Acta vet. scand. 1980, *21*, 625—632.

From the State Veterinary Serum Laboratory, Ringsted, Denmark.

VARIATION OF TYPE ANTIGENS OF GROUP-B STREPTOCOCCI

III. VARIATION OF THE PROTEIN ANTIGEN Ibc

By

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JENSEN, N. E.: *Variation of type antigens of Group-B streptococci. III. Variation of the protein antigen Ibc.* Acta vet. scand. 1980, *21*, 625—632. — During the natural course of bovine mastitis caused by Group-B streptococci loss or gain of the Ibc-protein antigen was demonstrated on examination of isolates from subsequent samplings. Thus, "genuine" Ibc-strains may arise from Ib, II Ibc, III Ibc and possibly from Ic-strains by loss of polysaccharide antigen or from non-typeable strains by acquisition of the Ibc-antigen. Variations were not provoked by ordinary laboratory procedures, but probably induced in vivo by specific antibodies.

Group-B streptococci; serological types; variation of type antigens.

The protein antigen Ibc (*Wilkinson & Moody* 1969, *Wilkinson & Eagon* 1971, *Wilkinson* 1975) is included in the present type classification system for Group-B streptococci (B-str.) together with the polysaccharide antigens Ia, Ib, II, III and Iabc (*Lancefield* 1934, 1938, 1975). Unlike antisera against the protein antigens R and X (*Pattison et al.* 1955 a, b), Ibc antisera have a protective effect in challenge experiments (*Lancefield* 1972, 1975). Besides its appearance in strains of types Ic (Ia, Iabc, Ibc) and Ib (Ib, Iabc, Ibc), the Ibc-antigen may occur together with the polysaccharide antigens II and III (*Wilkinson & Eagon, Baker & Kasper* 1977, *Jelínková* 1977, *Bevanger & Mæland* 1977, *Jensen* 1980 a, b) and with the protein antigens R and X (*Jelínková, Jensen* 1980 b).

According to *Jelínková* strains typeable by the Ibc-antigen are met with very rarely. She designates such strains "Ibc only". In

a Danish human urogenital material 2.6 % of strains (3/114) were of this "type" (*Jensen* 1980 a). Such strains were also found widely distributed among Danish bovine B-str. isolates (8.6 %, 118/1369) (*Jensen* 1980 b).

Ibc may be the only "type" discovered in a herd infected with B-str., but in most herds (17/28) Ibc-strains are found simultaneously with strains of a different antigenic structure.

The new-infection rate with B-str. in herds in the area covered by SVS, Ringsted, being low (0.8 % per year) (*Jensen* 1980 b), it was felt unlikely that so many herds should have become infected from 2 sources. Therefore, the stability/variability of the Ibc-antigen was studied in vivo and in vitro.

MATERIAL AND METHODS

During the period from April 1st 1976 through March 31st 1979 a total of 129 herds were found infected with B-str. in the above-mentioned area. In 28 of these herds the infection was caused by B-str. of Type Ibc ("Ibc only"). Of a total of 222 quarters infected with B-str., 118 yielded isolates of Type Ibc on at least 1 occasion. Isolates from 93.2 % (207/222) of the infected quarters were serologically typed. Bacteriological and diagnostic procedures were as described by *Jensen* (1976).

Isolates from quarters persistently infected with B-str. were typed whenever a herd examination was carried out. Most retypings took place at intervals of 4—8 weeks. In a few instances the interval was several months.

The procedures for demonstrating variability in vitro and for disclosing double infections were those described by *Jensen & Berg* (1980). Serological type determinations were made by double diffusion in agarose gel (*Jensen* 1979). By this method all recognized type antigens of B-str. will be revealed. A strain is designated by its antigenic composition. Antigen shift is indicated by $\rightarrow$.

RESULTS

In 11 herds (Table 1) Type Ibc was the only type demonstrated. In 17 herds strains of a different antigen composition were found as well: 8 herds had 1, 6 herds 2, and 3 herds respectively 3, 4, and 5 additional types.

Table 1. Total of quarters, number of Ibc-infected quarters and number of retyped quarters in 28 B-str.-infected herds arranged in 4 categories according to the number of B-str. "types" present.

Herd category	Number of herds	Number of B-str.-infected quarters			Number of retyped Ibc-infected quarters	
		typed	not typed	type "Ibc only"	total	with variation
"Ibc only"	11	35	3	35	7	—
2 types	8	59	3	50	15	3
3 types	6	64	1	28	8	3
> 3 types	3	49	8	5	4	4
	28	207	15	118	34	10

On retyping of isolates from Ibc-infected quarters, the antigenic pattern was unchanged in 24 of 34 quarters. The remaining 10 quarters, belonging in 7 different herds, showed a shift of type on retyping. Results of typing and retypings of these 10 quarters are listed in Table 2.

As will appear, the variations consisted in either loss or gain of the Ibc-antigen. The variations involved strains with the protein antigen X as well as such with polysaccharide antigens Ia, Ib, II or III. It is noteworthy that 1 strain shifted from Ibc to Ia, Ibc, i.e. gained the Ia-antigen but not the Iabc-antigen, which is

Table 2. Results of typings and retypings of 10 "Ibc"-infected quarters in which variations were demonstrated.

Results of			
initial typing	1st retyping	2nd retyping	3rd retyping
Ibc	Ibc	NT	Ibc
Ibc	Ibc	NT	NT
Ibc	NT	Ibc	—
X	X	Ibc	—
II Ibc	Ibc	—	—
Ibc	Ia Ibc*	—	—
Ib	Ibc	Ibc RX	—
III Ibc	Ibc	—	—
IIIX	Ibc	Ib	—
IIIX	Ibc	—	—

— = no retyping performed.
* = not Iabc.

generally considered to be constant in Ic-strains (*Lancefield* 1975). A Ic-strain with the complete antigen pattern (Ia Iabc Ibc) was present in the same herd.

To supplement the information given in Table 2 it should be mentioned that Ib-strains (Ib, Iabc, Ibc) occurred in 6 of 17 herds in which strains of types other than Ibc were found. In 1 of these herds a "complete" Ib-strain (Ib, Iabc, Ibc) was found. Ic-strains (Ia, Iabc, Ibc) were diagnosed in 4 of the 17 herds, and in 2 herds Ic-strains devoid of the Iabc-antigen (Ia, Ibc) were found in addition.

To establish whether an initial type and its variant occurred simultaneously in the infected quarter, isolates from 10 quarters in which such double infections could be anticipated were typed. Eight single colonies from the primary culture from each of the 10 infected quarters were the starting point for the type determinations. All 80 type determinations gave the same result (Ibc).

Variability in vitro was searched for among 8 strains, namely 6 Ibc-strains, 1 Ib-strain and 1 NT-strain. No variations were detected through 4 laboratory "generations".

DISCUSSION

The data reported above suggest that the B-str. protein antigen Ibc is constant in vitro, but that some 25 % of bovine Ibc-strains (8/34) will vary in vivo.

Previous experiments (*Jensen & Berg* 1980) have shown that the type antigens of B-str. are demonstrable by the routine method of extraction and type determination even when present in minute amounts. The fact that the antigenic structure of 8 strains (7 of which possessed the Ibc-antigen) remained unchanged through 4 generations would seem to indicate that the laboratory procedures used do not influence the Ibc-antigen.

As previously mentioned, the new-infection rate with B-str. in the area covered by SVS, Ringsted, is very low. This means that the possibility for a herd to contract a new infection with these microorganisms within a period of a few months is negligible. In a study on the variation of the X-antigen (*Jensen* 1980 c, *Jensen & Berg*) herds in which uncontrolled purchase of cows took place were omitted. In the present study this was not the case. Accordingly, 2 of the in-vivo variations observed (IIIX → Ibc) may well have been the result of double infection.

In-vivo variations may be induced by type-specific antibodies. Except for the fruitless effort to discover double infections among 10 Ibc-infected quarters, investigations to prove this hypothesis, discussed in a previous paper (*Jensen & Berg*), have not been carried out.

Wilkinson & Eagon (1971) characterized the Ibc-antigen as stable and held it to be present in all strains of Type Ib but not in streptococci of other Lancefield groups. Later on, however, *Wilkinson et al.* (1973) did report on Ib-strains devoid of the Ibc-antigen. One such strain was found in the present investigation. Two Ic-strains devoid of the Iabc-antigen (Ia Ibc) represented another unusual antigen composition.

In general, literature on the stability of bovine B-str. antigens is sparce, and no information at all seems to be available on the Ibc-antigen.

Two conflicting views have been put forward. According to *Stableforth* (1938) the infection of a given quarter with Str. agalactiae is, with rare exceptions, pure in a serological sense, and it remains pure over a period of at least 12 months, the last fact also connoting the stability of the serological type.

In contrast to *Stableforth, Müller* (1967) found that isolates of B-str. obtained successively during the natural course of udder infections varied at the rate of 21 % (12/58). The variations were due to loss or gain of either polysaccharide or protein antigens or both. Strains excreted from the remaining 46 quarters had a stable structure throughout the infection period.

In connection with phage-typing of isolates of human B-str. *Sanderson et al.* (1979) found an apparent change of serotype in 3 cases. Multiple isolates from the same patients, though constant with respect to phage-type, showed variations in the serotype pattern (e.g. a shift from Ib to Ibc).

CONCLUSION

Genuine Ibc-strains "Ibc only" (*Jelínková* 1977) may arise from Ib-, II Ibc-, III Ibc- and possibly from Ic-strains by loss of polysaccharide antigens. Through loss of the Ibc-antigen strains may become non-typeable (NT), but later on they may regain the Ibc-antigen.

Such variations of B-str. antigens, as well as other variations reported on separately (*Jensen & Berg* 1980) make it essential

to work with "herd types" (*Jensen* 1980 b) in epidemiological studies on bovine infections with B-str.

ACKNOWLEDGEMENT

The author wishes to acknowledge his indebtedness to Mrs. Burghild Berg, technical assistant, the State Veterinary Serum Laboratory, Ringsted, for her skilled assistance.

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SAMMENDRAG

Variation af gruppe B-streptokok typeantigener. III. Variation of the Ibc protein-antigenet.

Indledningsvis redegøres for Ibc-proteinantigenets placering i det officielle gruppe B-streptokok (B-str.) typeklassificeringssystem. Prævalensen af denne type („Ibc only", *Jelínková* 1977) blandt danske humane (2,6) og bovine (8,6) B-str. isolater anføres.

I 3-årsperioden april 1976—april 1979 diagnosticeredes B-str. infektion i 129 besætninger. I 28 af disse forekom B-str. af Type Ibc. Ialt var 222 kirtler B-str. inficerede og 118 kirtler udskilte B-str. af Type Ibc. Ovennævnte materiale dannede grundlag for studier over Ibc-proteinantigenets stabilitet in vivo og in vitro.

Materialet er anskueliggjort i Tabel 1. I 11 besætninger var Type Ibc den eneste forekommende type, i 17 forekom tillige B-str. af anden antigenstruktur.

Hvor en kirtel fortsat fandtes inficeret ved en gentagen besætningsundersøgelse (interval 4—8 uger) foretoges „retypisering". Ti af 34 stammer udviste variation. To af disse kunne imidlertid henføres til regulær dobbeltinfektion i besætningen, således at 8 af 34 (25 %) var egentlig variable. Variationernes art er anført i Tabel 2. Der kunne ikke påvises dobbeltinfektion af kirtler (10 undersøgte). Otte stammer undersøgtes for variabilitet in vitro gennem 4 laboratoriegenerationer. Variation påvistes ikke.

I de undersøgte besætninger påvistes to usædvanlige B-str. antigenkombinationer: Ia Ibc (mangler Iabc) og Ib Iabc (mangler Ibc).

Det konkluderes, at Type Ibc („Ibc only") er stabil in vitro, men at denne type kan opstå in vivo fra stammer af Type Ib, II Ibc, III Ibc og muligvis Type Ic grundet tab af polysakkaridantigenet. Type Ibc kan blive ikke-typiserbar (NT) ved tab af Ibc antigenet, men reversion kan forekomme.

(Received September 9, 1980).

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