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Correspondence

Streptococcus pyogenes pharyngitis & impetigo in a rural area of Panchkula district in Haryana, India

Sir,

To justify development of a vaccine against *Streptococcus pyogenes* [Group A Streptococcus (GAS)], the burden of streptococcal diseases needs to be estimated. There are several reports on the occurrence of GAS infection in India but information on the incidence of pharyngitis and impetigo is still limited[1-4]. Hence, incidence of GAS pharyngitis and impetigo was determined and GAS *emm* types were identified in rural children aged 7-11 yr in four primary schools of Raipur Rani Community Development Block of Panchkula district in Haryana, India. These schools were selected in villages where health care workers were available to assist project field staff. Permission from the respective schools authorities was taken to conduct the study.

After approval of study protocol by Institute Ethics Committee of Postgraduate Institute of Medical Education and Research (PGIMER), Chandigarh and the Institute Review Board of National Institute of Allergy and Infectious Diseases (NIAID), USA, a general physical examination of the registered children was done at each weekly visit by a physician except during holidays. The student volunteers were asked about symptoms of upper respiratory infections, *i.e*., sore throat, cough, nasal discharge, and the common cold. A diagnosis of pharyngitis was made if there were symptoms of sore throat, and on physical examination there was tonsillar and/or pharyngeal exudate, and/or pharyngeal erythema. Similarly, all children were examined for skin rash or eruption/lesion. If present, the nature of the lesion was noted, and cultured for a bacteriologic diagnosis. If the throat or skin cultures were positive for GAS, these patients were given a diagnosis of GAS pharyngitis and GAS impetigo, respectively. Pharyngitis patients were given a 10-days course of oral penicillin and impetigo patients were given betadine ointment for local application.

Standard methodology[5] was employed for transportation of swabs, identification of ß-haemolysis of streptococci on sheep blood agar plates, and to determine Group A streptococcus using a Streptex Murex test kit (Remel Europe Ltd, UK). GAS was further processed for *emm* typing in the Molecular Genetics Laboratory at Department of Experimental Medicine and Biotechnology, PGIMER, Chandigarh using methods described earlier[6]. The *emm* gene sequence was searched for homology by BLAST (Basic Length Alignment Search Tool) search analysis (*http://www.ncbi.nlm.nih.gov/BLAST/Blast.cgi*). To be more precise the sequences were also submitted to CDC website (*http://www.cdc.gov/ncidod/biotech/strep/strepblast.htm*). Strains showing > 95 per cent sequence homology having maximum alignment with the reference strain in the CDC Gene Bank database were selected and designated particular parental *emm* type.

Weekly surveillance was conducted from July 2002 to April 2004 on 241 student volunteers aged 7 to 11 yr. Sample size calculations had assumed incidence of *S. pyogenes* pharyngitis to be at least 15/100 child-year with absolute precision of 5/100 child-year[7]. There were a total of 13,168 weekly observations done, out of a possible 18,222 due, with a participation rate of 72 per cent. The child-weeks at risk were derived from the number of weekly observations carried out among cohort children over the study period. The incidence (of GAS pharyngitis/impetigo) was estimated by dividing the number of (GAS pharyngitis/ impetigo) cases that occurred during the study period with the number of child-weeks at risk [Incidence per 1000 child-weeks (A) = GAS cases/13168 child-weeks X 1000 and incidence per 1000 child-years (B) = AX52 wk]. Isolation of GAS with the same *emm* type within a period of 14 days from the same child was considered

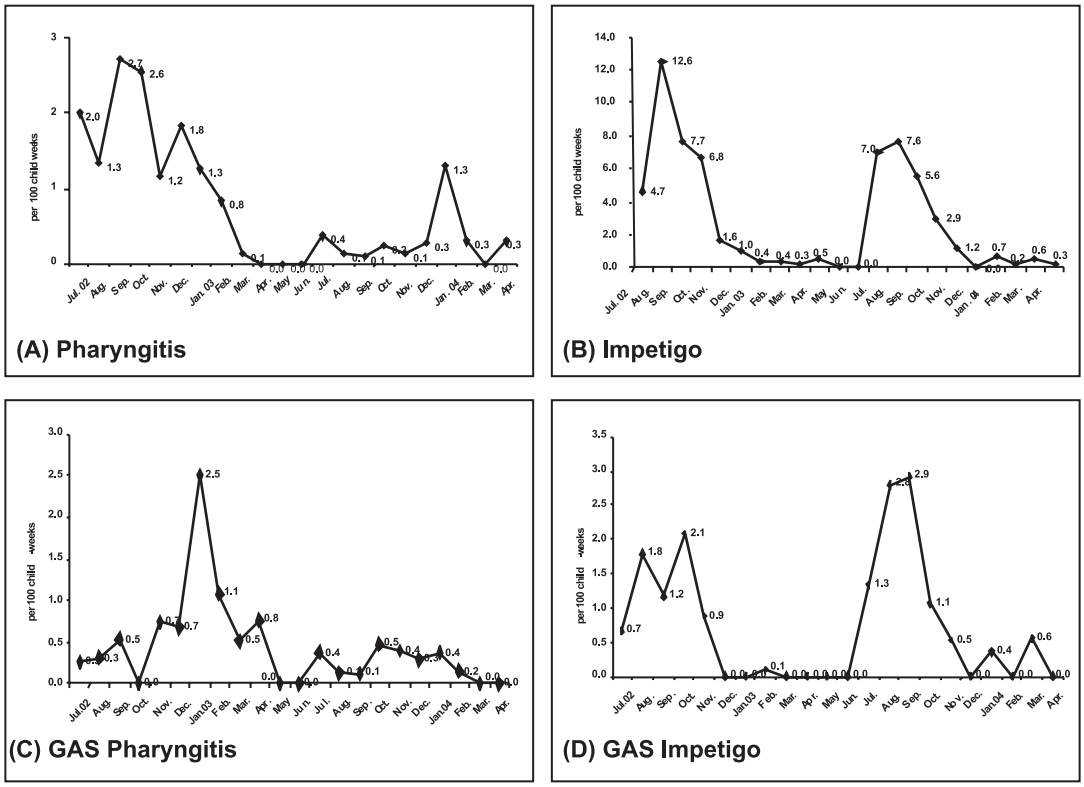

Fig. Monthly incidence of pharyngitis, impetigo and Group A streptococcal infections during 2002-2004.

a single infection (from one child same GAS *emm* type was identified within 14 days). Epi Info version 6 software (CDC, Altanta, USA & WHO, Geneva) was used to calculate 95 per cent confidence interval (CI) and odds ratio (OR).

There were 42 cases of GAS pharyngitis. The incidence of GAS pharyngitis was 3.2 cases/1000 child-weeks or 166 cases/1000 child-years (95% CI 122, 218). There were 97 GAS impetigo cases. The incidence of GAS impetigo was 7.4 cases/1000 child-weeks or 383 cases/1000 child-years (95% CI 323, 446)**.** GAS impetigo cases were more common in summer, whereas GAS pharyngitis was higher during winters (Fig.).

Approximately 15 per cent of school age children (150/1000 child-years) are estimated to have GAS pharyngitis each year[7]. For example, in an affluent population of Melbourne, the incidence of pharyngitis was 0.14 cases/child/ year (140 cases/1000 child-years), a rate similar to that seen in this study[8]. The incidence is estimated to be appreciably greater in developing countries[7]. Although India is a less developed country, the school children of our study lived in villages in a relatively prosperous rural region about 40 km from Chandigarh. In contrast, in an earlier study on school age children in a crowded slum area of Chandigarh, the incidence of GAS pharyngitis was reported to be 950 cases/1000 child-years[3].

There is a consensus that it is difficult to make a diagnosis of GAS pharyngitis on the basis of signs and symptoms and physical examination alone, because virus infections are a frequent cause of pharyngitis. Also, there is an appreciable pharyngeal carriage of GAS in the general population. Recovery of GAS at the time of viral pharyngitis may lead to an erroneous diagnosis and give a false estimate of the incidence of GAS pharyngitis. For these reasons an alternative approach can be employed to estimate the incidence that relies on an estimate of the GAS pharyngeal carrier rate. To illustrate this strategy, the number of GAS positive pharyngitis patients (n=42) in 1209 pharyngitis cases that occurred during the study, were compared to the GAS pharyngeal carriage rate (n=26) in a monthly survey of children without symptoms (n=2016). This carrier study was

conducted a year earlier in the same region as the four study villages[6]. Comparison revealed the odds ratio of GAS pharyngitis to be 2.62 (95% CI 1.55 - 4.04). While this suggests that patients with GAS positive pharyngitis were likely to have had a GAS infection, the conclusion is only tentative because the carrier survey was not done during the same year (although in the same locale) and not on the same children.

Twenty two of the student volunteers in our study had only one attack of pharyngitis. Seven children had GAS pharyngitis more than once; four children had twice, two had thrice, and one had GAS pharyngitis four times. In most instances, different *emm* types of GAS were isolated from children who had more than one episode of pharyngitis. GAS impetigo occurred a single time in 62 students. Seventeen children had GAS impetigo on two occasions. The same *emm* type was isolated from two of these children, but the second occurrence of impetigo was greater than 100 days. It is likely these were re-infections. There were 13 children who showed signs and symptoms of both GAS pharyngitis and impetigo during the two year follow up. In eight children, the impetigo and the pharyngitis were due to the same *emm* type, but the interval between the two events was between 68 and 271 days. Pharyngitis and impetigo due to the same *emm* type was uncommon. This was not surprising, because almost all impetigo occurred in the summer, when the attack rate of GAS pharyngitis was low, and one-third of the impetigo cases were due to *emm* types of GAS that did not cause pharyngitis.

Wannamaker had first recognized that impetigo was due to multiple M types that were often different from those that cause pharyngitis, although the same M type can cause both infections in children[9]. GAS M protein serotypes like M 1, 3, 4, 5, 12, 14, 18, 19, and 24 were found to be associated with throat infection, while M serotypes 2, 49, 57, 59, 60 and 61 are considered to be associated with impetigo[10]. In our study GAS *emm*44 and *emm*112 were predominantly associated with skin infection. The GAS *emm*1-2.2, first time identified in India was also from skin infected patients. Certain *emm* types have the potential for infecting both throat and skin sites[11]. There are numerous cases in which the same M type can be isolated simultaneously from both the pharynx and the impetigo lesion.

The *emm* types are not always adequate strain markers, as these can be shared by unrelated clonal types also. More so the strain variations have been noted within particular M types, and virulence has been linked with a particular isolate rather than being broadly related to a given serotype. GAS *emm* type 81.1 (23.8%, 10/42) was the most prevalent type recovered from pharyngitis. GAS *emm* type 81.1 and *emm* type 112.2 were recovered from 33% (32/97) of the patients with impetigo. Fourteen *emm* types/subtypes that caused impetigo did not cause pharyngitis, and six *emm* types/subtypes of GAS caused pharyngitis that did not cause impetigo (Table). The distribution of *emm* types of GAS isolated from impetigo was similar

Table. *emm* types/subtypes of *S. pyogenes* isolated from a rural area of Panchkula District in Haryana, India

emm type/ subtype	Pharyngitis (n=42)			Impetigo (n=97)		
	July 2002 - June 2003	July 2003 - April 2004	Total	July 2002 - June 2003	July 2003 - April 2004	Total
81.1	8	2	10	7	7	14
112.2	3	1	4	10	8	18
15.1	1	4	5	2	5	7
11.1	3	0	3	1	0	1
49.4	3	0	3	1	0	1
11	2	1	3	1	2	3
42	0	2	2	1	2	3
NT	3	0	3	2	2	4
118	1	0	1	-	-	-
111.1	1	0	1	1	0	1
92	1	0	1	-	-	-
90.2	1	0	1	1	0	1
81.4	1	0	1	-	-	-
81.2	0	1	1	4	2	6
74	1	0	1	-	-	-
13.1	1	0	1	-	-	-
3	1	0	1	-	-	-
44	-	-	-	1	9	10
85	-	-	-	2	6	8
1-2.2	-	-	-	2	1	3
103	-	-	-	2	1	3
70	-	-	-	1	2	3
82.1	-	-	-	1	1	2
ST212	-	-	-	2	0	2
1	-	-	-	0	1	1
11.2	-	-	-	1	0	1
55	-	-	-	0	1	1
77.1	-	-	-	0	1	1
ST1389.1	-	-	-	1	0	1
ST1731	-	-	-	1	0	1
ST95050	-	-	-	1	0	1
Total	31	11	42	46	51	97

in both years. There was no evidence of clustering of pharyngitis or impetigo cases in any of the four schools under observation. The total number of pharyngitis patients for each school was similar.

In our study, 23.8 per cent of the pharyngitis cases were caused by GAS *emm* type 81.1, with 76.2 per cent due to 16 different *emm* types/subtypes. Our findings support the earlier observations about the genetic heterogeneity among Indian skin and throat isolates[12,13]. Such diversity was also seen in other settings[14]. It appears that serotypes present within a population vary between distinct geographical locations and may also change with passage of time. Kaplan *et al*[15] reported displacement of M1 GAS with M6 in USA. Thus, surveillance is needed to identify the diversity of M types of GAS, and the emergence of M types not previously present in the population.

There are a few instances today where only several predominant GAS M types cause the majority of infections. Notable exceptions are: M 18 causing the majority of endemic ARF in the Salt Lake City area[16], and M 3 as the primary cause of necrotizing fascitis in Canada and elsewhere[17].

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