Beta-haemolytic streptococci in School Children 5–15 years of Age with an Emphasis on Rheumatic Fever, in the Tri-island state of Grenada

TP Noel¹, J Zabriskie², CNL Macpherson¹, G Perrotte²

ABSTRACT

The objective of this study is to determine the prevalence of β-haemolytic streptococci in the pharynx and antibodies to β-haemolytic streptococci in school children 5–15 years of age in the tri-island state of Grenada. Blood samples and throat swabs were obtained from 1388 school children, aged 5–15 years old attending randomly selected schools in each parish of the tri-island state of Grenada. Serum samples were subjected to antistreptolysin O testing (ASOT) while throat swabs were cultured on sheep blood agar plates. The prevalence of positive throat swab was 15.4% (95% CI = 13.4%, 17.2%). The rate was highest in St Patrick (21.8%) and lowest in Carriacou (5.7%). The prevalence of antibodies was 38.6% (95% CI = 37.6%, 42.8%). It was highest in St Patrick (54.4%) and lowest in Petit Martinique (26.8%). In St Patrick, males were significantly more likely than females to have a positive ASOT (p = 0.0084). In St George’s, males were significantly more likely than females to have a positive throat culture (p = 0.0172). Thirty-four per cent of the positive cultures were type A, 10% were type C and 56% type G. The data illustrate that there is a high prevalence of β-haemolytic streptococci in school children in certain parishes in Grenada. Public health measures should address prevention and control of β-haemolytic streptococcal infection in order to prevent the possible sequelae of this disease.

Estreptococos Beta-hemolíticos en Escolares de 5 a 15 años de edad con Énfasis en la Fiebre Reumática en el Estado Triinsular de Granada

TP Noel, J Zabriskie, CNL Macpherson, G Perrotte

RESUMEN

El objetivo de este estudio es determinar el predominio de estreptococos β-hemolíticos en la región faríngea y los anticuerpos de los estreptococos β-hemolíticos en escolares de 5 a 15 años de edad en el Estado tri-insular de Granada. Se obtuvieron muestras de sangre e hisopos de garganta de 1,388 escolares comprendidos en las edades de 5 a 15 años, que asistían a escuelas seleccionadas de manera aleatoria, en cada parroquia del Estado tri-insular de Granada. Muestras de suero fueron sometidas a la prueba de antistreptolisina O (ASTO), mientras que las muestras tomadas de los hisopos de garganta fueron cultivadas en placas de agar de sangre bovina. La prevalencia de muestras de garganta positivas fue 15.4% (95% CI = 13.4%, 17.2%). Los por cientos más elevados correspondieron a Saint Patrick (21.8%) y los más bajos a Carriacou (5.7%). El predominio de anticuerpos fue de 38.6% (95% CI = 37.6%, 42.8%). El más alto correspondió a Saint Patrick (54.4%) y el más bajo a Petit Martinique (26.8%). En Saint Patrick, la probabilidad de tener un ASTO positivo (p=0.0084) fue significativamente más alta en los hombres que en las mujeres. En Saint George’s, la probabilidad de tener un cultivo de garganta positivo, fue significativamente más alta en los hombres (p = 0.0172) que en las mujeres. El treinta y cuatro por ciento de los cultivos positivos fueron del tipo A, el 10% fue del tipo C y el 56% del tipo G. Los datos indican que hay un alto predominio de estreptococos β-hemolíticos en los niños escolares en ciertas parroquias de Granada. Las medidas de salud públicas deben dirigirse a la prevención y control de la infección estreptocócica β-hemolítica, a fin de prevenir las posibles secuelas de esta enfermedad.

INTRODUCTION

People living in developing regions of the world experience 90% of the world’s disease burden but have only 10% of the global healthcare funds at their disposal. In many developing countries, which account for almost two-thirds of the world’s population, streptococcal infections, rheumatic fever and
rheumatic heart disease remain a very significant public health problem. The magnitude of the problem in these countries today is similar to that in North America 50 years ago (1).

In Grenada, the morbidity caused by rheumatic fever/ rheumatic heart disease has been challenging, a reduction in the number of cases will increase overall productivity for the children and adults afflicted. This calls for the public health task of reducing cases of rheumatic fever through the use of primary and secondary prevention. The key concept is to prevent the progression of streptococcal infections in the pharynx to rheumatic fever and the potential worst case scenario of rheumatic heart disease.

SUBJECTS AND METHODS
A streptococcal surveillance programme was conducted in which 50 children of different age groups (5–15 years) from each of 27 primary schools were randomly selected. Throat swabs and five millilitres of blood were collected from each child by a qualified community nurse.

The blood was collected using red/gray brand SST tubes and serum and butterfly needles 21G3/4 with 12 inch tubing with multiple sample luer adapters. The blood samples were spun in a centrifuge and the sera were extracted. The serum from each student was then subjected to a rapid agglutination test for the qualitative measurement of antistreptolysin – O antibodies in serum. The test kits used were manufactured by Akudex, Akucheck in Chemindex, Florida.

A quantity of 0.03ml of serum was added to a stabilized buffered suspension of polystyrene latex particles coated with Streptolysin O. This process was carried out on a six ring glass slide. The slide was rocked for two minutes and was observed for agglutination while holding it under a high intensity lamp. Agglutination after two minutes was a positive result and indicated a content of Antistreptolysin – O antibodies in serum. The test kits used were manufactured by Akudex, Akucheck in Chemindex, Florida.

The results of this research allowed identification of potential high risk areas for prevalence of streptococcal infections and assisted in the prioritization of allocating scant resources to areas requiring educational intervention. The research provided a starting point for the education programme. The education programme was introduced to various focus groups including the children, teachers and principals of 53 primary schools on the island of Grenada. Institution of a social mobilization programme was carried out to investigate the differences between parishes.

All throat culture samples that were positive were frozen and forwarded to Rockefeller University laboratories. All results for these throat culture samples were re-examined and reproduced by international collaborators using similar procedures. Additionally, samples were further analyzed for sub-grouping purposes. They were separated into three groups, A, C and G.

Ethical responsibilities
The project was reviewed and passed through an Institute Review Board. Informed consent forms were circulated and signed by the parent or guardian of each student sampled. All children, screened through the target school surveillance, with positive ASO titers and throat swab samples were referred to the rheumatic fever (RF) clinic.

Consultation and medication were given to each positive child free of cost through the clinic. Two local paediatricians and a community nurse managed the RF clinic. This clinic was held on the fourth Thursday of every month. All children testing positive for streptococcal infection in this study, either serologically using ASO titres, greater than or equal to 200 Iµ/ml or by positive Type A β-haemolytic throat culture, upon examination by a physician, were treated either in the district medical office in their parish or at the RF clinic in St George’s. All results were forwarded to the paediatrician and treatment was assigned accordingly. This ensured that children with positive throat cultures did not have to wait four weeks to receive treatment. The treatment that was prescribed was penicillin/penicillin derivatives and for persons allergic to penicillin, erythromycin was given.

The number of positive sera samples and throat swabs were then analyzed and represented in both tabular and figure form using Microsoft Excel. Chi Square analysis was carried out to investigate the differences between parishes.

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The pharyngeal throat swab samples were collected and transported at room temperature using BBL CultureSwab for in vitro diagnostic’s manufactured by Becton Dickinson. These swabs were in individual containers impregnated with Stuart solution. Each throat swab was streaked within a 12-hour period from the time of collection to the time of plating. Vigorous swabbing of the tonsillar pharyngeal region was done on each student. These swabs were streaked under the flow hood in the laboratory on plates made with trypticase soy agar with 5% sheep blood. They were placed in an incubator at 35 degrees celsius for a 24 hour period. Clear halos where haemolysis has occurred due to β-haemolytic streptococci presence were noted 24 hours later. The samples responsible for this were selected and were further streaked and cultured to ensure that colonies that are positive could be further sub-grouped.

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**β-Haemolytic Streptococci in Grenada**

Fig. 1: Map of the tri-island state of Grenada displaying areas that samples were drawn from in 27 primary schools in 2001/2002.

Fig. 2: Antistreptolysin O test positive percentage by parish

Fig. 3: Throat swab percentage by parish

Fig. 4: Prevalence of positive Antistreptolysin O titres in 1387 school children aged 5–15 years by gender, in parish's throughout Grenada 2001/2002.

Fig. 5: Prevalence of positive, ASOT and β-haemolytic streptococci by parish in Grenada 2001/2002

Fig. 6: Prevalence of positive β-haemolytic streptococci in 1388 school children ages 5–15 years by gender and parish in Grenada 2001/2002
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RESULTS

In total, 1388 schoolchildren (637 males, 751 females) gave blood samples and throat swabs from the six Grenadian parishes, Carriacou and Petit Martinique (Fig. 1). The sera samples that were positive for antibodies to streptococci and the throat cultures that were positive for β-haemolytic streptococci in Grenada in 2001 can be seen in the Table.

St Patrick’s parish (parish 6) has the highest ASOT positive percentage. Because the 95% confidence interval for St David’s, St Andrew’s, St Mark’s, St John’s and St Patrick’s parish, The throat swab positive percentage for Carriacou is significantly different from the positive percentage for parishes: St David’s, St Andrew’s, St Mark’s, St John’s and St Patrick’s (Fig. 3). Therefore, the differences between Carriacou compared to St David’s, St Andrew’s, St Mark’s, St John’s and St Patrick’s were probably not due to chance. For ASOT, in St Patrick’s parish, the positive percentage is significantly higher for males than for females with 99 % confidence. This was seen using chi-square analysis with 1 degree of freedom at alpha = 0.01. The p-value was 0.0084 (Fig. 4).

The parish with the largest percentage of throat cultures that were positive for β-haemolytic streptococci was St Patrick’s where 21.62 % of the parish population sampled tested positive. The parish with the lowest percentage of positive throat cultures for β-haemolytic streptococci was Carriacou, one of the smaller island’s which comprises Grenada, where 5.68% of the population sampled tested positive (Fig. 5).

For throat cultures, the positive percentage is significantly higher for males than for females with 98% confidence only in St George’s parish. This was seen using chi-square analysis with one degree of freedom at alpha = 0.01. The p-value was 0.0172 (Fig. 6).

The average percentage of throat cultures that were positive for β-haemolytic streptococci for the entire country was 15.35 %.

The fifteen per cent (15%) that were positive were divided into the following subtypes: Type A, thirty-four per cent (34 %), Type C, ten per cent (10%) and Type G, fifty-six per cent (56%) (Fig. 7).

Table: Results of the streptococcal survey in Grenadian school children 5–15 years of age carried out between 2001 – 2002

<table>
<thead>
<tr>
<th>Parish</th>
<th># of Schools</th>
<th># of Students</th>
<th>% ASOT positive (95% Confidence Interval)</th>
<th>% Throat Swab positive (95% Confidence Interval)</th>
</tr>
</thead>
<tbody>
<tr>
<td>St George’s</td>
<td>6</td>
<td>221</td>
<td>39.82 (33.4, 46.3)</td>
<td>11.3 (7.1, 15.5)</td>
</tr>
<tr>
<td>St John’s</td>
<td>3</td>
<td>147</td>
<td>32 (24.4, 39.5)</td>
<td>19.7 (13.3, 26.2)</td>
</tr>
<tr>
<td>St Mark’s</td>
<td>2</td>
<td>102</td>
<td>40.6 (30.7, 49.7)</td>
<td>20.6 (12.7, 28.4)</td>
</tr>
<tr>
<td>St Patrick’s</td>
<td>3</td>
<td>147</td>
<td>54.42 (46.4, 62.5)</td>
<td>21.8 (15, 28.3)</td>
</tr>
<tr>
<td>St Andrew’s</td>
<td>5</td>
<td>404</td>
<td>42.43 (37.5, 47.1)</td>
<td>14.9 (11.4, 18.4)</td>
</tr>
<tr>
<td>St David’s</td>
<td>3</td>
<td>150</td>
<td>29.33 (22.6, 36.6)</td>
<td>21.3 (14.7, 27.7)</td>
</tr>
<tr>
<td>Carriacou</td>
<td>4</td>
<td>176</td>
<td>43.18 (35.9, 50.5)</td>
<td>5.7 (2.3, 9.1)</td>
</tr>
<tr>
<td>Petit Martinique</td>
<td>1</td>
<td>41</td>
<td>26.8 (13.3, 40.4)</td>
<td>10 (0.7, 19.3)</td>
</tr>
<tr>
<td>Total</td>
<td>27</td>
<td>1388</td>
<td>38.6 (37.6, 42.8)</td>
<td>15.4 (13.4, 17.2)</td>
</tr>
</tbody>
</table>

DISCUSSION

Streptococcal upper respiratory tract infections remain common but with reduced severity and fewer subsequent cases of rheumatic fever (3). Whether recent sporadic outbreaks of rheumatic fever due to known rheumatogenic strains of group A streptococci will have a significant effect

Fig. 7: Positive β-haemolytic streptococci by subtype from 1388 Grenadian students ages 5–15 years in 2001/2002

Fig. 8: Annual incidence of ARF (1997–2003) in Grenada, Carriacou and Petit Martinique
on morbidity and mortality trends remains to be seen (3). Relatively high rates of rheumatic fever and rheumatic heart disease in developing countries are difficult to evaluate because of irregularities in reporting and investigative procedures (3).

Studies have shown that socially and economically disadvantaged populations worldwide, including some indigenous and minority populations living in affluent countries, continue to have high rates of rheumatic fever and rheumatic heart disease (4). The current high rates of rheumatic fever in one such population, the Aboriginal population of the Top End of Australia, are not related to ethnicity, but are likely to reflect high levels of exposure to group A streptococci, which, in turn, are related to overcrowding and continuing poor living conditions (5,6).

Taking into account these past contributing factors from Australia and the results from the Grenada study, certain aspects of unequal access to healthcare and general living conditions must be considered. A clear example of this is seen in the parish of St Patrick’s which was noted as having both the greatest percentage of ASOT positive and β-haemolytic streptococci positive. The noted significance both by parish and male gender may be attributable to levels of personal hygiene. It may be possible that girls are more prone to seek medical attention than boys.

St Patrick’s is a more rural part of the island, associated with low socio-economic status, the number of clinics, access to physicians and pharmacies and medication are relatively limited in contrast to the capital St George’s. The probable cause for the high prevalence of streptococci relates to all the potential contributing factors confounded by poor living conditions.

An understanding of the impact of social relationships on health status, health behaviours and health decision making contributes to the design of effective interventions for preventing the onset of and reducing the negative consequences of a wide array of diseases (7). Treatment of streptococcal infection and educational intervention assist in a reduction in the occurrence of rheumatic fever. While there was an initial reduction prior to this study, the initial sensitization was carried out by members of our collaborative team. This comprised the Grenada Heart Foundation and local paediatricians sensitizing both the public and their colleagues through different means eg Grenada Medical Association meetings, and initial use of multimedia in the form of television, newspaper and radio. The continuing educational campaign began before 1999. The continuing educational campaign in conjunction with this prevalence study’s educational programme component probably contributed largely to the decline of index cases of acute rheumatic fever (Fig. 8).

The knowledge that streptococcal infection is correlated with socio-economic status allows a country to allocate limited healthcare funds to areas with the greatest need. Furthermore, prevention through education can be integrated into existing educational and healthcare systems, to reduce the amount of additional funding necessary for sustainability.

As has been noted by Nordet, a successful programme has key components which are community mobilization, supportive policy decisions, health reforms, NGOs and industry, a multi-sectoral action integrated with policies of other governmental departments and integration of appropriate healthcare into the national health system (8).

In this study, participation and ownership of the programme locally was essential and was obtained by involving the Ministry of Health, Ministry of Education, public health personnel, physicians, nurses, teachers and principals. This is similar to the successful programme that was run by Poon King in Trinidad and Tobago in the 1970s; the creation of a clinic for streptococcal follow-up was invaluable and the rate of cases of rheumatic fever dropped from 150 cases per year to under 10 per year (personal communication).

Similar programmes using combinations of primary and secondary prevention were conducted in Cuba, Costa Rica, Guadeloupe and Martinique with resounding success (8).

This programme was run at a cost of US$30 000 per year. However, this programme was not sustainable at the time for continuity by government. Different organizations including the World Heart Federation have expressed an interest in funding a surveillance programme. The educational and research component will not be included.

There were limitations to this study. The source of the incidence figures was the reported figures from the medical records department from all three public hospitals, private physicians and clinics confirmed by records compiled by the consultant paediatrician and her junior staff on the island. It is important to note that reporting was carried out during 1997 – 2003 inclusive, but it was not mandatory. In the target population of school children, some children with active streptococcal infection would not have been attending school during the acute stages of the infection and would not have been sampled.

In summary, β-haemolytic streptococci is prevalent among school children (age 5–15 years) in Grenada, Carriacou and Petit Martinique. Further research and investigation is required in the parish of St Patrick’s due to the high prevalence of streptococci, and in both St George’s and St Patrick’s due to the high prevalence in males (Fig. 6). Carriacou had low streptococcal throat swab positive rates but the second highest positive ASO titre rates. Public health measures should address prevention and control of β-haemolytic streptococci infection in order to prevent the possible sequelae of rheumatic fever/rheumatic heart disease.

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REFERENCES