

Beta-Haemolytic Streptococci Isolated from Acute Sore-Throat Patients: Cause or Coincidence? A Case–Control Study in General Practice

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As beta-haemolytic streptococci can be cultured in people with and in those without a sore throat, a case–control study was set up in 43 family practices in The Netherlands. The association was tested between the number of colony counts, specific T/M types and exotoxin genes and an acute sore throat. Duplicate throat swabs were taken from 663 sore-throat patients, selected by clinical criteria, and from 694 healthy controls. They were cultured for beta-haemolytic streptococci by combining several updated laboratory methods. Approximately 40% of the controls and 80% of the patients had beta-haemolytic streptococci-positive cultures. When focusing on cultures with high colony counts, not only group A (46%), but also non-group A streptococci (20%), predominated significantly in adult patients compared with controls. No T/M or exotoxin gene type was significantly more prevalent in patients than in controls. Thus, semiquantitative analysis, but not T/M and exotoxin gene typing, showed an association between beta-haemolytic streptococci and active disease. Groups A, C and G streptococci were found to be potentially pathogenic in adult sore-throat patients, and should be included in the discussion on the use of rapid antigen detection tests and penicillin treatment in primary care.

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INTRODUCTION

Three to five per cent of all visits to the general practitioner (GP) occur because of an acute sore throat (1, 2). Most cases are of viral origin as part of an upper respiratory tract infection (3). Of the bacterial causes of sore throat group A beta-haemolytic streptococci (BHS) are the most important (4). The prevalence of group A BHS may vary with population characteristics (age, socioeconomic background), signs and symptoms, and underlying clinical conditions (5–7). Since only group A BHS-positive patients can be offered causative treatment, attempts have been made to pinpoint them by several combinations of signs and symptoms (8). In adults, Centor showed that through applying 4 clinical criteria, i.e. history of fever, absence of cough, anterior cervical lymphadenitis and (tonsillar) exudate, the group A BHS prevalence could be increased from approximately 15% to 50% (6, 9). However, group A BHS have also been cultured in 5–10% of people not suffering from an acute sore throat (1, 7). It is therefore questionable whether each group A BHS strain isolated from a patient with an acute sore throat is invariably responsible for the symptoms. It may well be an asymptomatic colonizer isolated coincidentally from a patient with a viral pharyngitis. Viral cultures (10) and serology (11) did not produce an unequivocal answer to this question. The problem of discriminating between cause and coincidence is even more delicate in non-group A BHS, because their pathogenic role in sore

throat is still under debate. Population-based studies have found only small differences in prevalence rates between cases and controls (5), while, groups C and G BHS have been shown to be implicated in small epidemics of sore throat (12,13).

Considering a therapy trial for sore throat it (14) was also decided, as a spin-off, to perform an epidemiological case–control study of BHS. BHS was examined in throat cultures of patients and healthy controls for 4 possibly discriminating factors, as potential markers of active disease: BHS colony counts (15–17), group A BHS T/M types (18, 19), and streptococcal pyrogenic exotoxin A and C genes (*speA* and *speC*) (20).

MATERIALS AND METHODS

Design

From October 1994 to August 1996, 55 GPs from 43 general practices in a semiurbanized area in the eastern part of The Netherlands participated in the study. Throat swabs were taken from 663 ambulatory patients with signs and symptoms of an acute sore throat and from 694 asymptomatic controls, all aged 4–44 y. The age range was restricted to 4–44 y, which comprises nearly all cases of streptococcal pharyngitis and group A BHS carriers (1). Since pharyngeal BHS are much more prevalent in children than in adults (1, 21), 2 age groups were studied separately (4–14 and 15–44 y) with regard to serogroups and colony counts. Inclusion of controls was regulated by frequency measures of age. Informed consent was requested from each patient or legal guardian. The study protocol was approved by the medical ethics committee of the Isala Clinics Zwolle.

Patients

Sore-throat patients. Inclusion criteria were an acute (≤ 7 d) sore throat and fulfilling at least 3 of the 4 criteria elaborated by Centor et al. (9): history of fever, absence of cough, anterior cervical lymphadenitis and (tonsillar) exudate. As these criteria are less sensitive in children, a minimum of 2 Centor criteria was required in the 4–14 y age group.

Exclusion criteria were antibiotic treatment in the preceding 14 d, penicillin intolerance, an imminent peritonsillar abscess or comorbidity demanding antibiotics. The latter criteria were used because of a concomitant penicillin trial. Of the 1,351 patients complaining of acute sore throat, 270 (20%) had insufficient clinical signs or symptoms to justify inclusion. Another 168 (12%) patients met 1 of the exclusion criteria [87 (6%) had an urgent need for penicillin] and 250 (19%) were not able or willing to participate because of the associated therapy trial, leaving 663 patients for the analysis. The 250 patients who were not able or willing to participate fulfilled the 4 Centor criteria significantly more often than the 663 included patients.

Asymptomatic controls. Every third patient aged 4–44 y attending the general practice for reasons unrelated to respiratory tract infection (cough, runny nose, earache, sinusitis, wheezing), antibiotic treatment in the preceding 14 d, or any throat infection or other suspected BHS disease in the preceding 3 months was asked to participate. Six of 700 persons refused, leaving 694 controls.

Age, gender, smoking habits, asthma/chronic obstructive pulmonary disease (COPD) or diabetes mellitus, a history of tonsillectomy, the number of household members, and the system of health-care insurance (private or collective, as an indicator of socioeconomic status) were recorded.

Bacteriological procedures

After training, the GPs took 2 throat samples for culture from both tonsils or tonsillar fossae and the posterior pharyngeal wall of each person, since previous research demonstrated that the bacteriological yield from 2 samples is significantly (7–30%) higher than from 1 sample (21). The samples were transported in a modified Stuart medium and processed in the laboratory within 48 h. Each throat sample was cultured semiquantitatively on sheep blood agar and ssA (Becton Dickinson, Leiden, The Netherlands). The loop was then stabbed several times into the agar to maximize the ability to detect beta-haemolysis (22). The agar plates were incubated anaerobically at 35°C for up to 48 h, and inspected daily for beta-haemolytic colonies. Catalase-negative, Gram-positive cocci in chains were serogrouped using Streptex (Murex Diagnostics, Utrecht, The Netherlands). Finally, a bacitracin susceptibility test was performed.

Colony counts of BHS on both agar plates were reported as follows: no growth, sporadic colony counts (1–10 colonies), 1+ or low colony counts (> 10 colonies, but limited to initial inoculation area), 2+ or medium colony counts, and 3+ or high colony counts (growth into the second or third inoculation area, respectively). In cases where 2 serogroups were isolated simultaneously (7 patients), the serogroup with the highest number of colony counts was taken for further analysis. Furthermore, the number of indigenous throat flora colonies per plate was assessed to control for the streaking technique of the doctors, who were not blinded to the difference between patients and controls.

BHS were stored at -85°C . Serogroup A isolates were investigated at the National Institute of Public Health and the Environment (RIVM) in Bilthoven for T serotyping and M and exotoxin gene typing (23).

Statistical procedures

Baseline characteristics are presented as means and actual numbers. Medians were used in cases of non-normality. $p < 0.05$ was considered significant. Associations were determined using odds ratios, computed by SPSS logistic regression analysis (SPSS 7.0, 1997; SPSS, Chicago, USA). Possible confounders, such as gender, health-care insurance, urbanization, season, number of household contacts, smoking, asthma/COPD or diabetes mellitus, and history of tonsillectomy, were examined, first in a univariate model and then for their unique distinction between patients and controls. All significant confounders were indicator variables in further logistic regression analyses. In order to find a clinically applicable prediction of infections, cut-off points in the semiquantitative 4-point scale of colony counts were assessed using the proportional odds model (23). Colony counts were dichotomized and the odds ratios, indicating the difference between patients and controls, calculated at each of the 4 possible cut-off points. The χ^2 test was used with continuity correction to measure the influence of tonsillectomy on the number of colony counts. The Wald statistic, with Bonferroni's correction, was used to compare the prevalence of T/M and exotoxin gene types in patients with that in controls.

RESULTS

Except for the season of culturing and history of tonsillectomy, patients did not differ from controls with regard to the baseline characteristics (Table I). All data were adjusted for these 2 confounding factors. Tonsillectomy had been performed significantly more often in controls, irrespective of age.

Prevalence

A significantly higher prevalence of BHS-positive cultures was obtained in wintertime. Medium or high colony counts were associated with tonsils in situ in both patients ($p < 0.001$) and controls ($p = 0.04$). Adjustment was made for age by analysing children and adults separately.

Group A BHS were significantly more prevalent in patients than in controls, irrespective of age (Table II). The prevalence of group A BHS in patients and controls did not correlate with a history of asthma/COPD or diabetes mellitus. In this multicentre study, the prevalence rates of the different BHS serotypes did not differ significantly between the 5 communities having at least 4 participating GPs. The prevalence of 3+ indigenous throat flora varied between 88% and 93%, irrespective of the participating doctor, patient or control.

Colony counts

The odds ratios of group A BHS-positive patients vs. controls, for adults at a higher level than for children, increased parallel with the number of colony counts (Table IIIa). A similar association was found in adult patients with non-group A BHS (Table IIIb). Because of these results, the analysis was repeated using 3+ cultures only (Table IV) and it was found that these cultures discriminated between patients and controls more clearly. The 3+ BHS cultures of groups A, C, and G were significantly more prevalent in adult patients than in adult controls. This

trend also applied to serogroups B and F, although not at a significant level.

T/M and exotoxin gene typing

Since the prevalences of T/M types and exotoxin genes *speA* and *speC* of group A BHS were not found to be significantly different between children and adults, the data were summated for both age groups. The 3+ cultures of group A BHS had the strongest association with acute sore throat. Therefore, the patients were divided into 2 sub-

groups (3+ cultures, $n = 319$; and <3+ cultures, $n = 36$) and compared with controls ($n = 89$). No T/M type, not even *T1M1* and *T3M3*, known to be associated with invasive streptococcal disease (19), was significantly more prevalent in patients with 3+ cultures than in controls (Table V).

The prevalence of *speA* exotoxin gene did not differ significantly between patients (25%) and controls (30%). The same observation was made when analysing the prevalence of *speC* exotoxin gene (patients 59% and controls

Table I. Baseline characteristics of sore-throat patients and asymptomatic controls, per age group

	Children (4–14 y)		Adults (15–44 y)	
	Patients ($n = 129$)	Controls ($n = 184$)	Patients ($n = 534$)	Controls ($n = 510$)
Age, median (SD) (y)	8.8 (3.4)	8.0 (3.0)	25.9 (7.6)	30.4 (8.2)
Gender (% male)	48.8	49.2	37.6	37.8
Health-care insurance (% Collective Health Plan)	63.3	58.4	72.4	73.9
Urbanization (>30,000 inhabitants)	47.3	58.7	52.8	58.0
Season (cultured in Oct–March)	58.1*	33.2	64.8*	28.2
>3 household members	86.5	91.3	57.8	51.8
<i>Medical history</i>				
History of tonsillectomy	4.7*	12.6	17.5*	42.5
Asthma/COPD or diabetes mellitus	10.1	7.1	5.6	6.8
Smoker (>4 cigarettes/d)	–	–	29.5	27.9

All values are percentages except where otherwise indicated.

* $p < 0.05$ compared with controls within the same age group.

Table II. Prevalence of beta-haemolytic streptococci (BHS) in sore-throat patients and in asymptomatic controls (per age group) (adjusted^a odds ratios)

BHS serogroup	Patients		Controls		OR (95% CI)
	n	%	n	%	
<i>Children (4–14 y)</i>					
Group A	87	67.4	56	30.4	4.3 (2.6–7.2)*
Group B	0	0.0	5	2.7	0.0 (–)
Group C	6	4.7	4	2.2	1.7 (0.4–6.3)
Group F	1	0.8	6	3.3	0.2 (0.0–1.9)
Group G	5	3.9	12	6.5	0.6 (0.2–1.8)
Not typeable	3	2.3	4	2.2	1.0 (0.2–5.1)
No growth	27	20.9	97	52.7	–
Total	129	100.0	184	100.0	–
<i>Adults (15–44 y)</i>					
Group A	268	50.2	33	6.5	14.0 (9.2–21.3)*
Group B	31	5.8	36	7.1	0.8 (0.5–1.3)
Group C	66	12.4	41	8.0	1.3 (0.8–2.0)
Group F	12	2.2	17	3.3	0.5 (0.2–1.2)
Group G	42	7.9	28	5.5	1.4 (0.8–2.5)
Not typeable	6	1.1	13	2.5	0.3 (0.1–0.9)*
No growth	109	20.4	342	67.1	–
Total	534	100.0	510	100.0	–

^a OR adjusted for season and history of tonsillectomy.

* $p < 0.05$: significant difference between patient and controls.

OR: Odds ratio; CI: confidence interval.

Table III. Colony counts of (a) group A and (b) non-group A beta-haemolytic streptococci (BHS) in sore-throat patients and in asymptomatic controls (per age group) (adjusted^a odds ratios for ordered categories^b)

	Children (4–14 y)				Adults (15–44 y)			
	Patients		Controls		Patients		Controls	
	<i>n</i>	%	<i>n</i>	%	<i>n</i>	%	<i>n</i>	%
(a) Colony counts of group A BHS								
Neg ^c	42	32.6	128	69.6	266	49.8	477	93.5
Sporadic	1	0.8	3	1.6	2	0.4	3	0.6
1+	0	0.0	14	7.6	4	0.7	11	2.2
2+	10	7.8	20	10.9	19	3.6	13	2.5
3+	76	58.9	19	10.3	243	45.5	6	1.2
Total	129	100.0	184	100.0	534	100.0	510	100.0
Dichotomized categories ^b								
	OR	(95% CI)			OR	(95% CI)		
	4.3	(2.6–7.2)*			14.0	(9.2–21.3)*		
	4.5	(2.7–7.4)*			15.4	(10.0–23.6)*		
	6.8	(3.9–11.6)*			24.8	(14.7–41.7)*		
	12.3	(6.6–22.0)*			71.7	(30.8–165.7)*		
(b) Colony counts of non-group A BHS								
Neg ^d	114	88.4	153	83.2	377	70.6	375	73.5
Sporadic	0	0.0	1	0.5	4	0.7	14	2.7
1+	0	0.0	8	4.3	12	2.2	31	6.1
2+	4	3.1	13	7.1	33	6.2	63	12.4
3+	11	8.5	9	4.9	108	20.2	27	5.3
Total	129	100.0	184	100.0	534	100.0	510	100.0
Dichotomized categories ^b								
	OR	(95% CI)			OR	(95% CI)		
	0.6	(0.3–1.2)			0.9	(0.7–1.3)		
	0.6	(0.3–1.2)			1.1	(0.8–1.4)		
	0.8	(0.6–1.7)			1.3	(0.9–1.8)		
	1.5	(0.6–3.9)			3.7	(2.3–6.0)*		

^a Adjusted for season and history of tonsillectomy.

^b See Materials and Methods.

Neg: no growth of ^c group A or ^d non-group A beta-haemolytic streptococci.

OR: Odds ratio; CI: confidence interval.

* $p < 0.05$: significant difference between patients and controls.

71%). Subanalysis of 3+ cultures in patients did not alter these results significantly. The *speA* exotoxin gene was isolated in 18 of the 19 *T1M1* and in all 25 *T3M3* isolates.

Bacteriological yield

The number of BHS-positive cultures increased by 3% in patients and 13% in controls when 2 throat samples were taken instead of 1. The number of 3+ cultures increased 13% and 59%, respectively. Of all 444 group A BHS positive cultures, 1 yielded growth only on the sheep blood agar and not on the ssA agar plates. One other culture yielded growth only on the ssA agar plates. Of all 338 non-group A BHS positive cultures, 175 (52%) yielded growth only on the sheep blood agar and not on the ssA

agar plates. Seven other cultures (2%) yielded growth only on the ssA agar plates.

DISCUSSION

In this case-control study, high prevalences of group A and non-group A BHS were found in patients and controls, in children as well as in adults. With regard to the patients, group A BHS were isolated in 67% of the children and 50% of the adults, while non-group A BHS were isolated in 12% and 29%, respectively. In other studies on acute sore throat, much lower BHS prevalences were found (group A BHS, 22–56%; non-group A BHS, 2–17%) (6, 17, 24–32). With regard to the controls, this study also showed high BHS prevalences: group A BHS in 30% of the children and 7%

Table IV. Prevalence of high colony counts (3+ cultures) of beta-haemolytic streptococci (BHS) in sore-throat patients and in asymptomatic controls (per age group) (adjusted^a odds ratios)

BHS serogroup 3+ cultures	Patients		Controls		OR (95% CI)
	n	%	n	%	
<i>Children (4–14 y)</i>					
Group A	76	58.9	19	10.3	12.3 (6.6–22.0)*
Group B	0	0.0	0	0.0	–
Group C	5	3.9	3	1.6	1.8 (0.4–7.8)
Group F	1	0.8	1	0.5	2.1 (0.1–35.2)
Group G	3	2.3	5	2.7	0.7 (0.2–3.4)
Not typeable	2	1.6	0	0.0	–
No/<3+ growth ^b	42	32.6	156	84.8	–
Total	129	100.0	184	100.0	–
<i>Adults (15–44 y)</i>					
Group A	243	45.5	6	1.2	71.7 (30.8–165.7)*
Group B	21	3.9	9	1.8	1.7 (0.7–4.1)
Group C	51	9.6	10	2.0	3.9 (1.9–8.1)*
Group F	7	1.3	1	0.2	4.7 (0.5–42.9)
Group G	26	4.9	4	0.8	7.5 (2.4–23.3)*
Not typeable	3	0.6	3	0.6	0.8 (0.1–4.8)
No/<3+ growth ^b	183	34.3	477	93.5	–
Total	534	100.0	510	100.0	–

^a OR adjusted for season and history of tonsillectomy.

^b No growth of BHS or sporadic/1+/2+ colony counts of BHS.

OR: Odds ratio; CI: confidence interval.

* $p < 0.05$: significant difference between patients and controls.

of the adults and non-group A BHS in 17% and 27%, respectively. Previous population-based studies reported lower prevalences: group A BHS in 6–16% of the children and 0.2–3% of the adults and non-group A BHS in 2–21% of both children and adults (1, 28–32).

The data on BHS prevalence would probably have even been more impressive if the patients who were unable or unwilling to participate in the study (and having a high number of Centor criteria) had also provided samples for culture. Inclusion of these 2 groups in the analyses may have increased the differences between patients and controls even further.

The combination of the following factors is likely to have contributed substantially to this high yield in the present study: the use of the Centor criteria in the patient group, which allowed for effective patient selection (6, 9); the preparatory training of the GPs; the sampling in duplicate of patients and controls (thus raising the number of 3+ cultures by 13% and 59%, respectively); and, finally, the use of extensive laboratory techniques (using selective and non-selective agar media, stabbing into the agar, and anaerobic incubation for up to 48 h), as has been absorbed into the recent issue of the Manual of Clinical Microbiology (22).

The extensive diagnostic procedures used in the present study differ from those used in earlier epidemiological studies and thus hamper direct comparison of results.

They do, however, offer the chance to study in more detail the associations between BHS and sore throat.

Without using semiquantitative analysis, only group A BHS was found to be significantly more prevalent in patients than in controls (Table II), which is concordant with other case-control studies (5, 28–32). However, semiquantitative analysis of both group A and non-group A BHS showed that the higher the number of colony counts, the more likely these BHS would be cultured in the patient group (Table IIIa, b). Groups A, C and G BHS 3+ cultures were significantly associated with acute sore throat in adults. Groups B and F BHS showed a similar trend but, because of the small numbers, not to a significant extent. Other authors also reported an association between high group A BHS colony counts and active disease, although not on a statistically significant level (1, 15–17, 29–31). One of them noted a non-significant trend of high colony counts being associated with serological evidence of infection (16). Three case-control studies differed from the present study with regard to patient selection (hospital-based) and/or diagnostic methods (29–31).

The causal role of group A BHS in acute sore throat has long been recognized. This study, therefore, does not yield surprises in that respect. It does, however, underline the importance of the number of colony counts in discriminating between group A BHS isolated as the cause of sore throat and group A BHS isolated as a coincidence.

Table V. Top 16 T and M types of group A beta-haemolytic streptococci (BHS) cultured in sore-throat patients with high (3+) or lower (<3+) colony counts and in asymptomatic controls (all ages)^a

T type	M type	Patients, 3+ colony counts		Patients, <3+ colony counts		Controls, all colony counts		Total	
		n	%	n	%	n	%	n	%
12	22-60	38	11.9	2	5.6	10	11.2	50	11.2
28	28	35	11.0	4	11.1	4	4.5	43	9.7
2-28	2	35	11.0	2	5.6	5	5.6	42	9.5
B 3264	100	26	8.2	3	8.3	8	9.0	37	8.3
12	12	23	7.2	3	8.3	8	9.0	34	7.7
4	4	21	6.6	2	5.6	10	11.2	33	7.4
9	9	25	7.8	1	2.8	3	3.4	29	6.5
3	3	21	6.6	2	5.6	2	2.2	25	5.6
11	60-101	18	5.6	3	8.3	1	1.1	22	5.0
6	6	5	1.6*	5	13.9	10	11.2	20	4.5
1	1	15	4.7	0	0.0	4	4.5	19	4.3
28	NT ^b	5	1.6	2	5.6	6	6.7	13	2.9
11	11	7	2.2	1	2.8	3	3.4	11	2.5
B 3264	9	8	2.5	0	0.0	0	0.0	8	1.8
25	NT	4	1.3	2	5.6	1	1.1	7	1.6
B 3264	9-104	4	1.3	2	5.6	0	0.0	6	1.4
Subtotal		290	90.9	34	94.4	75	84.3	399	90.0
Other types ^c		29	9.1	2	5.6	14	15.7	45	10.0
Total		319	100.0	36	100.0	89	100.0	444	100.0

^a Wald statistic adjusted for season and history of tonsillectomy, pairwise comparison procedure, with Bonferroni's correction.

^b NT: not typeable.

^c Other T/M types found were: 3-B3264/3, 11/60, 3264/9-104, 5-27-44/5, 5-27-44/61, 3/NT, 6/NT, 8/NT, 9/NT, 13/NT, 5-27-44/NT, 8-25/NT, B3264/NT, IMP19/NT, AUTO/NT, NT/3, NT/6, NT/12 and NT/NT.

Nine strains could not be recultured after storage at -85°C .

* $p < 0.05$ compared with patients with lower (<3+) colony counts.

Low colony counts, occurring in the same frequency in patients as in controls, probably represent an asymptotically acquired colonization or a late sequela of infection.

With regard to the non-group A BHS, the picture seems quite similar, although weaker, and in adults more distinctly than in children. High colony counts were significantly associated with active disease, which raises the question of whether this association is causal, as in group A BHS, or due to some other mechanism. Apart from the present study results, which suggest a causal role of non-group A BHS, there are other data, derived from epidemics in semiclosed communities and from complications (12, 13), that support a causal role of group C and G BHS in pharyngitis. Perhaps treatment studies, which specifically include high-count non-group A BHS patients, will lead to more definite conclusions.

Our findings on T/M typing are concordant with a recent study in the USA, which suggested that the prevalent M1, M3 and M28 subtypes from patients with severe infections were equally common in the general population (33). The finding that *TIM1* and *T3M3* isolates are accompanied by *speA* exotoxin gene in nearly 100% of the healthy controls and sore-throat patients (present study), as well as in severe streptococcal infections, stresses (34) the importance of host-related factors, apart from virulence, in deter-

mining who actually develops an invasive streptococcal infection.

Doubt has been expressed as to whether tonsillectomy significantly reduces the frequency of streptococcal infections (4). The present results indicate that tonsillectomy seems to protect not only against acute sore throat, but also against asymptomatic colonization with BHS. This finding is concordant with the conclusion of earlier studies, indicating an association between tonsils in situ (35), or even the size of the tonsils (36), and the high rate of recurrent throat infection. The association found between tonsils in situ and high BHS colony counts supports the concept that BHS prefer to adhere to tonsillar tissue, in patients more than in healthy controls (37).

Clinical criteria are the clinician's main tool in selecting group A BHS-positive sore-throat patients. The rapid group A antigen-detection test was developed as a more precise instrument to distinguish between streptococcal and non-streptococcal pharyngitis (4). To date, the rapid test kits only aim at detecting group A BHS and often fail to detect low inocula, which is often regarded as a disadvantage of these tests (38). Missing low inocula, however, could mean rejecting coincidentally present BHS. Perhaps new rapid test kits for adults should also include non-group A serogroup antigens and react only to high bacterial counts.

In conclusion, high colony counts of group A BHS, but not T/M and exotoxin gene types, were significantly associated with acute sore throat. In adults, high colony counts of non-group A BHS were also associated with active disease, suggesting a causal role.

ACKNOWLEDGEMENTS

No conflict of interest was perceived by any of the authors. This study was funded by Groene Land Health Insurances (Achmea Group) and the Stichting Gezondheidszorgonderzoek Ysselmond in Zwolle. Special thanks are given to all participating patients, general practitioners and their assistants, Y. Mulder and G. Kajim for administrative assistance, H. Moeys, M. Borggreve (Zwolle), K. Elzenaar and H. Brunings (RIVM) for technical assistance, L. Cobb for correcting the English text, and C. Dagnelie, MD, Professor D. Post, J. Schellekens, MD, Professor F. Touw (✉), and Professor J. Verhoef for their contribution to the study design.

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Submitted June 21, 1999; accepted December 14, 1999