Acta vet. scand. 1980, *21*, 617—624.

From the State Veterinary Serum Laboratory, Ringsted, Denmark.

VARIATION OF TYPE ANTIGENS OF GROUP-B STREPTOCOCCI

II. STUDIES ON THE IN-VITRO VARIATION OF THE X-ANTIGEN AND OTHER TYPE ANTIGENS

By

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JENSEN, N. E. and B. BERG: *Variation of type antigens of Group-B streptococci. II. Studies on the in-vitro variation of the X-antigen and other type antigens.* Acta vet. scand. 1980, *21*, 617—624. — When occurring together with the polysaccharide III-antigen the X-antigen displayed a phenotypic variation in vitro, and so did 1 R-strain. Genuine X-strains, and other types as well, seemed to remain unaffected by commonly used laboratory procedures. The hypothesis is advanced that previously described in-vivo variations of these antigens are induced by antibodies against the antigen in question.

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

Pattison et al. (1955 a) reported that when found together with a polysaccharide antigen the X-antigen of Group-B streptococci (B-str.) seemed to vary randomly during subculture. *Jensen* (1980 b) found that such variations took place also in vivo. *Pattison et al.* (1955 b) took the view that genuine X-strains would show sufficient stability through subculture and animal passage to be identified by this antigen. *Jensen*'s results (1980 b) demonstrated, however, that such strains were far from stable in vivo, and also the B-str. polysaccharide antigens and the protein antigen Ibc were shown to vary.

The reasons for these variations were not elucidated, but the following factors may be instrumental in the shift of types.

1. Quantitative differences between strains in the output of type antigens which are not demonstrable by the typing method employed.

2. Quarters may have been infected initially by strains of 2 serotypes of B-str. The routine of using cells from only 1 colony for typing will leave such a condition undiscovered.
3. The laboratory procedures and media, per se, may induce changes of the type antigens of B-str. (phase variation).
4. The initial infection may disappear and the quarter become reinfected with a different serotype.

Investigations were initiated to elucidate the influence of these factors on the variations observed.

MATERIAL AND GENERAL PROCEDURE

The strains examined originated from cases of bovine B-str. mastitis. Diagnostic procedures and the laboratory methods employed were those described by *Jensen* (1976, 1979). A strain is designated by its antigenic composition. Variation of antigens is indicated by →.

Experimental

Modification of the routine method of typing in an attempt to demonstrate small amounts of antigen

Twenty-eight strains of B-str. of different antigenic composition were

a) typed routinely twice (cells from 10 ml Todd-Hewitt(TH)-broth),
b) typed by the ordinary extraction procedure (0.5 ml, 0.05 N-HCl, 100°C, 16 min) with the modification that cells from 60 ml instead of 10 ml TH-broth were extracted and
c) typed under employment of the volume of cells and the extraction procedure indicated by *Jelínková* (1977), i.e. 40 ml TH-broth, 0.35 ml 0.2 N-HCl, 52°, 2 h.

All serological typings were performed by double diffusion in agarose gel. For all of the 28 strains the result was the same no matter what method was used.

In view of the fact that excess of either antigen or antibody may cause a serious imbalance of a double diffusion system and ultimately prevent the formation of visible precipitates, routine extracts of 2 X-strains and 2 NT-strains (non-typeable) were tested in dilution 1:10, 1:20, 1:40 and 1:80 against undiluted

sera. Additional precipitates were discovered neither by this procedure nor by testing the reverse procedure, i.e. testing diluted antisera (1:10, 1:20, 1:40 and 1:80) against undiluted extracts.

The occurrence of double infections

Routinely 1 colony from the primary plate was typed from each infected quarter. To see if typing of a number of colonies from each infected quarter would disclose double infections, 8 colonies were examined from each of 29 different quarters from 7 different herds in which more than 1 type was found.

Types III and IIIX were found in 3 quarters, Type II and NT in 1 (Table 1).

Table 1. Type determination of isolates from 29 B-str.-infected quarters. Eight colonies from each primary culture were examined.

Number of quarters	Results of type determination of 8 colonies
6	8 IIIX
5	8 III
2	6 IIIX / 2 III
1	2 IIIX / 6 III
4	8 IIIR
1	8 R
2	8 X
2	8 NT
3	8 Ic
1	6 II / 2 NT
2	8 IaX

Variations in vitro

It is well known that the R-antigen of Group-L streptococci is subject to phase variation (*Perch & Olsen* 1964). To see if this might be the case also with B-str. antigens, more especially the X-antigen, the following investigations were carried out.

Fresh isolates as well as strains kept for some time were examined.

From a typed primary culture or a fresh culture of a laboratory strain (1st generation) 8 individual colonies picked from the surface-inoculated 1st generation broth were subcultured and typed (2nd generation). If all typings gave identical results, 8 colonies were picked from a plate culture from 1 of the 2nd

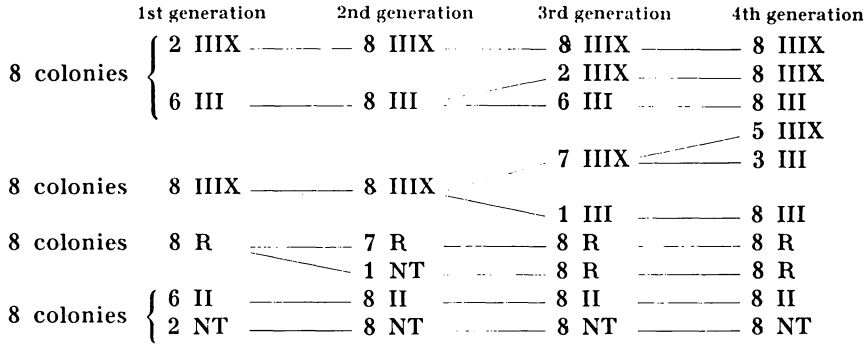
generation broths, and if more than 1 type was demonstrated, 8 colonies were picked from 1 broth culture of each type (3rd generation). A total of 4 generations were examined within this scheme.

Isolates from 20 quarters initially typed as III or IIIX, or with double infections of III/IIIX, were examined. Fourteen of these strains either lost or gained the X-antigen, apparently at random, through the 4 laboratory generations. Among the 6 stable strains both Type III (4) and Type IIIX (2) were represented. The polysaccharide III-antigen remained stable in all strains.

Four NT-strains, 4 X-strains and 1 IIIR-strain showed no variation through the 4 laboratory generations. One R-strain showed the variation R → NT → R.

A II-strain and an NT-strain originating from an initially double-infected quarter (6 colonies: Type II, 2 colonies: NT) showed no variation in vitro.

The diagram below shows examples of the variations found:



DISCUSSION

The present study has confirmed that the same results are obtained with the method of extraction used in routine type determinations as with an internationally approved extraction procedure, and that extraction of larger amounts of cells are unlikely to lead to the demonstration of additional antigens.

While *Jelínková* (1977) performed the precipitation as a "ring-precipitation" in conical capillaries, all precipitations in this investigation were performed by double diffusion in agarose gel. Though not investigated, it seems unlikely that this difference in method can have influenced the results.

The common occurrence of double infections with Types III and IIIX is consistent with the previous finding that the X-antigen would vary in vivo when occurring together with the III-antigen (*Jensen* 1980 b). The present investigation has shown this combination of antigens to be extremely labile also in vitro: 14 of 20 III/IIIX strains examined displayed variations.

"Genuine" X-strains and NT-strains were not found in double infections and did not vary in vitro.

In all 20 strains tested the polysaccharide III-antigen was stable through 4 laboratory generations, and so was the II-antigen in 1 strain. The observed in-vitro stability of the protein X-antigen and of the polysaccharide III- and II-antigens tends to confirm the views expressed by *Pattison et al.* (1955 a, b). On the other hand, the in-vivo variations previously demonstrated (*Jensen* 1980 b) contrast with these views.

The observed in-vivo type variations of B-str. can hardly be ascribable to elimination of an initial infection followed by reinfection with a different type, since the herds in which the in-vivo variations were found were selected in such a way that the usual source of new infections with B-str. (i.e. purchased infected cows) could be disregarded. Furthermore, in herds within the control area covered by SVS, Ringsted, the new-infection rate with B-str. is very low, namely 0.8 % per year (*Jensen* 1980 a). Thus, it is very unlikely that within a short time the same quarter should have contracted B-str. infection from 2 or more different sources.

The observed variation of the protein antigen R suggests that this antigen may be subject to a phase variation comparable to that of the R-antigen of Group-L streptococci (*Perch & Olsen* 1964).

Reversibility of the antigenic shifts in the examined R-strains was demonstrated ($R \rightarrow NT \rightarrow R$).

In-vitro variation of the X-antigen has so far been demonstrated only when this antigen occurs together with a polysaccharide antigen (III). In-vitro variation was also found in 1 R-strain. Variations of other antigens have been observed only in vivo (*Jensen* 1980 b) and might have been induced by antibodies against the type antigen in question. Some evidence exists that antibodies may occur in bovine intramammary B-str. infection (*Spencer & Angevine* 1950, *Yokomizo & Norcross* 1978) and a

type-specific antibody response is certainly provoked in the course of human B-str. infections (*Christensen* 1979).

The antibodies in question, if existing, are not of the precipitating kind, in that whey and serum samples from infected cows gave rise to no formation of precipitates when reacted against the different B-str. type reference strains in the normally used double diffusion system (*Jensen*, unpublished data). They may very well, however, be of a different nature, but owing to lack of technical possibilities the possible occurrence of non-precipitating B-str. antibodies in whey or serum could not be demonstrated in this study.

Lancefield et al. (1975) were able to deprive a Ia-strain (090S) of its type-specific antigen by serial subculture in broth with 10 % type-specific antiserum, but this variant (090R) tended to revert to the type-specific form (090S) under ordinary laboratory conditions.

If in-vivo variations of type antigens may be caused by immunological "pressure" and subsequent selection of a new type, one would expect both types to be present simultaneously at a certain stage of the infection. This situation did in fact occur in 1 quarter. Type determination of 8 colonies picked from the primary culture from a B-str. infected quarter revealed the presence of 6 colonies of Type II and 2 colonies of NT.

Reversion of the NT-strain to Type II, comparable to what was reported by *Lancefield et al.* had not taken place after 4 subcultivations.

CONCLUSION

In vitro, the X-antigen of B-str. shows random variation when it occurs together with the polysaccharide III-antigen and probably also when it occurs with other B-str. polysaccharide antigens. When occurring as the only antigen, however, the X-antigen has so far proved stable in vitro. Since Danish strains of B-str. carrying the X-antigen seem almost exclusively related to the bovine reservoir, the X-antigen may be of some epidemiological significance (*Jensen* 1980 a).

Strains of other types of B-str. vary in vivo, but usually remain stable in vitro.

Hence, as previously pointed out (*Jensen* 1980 a) in epidemiological studies it is essential to be able to deal with "herd types".

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SAMMENDRAG

Variation af gruppe B-streptokok typeantigener. II. Undersøgelse af X-antigenets og andre typeantigeners variation in vitro.

Mulige årsager til de tidligere påviste variationer in vivo af gruppe B-streptokok (B-str.) typeantigener er undersøgt.

Det blev fundet:

1. At den anvendte typebestemmelsesmetode er følsom og specifik nok til at registrere kvantitative forskelle i B-str. stammernes antigenoutput.

2. At kirtler fra starten kan være inficerede med to serotyper. Ved undersøgelse af 29 kirtler fandtes i 3 kirtler både Type III og Type IIIX, i 1 kirtel både Type II og NT (Tabel 1).
3. At laboratorieprocedurer kan medvirke ved typeskift (fasevariation).

Af 20 stammer — oprindelig typebestemt til III eller IIIX — varierede 14. Både Type III (4) og IIIX (2) var blandt de 6 konstante stammer. Polysakkarid III-antigenet var stabilt.

Fire NT-stammer, 4 X-stammer og 1 IIIR-stamme varierede ikke. En R-stamme varierede: R → NT. Polysakkaridantigenet II var stabilt in vitro (1 stamme).

Det konkluderes, at X-antigenet varierer tilfældigt når det optræder i forbindelse med III-antigenet. Det synes derimod at være stabilt in vitro når det forekommer som eneste antigen. Andre undersøgte stammer (II, IIIR, NT) var ligeledes stabile in vitro, men en R-stamme varierede.

På trods af variationerne kan X-antigenet have epidemiologisk betydning, da dette antigen, hvad danske B-str. stammer angår, næsten selektivt synes knyttet til det bovine reservoir.

Variationerne — in vivo og in vitro — nødvendiggør, at man ved epidemiologiske undersøgelser af bovin B-str. mastitis vurderer muligheden for sammenhæng mellem forskellige „typer", altså arbejder med besætningstypebegrebet.

(Received September 1, 1980).

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