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Characterization of *Streptococcus zooepidemicus* (Lancefield group C) from human and selected animal infections

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SUMMARY

We assembled an international collection of strains from sporadic and epidemic human infection with *Streptococcus zooepidemicus* (Lancefield group C) for laboratory study. Cultural and physiological characteristics of the isolates were determined, including biotyping with the API 20 STREP test kit and susceptibility testing with penicillin, erythromycin and tetracycline. The strains were examined for bacteriocin production and sensitivity and typed with a specially developed group-C streptococcal bacteriophage system incorporating a panel of 14 phages. Results of these tests gave useful discrimination between many of the strains: differences were shown between each of the major outbreak strains, including those complicated by post-streptococcal glomerulonephritis.

Serious group C streptococcal infection may be caused by *S. zooepidemicus* and isolates should be identified to species level; the application of a typing scheme such as this may help to distinguish epidemiological patterns of infection.

INTRODUCTION

Streptococcus zooepidemicus causes infection in a wide range of animals but it has been found rarely in man (Parker, 1983). The few reports of human infection include upper respiratory tract infection, cervical lymphadenitis, pneumonia, septicaemia, endocarditis and meningitis, usually in patients in close contact with

horses or drinking unpasteurized milk (Barnham, Thornton & Lange, 1983). It seems that the infection should, in general, be regarded as a zoonosis.

Human infection is often sporadic and may be complicated by post-streptococcal glomerulonephritis (PSGN) (Barnham, Ljunggren & McIntyre, 1986). Recent reports of outbreaks in communities where unpasteurized dairy products are consumed featured pharyngitis, complicated in a proportion of patients by PSGN (Duca *et al.* 1969; Barnham, Thornton & Lange, 1983), or severe invasive disease with a high mortality rate (Ghoneim & Cooke, 1980; Morbidity and Mortality Weekly Report, 1983; PHLS Communicable Disease Surveillance Centre, 1984, unpublished) although some patients at risk of infection remained well.

We have gathered together an international collection of strains of *S. zooepidemicus* from human infection, together with some associated isolates from animals. We present here the results of laboratory studies to show the characteristics of the isolates, and the development of a typing system based on bacteriocin, bacteriophage and biotyping tests. We hope that the application of this system will help in the investigation of future incidents.

MATERIALS AND METHODS

Collection of organisms. Isolates of *S. zooepidemicus* were collected from sporadic and epidemic human infections, and from related animal sources, as shown in Table 1. Organisms were isolated locally in North Yorkshire or kindly donated by doctors and laboratories as shown in the table. Index strains from the Halifax outbreak (isolate number 8a) and the Northallerton outbreak (10c) have been laid down in the National Collection of Type Cultures at the Central Public Health Laboratory, Colindale, code numbers NCTC 11854 and 11606 respectively. Altogether 46 isolates were assembled: 31 from human infection, 11 from animals and 3 from dairy products, plus 1 from a carrier (whether human or animal unknown) in the follow-up studies to the Romanian outbreak of 1968.

Isolates numbered 1 and 6 were from human infections related to horses (see the references in Table 1) and the series of isolates numbered 8, 9, 10 and 11 were from infections considered to be due to the consumption of unpasteurized cow's milk or its products. Isolate 7 was from a cat fancier with an infected finger who also kept poultry and donkeys. Series 13 was from an episode of bovine mastitis which did not lead to human infection. In series 8 and 13 isolates from horses on the farms were included in the collection as these animals were thought to be possible original sources of the bovine infection. Isolate 12 was from one of a litter of piglets that died of septicaemia in North Yorkshire while our study was in progress; there was no related human infection.

Acute PSGN was seen in man as a complication of infection with isolate numbers 5, 10a, 10c and 11a.

Colonial morphology. Organisms were cultured on Columbia agar (Oxoid Ltd., Basingstoke, Hants., code CM331) containing 5% defibrinated horse blood, incubated at 37 °C in air for 24 h and examined with a plate microscope.

Identification. The Lancefield group C antigen present in all strains was detected initially with the Streptex grouping kit (Wellcome Diagnostics, Dartford, Kent) and confirmed by testing acid extracts, in parallel with a reference strain, against

Table 1. *Origin of the study strains of* S. zooepidemicus

Isolate no.	Patient/ animal	Source	Date	Given by/reference
Sporadic infections				
1	Patient (neonate)	C.s.f.	1976–82	Prof. Zanen/Mulder *et al.* (1984)
2	Patient	Leg ulcer	Oct. 82	—/Barnham (1987)
3	Patient	Sore throat	Jan. 83	—/Barnham (1987)
4	Patient	Knee aspirate	May 85	—/Barnham *et al.* 1987)
5	Patient	Blood culture	July 85	Dr Lunggren/Barnham *et al.* 1987)
6a	Patient	Blood culture	Sept. 85	Dr Skirrow/Barnham *et al.* 1987)
6b	Horse	Tracheostomy	Sept. 85	Worcester VIC/Barnham *et al.* (1987)
6c	Horse	Tracheostomy	Sept. 85	Worcester VIC/Barnham *et al.* (1987)
7	Patient	Finger	Feb. 86	—/Barnham (unpublished)
Halifax (Yorkshire) outbreak				
8a	Patient	Blood culture	Mar. 84	Dr Edwards/PHLS CDSC (1984)
8b	Patient	Blood culture	Apr. 84	Dr Edwards/PHLS CDSC (1984)
8c	Patient	Blood culture	Apr. 84	Dr Edwards/PHLS CDSC (1984)
8d	Patient	Aneurysm	Apr. 84	Dr Edwards/PHLS CDSC (1984)
8e	Patient	Blood culture	Apr. 84	Dr Edwards/PHLS CDSC (1984)
8f	Patient	Blood culture	Apr. 84	Dr Edwards/PHLS CDSC (1984)
8g	Patient	Blood culture	Apr. 84	Dr Edwards/PHLS CDSC (1984)
8h	Patient	Blood culture	Apr. 84	Dr Edwards/PHLS CDSC (1984)
8i	Patient	C.s.f.	May 84	Dr Edwards/PHLS CDSC (1984)
8j	Patient	Blood culture	June 84	Dr Edwards/PHLS CDSC (1984)
8k	Patient	Necropsy	May 84	Dr Edwards/PHLS CDSC (1984)
8l	Cow T66	Udder	May 84	Leeds VIC/PHLS CDSC (1984)
8m	Cow B278	Milk	May 84	Leeds VIC/PHLS CDSC (1984)
8n	Cow Y246	Milk	May 84	Leeds VIC/PHLS CDSC (1984)
8o	Bulk milk	Milk	May 84	Leeds VIC/PHLS CDSC (1984)
8p	Horse	Vagina	May 84	Leeds VIC/PHLS CDSC (1984)
New Mexico outbreak				
9a–h	Patients	Blood	Jul–Sept. 83	Dr Facklam/MMWR (1983)
9i	Food	Cheese	Jul–Sept. 83	Dr Facklam/MMWR (1983)
9j	Food	Milk	Jul–Sept. 83	Dr Facklam/MMWR (1983)
Northallerton (Yorkshire) outbreak				
10a	Patient	Sore throat	Apr 82	—/Barnham *et al.* (1983)
10b	Patient	Sore throat	Apr 82	—/Barnham *et al.* (1983)
10c	Patient	Sore throat	Apr 82	—/Barnham *et al.* (1983)
10d	Patient	Throat	July 82	—/Barnham *et al.* (1983)
Romania nephritis outbreak				
11a	Patient (L.V.)	Sore throat	1968	U of M 73–112/Duca *et al.* (1969)
11b	Outbreak collection,carrier		1968	Prof Duca/Duca *et al.* (1969)
Veterinary infections				
12	Piglet	Necropsy	Nov. 84	Thirsk VIC (farm, Darlington)/—
13a	Cow	Udder (mastitis)	Feb. 85	Thirsk VIC (farm, Ravenscar)—
13b	Same cow	Udder (mastitis)	Mar. 85	Thirsk VIC (farm, Ravenscar/—
13c	Mare A	Vagina	Mar. 85	Thirsk VIC (farm, Ravenscar)/—
13d	Mare B	Vagina	Mar. 85	Thirsk VIC (farm, Ravenscar)/—

C.s.f, cerebrospinal fluid; VIC, Ministry of Agriculture Fisheries and Food, Veterinary Investigation Centre; PHLS CDSC, Public Health Laboratory Service, Communicable Disease Surveillance Centre; MMWR, Morbidity and Mortality Weekly Report, Center for Disease Control, Atlanta; U of M, University of Minnesota streptococcal collection no.; Thirsk is in North Yorkshire.

serum (prepared at the Streptococcus Reference Unit, CPHL, Colindale) in a double diffusion precipitation test in agarose gel (Lancaster & Sherris, 1960).

The biochemical methods used were the same as those employed by Colman & Ball (1984). The API 20 STREP kit was used (API Laboratory Products Ltd., Basingstoke, Hants), supplemented with tests for resistance to optochin (5 μg disk) and bacitracin (0·1 unit disk) and also for production of an extracellular polysaccharide in a sucrose medium (TYC agar, Lab M, Salford, Lancs).

Surface T-antigen typing. All strains were screened in the Colindale laboratory for the presence of T-protein antigens according to the scheme developed by Efstratiou (1983) for typing human strains of group C and G streptococci.

Bacteriocin typing. Inhibitor 'fingerprinting' was performed in the Dunedin laboratory essentially as described by Tagg & Bannister (1979). The test medium was Columbia agar base (Gibco Laboratories) containing 5% (v/v) human blood and poured on a base layer of saline agar. For producer (P)-typing the test strain was grown as a diametric streak culture at 32 °C for 18 h before removing the growth, sterilizing the surface by exposure to chloroform vapours and then cross-inoculating the nine standard indicator cultures.

Six standard producers (P1–P6) were used for sensitivity (S)-typing. Producers P1–P5 were grown as streak cultures for 24 h at 32 °C. In this study P1 was incubated anaerobically, since this has been found (Tagg & Bannister, 1979) to significantly enhance its inhibitory activity and overcomes some problems of variable production from run to run. P6 was incubated at 37 °C in 5% CO_2 in air. The test strains were cross-inoculated after scraping and chloroforming the producer streaks.

The P-type and S-type results of the test strains represent in code form the patterns of inhibition of the nine indicators and sensitivity to the six producers respectively.

Bacteriophage typing. A group-C bacteriophage typing system was specially developed for this study in the Minneapolis laboratory. Mitomycin C induced lysate from 72 group-C cultures representing 16 areas of the world were examined for the presence of phage in the form of plaques or lysis on 12 group-C indicator strains. Thirty of the cultures produced lysis or plaques. Twelve of these lysogenic strains were then selected for phage typing on the basis of their lysates yielding phage plaques upon dilution and demonstrating a unique pattern of lysis. Two virulent bacteriophages were propagated by infection on indicator strains and added to the panel to make a total of 14 phages.

Bacterial cultures were grown in 549 broth which consisted of 8% Proteose Peptone No. 3 (Difco Laboratories, Detroit MI 48232), 0·2% Yeast Extract (Difco), 40 mM Hepes buffer (U.S. Biochemical Corp., Cleveland OH 44128) and adjusted to pH 7·7 with 5 N-NaOH. After autoclaving the broth was completed by the addition, to a final concentration, of 14 mM glucose and 2·7 mM-$CaCl_2$.

Lawns for the detection of phage plaques were grown on 749Y plates consisting of 4% Proteose Peptone No. 2 (Difco) 80 mM Hepes buffer, 130 mM-NaCl, adjusted to pH 6·9 with 5 N-NaOH and 1% Noble Agar (Difco). The agar medium after autoclaving was completed with the addition of 6 mM glucose, 1·8 mM-$CaCl_2$, 1 mM-$MgSO_4$, 5% horse serum and 37 units/ml hyaluronidase (Sigma).

The method for phage typing group-C streptococci was essentially as described

Table 2. *Biochemical identification of* S. zooepidemicus *isolates*

Isolate no.	VP	HIP	ESC	PYR	AGL	GUR	BGL	PAL	LAP	ADH	RIB	ARA	MAN	SOR	LAC	TRE	INU	RAF	AMD	GLY	BHS	API profile no.
Sporadic infections																						
1	−	−	+	−	−	+	−	+	+	+	+	−	−	+	+	−	−	−	+	+	+	4463607
2	−	−	+	−	−	+	−	+	+	+	+	−	−	+	+	−	−	−	+	+	+	4463607
3	−	−	+	−	−	+	−	+	+	+	+	−	−	+	+	+	−	−	+	+	+	4463617
4	−	−	+	−	−	+	−	+	+	+	+	−	−	+	+	−	−	−	+	+	+	4463607
5	−	−	+	−	−	+	−	+	+	+	+	−	−	+	+	−	−	−	+	+	+	4463607
6a–c	−	−	+	−	−	+	−	+	+	+	+	−	−	+	+	−	−	−	+	+	+	4463607
7	−	−	+	−	−	+	−	+	+	+	−	−	−	+	+	+	−	−	+	+	+	4461617
Halifax outbreak																						
8a–o	−	−	+	−	−	+	−	+	+	+	+	−	−	+	+	−	−	−	+	+	+	4463607
8p	−	−	+	−	−	+	−	+	+	+	−	−	−	+	+	−	−	−	+	+	+	4461607
New Mexico outbreak																						
9a–j	−	−	+	−	−	+	−	+	+	+	+	−	−	+	+	−	−	−	+	+	+	4463607
Northallerton outbreak																						
10a–c	−	−	−	−	−	+	+	+	+	+	−	−	−	+	+	−	−	−	+	+	+	0471607
10d	−	−	+	−	−	+	−	+	+	+	−	−	−	+	+	−	−	−	+	+	+	4461607
Romania outbreak																						
11a	−	−	+	−	−	+	−	+	+	+	+	−	−	+	+	−	−	−	+	+	+	4463607
11b	−	−	+	−	−	+	−	+	+	+	+	−	−	+	+	−	−	+	+	+	+	4463647
Veterinary infections																						
12	−	−	+	−	−	+	−	+	+	+	+	−	−	+	+	−	−	−	+	+	+	4463607
13a–d	−	−	+	−	−	+	−	+	+	+	+	−	−	+	+	−	−	−	+	+	+	4463607

VP, Acetoin production.
HIP, Hippurate hydrolysis.
ESC, Aesculin hydrolysis.
PYR, Pyrrolidonylarylamidase.
AGL, Alpha galactosidase.
GUR, Beta glucuronidase.
BGL, Beta galactosidase.
PAL, Alkaline phosphatase.
LAP, Leucine aminopeptidase.
ADH, Arginine hydrolysis.
RIB, Fermentation of ribose.
ARA, Fermentation of L arabinose.
MAN, Fermentation of mannitol.
SOR, Fermentation of sorbitol.
LAC, Fermentation of lactose.
TRE, Fermentation of trehalose.
INU, Fermentation of inulin.
RAF, Fermentation of raffinose.
AMD, Fermentation of starch.
GLY, Fermentation of glycogen.
S, Beta haemolysis on Columbia agar (Oxoid CM 331) with 5% horse blood.

by Skjold & Wannamaker (1976) and Skjold *et al.* (1983) for group-A M49 streptococci, with a few modifications. The streptococcal lawns were made on 749Y plates with a 1 in 5 dilution of culture prepared by making two consecutive 1% 18 h transfers in 549 broth at 35 and 26 °C respectively. *S. zooepidemicus* was phage-typed with 14 bacteriophages by applying two dilutions of phage on lawns at RTD (near confluent lysis) and at 10 × RTD (confluent lysis). Lawns which demonstrated 50 or more plaques at either dilution of phage were considered positive.

Minimum inhibitory concentrations (MIC) of three antibiotics. MIC's of penicillin, tetracycline and erythromycin were determined by inoculation of the isolates of *S. zooepidemicus* on to Petri dishes containing Iso-sensitest Agar (Oxoid, code CM471) with 5% defibrinated horse blood, incorporating doubling dilutions of antibiotic (Mast Adatabs; Diamed Dignostics Ltd, Merseyside). Organisms were grown for 24 h in Todd Hewitt Broth (BBL 11736; Beckton Dickinson UK Ltd,

Table 3. *Results of bacteriocin typing of* S. zooepidemicus

Isolate no.	P-type	S-type
Sporadic infections		
1	000	57
2	000	57
3	000	57
4	000	53
5	000	53
6a–c	000	53
7	004	57
Halifax outbreak		
8a–p	266	57
New Mexico outbreak		
9a–f, i, j	000	53
9g, h	226	52
Northallerton outbreak		
10a	000	53
10b–d	000	57
Romania outbreak		
11a	407	53
11b	000	57
Veterinary infections		
12	000	53
13a–d	000	53

Oxford), cultures were well shaken, diluted 1 in 50 in sterile saline solution and dispensed to the dishes in 10 μl amounts using a multipoint inoculator (Mast Scan 100; Diamed). The dishes were incubated for 24 h in air and MIC's read and recorded as the lowest antibiotic concentrations completely inhibiting growth.

RESULTS

Colonial morphology

At 24 h, colonies varied between 0·5 and 1·5 mm diameter, were typically opaque and circular, with an entire edge, convex elevation and smooth surface. A few strains showed umbonate colonies. Isolate no. 1 gave a mixed appearance with some very mucoid colonies spreading along the lines of inoculation. The colonies of all isolates were surrounded by wide zones of beta-haemolysis.

Identification/biotype

Extracts of each isolate gave positive results with the group-C reagent in agglutination tests and reactions of identity with stock extracts of Lancefield group C.

Results of the biochemical identification tests are given in Table 2. Reactions that were generally given by the species included the fermentation of sorbitol, lactose, starch and glycogen, production of beta-glucuronidase and phosphatase

Table 4. *Patterns of bacteriophage susceptibility of* S. zooepidemicus

Isolate no.	Susceptibility to phage no. 1	2	3	4	5	6	7	8	9	10	11	12	13	14
Sporadic infections														
1	.	+	.	+	.	+	.	.	.	+	.	+	.	+
2	+	.	.	.	.	.	.	.	.	.	.	+	.	.
3	.	.	.	.	.	.	.	.	.	.	.	+	.	.
4	.	.	.	.	.	.	.	.	.	.	.	+	.	.
5	.	+	+	+	.	+	.	+	.	+	+	+	+	+
6a	.	.	.	+	.	+	.	.	+	.	.	+	.	.
6b,c	No lysis													
7	.	.	.	+	.	+	.	.	.	.	.	.	.	+
Halifax outbreak														
8a–d, g, l, p	.	+	.	.	.	.	+	.	.	.	.	+	.	.
8e, f, i–k, o	.	+	.	.	.	.	+	.	.	.	+	+	.	.
8h	.	+	.	.	.	.	+	.	.	.	.	.	.	.
8m,n	No lysis*													
New Mexico outbreak														
9a, b, d–f, i	.	+	+	+	.	+	.	.	.	.	.	+	.	+
9c	.	.	+	+	.	+	.	.	.	.	.	+	.	+
9j	.	+	.	.	.	.	.	.	.		.	+	.	.
9g, h	.	.	.	.	.	.	+	.	.	.	+	+	+	.
Northallerton outbreak														
10a	.	.	.	+	.	+	.	.	.	.	.	.	.	.
10b–d	.	.	.	.	.	.	.	.	.	.	.	+	.	.
Romania outbreak														
11a	.	.	.	.	.	.	.	.	.	+	.	.	.	.
11b	No lysis													
Veterinary infections														
12	.	.	.	.	.	.	.	.	.	.	.	+	.	.
13a, c	.	.	.	.	.	.	.	.	.	.	.	+	.	.
13b	No lysis													
13d	.	.	.	+	.	+	.	.	.	.	.	+	.	+

*, 8n possibly type 7/12.

and the failure to produce pyrrolidonylarylamidase, hydrolyse hippurate or give the Voges–Proskauer reaction. All but 8 of the isolates gave the API profile number 4463607; unusual reactions included a failure to ferment ribose (seen in 6 isolates), positive trehalose fermentation (2 isolates) and late raffinose fermentation (1 isolate).

All the isolates were resistant to disks containing 0·1 unit bacitracin and 5 μg optochin, and none produced dextran or levan from sucrose.

T-antigen typing

T-protein antigens were not detected on any isolate using the collection of antisera prepared with human isolates of the Lancefield groups A, C or G.

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Table 5. *Minimum inhibitory concentrations (MIC) of three antibiotics against* S. zooepidemicus

Isolate no.	MIC (mg/l) of antibiotic: Penicillin	Tetracycline	Erythromycin
Sporadic infections			
1	0·008	1·0	0·008
2	0·008	4·0	0·03
3	0·015	16·0	0·06
4	0·008	1·0	0·06
5	0·008	2·0	0·06
6a–c	0·015	4·0	0·06
7	0·015	4·0	0·06
Halifax outbreak			
8a, b, d–h, j, k, p	0·008	4·0	0·06
8c, i	0·008	4·0	0·03
8m, n	0·008	2·0	0·06
8l	0·008	2·0	0·03
8o	0·008	0·5	0·015
New Mexico outbreak			
9c–f, j	0·015	8·0	0·06
9a, b, i	0·015	4·0	0·06
9g, h	0·015	2·0	0·06
Northallerton outbreak			
10a, b	0·015	4·0	0·06
10c, d	0·008	8·0	0·06
Romania outbreak			
11a	0·008	8·0	0·06
11b	0·008	4·0	0·06
Veterinary infections			
12	0·015	8·0	0·06
13a, b	0·008	4·0	0·03
13c	0·008	4·0	0·06
13d	0·015	4·0	0·06

Bacteriocin typing

Results of the bacteriocin typing tests are shown in Table 3. The scheme revealed 5 P-type and 3 S-type patterns in the collection of *S. zooepidemicus*. There were differences between each of the major outbreak collections (series 8, 9, 10 and 11). Isolates 9g, h were distinct in both P- and S-type from the other isolates in the New Mexico series; in the Northallerton series isolate 10a was distinct from the others in S-type.

Bacteriophage typing

Bacteriophage typing results are shown in Table 4. As with the bacteriocin typing, there were differences between each of the major outbreak collections; the scheme showed a difference between isolates 9g, h and others in the New Mexico series, and between isolate 10a and the others in the Northallerton series. Most of the isolates were susceptible to phage number 12 but none to number 5 in the panel.

Table 6. *Predominant patterns of combined bacteriocin, bacteriophage and biotyping results in the* S. zooepidemicus *collection*

Combined results of typing by bacteriocin: bacteriophage: API profile	Isolate numbers showing the pattern
P000, S53: 12: 4463607	4, 12, 13a, c
P000, S53: 2/3/4/6/12/14 complex: 4463607	9a–f, i
P226, S52: 7/11/12/13: 4463607	9g, h
P266, S57; 2/7/11/12 complex: 4463607	8a–g, i–l, o
P000, S57: 12: variable	3, 10b–d

Antibiotic MIC studies

MIC's of penicillin, tetracycline and erythromycin against the isolates of *S. zooepidemicus* are shown in Table 5. All isolates were susceptible to penicillin (MIC range 0·008–0·015 mg/l) and erythromycin (range 0·008–0·06 mg/l) but resistant to tetracycline (range 0·5–16·0 mg/l).

Combined typing patterns

Combining the results of typing by bacteriocin, bacteriophage and the API profile, isolates in the collection were grouped into five main patterns, as shown in Table 6. Other combined patterns were seen with individual isolates.

DISCUSSION

Human infection with *S. zooepidemicus* appears to be a rare event and has mostly followed close exposure to horses or the consumption of contaminated dairy products (Barnham, Thornton & Lange, 1983). When it does occur the infection may be overwhelming, as in many patients in the recent milk- and cheese-borne outbreaks (Ghoneim & Cooke, 1980; Morbidity and Mortality Weekly Report, 1983; PHLS Communicable Disease Surveillance Centre, 1984, unpublished). In view of its severity, *S. zooepidemicus* infection has been considered the most notable milk-borne disease of the last few years in Britain (Sharp, Paterson & Barrett, 1985). The infection is also of special interest as a cause of PSGN, which until recently was thought only to follow infection with *S. pyogenes* (Duca *et al.* 1969; Barnham, Thornton & Lange, 1983).

We put together an international collection of isolates from human infection in order to study and compare the organisms, and to develop a typing system that might help in epidemiological studies. Organisms from the first recorded outbreak of systemic infection, in Leeds in 1979 (Ghoneim & Cooke, 1980), were unfortunately not saved but we have assembled isolates from all the other recorded outbreaks and from a range of sporadic infections. Yorkshire has been a good area to gather the organisms as the practice of drinking raw milk is particularly common here (Sharp, Paterson & Barrett, 1985).

We found that the API 20 STREP profile led to the identification of the organisms but was less helpful as a biotyping tool by itself, as most of the strains gave the same profile, 4463607. Isolates from patients and cowman in the Northallerton outbreak were indistinguishable by bacteriocin and bacteriophage

typing although there were differences in two biochemical reactions between them (profiles 0471607 and 4461607 respectively).

A serotyping scheme for isolates of *S. zooepidemicus* from horses was reported by Bryans & Moore (1972), but this work was discontinued and the sera are no longer available (personal communication). They were able to detect a series of 15 type-specific protein antigens, acid extracts of which were labile to trypsin or pepsin. Mihalcu *et al.* (1982) raised three antisera which typed 42% of a collection of strains of Lancefield group C *S. equisimilis* but they completely failed to type *S. zooepidemicus*. We applied the T-protein antigen serotyping scheme developed by Efstratiou (1983) for human isolates of Lancefield group C and G streptococci but obtained no positive results. The system achieves 76% typability with human strains of *S. equisimilis* and the failure to type any of the strains of *S. zooepidemicus* emphasizes the antigenic differences between these species.

The only previous study of bacteriocin typing of group-C streptococci was that of Schofield & Tagg (1983), who found that certain strains of *S. zooepidemicus*, *S. equisimilis* and *S. dysgalactiae* produced bacteriocin-like inhibitors. Four of 8 strains of *S. zooepidemicus* produced inhibitors, all giving different P-types.

In the present study a new P-type (266), not seen with any previously tested streptococcus, was found in the organisms from the Halifax outbreak (series 8). Isolates 9g, h in the New Mexico collection gave a P-type (226) identical to the strain 4881 in the earlier study of Schofield & Tagg (1983), an isolate from an aborted foal in New Zealand. S-typing gives some useful further discrimination, particularly amongst the non-producer strains.

The group-C phage-typing system described here was developed specially for the purpose and modelled on the group-A type-49 phage-typing system of Skjold & Wannamaker (1976). The system appears to give useful discrimination, with differences shown between each of the main outbreak collections. Within outbreaks the differences were minor, as in series 8, or major, as in series 9 (where isolates 9g, h appeared quite different) and 10 (where 10a was different); these results concurred with the findings of bacteriocin typing and DNA fingerprinting (see Skjold *et al.* 1987), suggesting that in both the New Mexico and Northallerton outbreaks more than one strain was involved. Additional phage may be needed in the typing panel to differentiate the strains which are susceptible only to phage number 12. Human and animal strains of *S. zooepidemicus* were not susceptible to phage in the lysotyping scheme developed for group C streptococci by Mihalcu *et al.* (1982).

Many strains that show the common API profile number 4463607 can be distinguished by the combination of bacteriocin and bacteriophage typing, as shown in Table 6. We do not know if these groups could be divided further by elaboration of the typing systems, but they already give helpful information about the collected strains. Acute PSGN followed infection with isolates 5, 10a, c and 11a and these have come out differently in our combined typing system. Indistinguishable strains were found within many of the epidemiological clusters: the cow and mare A at Ravenscar (series 13), the patients, cow and milk at Halifax (series 8; the equine strain here varied only in the failure to ferment ribose), and many patients, the cheese and the milk from New Mexico (series 9). DNA fingerprinting of the organisms (Skjold *et al.* 1987) confirms many of these

distinctions and further discriminates between sporadic isolates of the same combined bacteriocin-, bacteriophage- and bio-type.

Carriage and infection with *S. zooepidemicus* is especially common in horses (Stableforth, 1959; Bryans & Moore, 1972; Erickson, 1980) and this might be a source of infection on a farm, perhaps by common grazing, environmental contamination or via handlers, to produce the unusual bovine mastitis that is such a hazard to man. The similar typing patterns of the equine and bovine isolates in the mastitis episodes (series 8 and 13) lend some support to this concept.

To gain more information on the nature and circumstances of *S. zooepidemicus* infection we suggest that isolates of group C streptococci from man should be identified to species level, at least in the following situations: when infection is severe, invasive or followed by PSGN, when there seems to be a link with animals, or when clusters occur. Examination of human and associated animal isolates by a typing scheme such as that described here, perhaps augmented with DNA fingerprinting, should then help to clarify the patterns of infection.

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