2 enzymes were used in combination.

In the human material 8 *Hind* III and 6 *Hha* I types were recorded (Table 3). The combined use of the 2

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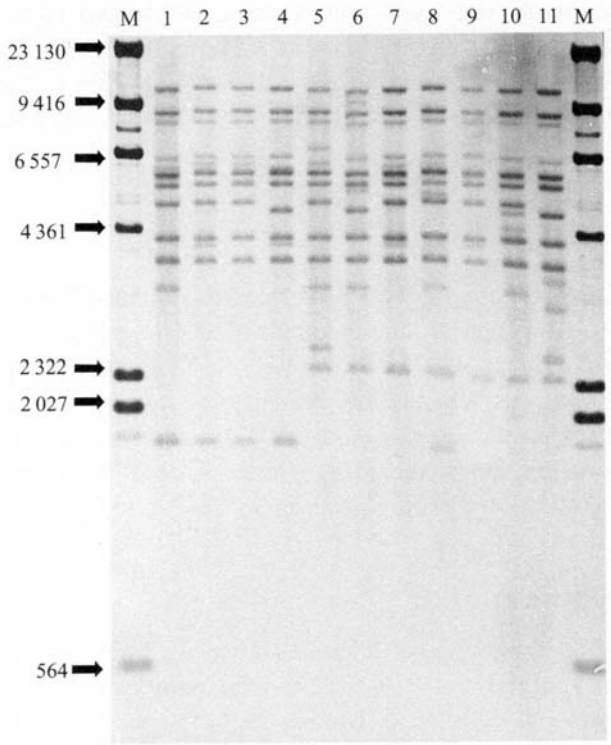

Fig. 1. *Hind* III ribotype patterns among 69 *Streptococcus agalactiae* strains of bovine (53) and human (16) origin and the 10 reference strains.

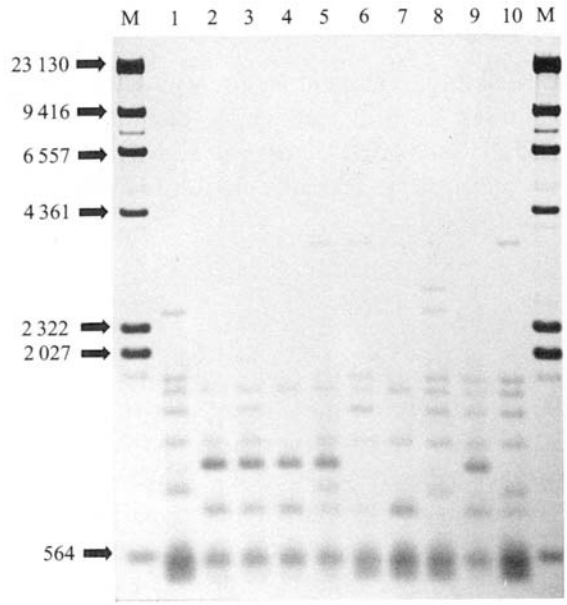

Fig. 2. *Hha* I ribotype patterns among 69 *Streptococcus agalactiae* strains of bovine (53) and human (16) origin and the 10 reference strains.

endonucleases yielded 8 types, and if serotypes were also considered, 11 types could be identified.

In the bovine material 4 *Hind* III and 3 *Hha* I types were observed (Tables 1 and 2). Combined use allowed further discrimination in six ribotypes. Epidemiologically related isolates displayed the same ribotype. Ribotyping could discriminate between herds infected with strains identical serologically (Table 1). Serologically different, but epidemiologically related strains had, in all cases, the same ribotype (Table 1). Likewise, antigenically dissimilar strains isolated from the same intramammary infection proved to be genetically similar as judged by ribotyping (Table 2).

DISCUSSION

DNA-based subtyping techniques have been used to subtype GBS strain collections of human origin [13–15], but to our knowledge such methods have only been applied to a small number of bovine isolates. Denning and colleagues [15] used digestion with *Eco*R I, *Bgl* II, *Hind* III and concluded, from a small survey, that restriction endonuclease analysis is a promising tool for detailed epidemiological study of GBS. The strains were serologically nontypable and differed from the human isolates investigated. However, epidemiologically linked isolates had identical REA patterns and phenotypic variation was observed with regard to colony type. If more bovine isolates from more sources had been examined, REA patterns identical with patterns among the human stains might have been disclosed. Wanger and Dunny [17] compared the genetic relationship of human and bovine strains of GBS by DNA–DNA hybridization and found a fairly high degree of relatedness among most strains in the species. However, strains isolated from a particular host (man or cow) generally showed a slightly closer relationship than strains from the different hosts. Musser and colleagues [18] found, by means of multilocus enzyme electrophoresis, that two ET types, indicating clones, could be recovered from cases of human disease and bovine mastitis. In this study some bovine and human isolates had identical *Hind* III and *Hha* I ribotypes indicating a close relationship between the two. Based on the results of Efstratiou and colleagues [12] and Blumberg and colleagues [11] we used *Hind* III and *Hha* I as restriction enzymes and found the banding patterns easily interpretable (Figs 1 and 2). Other restriction enzymes such as *Eco*R I or *Bgl* I, as used by Denning and colleagues [15], or the enzymes used by Fasola and colleagues [13] or Gordillo and colleagues [14] might have worked equally well. Despite the limited number of strains and serotypes in the human material

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the combined use of serotyping and ribotyping produced 11 types among the 16 isolates. The bovine material included epidemiologically related strains and, naturally, fewer types were detected. However, ribotyping proved efficient in discriminating between epidemiologically unrelated strains and indicated identity between those that were epidemiologically related. Furthermore, the ribotyping validated the concept of 'herd types' [19] which was developed to overcome difficulties in explaining the epidemiology of GBS infections within herds in which different antigenic patterns may be found among strains isolated. In most cases this does not represent a new infection but rather antigenic variation which is a common phenomenon regarding both polysaccharide- and protein antigens [6–8] and has been described 'phaseshift' by Sellin and colleagues [10]. Within the three herds in which GBS with different antigen combinations were isolated all strains displayed the same ribotype patterns (Table 1). That phenotypic variation takes place within the persistently infected mammary gland with time was supported by the fact that subsequent isolates from the same quarter showed varying antigenic composition despite the fact that the ribotypes remained the same (Table 2).

None of the 16 human strains fermented lactose. Four lactose-negative strains (serotype Ia/c) were present in the bovine isolates (one herd). With respect to serotype and ribotypes these lactose-negative strains proved identical to two lactose-positive strains recovered from the same herd, indicating a close relationship. Three lactose-positive strains (serotype Ib/c) were isolated from two workers attending a herd from which lactose-positive strains were isolated from quarter milk (serotype Ib/c) and bulk milk (serotype NT/c). The isolates from milk and dairy workers displayed identical ribotypes suggesting a common origin. It remains unknown whether the dairy workers infected the cows or the opposite was the case. Regarding biotyping in general, GBS strains isolated from bovine intramammary infections are practically all lactose fermenters, but only a minority of human strains have this capacity [3, 4, 20]. However, the usefulness of the lactose-fermenting capability appears to be limited, since this characteristic is subject to phenotypic variation [4] and may only indicate an adaptation to one or other of the two host species. Devriese [3] did not acknowledge the significance of phenotypic variation when claiming that GBS is not a zoonotic agent. Presumably, the bovine lactose positive biotype may colonize human beings, and human lactose negative strains have definitely been shown to cause bovine mastitis [21].

In conclusion, ribotyping appears to be a useful typing technique for GBS strains of bovine origin. It has the capability of discriminating between serologically identical types and it can be used to place a serologically nontypable strain in a common 'herd type'. Furthermore, it seems to be a valuable tool in the study of phenotypic variations which commonly appear among bovine GBS.

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