

## Antistreptolysin O titres amongst children in a rural area of Ceylon

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### SUMMARY

Antistreptolysin O titres in the sera of children in a rural population of Ceylon were determined, to establish the upper limit of the normal ASO titres in non-rheumatic children, and to compare these ASO titres with results of surveys done in other countries.

Swabs were taken from throats and ulcers of the children examined and cultured specifically for group A haemolytic streptococci. Of a total of 257 children, 29.5% had ASO titres of over 166 Todd units. The greatest number of children showed ASO values between 100 and 166 units. The percentage of children showing values over 166 units increased with age until a maximum of 54% was reached between 9 and 10 years. Group A haemolytic streptococci were isolated from five throat swabs, but there were no isolations from ulcers. The values obtained from this survey have been compared with those from a few other developing countries.

### INTRODUCTION

Group A haemolytic streptococci, which may be associated with rheumatic fever, produce a toxin, streptolysin O, which evokes the production of streptolysin O antibodies in the host (Todd, 1932). The antistreptolysin O (ASO) titre in the blood can be accurately measured with reliable and reproducible results (Hollinger, 1953) and high values indicate past infection with group A haemolytic streptococci.

An increase in the incidence of rheumatic fever and chronic rheumatic heart disease has been observed in Ceylon during the last few years (Reports of the Epidemiologist, Ceylon, release no. 23). Of 8094 blood samples sent from suspected cases of rheumatic fever from various parts of the island for the determination of antistreptolysin O titres, 66% showed values of over 300 Todd units. No survey has been carried out in Ceylon to determine the ASO titres in healthy (non-rheumatic) children. It was therefore decided that the determination of the ASO titres in the sera of healthy children of various age groups would be helpful in obtaining evidence of the incidence of past streptococcal infections, and also in establishing the upper limit of the 'normal' ASO titre in non-rheumatic children.

## MATERIALS AND METHODS

A rural area (Udahamulla) close to Colombo was chosen for the purpose of this survey. This village has a population of *ca.* 2800 in an area of about 1 square mile. Children with any history of swelling of joints, swelling or puffiness of the face and feet, or any evidence of genito-urinary disease were excluded from the survey after information was obtained from the parents. Those with any cardiac murmurs were also excluded. Blood samples were collected at the infant clinic, preschool clinic, milk feeding centre and in the junior school of the area. Throat swabs were taken on dry, sterile, cotton-wool swabs from all the children at the time of blood sampling. Swabs were also taken from any ulcers found on the legs or hands. A note was also made of any healed, multiple ulcers on the body. Sera from the blood samples were frozen on the day of collection and stored at  $-20^{\circ}\text{C}$ . The throat swabs were plated on blood-agar within a few hours.  $\beta$ -haemolytic streptococcal colonies were picked up after overnight aerobic incubation of the plates at  $37^{\circ}\text{C}$ . and tested for sensitivity to Bacitracin using 2  $\mu\text{g}$ . strength Bacitracin disks on blood-agar plates.

The antistreptolysin O titre determinations were carried out using 'Wellcome' brand of reduced streptolysin O (dried) by the method based on that of Rantz & Randall (1945). Sheep cells were used as indicator. A control containing a known strength of antistreptolysin O ('Wellcome' brand) was used in parallel with every batch of sera tested.

## RESULTS

The results of the antistreptolysin titre determinations are shown in Table 1 and Fig. 1. It is seen that 29.5% of a total number of 257 children tested showed ASO titres of over 166 Todd units. Only 7% showed values higher than 250 units. There were none over 333. The greatest number of children (40%) were between the levels 100- < 166 units. None of the infants below 1 year showed a titre of over

Table 1. *Results of the antistreptolysin O survey*

Age in years	Total no. examined	ASO titres, Todd units/ml.						Percentage $\geq 166$
		Below 50	50- < 100	100- < 166	166- < 250	250- < 333	$\geq 333$	
0- < 1	16	11	3	2	0	0	0	0
1- < 2	22	11	3	5	2	1	0	14
2- < 3	22	12	2	7	0	1	0	5
3- < 4	24	4	2	11	5	2	0	29
4- < 5	17	3	4	8	2	0	0	12
5- < 6	23	3	1	15	4	0	0	17
6- < 7	22	5	1	9	5	2	0	32
7- < 8	23	2	2	11	5	3	0	35
8- < 9	20	3	1	6	10	0	0	50
9- < 10	26	0	1	11	9	5	0	54
10- < 11	22	2	0	10	5	5	0	45
11- < 12	20	0	3	7	10	0	0	50
Total	257	56	23	102	57	19	0	29.5
%	—	22	9	40	22	7	—	—

166 units. In fact most of these showed values below 50 units. The percentage of children showing values over 166 units increased with age until a maximum (54%) was reached between 9 and 10 years. (See Fig. 2). After an erratic beginning the graph showed a steady rise after the 5 year age group.

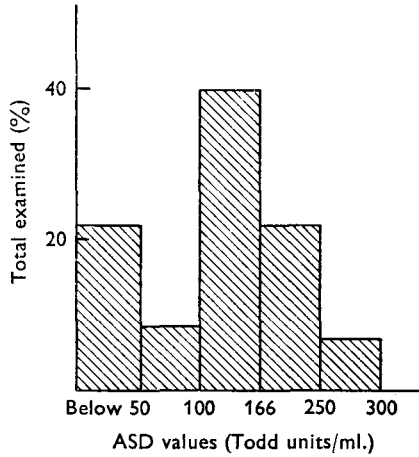


Fig. 1. Percentage of children showing different ASO values.

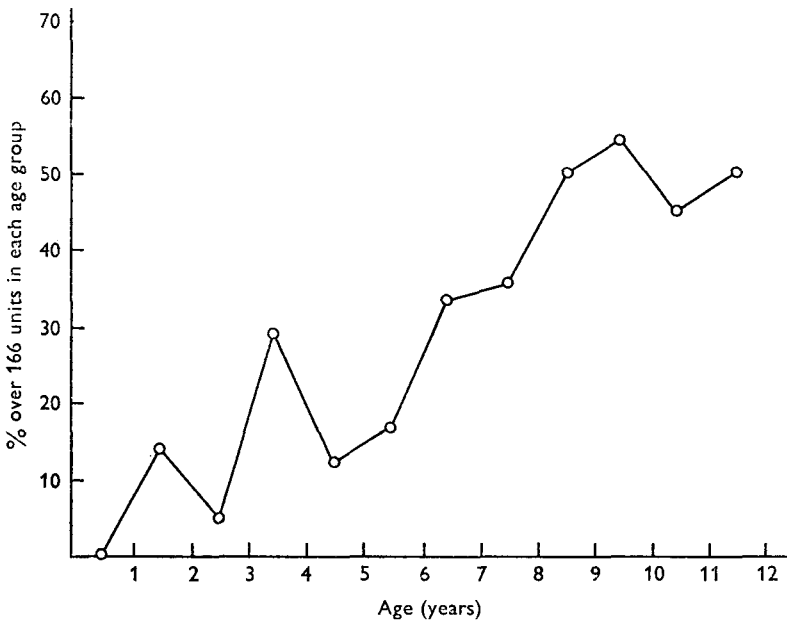


Fig. 2. Percentage of children in each age group showing ASO values of over 166 units/ml.

Group A haemolytic streptococci were isolated from five throat swabs taken from the children. The ASO titres of these five children were, however, below 166 units. There were no streptococci isolated from five swabs taken from ulcers.

## DISCUSSION

Following an acute streptococcal infection, the ASO titre rises to a maximum within 2–4 weeks. The return to normal is, however, much slower and may take 6–12 months (Stollerman, Lewis, Schultz & Taranta, 1956). The sera of most healthy adults contain a certain amount of antistreptolysin O due to past infection. Most workers are agreed that the upper limit of normality in adults is about 200 Todd units (Williams, 1958). Different writers have, however, quoted widely different values as being normal. Coburn & Pauli (1935) who carried out a survey among adults in a New York hospital found a 'natural human level' of *ca.* 50 units. Rantz, Di Caprio & Randall (1952) working in San Francisco found a mean value of 150 units in healthy children between the ages of 5 and 7 years. The mean ASO titre in children between 8 and 12 years was 184. The results of the present survey are almost in complete agreement with those of Rantz *et al.* (1952). Thus the mean ASO value amongst the children in the 5–7 age group is 166 units, while those between 8 and 12 years show a mean value of 207 units. The highest mean value appears in late childhood (9–10 years of age). This is also in agreement with the findings of Rantz *et al.*

An analysis of the cases showing an antistreptolysin titre of over 166 units in each age group is shown in Table 1. None of the children below the age of 1 year showed ASO titres of over 166 units. Being a  $\gamma$ -G globulin, antistreptolysin O would pass the placental barrier and one would expect at least a few infants in the neo-natal period to show a raised titre in their blood. Unfortunately the cases included under 1 year did not have any infants below 6 months of age. The steady rise in the graph after 5 years may be due to the fact that children in Ceylon start school at 5 years, and that the crowded schools provide a suitable environment for the spread of infection. The highest value of 54% is found in children between 9 and 10 years. Although there is a slight drop after 10 years, the values in adults were not measured to determine whether this high titre is maintained throughout adult life.

These figures may be compared with surveys done in a few other developing countries. Rotta *et al.* (W. H. O. Surveillance Reports, CES/SR/66-4) found that in an urban area of Pakistan, 13% of children under 10 years showed ASO values of over 199 units. The same workers found the rate amongst a rural population in Thailand to be 17%, whilst a rate of 56% was found in eastern Nigeria in children of the same age group. The present survey shows that 26% of the children below 10 years have ASO values of over 166 units. Dunbar & Erwa (1967) in a recent study in Khartoum (Sudan) found that 48% of children between 7 and 11 years of age had values of over 166 units. The present survey where 46% of the children in the corresponding age group show values of over 166 units, is in agreement with their findings.

The haemolytic streptococci from the swabs taken from the children were isolated from blood-agar plates grown aerobically at 37° C., and only the group A streptococci were selected by the bacitracin test.  $\beta$ -haemolysis itself is a variable character and anaerobic culture of the plates might have yielded more haemolytic

colonies (Wilson & Miles, 1966). Variant type 12 of group A which produce haemolysis at 22° C. only, isolated by Coburn & Pauli (1941) from an epidemic of respiratory infection, produce O streptolysin. These were reported to have a higher degree of infectivity than the normal haemolytic streptococci. More positive cultures of streptococci might have been obtained if primary cultures were done in an enrichment medium (Williams, 1958). Apart from group A streptococci, for which a specific search was made, there may have been other groups which produce streptolysin O. Group C and G streptococci which are known to infect human beings produce streptolysin O. Mallen, Evans & Balcazar (1957) report that they isolated group G haemolytic streptococci from 11 % of people examined in a tropical climate.

The problem of high ASO titres in populations with a low rate of isolation of haemolytic streptococci has been observed by other workers. Dunbar & Erwa (1967) found that less than 1 % of children amongst whom 48 % had ASO titres of over 166 units had haemolytic streptococci in their throats. Mallen *et al.* (1957) could isolate group A haemolytic streptococci from throat swabs of only three out of 102 people whereas 55 % showed high ASO values.

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