J. Hyg., Camb. (1969), **67**, 691
Printed in Great Britain

A new nephritogenic streptococcus

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(*Received* 21 *May* 1969)

INTRODUCTION

According to our present state of knowledge, only certain types of group A streptococci are usually associated with acute glomerulonephritis as a late non-suppurative complication of sore throat or pyoderma.

We recently had an opportunity of studying a summer outbreak of sore throat in which the infecting organism was a streptococcus of group C, *Streptococcus zooepidemicus*; a third of those infected later developed acute glomerulonephritis. Epidemiological inquiry revealed all the features of a milk-borne epidemic connected with a temporary fault in pasteurization (Teodorovici, 1969).

Since human infections with *S. zooepidemicus* have been rarely, if ever, described, and there is no record of any such infections being complicated with glomerulonephritis, an account of the findings in this outbreak may be of interest to streptococcal workers.

MATERIALS AND METHODS

Clinical material

Between 20 May and 12 July 1968, in the town of P. Neamtz (Romania), 85 cases of sore throat occurred, nearly all of them associated with cervical lymphadenitis and pyrexia. Seventy-four (87 %) of the patients were adults; about a third of them showed acute glomerulonephritis as a late complication. Signs of renal involvement appeared in the second or third week of illness and consisted of oedema of eyelids and feet, oliguria which in a few cases became anuria, haematuria, proteinuria frequently associated with cylindruria, and arterial hypertension.

From two of three patients on whom renal biopsy was performed sufficient material for histological examination was obtained, and typical lesions of subacute glomerulonephritis were observed, consisting of proliferative glomerulitis associated with neutrophil and eosinophil infiltration, thickening of Bowman's capsule and capsular synechia, periglomerular polymorphonuclear and lymphocytic infiltration, and protein exudate in some proximal convoluted tubules.

The clinical, physiological and histological aspects of the outbreak are discussed in detail in a separate paper (Vîţă & Niculescu-Talaşman, 1969).

Because an outbreak of tularaemia with cervical adenitis had occurred in the same region in the previous year (Pencea & Vita, 1968) the early clinical diagnosis

and treatment were at fault in the early cases, and penicillin was used in only a few cases. For the same reason complete bacteriological examinations were requested rather late, 30 days after the first cases occurred, at a time when 15 patients only could still be investigated.

Microbiological investigations

Material for culture

From each patient throat swabs were examined, as this was the probable site of entry; urine and faeces were also cultured as possible routes of excretion. In four patients biopsy specimens from an inflamed cervical lymph node were also cultured.

Methods of culture

Since in the early stages the possible diagnosis might have been any one of a number of bacterial and viral infections, all specimens were inoculated on suitable culture media and into animals. Each throat swab was inoculated on a blood-agar plate, containing 10% defibrinated sheep blood, and a second swab was washed out in 2 ml. of sterile saline which was immediately inoculated subcutaneously into two young Swiss albino mice of 16–18 g. each. Biopsy specimens from lymph nodes were also inoculated intraperitoneally into rabbits and guinea-pigs.

Urine samples were cultured quantitatively on blood-agar plates. Faeces specimens were cultured on Leifson's deoxycholate citrate agar.

Cow's milk samples, after sedimentation, were inoculated on blood-agar plates and also subcutaneously into mice.

All plates were examined after 24 and 48 hr. incubation at 37° C. and the colonies were identified by the usual tests.

Another set of throat swabs and faeces samples were frozen in Hanks's solution containing 1% lactalbumin hydrolysate, penicillin 1000 u./ml. and streptomycin 500 u./ml.; 24 hr. later they were inoculated, after thawing, into HeLa and KB cells and into 10-day-old chick embryos.

Other diagnostic methods

Blood was collected from patients for serological tests. The group-specific precipitating sera for streptococci of groups A, C and G were those obtained from the Institute 'Dr I. Cantacuzino', Bucharest.

Fermentative activity of streptococci was determined in peptone water containing 1% lactose, sorbitol or trehalose, using bromthymol blue as indicator.

Autoserodiagnosis was performed by the ring precipitation test, using antigens prepared from the strain Bi.Gh. This was considered the most representative strain for the outbreak since it was isolated in pure culture from a biopsy specimen from an untreated patient. Two antigens were prepared according to Lancefield's techniques; carbohydrate C, by extracting with N/5 hydrochloric acid for 10 min. at 100° C., without further purification, and 'protein antigen' by extracting with N/20 HCl for 15 min. at 100° C. without further purification. The latter is the technique used for the M protein of group A streptococci (Lancefield, 1928*a*, *b*; 1933).

The antistreptolysin-O titration in patients' sera was performed by the method of Rantz & Randall (1945), using streptolysin-O supplied by the Institute 'Dr I. Cantacuzino', Bucharest.

RESULTS

After 24 hr. incubation the urine and faeces cultures showed nothing significant. From the throat swabs some streptococcus viridans, pneumococci, some Gram-negative diplococci, some yeasts, and two haemolytic streptococci (specimens I.St. and P.Gh.) were isolated (Table 1).

Table 1. *Strains of streptococci isolated from untreated and treated patients, and from convalescents*

				Specimens			Isolated on or in			Biochemical type		
Name	Age (yr.)	Sex	Anti-biotics	TS*	CL*	Days from onset	Blood agar	Mouse	Group	Tr†	So†	La†
Untreated patients												
I.St	56	M	None	+	.	16	+	+	C	−	+	+
P.Gh.	35	M	None	+	.	10	+	+	C	−	+	+
L.V.	39	M	None	+	.	9	−	+	C	−	+	+
M.A.	30	M	None	+	.	12	−	+	C	−	+	+
Bi.Gh.	31	M	None	+	+	7	+‡	+§	C	−	+	+
B.M.	25	F	None	+	.	6	−	+	−	.	.	.
P.M.	8	F	None	.	+	15	+	.	A	+	+	+
V.I.	14	M	None	+	.	8	+	.	G	.	.	.
G.A.	28	F	None	+	.	14	+	.	C	−	+	+
I.E.	36	F	None	+	.	11	+	.	C	−	+	+
D.V.	10	M	None	.	+	23	−	−	−	.	.	.
Treated patients												
Bu.Gh.	44	M	T	.	+	23	+‡	+‖	C	−	+	+
C.V.	31	M	P,T	+	.	19	−	−	−	.	.	.
F.M.	13	M	P,T	+	.	7	−	−	−	.	.	.
R.M.	25	F	P,T	+	.	6	−	−	−	.	.	.
Convalescents												
S.A.	34	M	T	+	.	124	+	.	C	−	+	+
G.M.	26	M	P,T	+	.	120	+	.	C	−	+	+

* TS = throat swab; CL = cervical lymph node.
† Tr = trehalose; So = sorbitol; La = lactose.
‡ Also positive in tissue culture and chick embryo.
§ Also positive in rabbit and guinea-pig.
‖ Also positive in rabbit, negative in guinea-pig.
Antibiotics: T = tetracycline; P = penicillin.

The mice inoculated with the pharyngeal exudate from patient I.St. died in 24 hr. of septicaemia with a capsulated strongly beta-haemolytic streptococcus; 96 hr. later, the mice inoculated with throat swabs L.V. and M.A. died also of septicaemia and intense intravascular haemolysis. In all smears of mouse blood or organs the same capsulated streptococci, often arranged in pairs, were found.

https://doi.org/10.1017/S0022172400042145 Published online by Cambridge University Press

A biopsy specimen from a cervical lymph node of the patient Bi.Gh. was available on the third day of the investigations, and the suspension of this was inoculated intraperitoneally in various animals and on culture media, with the following results: a 3 kg. rabbit died after 24 hr. and his spleen was passed to another rabbit which died after 17 hr. Inoculated mice died after 48 hr. and one of two guinea-pigs after 72 hr. All the dead animals showed intense hyperaemia of the abdominal wall and peritoneum, intense haemolysis and heavy streptococcal septicaemia.

A second lymph-node biopsy was carried out, on patient Bu.Gh. on the 23rd day of illness, while he was being treated with tetracycline and chloramphenicol. Smears showed pus cells and a few streptococci. Both young and adult mice inoculated with this material died after 48 hr. and a rabbit after 96 hr. Inoculated guinea-pigs survived.

Cultures from both these biopsy specimens, on solid and in liquid media and in chick embryos and tissue culture, showed a pure growth of the same beta-haemolytic capsulated streptococcus. Six strains of this streptococcus were isolated from the first ten patients investigated (I.St., P.G., L.V., M.A., Bi.Gh., B.M., Bu.Gh., C.V., F.V., R.M.), a high proportion since most were in the second to the fourth week of illness and four were receiving antibiotic treatment. Three of these, who were receiving penicillin, showed negative cultures.

All attempts to isolate another pathogenic agent were negative, as well as serological investigations for infective mononucleosis, tularaemia, adenovirus and other virus infections.

Streptococci of group A (*Streptococcus pyogenes*), which are responsible for most severe human streptococcal infections, show a low and variable pathogenicity for mice and rabbits, and it was therefore remarkable to isolate six strains from a human epidemic which showed a high initial pathogenicity for these animals, which were strongly haemolytic *in vitro* and *in vivo*, and which predominantly appeared as capsulated diplococci. The explanation was provided by the results of serological grouping of these strains, which all proved to belong to Lancefield's group C.

Group C streptococci occasionally isolated from humans are found as a commensal of pharyngeal and vaginal mucosa, and cause a small proportion of sporadic benign streptococcal infections without delayed non-suppurative complications. Such streptococci belong to the *Str. equisimilis* type, and never appear to cause epidemics. Biochemical typing showed that the strains in this epidemic all fermented sorbitol but not trehalose and therefore belonged to the animal type of group C (*Str. zooepidemicus*). They were all of the lactose-positive subtype. Cottoni & Floch (1939), in their study on the experimental pathogenicity of beta-haemolytic streptococci, pointed out the high virulence of the animal strains for mice and even more for rabbits.

The serological and biochemical typing of these strains thus confirmed the animal origin of this epidemic; such an origin had appeared certain as a result of the epidemiological investigation (Teodorovici, 1969).

During the next few days two more strains of the same group and type were isolated by the local P. Neamtz laboratory (specimens G.A. and I.E.) together

with one strain of group G (V.I.), and one of group A from a lymph node (P.M.). Specimen D.V. was negative (Table 1).

Since *Str. zooepidemicus* has not been previously implicated as the cause of an outbreak with glomerulonephritis as a complication, it is fortunate that we were able to isolate the same agent from uncontaminated specimens, i.e. biopsies of lymph nodes from patients Bi.Gh. and Bu.Gh., thus strengthening the evidence for it being the causal agent. Once this had been established it was possible to prevent further cases of glomerulonephritis by early penicillin treatment of new cases of sore throat; consequently, new attempts to isolate strains of streptococci from patients gave negative results.

Table 2. *Strains of streptococci isolated from throat swabs of carriers at the dairy farm, and from samples of cow's milk*

Name	Age (yr.)	Sex	Isolated on or in: Blood agar	Isolated on or in: Mouse	Group	Biochemical type: Tr*	Biochemical type: So*	Biochemical type: La*
			Carriers					
N.Gh.	22	M	+	.	A	+	−	+
S.M.	45	M	+	.	A	+	−	+
M.C.	29	M	+	.	C	−	+	+
C.I.	27	M	+	.	G	.	.	.
N.I.	34	F	+	.	C	−	+	+
N.L.	39	F	+	.	A	+	−	+
L.M.	23	F	+	.	A	+	−	−
D.M.	41	F	+	.	G	.	.	.
T.C.	22	M	+	.	A	+	−	+
B.V.	29	M	+	.	A	+	−	−
A.V.	37	M	+	.	A	+	−	+
P.A.	40	F	+	.	C	−	+	+
A.V.	25	M	+	.	C	−	+	−
			Cow's milk					
Sample 1	.	.	+	+	C	−	+	+
Sample 2	.	.	+	+	C	−	+	+
Sample 3	.	.	+	+	C	−	+	+

* Tr = trehalose; SO = sorbitol; La = lactose.

Three other strains of *Str. zooepidemicus* were isolated by the local veterinary laboratory from 277 samples of cow's milk from animals with mastitis in one of the great dairy farms supplying the town of P. Neamtz. At the same time, adult workers at this dairy farm were examined in a search for streptococcal carriers. Thirteen carriers were found, and four of these were carrying group C strains, a very high proportion. Of these four, one was of the human type, and the other three were identified as *Str. zooepidemicus* of the lactose-positive subtype similar to the strains isolated from our patients (Table 2). From one of these carriers (P.A.) the same type of streptococcus was isolated four times during 3 months of supervision.

Two further strains of *Str. zooepidemicus* were isolated from convalescent patients (S.A. and G.M.) 4 months after the acute stage of their illness (Table 1). It should be pointed out that two patients show chronic sequelae, nephrotic syndrome and hypertension, 7 months after the acute phase.

Because of the great variety of streptococcal groups and types, we made use of autoserodiagnosis, as a ring precipitation test, as further evidence of the aetiologic role of the agent we had isolated. As shown in Table 3, all the precipitation reactions with patients' sera were positive with both the antigens prepared from the strain Bi.Gh. by the Lancefield techniques.

Antistreptolysin-O (ASO) titres were generally low.

Table 3. *Serological results with sera from untreated and treated patients, and from convalescents*

Name of patient	Strepto-coccal group	Precipitation with patient's serum using Lancefield's 'C' extract	Precipitation with patient's serum using Lancefield's Protein extract	Antistrepto-lysin-O titre
		Untreated patients		
I.St.	C	+	+ +	125
P.Gh.	C	+ +	+ + +	125
L.V.	C	+	+	50
M.A.	C	.	.	125
Bi.Gh.	C	+ +	+ + +	100
B.M.	—	±	.	166
P.M.	A	−	−	125
G.A.	C	+ +	−	166
I.E.	C	+ +	+ + +	100
D.V.	—	+	+	50
		Treated patients		
Bu.Gh.	C	+ + +	+ +	333
C.V.	—	.	.	12
F.M.	—	+ + +	+ + +	125
R.M.	—	+ + + +	+ + + +	250
		Convalescents		
S.A.	C	+ + +	+ + + +	120
G.M.	C	+ + +	−	12

DISCUSSION

The evidence for the part played by the group C streptococcus (*Str. zooepidemicus*) of lactose-positive subtype in the outbreak studied was based on the isolation of strains with very similar characteristics from (*a*) two biopsies of inflamed cervical lymph nodes from patients Bi.Gh. and Bu.Gh., (*b*) four out of five throat swabs from untreated patients, (*c*) two convalescent carriers, (*d*) three apparently normal carriers among people in close contact with cows in one dairy farm, (*e*) throat swabs from two other patients, examined by the P. Neamtz laboratory, and (*f*) three samples of cow's milk from the dairy farm in which the carriers under (*d*) worked, isolated by the local veterinary laboratory. All these

strains, isolated from various sources by three different laboratories, belong to the same biochemical type and subtype of group C streptococci, a type which is not usually found in man and which has not yet been shown to cause nephritis in man.

In this outbreak the autoserodiagnostic test proved to be efficient. Despite the fact that the acid extraction techniques used did not furnish pure carbohydrate and protein antigens, we are of the opinion that the positive results obtained with patients' sera were, in this investigation, more valuable than the ASO titration as evidence of infection.

Chronic mastitis in cows is generally produced by *Streptococcus agalactiae* (group B), and the subacute mastitis affecting one-quarter of the udder is often the result of infection with *Str. dysgalactiae*, a non-haemolytic type of group C; other streptococci, such as *Str. pyogenes* (group A) or *Str. uberis* (group D), are seldom encountered. However, Buxton (1949) described two small epizootics of a very severe form of mastitis caused by group C streptococci type *zooepidemicus* subtype lactose-positive, corresponding to the serological type 2 of Bazeley & Battle (1940) which, because of its characteristic appearance as diplococci, was also called 'diplostreptococcus' (Haupt, 1964). The streptococci isolated in the outbreak described here appear to fall in this subtype.

Almost every new epidemic of acute glomerulonephritis reported reveals new types of nephritogenic streptococci of group A (Dillon, Moody, Maxted & Parker, 1967; Dillon, Reeves & Maxted, 1968; Perlman, Herdman, Kleinman & Vernier, 1965; Top, Wannamaker, Maxted & Anthony, 1967). In the outbreak of 1966 in Trinidad, from patients with typical acute glomerulonephritis, in addition to 25 strains of group A, types Trinidad A and B, three strains of group G and one of group C were also isolated; the authors point out that the group G strain possesses the M 12 antigen characteristic of the well known nephritogenic type 12 of group A (Maxted & Potter, 1967; Poon-King *et al.* 1967). It is clear that the list of streptococcal types in group A is not yet closed, and the same is probably true of the nephritogenic streptococci. The search for a nephritogenic factor could probably bring new light into this field.

SUMMARY

A local milk-borne outbreak of 85 cases of sore throat with cervical lymphadenitis, a third of whom later developed acute glomerulonephritis as a complication, is described. Renal involvement was shown by Volhard's criteria, filtered fraction data and renal biopsy findings. From lymph-node biopsies from patients, from the pharyngeal exudate of patients and carriers, and from three samples of cow's milk, 16 strains of beta-haemolytic streptococci of Lancefield group C, type *zooepidemicus* subtype lactose-positive, were isolated.

The aetiological role of the streptococcus isolated in this outbreak was confirmed by autoserodiagnosis (precipitation tests with Lancefield's antigens and patients' sera) which in this case proved more valuable than antistreptolysin-O titration as evidence of infection.

Two of the 85 patients, 7 months after the acute phase of illness, show nephrotic oedema and hypertension as chronic sequelae.

We are pleased to express our gratitude to Dr M. T. Parker and Dr W. R. Maxted for much advice, and confirmation of our serological and biochemical findings.

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