The epidemiology of beta-haemolytic non-Group A streptococci isolated from the throats of children over a one-year period

N. CIMOLAI^{1,2}, L. MACCULLOCH² AND S. DAMM²

¹ *Division of Medical Microbiology, Department of Pathology, University of British Columbia*

² *Program of Microbiology, Department of Pathology, British Columbia's Children's Hospital*

(Accepted 21 September 1989)

SUMMARY

The incidence of beta-haemolytic non-Group A streptococci (BHNAS) in the throats of a paediatric population was examined over a 1-year period. There was minimal seasonal fluctuation of Lancefield groups including species and biotypes within Groups C and G streptococci. A trend of increasing incidence with age of *Streptococcus anginosus* ('*Streptococcus milleri*') (possessing Groups C and G Lancefield antigens) was evident. A clinical impression of streptococcal pharyngitis was more common in patients with large-colony Groups C or G streptococci isolated from their throats compared with those patients where other BHNAS were isolated. This study is requisite to the planning of case control studies which are required to test the association of BHNAS (especially Groups C and G subgroups) and pharyngitis.

INTRODUCTION

Although beta-haemolytic non-Group A streptococci (BHNAS) have been associated with pharyngitis, their role in sporadic community-acquired pharyngitis is not confirmed (1). Several outbreaks have particularly implicated Groups C and G streptococci (2–7) and these reports are among the best of examples which support the role of these organisms as a etiologic agents in pharyngitis. The evidence from outbreaks is contrasted, however, to the significant carrier rates of BHNAS in asymptomatic populations (1). Many comparative studies of carrier rates in asymptomatic and symptomatic populations have been published, but there is a lack of well designed case-control studies which address the association of these bacteria with pharyngitis. The potential importance of finding BHNAS in throat swabs is emphasized by physicians' bias towards treatment in the context of positive reports (8).

Our understanding of the BHNAS has been complicated by the recognition of

major subsets within certain Lancefield groups, especially Groups C and G streptococci (1, 9). Beta-haemolytic Group C streptococci include *Streptococcus equisimilis*, *S. zooepidemicus*, and *S. anginosus* ('*S. milleri*'). Beta-haemolytic Group G streptococci include large colony Group G and *S. anginosus*. *S. anginosus* containing either Lancefield Groups C or G antigens are genetically homogeneous and can be distinguished practically from the other Group C and Group G streptococci by morphologic and biochemical criteria. The frequency of these species in clinical specimens including throat swabs has been examined in a few limited studies and their prevalences are considered significant enough to complicate therapy should only certain species be truly implicated in disease (10-12). Outbreak studies have not detailed the speciation of the implicated streptococci beyond Lancefield grouping, except for two reports which incriminated *S. zooepidemicus* (2, 13).

In order to conduct a case control study which we believe is requisite to establishing further the association between BHNAS and pharyngitis, the issue of seasonal prevalence of infection requires clarification. In symptomatic pharyngitis, there are opposite reports of seasonal variation (14, 15). In an asymptomatic population, seasonal variation has also been reported (16). Given our current understanding of taxonomic diversity within the BHNAS, such epidemiologic studies need to be reevaluated. We document here the incidence of these bacteria in a paediatric population over a 1-year period and focus especially upon the Groups C and G streptococci for which the current evidence of association with pharyngitis is most convincing.

METHODS

Throat swab specimens were obtained from 1730 paediatric patients who were admitted to British Columbia's Children's Hospital as out-patients between January and December, 1988. Patient ages ranged from 1 month to 18 years. These specimens were submitted by a variety of paediatricians primarily subserving Emergency, General Pediatric, and Surgical Clinics, and were obtained from patients in whom the diagnosis of streptococcal pharyngitis was to be excluded.

Swabs were transported in either Amies or Stuart's media. Upon arrival within 24 h at the laboratory, specimens were cultured on 5% sheep blood agar, incubated in anaerobic conditions for 48 h, and inspected at 24 and 48 h intervals. Semi-quantitation of beta-haemolytic streptococci was recorded as 1+, 2+, or 3+ corresponding to growth in primary, secondary, or tertiary areas of culture plates. BHNAS were Lancefield grouped by latex agglutination reagents (Streptex, Wellcome). Beta-haemolytic Groups C and G streptococci were further characterized after obtaining purity by one or two subcultures. Quality of haemolysis and colony size were documented. Group C streptococci were biochemically characterized using a rapid Voges-Proskauer (VP) test, trehalose fermentation, and sorbitol fermentation (11, 17). Group G streptococci were biochemically characterized by the rapid VP test, trehalose fermentation, lactose fermentation, raffinose fermentation and esculin hydrolysis (18). Weakly haemolytic, small-colony VP-positive Groups C and G streptococci were termed *S.*

anginosus. *S. equisimilis* was defined as beta-haemolytic large-colony VP-negative Group C streptococci which fermented trehalose but not sorbitol. *S. zooepidemicus* was defined as beta-haemolytic large-colony VP-negative Group C streptococci which fermented sorbitol but not trehalose. Beta-haemolytic large-colony VP-negative Group G streptococci were biotyped on the basis of a scheme previously proposed (18) BHNAS other than Groups B, C, and G, were pooled into a category of 'other'. The 'other' category therefore included Group F and non-groupable isolates.

Patient data was obtained from retrospective chart review.

RESULTS

Beta-haemolytic streptococci were found in 24.4% of the 1730 specimens. Group A was the predominant Lancefield group identified among the beta-haemolytic streptococci (15.8%) and BHNAS were identified in 8.6% of all specimens. Figure 1 details the distribution of the BHNAS including the two major subgroups of Groups C and G streptococci. The incidence of isolates among the VP-negative and VP-positive streptococci are similar. *S. equisimilis* was most frequently identified among the beta-haemolytic large-colony VP-negative Group C streptococci and biotypes 2 and 3 were predominant among the beta-haemolytic large-colony VP-negative Group G streptococci. (Table 1). Within the category labelled 'other', 22/47 isolates were Group F.

The seasonal incidence of BHNAS is relatively uniform (Fig. 2). There was a consistently low percentage of isolation from specimens per month (0-7.2%) throughout the year. Although there was a trend towards greater isolation of Group G *S. anginosus* in the early winter months, relatively minor fluctuations were noted for the Group C streptococci (both VP negative and VP positive) and the large-colony Group G streptococci (Table 2). Significant seasonal variation was not apparent for the large-colony Group G streptococcal biotypes.

Significant differences in the distribution of quantitation for positive cultures were not apparent for Group B, Group C, and large-colony VP-negative Group G streptococci where results were available (Table 3). A trend towards light growth was evident, however, for Group G *S. anginosus* and BHNAS other than Groups

Table 1. *Distribution of species and biotypes among beta-haemolytic large-colony Voges-Proskauer-negative Groups C and G streptococci*

Group C	
<i>Streptococcus equisimilis</i>	13 (92.9)*
<i>Streptococcus zooepidemicus</i>	1 (7.1)
Group G	
Biotype 1	1 (3.4)
Biotype 2	15 (51.7)
Biotype 3	12 (41.4)
Biotype 4	0 (0)
Biotype 5	1 (3.4)
Biotypes 6-8	0 (0)

* Figures within parentheses are percentages.

Fig. 1. Frequency of beta-haemolytic non-Group A streptococci (BHNAS) in pharyngeal specimens as percentage. Numbers in parentheses indicate absolute numbers of positive specimens. VP+, positive Voges-Proskauer test indicating *S. anginosus*. VP-, negative Voges-Proskauer test indicating large-colony Groups C or G streptococci.

Table 2. Incidence of beta-haemolytic Groups C and G streptococci by month (absolute members)

Streptococcal group	Month											
	J	F	M	A	M	J	J	A	S	O	N	D
Group C (total)	4	1	3	0	3	4	2	0	4	4	2	2
Group C (VP-)	2	1	2	0	1	3	0	0	2	1	2	0
Group C (VP+)	2	0	1	0	2	1	2	0	2	3	0	2
Group G (total)	1	4	2	2	1	5	2	1	7	2	8	7
Group G (VP-)	1	4	2	1	1	4	1	1	5	1	5	3
Group G (VP+)	0	0	0	1	0	1	1	0	2	1	3	4
Total C or G (VP-)	3	5	4	1	2	7	1	1	7	2	7	3
Total C or G (VP+)	2	0	1	1	2	2	3	0	4	4	3	6

VP+, positive Voges-Proskauer test. VP-, negative Voges-Proskauer test.

B, C, or G. The two commonly isolated biotypes (2 and 3) of large-colony Group G streptococci were evenly represented among the quantitation gradings.

The distribution of positive cultures for age group was determined (Table 4) where age data was available. The overall mean age for patients with BHNAS was 7.5 years. A trend towards increased isolation of Group B and large-colony VP-negative Group G streptococci in younger age groups was apparent. A trend towards isolation in older age groups was noted for Group G *S. anginosus*. Biotypes 2 and 3 of the large-colony Group G streptococci were evenly distributed over the age groups.

Clinical information was reviewed retrospectively for all patients with BHNAS where charts were available, and the frequencies of diagnosis of streptococcal or primary pharyngitis versus other illnesses were determined (Table 5). A diagnosis

Fig. 2. Frequency of beta-haemolytic non-Group A streptococcus in pharyngeal samples as a percentage of total monthly specimens.

Table 3. Quantitation of positive cultures for beta-haemolytic non-Group A streptococci (% of respective streptococcal group)

Streptococcal group	Quantitation		
	1+	2+	3+
Group B	14 (48)	6 (21)	9 (31)
Group C (total)	11 (39)	6 (21)	11 (39)
Group C (VP-)	6 (46)	2 (15)	5 (38)
Group C (VP+)	5 (33)	4 (27)	6 (40)
Group G (total)	16 (39)	14 (34)	11 (27)
Group G (VP-)	9 (31)	11 (38)	9 (31)
Group G (VP+)	7 (58)	3 (25)	2 (17)
Total C or G (VP-)	15 (36)	13 (31)	14 (33)
Total C or G (VP+)	12 (44)	7 (26)	8 (30)
Other	26 (63)	9 (22)	6 (15)

VP+, positive Voges-Proskauer test. VP-, negative Voges-Proskauer test.

Table 4. *Age grouping of patients positive for beta-haemolytic non-Group A streptococci (% of respective streptococcal group)*

Streptococcal group	Age group (years)			Mean age (yrs)
	0-5	6-9	> 10	
Group B	16 (49)	11 (33)	6 (18)	5.9
Group C (total)	14 (48)	3 (10)	12 (41)	8.0
Group C (VP-)	7 (50)	1 (7)	6 (43)	7.9
Group C (VP+)	7 (47)	2 (13)	6 (40)	8.0
Group G (total)	15 (37)	9 (22)	17 (42)	7.6
Group G (VP-)	15 (52)	7 (24)	7 (24)	5.5
Group G (VP+)	0 (0)	2 (17)	10 (83)	12.8
Total C or G (VP-)	22 (51)	8 (19)	13 (30)	6.7
Total C or G (VP+)	7 (26)	4 (15)	16 (59)	10.1
Other	15 (32)	17 (36)	15 (32)	7.6
Any BHNAS	60 (40)	40 (27)	50 (33)	7.3

VP+, positive Voges-Proskauer test. VP-, negative Voges-Proskauer test. *Note*: age data not available for one patient.

Table 5. *Clinical impression of illness associated with oropharyngeal isolation of beta-haemolytic non-Group A streptococci (% of respective streptococcal group)*

Streptococcal group	Diagnosis	
	Streptococcal or other primary pharyngitis	Presumed viral illness, other
Group B	8 (26)	23 (74)
Group C (total)	12 (44)	15 (56)
Group C (VP-)	8 (61)	5 (39)
Group C (VP+)	4 (29)	10 (71)
Group G (total)	16 (40)	24 (60)
Group G (VP-)	14 (50)	14 (50)
Group G (VP+)	2 (17)	10 (83)
Total C or G (VP-)	22 (54)	19 (46)
Total C or G (VP+)	6 (23)	20 (77)
Other	6 (14)	37 (86)

VP+ positive Voges-Proskauer test. VP-, negative Voges-Proskauer test.

of streptococcal or primary pharyngitis was more likely for large-colony VP-negative Groups C or G streptococci. The one patient with *S. zooepidemicus* had experienced pharyngitis accompanied by fever and torticollis. Biogroups 2 and 3 of large-colony VP Groups G streptococci were equally represented among the two diagnostic groupings.

DISCUSSION

Although exceeded in number by the Group A streptococci, the BHNAS were sufficiently common to potentially affect treatment plans in this paediatric population. If one were to assume, on the basis of previous evidence, that only

beta-haemolytic Groups C and G streptococci among the BHNAS are pharyngeal pathogens, there is a sufficient incidence of either large colony or *S. anginosus* groups to justify the laboratory in performing further biochemical characterization after Lancefield grouping. Our finding of *S. equisimilis* as the predominant large-colony Group C streptococcal species is consistent with the preponderance of this species in other body sites (17). *S. zooepidemicus*, although rare in this study, is most clearly associated with pharyngitis based on previous reports (2, 13), and the clinical importance of this animal pathogen is reemphasized by a recent description of serious illnesses following milk-borne spread (19). Large-colony VP-negative Group G streptococci were predominantly represented by two biotypes. Although these two biotypes are among the 'human' biotypes proposed by Clark and co-workers (18) the pattern of human biotype distribution is unlike that recognized in throat or other specimens reported by the same investigators. One might infer that *S. anginosus* would be the more unlikely pharyngeal pathogen among Groups C and G streptococci given the high frequency of these bacteria in the normal gastrointestinal tract. However, this lower degree of likelihood has not been clearly substantiated.

Our study did not find significant seasonal variation in the incidence of BHNAS or specifically the subgroups of Groups C and G streptococci. Although these findings might relate only to our locality, they are important in the temporal planning of a case control study. Overall, the incidences of BHNAS throughout the year were similar to those reported by Murray and co-workers (15).

Semi-quantitation of Group A streptococci from throats does correlate reasonably well with the likelihood of these bacteria being associated with active disease. Carrier states and active pharyngitis are typically, although not always, at opposite poles of the semiquantitative scale. Semi-quantitation frequencies of BHNAS are considerably heterogeneous and, in part, argue against the laboratory reporting only moderate to heavy quantities of these bacteria.

Hamrick (20) described in preliminary form evidence of an increasing incidence of BHNAS along with increasing paediatric age groups. Age-related differences in BHNAS isolation in our study are reflected by the discrepancy in mean ages for the beta-haemolytic large-colony Groups C and G streptococci, and Group C and G *S. anginosus*. Therefore, age variation has the potential to significantly affect the outcome of a case control study if patients in the study are not age matched.

The higher likelihood of ascribing the diagnosis of streptococcal pharyngitis or primary pharyngitis to patients with beta-haemolytic large-colony Groups C or G streptococci represents further circumstantial evidence that these subsets may be the more important among the BHNAS. These findings contrast to the lower and almost equal incidence of streptococcal or primary pharyngitis diagnoses among Group B streptococci, Groups C and G *S. anginosus*, and the BHNAS other than Groups B, C or G.

A case-control study is required to test the association of BHNAS and pharyngitis, especially given our current knowledge of the complexity within Groups C and G. This requisite study would not, however, prove causation, since it is possible for these agents to be merely associated with other etiologic agents of pharyngitis, e.g. viruses. Given that an association is shown, further microbiological, serological, and treatment studies would be warranted. Should a

lack of association be determined between bacteria and disease, investigators should be aware that pooling of subgroups may be associated with the loss of statistical power for association if in fact pathogenic subgroups exist.

ACKNOWLEDGEMENTS

We thank the technical staff in the Program of Microbiology, B.C.'s Children's Hospital for their assistance.

We appreciate the assistance of Therese Lemmens and Luisa Bravin in the manuscript preparation.

REFERENCES

1. Cimolai N, Elford RW, Bryan L, Anand C, Berger P. Do the beta-hemolytic non-group A streptococci cause pharyngitis? *Rev Infect Dis* 1988; **10**: 587-601.
2. Duca E, Teodorovici G, Radu C, *et al.* A new nephritogenic streptococcus. *J Hyg* 1969; **67**: 691-8.
3. Benjamin JT, Perriello VA Jr. Pharyngitis due to group C hemolytic streptococci in children. *J Pediatr* 1976; **89**: 254-6.
4. Hill HR, Caldwell GG, Wilson E, Hagar D, Zimmerman RA. Epidemic of pharyngitis due to streptococci of Lancefield group C. *Lancet* 1969; **ii**: 371-4.
5. Stryker WS, Fraser DW, Facklam RR. Foodborne outbreak of group G streptococcal pharyngitis. *Am J Epidemiol* 1982; **116**: 533-40.
6. McCue JD. Group G streptococcal pharyngitis: analysis of an outbreak at a college. *JAMA* 1982; **248**: 1333-6.
7. Cohen D, Ferne M, Rouach T, Bergner-Rabinowitz S. Food-borne outbreak of group G streptococcal sore throat in an Israeli military base. *Epidemiol Infect* 1987; **99**: 249-255.
8. Berger PC, Elford RW, Yeo M, Cimolai N, Anand CM. Pharyngitis 1987: a survey of physicians' attitudes and practices in southern Alberta. *Canad J Pub Health* 1989; **80**: 38-41.
9. Facklam RR. The major differences in the American and British streptococcus taxonomy schemes with special reference to *Streptococcus milleri*. *Eur J Clin Micro* 1984; **3**: 91-3.
10. Ruoff KL, Kunz LJ, Ferraro MJ. Occurrence of *Streptococcus milleri* among beta-hemolytic streptococci isolated from clinical specimens. *J Clin Microbiol* 1985; **22**: 149-51.
11. Lawrence J, Yajko DM, Hadley WK. Incidence and characterization of beta-hemolytic *Streptococcus milleri* and differentiation from *S. pyogenes* (group A), *S. equisimilis* (group C), and large-colony group G streptococci. *J Clin Microbiol* 1985; **22**: 772-7.
12. Bucher C, von Graevenitz A. Differentiation in throat cultures of group C and G streptococci from *Streptococcus milleri* with identical antigens. *Eur J Clin Microbiol* 1984; **3**: 44-5.
13. Barnham M, Thornton TJ, Lange K. Nephritis caused by *Streptococcus zooepidemicus* (Lancefield group C). *Lancet* 1983; **i**: 945-7.
14. Margileth AM, Mella GW, Zilvetti EE. Streptococci in children's respiratory infections: diagnosis and treatment. *Clin Pediatr* 1971; **10**: 69-77.
15. Murray PR, Wold AD, Washington JA II. Recovery of group A and non-group A beta-hemolytic streptococci from throat swab specimens. *Mayo Clin Proc* 1977; **52**: 81-4.
16. Quinn RW, Denny FW, Riley HD. Natural occurrence of hemolytic streptococci in normal school children. *Am J Public Health* 1957; **47**: 995-1008.
17. Facklam RR, Carey RB. Streptococci and aerococci. In Lennette EH, Balows A, Hausler Jr. WJ, Shadomy HJ, eds. *Manual of clinical microbiology*. Washington, D.C.: American Society for Microbiology, 1985: 154-75.
18. Clark RB, Berrafati JF, Janda JM, Bottone EJ. Biotyping and exoenzyme profiling as an aid in the differentiation of human from bovine group G streptococci. *J Clin Microbiol* 1984; **20**: 706-10.
19. Edwards AT, Roulson M, Ironside MJ. A milk-borne outbreak of serious infection due to *Streptococcus zooepidemicus* (Lancefield Group C). *Epidemiol Infect* 1988; **101**: 43-51.
20. Hamrick HJ. The throat culture reconsidered. *J Pediatr* 1986; **108**: 416-7.