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VARIATION OF TYPE ANTIGENS OF GROUP-B STREPTOCOCCI

I. VARIATION OF THE X-ANTIGEN AND OF OTHER TYPE ANTIGENS IN HERDS WHERE THE X-ANTIGEN OCCURS

By

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JENSEN, N. E.: *Variation of type antigens of Group-B streptococci. I. Variation of the X-antigen and of other type antigens in herds where the X-antigen occurs.* Acta vet. scand. 1980, *21*, 367—374. — Serological typing of Group-B streptococci isolated on different occasions during the course of natural intramammary infections revealed no shifts between polysaccharide types, but showed that polysaccharide antigens and the protein Ibc antigen may be lost and regained. Pure X-strains may arise from strains previously typed as IIIX or III, or from non-typeable strains, and possibly from Ibc, Ia and Ib strains as well. Such strains may later revert to their original types.

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

On the basis of different capsular polysaccharide antigens (Ia, Ib, II, III and Iabc) (*Lancefield* 1934, 1938, *Lancefield et al.* 1975) and a protein antigen (Ibc) (*Wilkinson & Moody* 1969, *Wilkinson & Eagon* 1971, *Wilkinson* 1975) Group-B streptococci (B-str.) are divided into 5 distinct serological types: Ia, Ib, Ic, II and III. These antigens seem related to virulence, since they give rise to protective antibodies (*Lancefield et al.*). Most B-str. isolated from human sources are typeable within this regimen. In the USA (*Wilkinson* 1977) 3 % of the strains were non-typeable (NT), in Denmark less than 1 % (*Jensen* 1980b). British investigators (*Pattison et al.* 1955a) found some 25 % of bovine strains non-typeable and *Pattison et al.* (1955b) arrived at the conclusion that such strains, apparently devoid of type polysaccharide antigens, were typeable by 2 protein antigens named R and X. A mutual relationship between R and X was described.

For typing of bovine B-str. strains apparently lacking polysaccharide antigens the use of anti X and anti R sera was recommended. Antibodies against R and X are not protective (*Lancefield* 1972) and the usefulness of these antigens in epidemiological studies is debatable. *Jelinkova* (1977), however, holds the view that any additional antigen should be included in a typing regimen.

Serological typing of strains of B-str. isolated from quarter milk samples collected within the area of mastitis control covered by the State Veterinary Serum Laboratory, Ringsted, revealed that the X-antigen was widespread, in that it was found in 40 % of the B-str.-infected herds (52/129). Most frequently the X-antigen was demonstrated along with 1 or more of the other B-str. type antigens (47/129) and very rarely (5/129) as the only B-str. antigen in a herd (*Jensen* 1980a).

Obvious variations in the antigenic pattern occurred in pure X-strains as well as in strains of a more complex antigenic composition.

The aim of the present work was to study the in vivo frequency of antigenic variations, especially such which involves the X-antigen, and to evaluate the significance hereof.

MATERIAL AND METHODS

During the period April 1976 through March 1979 practically all strains of B-str. isolated from quarter milk samples from cows within the area concerned were serologically typed.

B-str. infection was diagnosed in 129 herds. The X-antigen was demonstrated in 52 of these herds, 16 of which were selected for further analysis, by the following criteria:

1) At least 5 quarters infected
2) At least 2 different antigen combinations represented
3) Variation demonstrated, primarily concerning the X-antigen
4) The herd maintained without uncontrolled purchase or treatment of cows.

In the 16 herds thus selected, B-str. infection was diagnosed in 588 different quarters, and isolates from 560 (95 %) were typed serologically. Of the 560 quarters 209 were persistently infected, as shown by subsequent examinations. The isolates from these re-examinations were typed and the quarters concerned are referred to as retyped quarters. In most cases retyping

was done after 6—8 weeks, but in a very few instances not till after several months.

The bacteriological and serological techniques employed have been described previously (*Jensen* 1976, 1979). The typing method used will reveal all known antigens of B-str. A strain is designated by its antigenic composition. Antigen shift is indicated by → or ←.

RESULTS

The herds could be divided into 4 categories according to the number of antigen combinations found.

The number of herds, typed and non-typed quarters, and the number of retyped quarters of the different categories are enumerated in Table 1.

Table 1. Division in categories of 16 selected herds with varying X-antigen.

Herd category	Number of herds	Number of B-str.-infected quarters		Number of retyped quarters	
		typed	not typed	same type	different type
2 types	8	322	4	86	41
3 types	4	58	10	6	6
4 types	2	22	0	1	7
>4 types	2	158	14	30	32
	16	560	28	123	86

As will be noticed, isolates from 123 quarters showed the same antigen combination on retyping as on the first typing, while a shift was demonstrated in isolates from 86 quarters. For these 86 quarters a total of 149 retypings are on record, 37, 16, 9, and 1 quarter being retyped on the 3rd, 4th, 5th and 6th herd examination, respectively. In 46 cases the same antigen combination was recorded at 2 successive retypings, while in most such cases (103/149) a change was revealed of 1 or more of the type antigens.

Thirteen quarters reverted later on to the original type. Four quarters yielded B-str. with 3 different antigen combinations.

The nature and number of the variations and their distribution among the herds appear from Table 2.

Table 2. Antigenic variation of Group-B streptococci from 16 selected herds.

Nature of variation		Number of variations recorded	Herd incidence among 16 herds	
III	⇆ IIIX	51 (33 ⇆ 18)	9	
IIIX	⇆ X	15 (12 ⇆ 3)	4	
III	→ X	1 (1 → 1)	1	5
IIIX	⇆ NT	5 (3 ⇆ 2)	3	
III	→ NT	1 (1 → 1)	1	
X	⇆ NT	19 (11 ⇆ 8)	2	
III	→ IIIRX	1 (1 → 1)	1	
III IbcX	⇆ IIIX	4 (3 ⇆ 1)	1	
III IbcX	→ III	1 (1 → 1)	1	
II Ibc	→ IbcX	1 (1 → 1)	1	
X	→ Ibc	1 (1 → 1)	1	
IaX	→ Ia	1 (1 → 1)	1	
Ia	→ X	1 (1 → 1)	1	
Ib	→ X	1 (1 → 1)	1	

The variations III → IIIX and IIIX → III (III ⇆ IIIX) occurred most frequently (51/103) and were demonstrated in more than 50 % of the herds examined (9/16). Naturally, most variations were recorded on the second typing (III ⇆ IIIX, 26 ⇆ 13). Results from quarters on which retyping was carried out repeatedly showed that variations, including reversions to previous type, might occur after periods of shorter or longer duration.

Loss of polysaccharide antigen III was a frequent variation (17/103), which was observed in 5 herds. Of isolates from the 17 quarters, 2 were initially typed as III, and 15 as IIIX. On retyping 13 were X and 4 NT. Later on an isolate from 1 of these quarters was found to have regained the III-antigen (III → X → IIIX). Likewise, 4 quarters originally yielding strains of Type X (2) or NT (2) were found later on to yield strains containing the III-antigen.

The variations X ⇆ NT, which were also seen rather frequently (19/103), concerned 13 quarters in 2 herds. The variation III → IIIRX was recorded for 1 quarter only. In 1 herd loss of both the Ibc and the X-antigen was seen (III IbcX → III). Results from 3 other quarters in this herd were: III IbcX → IIIX, and from 1 quarter in another herd: X → Ibc.

The variations of the Ibc antigen will be reported on in a subsequent paper.

Finally, the analysis revealed variations of the polysaccharide antigens, II, Ia, and Ib, as well as of the X-antigen occurring along with the Ia antigen.

DISCUSSION

Retyping of isolates from B-str. infected quarters has shown that in the majority of cases the infecting strains will retain their antigenic structure (59 %, 123/209) and that in these cases the X-antigen is constant whether it occurs in combination with other type antigens or as the only antigen. Isolates from a considerable number of quarters, however, did not show such stability, in that variation, mainly of the X-antigen, was observed in 41 % (86/209).

In 51 cases the variation consisted in loss or gain of the X-antigen associated with constant polysaccharide III-antigen, or, in 1 quarter, with constant Ia antigen.

These variations are consistent with the findings of *Pattison et al.* (1955a): „In the course of experimental infections of the mammary gland of goats and cows with strains of known polysaccharide type it was frequently noted that isolations from the infected milk "gained" or "lost" protein antigens in an unpredictable manner".

For strains in which no polysaccharide antigen was detectable, *Pattison et al.* (1955b) took the view that "if a strain belongs clearly to Group B it will contain either X or R sufficiently consistently through laboratory subculture and animal passage to allow it to be identified by these antigens". This is contrary to the results here presented, where isolates from 13 quarters (19 cases) in 2 herds displayed the variations X $\leftrightharpoons$ NT. A number of other "X-quarters" (28) in these 2 herds have, however, shown a constant picture on retyping.

Also *Pattison et al.* (1955a) held the opinion that the polysaccharide antigens were stable. In the present work, loss or gain of the polysaccharide III-antigen was not unusual, being demonstrated in 5 herds (out of 16) in a total of 17 cases (out of 103). This feature is therefore not likely to be connected with a single or a few strains only. The other polysaccharide antigens in the material, i.e. II, Ia and Ib, were each lost in 1 strain only.

Apart from the reports of *Pattison and co-workers* (1955 a, b) literature on variation of B-str. type antigens is sparce. A shift

of type during natural infection was demonstrated by *Stableforth* (1938) in 4 of 125 quarters examined. In 24 experimentally infected quarters, however, he found no type variations.

The observations here reported on the variation of B-str. antigens are in agreement with those of *Müller* (1967) who found that B-str. may loose polysaccharide antigens (Ia and II) during natural infections and that strains initially typed as R, X or NT may acquire such antigens (Ia, II or III).

To overcome difficulties in explaining the epidemiology of bovine B-str. infections within herds, *Jensen* (1980a) introduced the notion of "Herd type". The results of the present study seem to justify this, but call for further examination of the in vitro induced variation of B-str. antigens.

CONCLUSION

The demonstration of B-str. with different antigenic structure within the same herd does not necessarily indicate that the herd has been infected from different sources. In fact, variations of B-str. antigens seem to occur quite frequently and a possible relatedness of "types" demonstrated should therefore be considered. In epidemiological studies the use of herd types would seem to be advantageous.

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SAMMENDRAG

Variation of gruppe B-streptokok typeantigener. I. Variation af X-antigenet og andre typeantigener i besætninger hvor X-antigenet forekommer.

Gruppe B-streptokokker (B-str.), hvor proteinantigenet X kunne påvises, forekom i 56 af 129 besætninger, hvor B-str. infektion diagnosticeredes i perioden 1/4 1976 — 1/4 1979.

Typebestemmelsesresultater fra 16 af "X-besætningerne" analyseredes nøjere med henblik på påvisning af art og hyppighed af antigenvariation.

I de 16 besætninger, der kunne opdeles i 4 kategorier (Tabel 1), fandtes 588 B-str. inficerede kirtler.

Retypisering (= gentagen typebestemmelse, når en kirtel viste sig fortsat inficeret ved en senere besætningsundersøgelse) blev foretaget for isolater fra 209 kirtler; 123 af disse udskilte fortsat B-str. af samme type, medens 86 kirtler viste typeskift ved retypisering. For de 86 varierende kirtler forelå ialt 149 typebestemmelser. To på hinanden følgende undersøgelser viste samme type i 46 tilfælde, medens 103 viste variation. Tretten kirtler reverterede efter variation til den oprindelige type. Fire kirtler udskilte B-str. af 3 typer.

Variationernes art, antal og udbredelse fremgår af Tabel 2. Variationen III ⇆ IIIX var hyppigst forekommende (51/103) og mest udbredt (9/16).

Tab af polysakkaridantigen III sås i 5 besætninger i ialt 17 tilfælde, og omvendt kunne isolater fra X eller NT kirtler (4) ved senere undersøgelse vise sig at have III antigenet. Variationen X ⇆ NT var almindelig (19/103). Andre antigenvariationer forekom sjældent (se Tabel 2) i dette materiale.

Undersøgelsen viste, at der ikke sker skift mellem polysakkaridtyper, men at såvel polysakkaridantigener som Ibc proteinantigenet er variabelt. X-stammer kan hidrøre fra stammer tidligere typebestemt til IIIX, III eller NT og antagelig også fra Ibc, Ia og Ib stammer.

Det konkluderes, at påvisning af B-str. af flere typer i en besætning ikke umiddelbart viser, at en sådan besætning er blevet inficeret fra forskellige kilder.

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