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DISTRIBUTION OF SEROTYPES OF GROUP-B STREPTOCOCCI IN HERDS AND COWS WITHIN AN AREA OF DENMARK

By

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JENSEN, N. E.: *Distribution of serotypes of Group-B streptococci in herds and cows within an area of Denmark.* Acta vet. scand. 1980, *21*, 354—366. — During a 3-year period strains of Group-B streptococci from 1227 (94.6 %) of a total of 1297 cases of bovine mastitis were serologically typed. Twenty-seven different antigenic combinations were found. Ab. 70 % of the strains carried polysaccharide antigens among which Type III was predominant (57 %). The protein antigen X was widespread (62 %), and 5 % of the strains were non-typeable.

Type Ic predominated among "false positive" bulk milk isolates (52.2 %, 12/23), but was rarely isolated from quarter milk samples (3 %).

Variation in the antigenic structure of infecting strains occurred to some extent and involved at least 10.8 % (132/1227) and probably 25.2 % (132/523) of the strains. Within a herd the antigenic variation was limited, however. Hence a definition of a herd type was possible. During the period of investigation infections caused by Herd Type III decreased numerically while the number of herds infected by other types remained almost stable.

Group-B streptococci; serological types; bovine mastitis.

For many years, bovine Str. agalactiae mastitis (B-str. mastitis) has been systematically controlled in Denmark. As a result the herd infection rate has fallen from 20—30 % in the fifties to 1.9 % in October 1979, and still shows a falling tendency.

At present the problems encountered in the control of B-str. mastitis are not so much related to the elimination of the infection from individual herds, but rather to new infections of inexplicable origin (*Jensen* 1976).

By biochemical typing of B-str. *Jensen* (1976) demonstrated that cows' milk may be contaminated with B-str. from non-bovine sources. Later (*Jensen* 1979a) it was found that the prevalence of B-str. in the human genital tract is high in Denmark and that numerically the human reservoir is much greater than the bovine reservoir.

The fermentation-type distribution has been elucidated for both the bovine and the human reservoir (*Jensen* 1976, 1979a), while serotyping has so far been carried out for the human material only (*Jensen* 1980).

MATERIAL AND METHODS

The material includes 129 herds in which B-str. mastitis was diagnosed during the period April 1st 1976 — April 1st 1979. A total of 1297 quarters in 774 cows were found infected. From 740 of these cows (95.6 %) 1750 isolates originating from 1227 quarters (94.6 %) were typed serologically. During the period concerned 23 isolations of B-str. were made from bulk milk samples originating from herds in which no B-str. could be found on subsequent examination of quarter milk samples.

Examination of quarter milk samples and identification of B-str. were performed as previously described (*Jensen* 1976). The serological typing included all known type antigens of B-str. and was performed by double-diffusion precipitation in agarose gel (*Jensen* 1979 b). Retyping of the "new" isolate was carried out when the same quarters were still found infected on re-examination of the herd. The type of a B-str. strain is defined by the antigen or combination of antigens it possesses.

RESULTS

1. *The stability/variability of strains of B-str. on propagation in naturally infected quarters*

Of the 1227 quarters examined 1095 showed 1 type only (retyping carried out in 381 cases). Of the rest of the quarters, 122 had 2 types (122 retypings carried out), while 10 had 3 types (20 retypings carried out). Thus, change of type was demonstrated in 132 of 1227 quarters (10.8 %) and different types found on first and subsequent typings in 132 of 523 cases (25.2 %).

2. *Serological identity/non-identity of different isolates from quarter milk samples in B-str. infected herds*

In accordance with the number of antigen combinations found, the 129 B-str. infected herds are divided into different categories (Table 1).

In 72 herds just 1 combination of antigens was demonstrated. However, in 39 of these 72 herds 1 quarter only was infected. When such herds are disregarded, the percentage of herds with more than 1 serological type of B-str. was 63 (57/90).

Table 1. Number of typed/not typed quarter milk isolates from 129 herds divided into categories according to number of antigen combinations.

Number of antigen combinations demonstrated	Number of herds	Number of quarter milk isolates typed	Number of quarter milk isolates not typed
1	72	270	22
2	34	547	13
3	14	151	11
4	4	34	1
5	1	5	0
6	3	199	23
7	1	21	0

3. *Serological types of B-str. in bovine mastitis within an area of Denmark*

Table 2 gives the type distribution of B-str. found in 1227 (94.6 %) of the total of 1297 infected quarters and thus allows of an almost complete analysis of B-str. involved in cases of mastitis observed in the area concerned during the period of investigation.

It should be noted that so far 10 main types and 28 antigen combinations have been recorded.

4. *Herd types*

The variability and scattering of types have initiated additional research as to the stability of B-str. antigens in vivo and in vitro (*Jensen,* to be published). It has been shown that no shifts occur between polysaccharide types. However, the polysaccharide antigens and the protein antigen Ibc are not invariably

Table 2. 1369 type determinations arranged according to type (antigen combination); 1227 different B-str. infected quarters from 129 herds.

Antigens demonstrated						Number	%
Ia	Iabc					6	
Ia	Iabc			R		1	6
Ia	Iabc			X		76	
Ib	Iabc		Ibc			14	1
Ib	Iabc					1	
Ia	Iabc		Ibc			37	
Ia			Ibc			2	3
Ia	Iabc		Ibc	R		2	
II						3	
II			Ibc			16	
II			Ibc	X		3	2
II				X		6	
II				R		5	
III						217	
III				R		14	
III				X		525	57
III			Ibc			13	
III			Ibc	X		8	
III				RX		1	
Ia	Iabc	III				12	1
Ia	Iabc	III		X		6	
			Ibc			118	
			Ibc	R		2	11
			Ibc	X		21	
			Ibc	RX		3	
				R		2	0.1
				X		190	14
					NT	65	5

NT = non-typeable.

present. The protein antigens X and R may be lost or acquired both when found along with polysaccharide antigens and when occurring as the only antigens.

Based on the observed variations of the antigens the following definition of a "herd type" is proposed:

Herd type denotes an antigen combination admitting shifts between antigens included in the combination but not between these and any other antigens. The herd type is specified by a principal antigen. First priority in this respect has been given to the polysaccharide antigens, since no shift between these has been demonstrated.

So far, 9 herd types have been established: Ia, Ib, Ic, II, III, Ia III, Ibc, X, NT.

Table 3. 1369 type determinations arranged according to herd type and antigen combination.

Antigen combination						Number of herds and herd type									Total
						6	8	14	17	59	3	13	5	4	129
						Ia	Ib	Ic	II	III	IaIII	Ibc	X	NT	
Ia	Iabc					4		1*		1*					
Ia	Iabc			R		1									
Ia	Iabc			X		76									
Ib	Iabc		Ibc				7		1*	6*					
Ib	Iabc						1								
Ia	Iabc		Ibc					33		4*					
Ia			Ibc					2							
Ia	Iabc		Ibc	R				2							
II									3						
II			Ibc						15	1*					
II			Ibc	X					3						
II				X					5	1*					
II				R					5						
III										215	2				
III				R		1*			2*	11					
III				X				1*		512	12				
III			Ibc							13					
III			Ibc	X						8					
III				RX						1					
Ia	Iabc	III									12				
Ia	Iabc	III		X							6				
			Ibc				16	29	22	5—4*		42			
			Ibc	R				2							
			Ibc	X					21						
			Ibc	RX			1		2						
				R						2					
				X					11	142			37		
					NT				5	45		5	2	8	
Total number conforming to herd type						81	25	68	92	954	32	47	39	8	1346
Total number of "foreign types" (*)						1		2	3	17					23

NT = non-typable.

Twenty-three quarter milk isolates originating from 11 herds did not conform with the respective herd types ("foreign types").

In Table 3, using the results from Table 2, the herds are divided in groups according to herd type.

It should be noted that 70 % (954/1369) of the typed isolates could be referred to Herd Type III, and that no other herd type accounted for more than 7 % of the material.

5. *The course of the control of B-str. mastitis during the period of investigation*

At the beginning of the period of investigation (April 1976 through March 1979) there were 29 infected herds, and 100 were new-infected during the period. The infection was eradicated from 110 herds, 85 of which were among those new-infected during the period. From January 1st, 1977 to January 1st, 1979 the rate of infected herds in the area investigated decreased from 0.98 % (41/4180) to 0.73 % (27/3714).

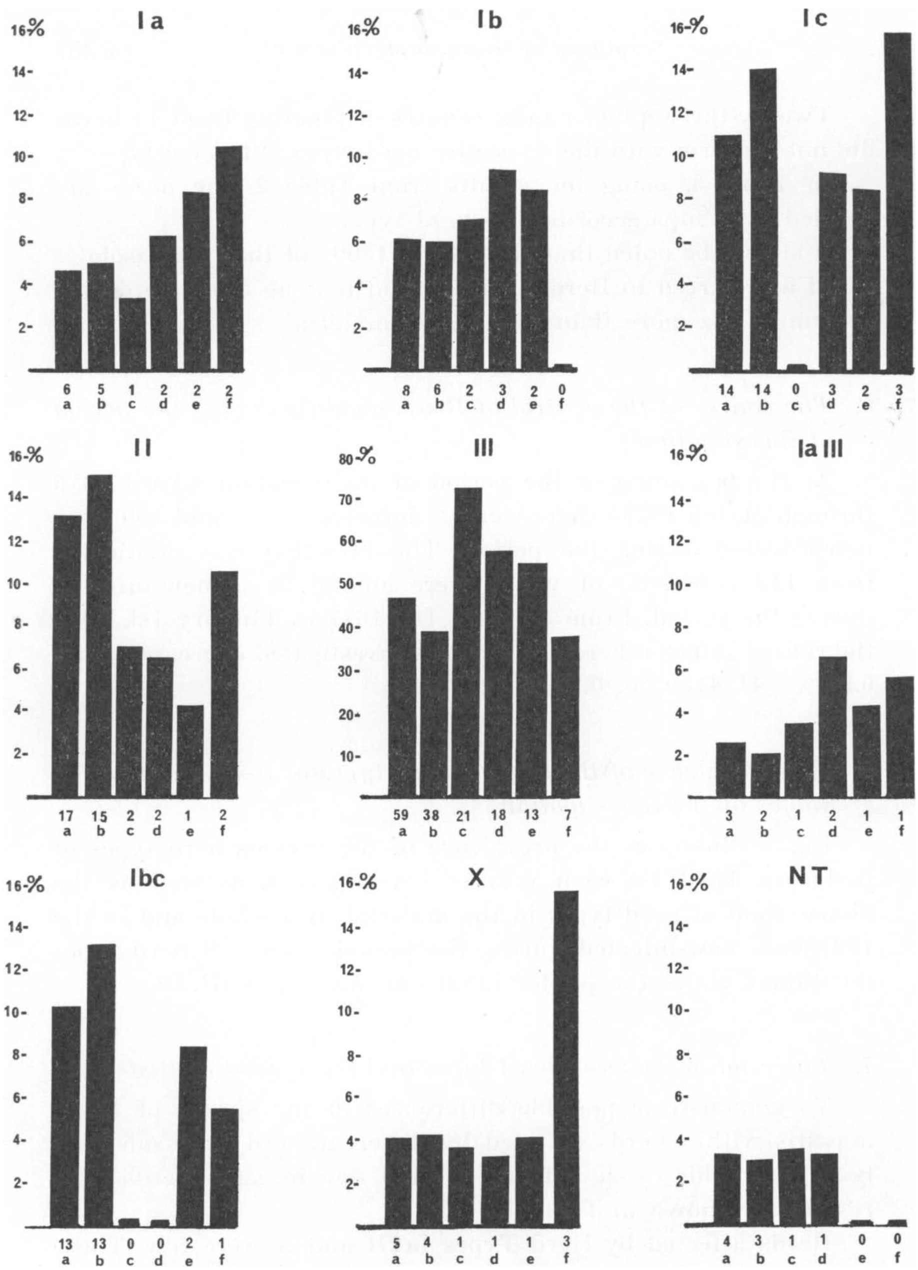
6. *The prevalence of the various herd types of B-str. at different stages of the investigation*

Fig. 1 illustrates the prevalence of the various herd types of B-str. on April 1st each year of investigation, as well as the distribution of herd types in the material as a whole and in the 100 herds new-infected during the period. Of the 9 herd types mentioned above the predominant one was Type III.

7. *The relation between herd types and the spread of B-str.*

To demonstrate possible differences in the spread of B-str. mastitis within herds affected by different herd types an analysis was made of data from the 100 new-infected herds. The results are shown in Table 4.

Herds affected by Herd Types Ia III and X were few. These herds being omitted from the analysis, B-str. mastitis was most widespread in Herd Type III herds with 3.0 infected cows per herd. In these herds 1.57 quarters were infected per infected cow, and the differences found between Herd Type III and the other herd types were significant ($P < 0.02$).



F i g u r e 1. Number and relative prevalence of herd types of B-str. in the control period April 1, 76 — April 1, 79.

a = total of herds (129) infected April 1, 76 — April 1, 79

b = total of herds (100) new-infected April 1, 76 — April 1, 79

c = 29 herds infected April 1, 76

d = 32 herds infected April 1, 77

e = 24 herds infected April 1, 78

f = 19 herds infected April 1, 79

Table 4. Number of cows and quarters infected in 100 new-infected herds divided according to herd type.

Herd type	Number of herds	Total number of cows	Infected cows			Infected quarters		
			number	%	per herd	number	%	per cow
Ia	5	131	6	4.6	1.2	6	1.1	1.00
Ib	6	124	16	12.9	2.7	18	3.6	1.13
Ic	14	376	25	6.6	1.8	29	1.9	1.16
II	15	324	27	8.3	1.8	33	2.5	1.22
III	38	1173	114	9.7	3.0	179	3.8	1.57
Ia III	2	59	8	13.5	4.0	11	4.7	1.38
Ibc	13	266	23	8.6	1.8	29	2.7	1.26
X	4	120	11	9.2	2.8	21	4.4	1.91
NT	3	107	3	2.8	1.0	3	0.7	1.00
Total	100	2680	233	8.7	2.3	329	3.1	1.41

NT = non-typeable.

8. *The distribution of serological types of B-str. found in bulk milk from herds with no B-str. infection demonstrable in cows*

During the investigation period examination of bulk milk samples led to false positive results in 23 cases. The results of the serological typing of these isolates are seen in Table 5. More than half of the isolates belonged to Type Ic.

Table 5. Distribution of serological types among 23 isolates from "false positive" bulk milk samples.

Number	Antigens demonstrated	Type	%
11	Ia Ibc Iabc	Ic	52.2
1	Ia Ibc		
1	II Ibc R	II	4.3
3	III	III	21.7
2	III R		
2	Ibc	Ibc	8.7
1	X	X	4.3
2	NT	NT	8.7

NT = non-typeable.

DISCUSSION

The evidence presented indicates that B-str. isolated from naturally infected quarters are most often constant in their antigenic structure. In ab. 10 % of infected quarters, however, an antigenic shift was observed and in quarters actually subjected to retyping such shift was demonstrated in ab. 25 %. In a material presented by *Müller* (1967) the corresponding figure was 20.7 % (12/58). Dealing with natural infections *Stableforth* (1938) found 3.2 % (4/125) of quarters with variable strains, but found no evidence of such variation in experimental infections.

The number of stable/variable strains will depend on the distribution of types in the material concerned, owing to differences in stability/variability between the individual B-str. antigens. These features will be further elucidated in subsequent articles, especially with regard to the X and Ibc-antigens.

The finding of strains of varying antigenic composition in 63 % (57/90) of herds with more than 1 infected cow need not necessarily mean that these herds have become infected from 2 or more sources.

The antigenic structure of the B-str. at present circulating in the cow population in question proved rather heterogeneous in that 27 different combinations of antigens and a small number of non-typeable strains were found. Some types were met with very rarely.

Polysaccharide antigens were predominant, being present in ab. 70 % of strains, and with Type III-antigen most frequently found (57 %).

Of the protein antigens, X was widespread, being demonstrated in 62 % of the material. Pure X-strains amounted to 14 %, while the protein antigen R was revealed in only 2 % of the examinations.

This distribution of types is entirely different from what has been reported from England (*Stableforth*), Italy (*Negretti & Lange* 1956), Japan (*Shimizu & Nakagawa* 1959), DDR (*Müller* 1967), Norway (*Haug* 1972) and Czechoslovakia (*Jelinkova* 1977). The distribution is, however, similar to a more recent investigation by *Spasic* (1974) for a Yugoslavian material of 412 bovine isolates where 81 % were of polysaccharide types, with Type III as the predominant one (50 %).

The difference noted between Denmark and other countries in the distribution of serological types may be real and, if so, attributable to the fact that owing to import restrictions the Danish cow population has for many years been rather secluded.

Jelinkova found the type distribution of human B-str. to be changing with time. The present investigation seems to suggest a similar tendency for bovine B-str., in that the prevalence of Herd Type III was relatively less at the end than at the start of the investigation period.

Finally, differences in type distribution may perhaps be related to the nature of the material examined. It may for example be of some significance whether it originates from clinical or subclinical cases, or whether it is selected or representative of a certain area.

The idea of herd types was adopted because operating with the more differentiated individual types would no doubt lead to confusion, considering the extensive type variation among B-str. within herds.

Most emphasis was placed on polysaccharide types. Strains hitherto non-typeable or typeable only by a protein-antigen can now be referred to a polysaccharide type. For example, as appears from Table 3, 29 Ibc and 2 IbcR quarters could be referred to Herd Type Ic. It also appears that by the introduction of Herd types the number of non-typeable strains could be "reduced" from 65 to 8.

Applying phage typing *Sanderson et al.* (1979) examined multiple isolates from the same patient and in 3 cases demonstrated an "apparent change of serotype in multiple isolates with the same phage pattern". This was not considered to indicate that the patient was carrying different strains.

This observation tends to confirm the expediency of introducing herd types.

During the period of investigation the number of B-str. infected herds was reduced from 29 to 19. Both absolutely and relatively (Fig. 1) the decrease pertained in particular to herds affected by Herd Type III. The other herd types were almost uniformly represented throughout and naturally showed a relative increase. It may be noted that during the investigation period 14 herds became infected with Type Ic and 13 with Type Ibc, and that among the "false positive" findings Type Ic was predominant.

By comparison of the type distribution in a Danish bovine reservoir of B-str. (represented by quarter milk samples) with that found in a human urogenital reservoir (*Jensen* 1980) appreciable differences are noticed. However, a trend towards a distribution resembling that seen in the human reservoir was apparent for the herd types in herds new-infected during the investigation period, suggesting that infection from human sources will increase in importance in the final stages of a program to control B-str. mastitis.

Presence of X-antigen in Danish B-str. seems to indicate a bovine origin. The X-antigen is very rarely found in isolates from false positive bulk milk samples and has so far never been discovered in isolates from the human urogenital tract (*Jensen* 1980).

CONCLUSION

B-str. strains associated with bovine mastitis in the area covered by the State Veterinary Serum Laboratory, Ringsted, show a diversity of antigen combinations which can be categorized in 9 different herd types.

The eradication of B-str. mastitis in the area is progressing, but the fall in the rate of B-str. infected herds is losing speed.

The reduction concerns mostly herds infected with Herd Type III, while the number of herds infected with other herd types has remained almost stable. On this account the distribution of herd types is shifting towards that applying to isolates of human urogenital origin. In this context it should be mentioned also that, as far as Danish B-str. strains are concerned, the X-antigen is almost exclusively linked with the bovine reservoir.

The present investigation does not answer the question whether all serological types may cause mastitis on such a scale that a herd problem is created, or whether some types will give rise to sporadic cases only. The latter may be the case, in that types other than Type III do not seem to be spreading as easily as Type III in new-infected herds.

Since in all cases a control program was initiated as soon as the infection was diagnosed, the natural course of the infection in the herds is unknown. Nevertheless, the main impression is that the course of B-str. mastitis in a herd varies with the serological type of the infecting strain, though it should still be borne in mind that it may also be influenced by other factors.

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SAMMENDRAG

Fordelingen af serologiske typer af gruppe B-streptokokker blandt køer og besætninger i et dansk område.

Serologisk typebestemmelse blev foretaget på isolater fra 95,6 % af køerne (740/774) og 94,6 % af kirtlerne (1227/1297) fra samtlige besætninger (129), hvor der i treårsperioden 1/4 1976 — 1/4 1979 diagnosticeredes gruppe B-streptokokmastitis (B-str. mastitis). Retypisering viste, at mindst 10,8 % (132/1227) og muligvis 25,2 % (132/523) af de inficerede B-str. stammer var antigent variable. I besætninger, hvor flere kirtler var inficerede (90/129), fandtes derfor hyppigst (63 %, 57/90) B-str. af mere end een „type" (Tabel 1).

Typebestemmelsesresultaterne (Tabel 2) viste, at B-str. af 28 forskellige antigenkombinationer forekom. 70 % af stammerne var i besiddelse af et polysakkaridantigen, blandt hvilke Type III var det mest udbredte (57 %). Af stammer af ren „proteintype" var der 14 % X; 11 % havde Ibc antigenet alene (8,6 %) eller sammen med X eller R. X-antigenet forekom alene og sammen med andre antigener meget udbredt (62 %); 5 % var ikke typiserbare.

Da den antigene variation blandt B-str. stammer var begrænset, kunne de 28 antigene kombinationer sammenfattes i 9 besætningstyper (Tabel 3).

Besætningstypefordelingen blandt 100 nyinficerede besætninger (Tabel 4) viste: Type III 38 %, Ic 14 %, II 15 % og Ibc 13 %. Type Ic var det hyppigste fund (52,3 %, 12/23) blandt de „falske positive" isolater fra leverandørmælkeprøver (Tabel 5).

Besætningstype III syntes hurtigst at kunne sprede sig i en besætning og især indenfor den enkelte ko (Tabel 4). Bekæmpelsesprogrammet har næsten udelukkende haft effekt overfor denne type, medens antallet af besætninger smittet med de forskellige øvrige typer holdt sig konstant gennem undersøgelsesperioden (Fig. 1).

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