

SHIGELLA TYPES ENCOUNTERED IN SHIGELLOSIS OF CHILDREN IN ANKARA

A Study of 108 Strains Isolated Within
the Past Three Years in Hacettepe Children's Hospital*

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Introduction

In this country, little investigative work has been done on human shigellosis. We have no data available, for example, on what strains are encountered in shigella infections in children. Although İrdelp,¹ Reşat Rıza,² Unat,³ Çelik,⁴ Braun and Unat,⁵ Braun and Özek,⁶ Alantar and Tümay,⁷ and Noyan⁸ have provided some data on the subject of shigellosis, much information is still lacking because those reports, in general, deal with rather small numbers of bacteria. To provide a complete understanding of the shigella types prevalent in Turkey, much more work and comprehensive studies from all over the country are necessary.

In our hospital, in a period of three years, (September 1957 to September 1960) we have isolated 108 strains of shigella from children. These are all single isolations; occasional repeated isolations are not included in this study, nor are clinically diagnosed shigellosis cases, not confirmed by isolation of the organism. From this information alone, we may draw no conclusions about shigella distribution in the country at large; however, it seemed useful to report our data on shigella types, distribution by age groups, seasonal variations, etc., as a contribution toward more complete understanding in the future.

Materials and Methods

Samples: For older children, feces specimens, for small infants, rectal swabs were used for the inoculations.

Media: (SS + Endo) or (Bismuth sulfite + Endo) combinations were used.

Procedure: Samples were directly streaked over the surfaces of plates and incubated 18-48 hours at 37 C. Suspected colonies were subcultured on agar

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slants and then transferred to TSI and Tryptone Broth tubes to determine the basic biochemical characteristics and the presence or absence of indol production. If the reactions were typical, these characteristics, together with a serological investigation of the strain, were considered to be satisfactory for identification. If the reactions remained doubtful, carbohydrate fermentation tubes containing phenol red indicator were also inoculated, using maltose, mannitol, dulcitol, xylose and salicin.

In each case, growths on the slant were tested first with polyvalent shigella diagnostic serum and with the corresponding group sera on slides to determine the type of bacteria.

For the culture medium, Difco's dehydrated material,⁹ and for agglutinations, Lederle's commercial diagnostic sera were used. All results obtained were confirmed by Dr. N. Aksoycan (National Salmonella and Shigella Center, Refik Saydam Central Institute of Hygiene, Ankara, Turkey).

Results

During the last three years we have isolated 108 strains of shigella from children, all under 15 years of age. All were sporadic cases as far as we could determine. Reportedly, there were some small epidemics in the city but since most of the cases were not seen in hospitals, no correlation between presumed epidemics and strains isolated is possible.

The monthly distribution of the shigella isolations is shown in figure 1.

It is evident in figure 1 that bacteria from each of the four groups of shigella were isolated during the period studied. One of the most interesting features of the results is that most of the strains belonged to group B. *Sh. flexneri*. This figure also illustrates the fact that even in the relatively short period under study there was considerable annual variation, the number of strains isolated in 1958 and 1960 exceeding the number in 1959. Since there have been no significant changes in materials and methods used in our laboratory nor in the number of fecal cultures performed during these three years, it seems reasonable to assume that the difference in the number of strains isolated was due merely to small epidemics in the above mentioned years in the city.

Without distinguishing among various types these findings are summarized in figure 2.

In this figure we see that the peak incidence occurred in July with a total of 35 isolations. June, August and May follow with 25,

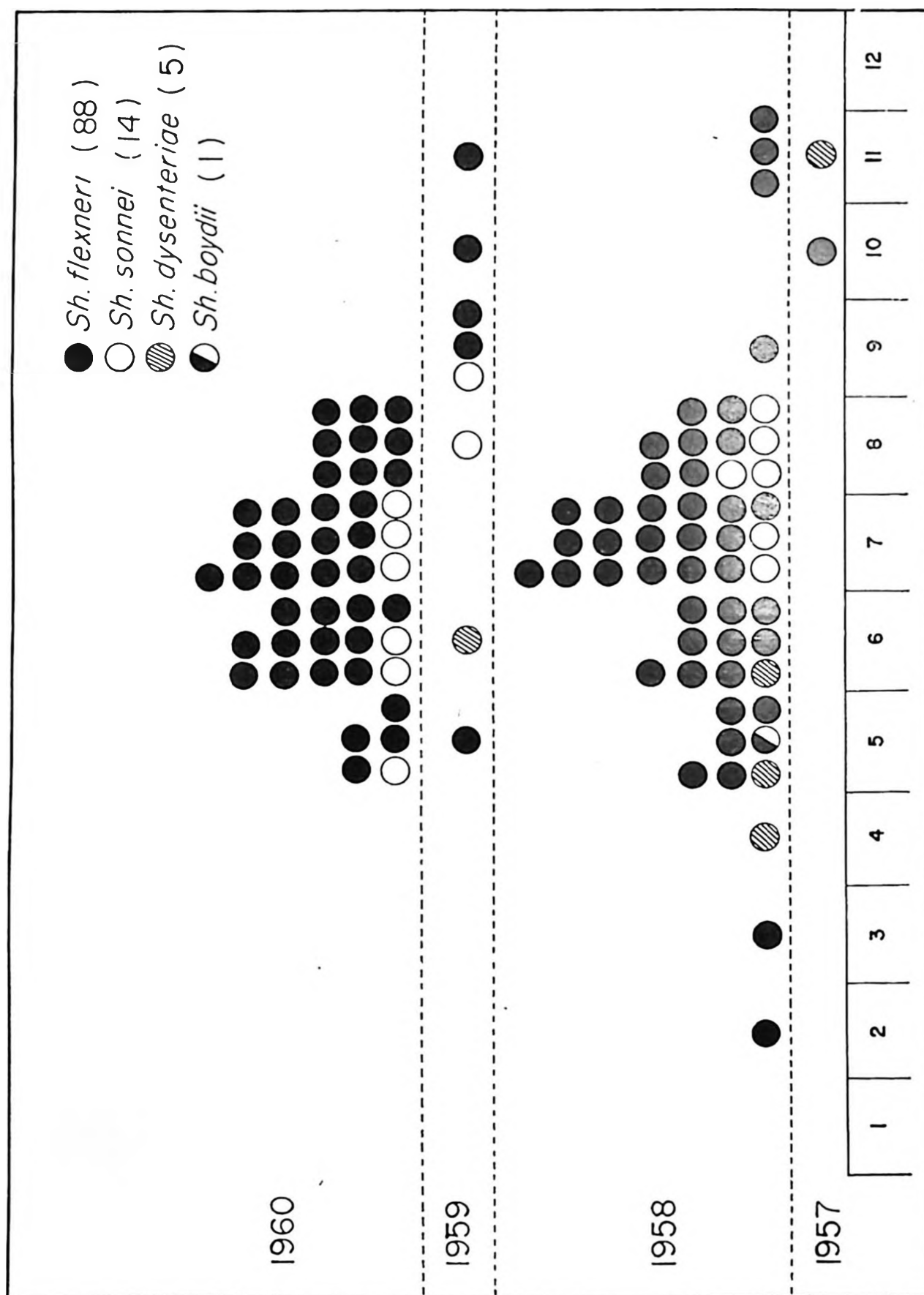


Fig. 1. — The monthly distribution of 108 strains of shigella isolated within the past three years from children in Ankara. Each dot represents a single bacterium. Numbers on the bottom of the figure indicate the months of the year.

21 and 13 isolations respectively. The increased incidence in these four months, with a peak in July and a rapid decrease after August, conforms very well with classical knowledge of the epidemiology of shigellosis in general.

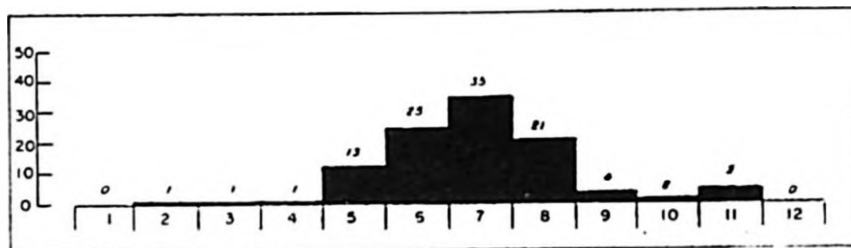


Fig. 2. — Monthly distribution of 108 strains of shigella isolated. Blocks indicate the total numbers isolated for each month, the figures at the bottom the months of the year.

We have seen in figure 1 that most of the strains isolated were *Sh. flexneri*. The incidence rates of the various types among these 108 strains are shown in figure 3.

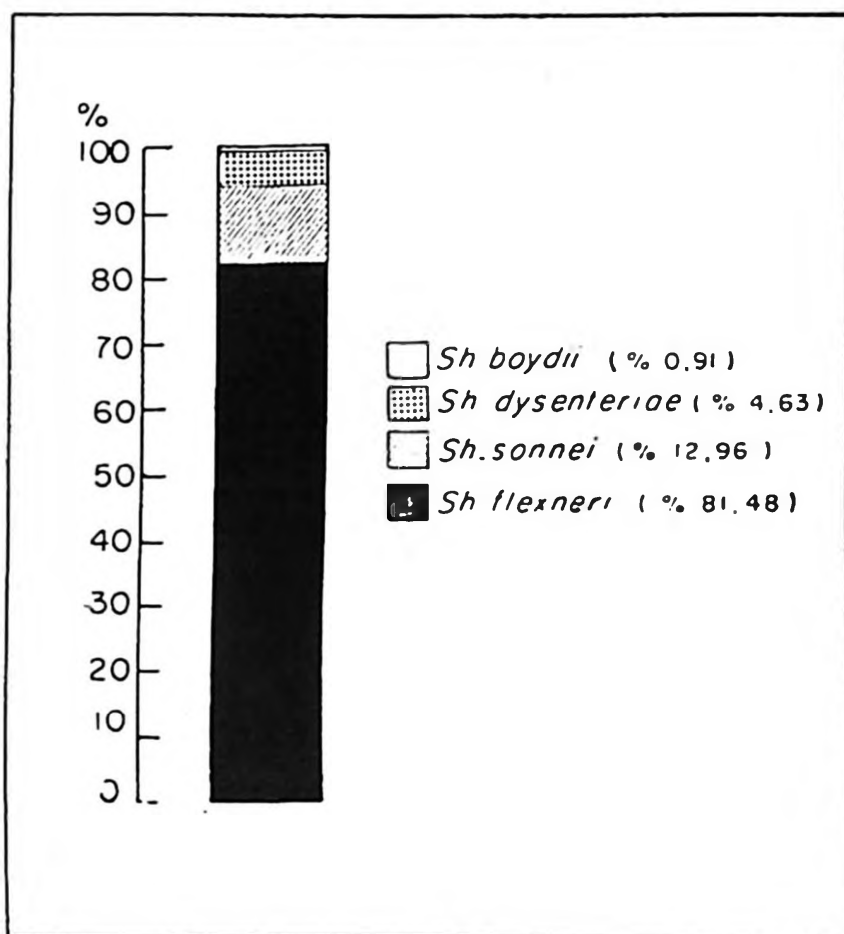


Fig. 3. — The incidence of various types found in 108 strains of shigella isolated from children within the past three years in Ankara.

As we see, out of 108 strains, 88 (or 81.48 per cent) are *Sh. flexneri*, 14 (or 12.96 per cent) *Sh. sonnei*, 5 (or 4.63 per cent) *Sh. dysenteriae* and

1 (or 0.9 per cent) *Sh. boydii*. (Since we isolated only one strain of *Sh. boydii*, we showed it as a thin, white line on the top of the column.)

From this we conclude that in shigellosis in children during the past three years in Ankara, *Sh. flexneri* played the most important etiological role. The number of *Sh. flexneri* strains is about three times the sum of the remainder.

Fifty-one strains of *Sh. flexneri*, which were isolated after obtaining the type specific agglutinating sera, were typed. The results of this work are shown in a diagram (fig. 4).

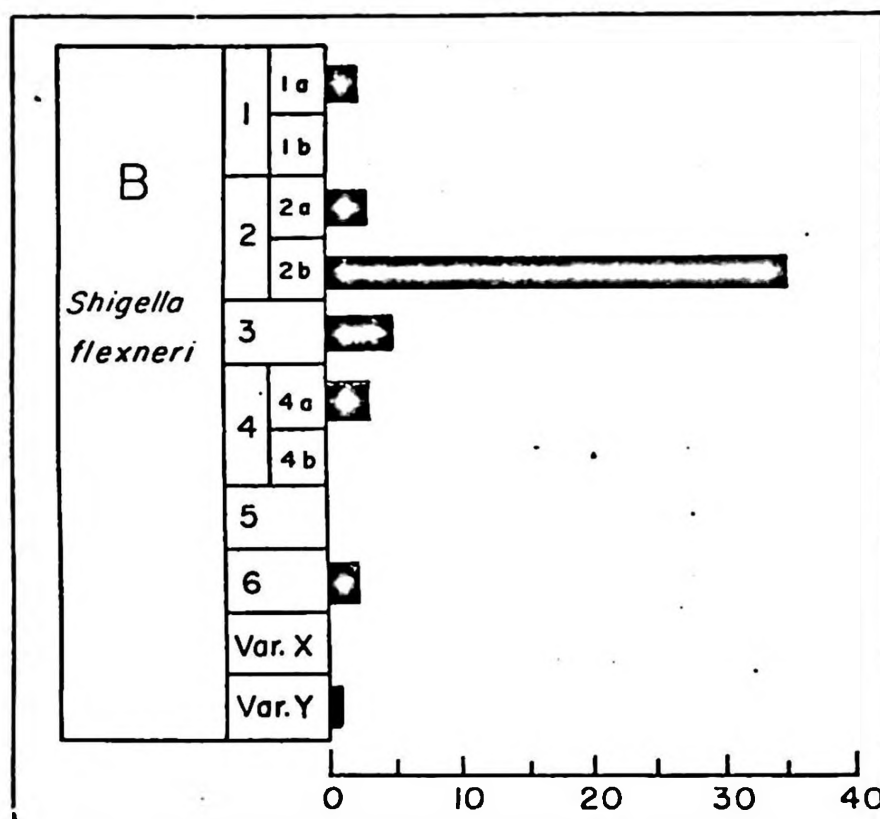


Fig. 4. — The types and sub-types of 51 strains of *Sh. flexneri* isolated from children. The figures on the bottom indicate the numbers of strains.

It will be seen from figure 4 that a great majority of *Sh. flexneri* strains (35 out of 51 or 68.6 per cent) were proved to be type 2b. This is followed by type 3 (5 strains), types 2a and 4a (3 strains each), types 1a and 6 (two strains each), and finally by Variant-Y (only a single strain). Among these 51 strains there were no specimens of types 1b, 4b, 5 or Variant-X. This is the first reported survey on types and sub-types of shigella strains in Turkey, as far as we know. We have no idea whether or not future investigations will show the existence of types not found among the strains tested and will demonstrate changes in the incidence rates among the types which we detected.

Out of 108 strains, 57 were isolated from males and 51 from females. Thus, as would be expected, there is no significant difference in the attack rate in the two sexes.

As for the age groups, out of 108 strains, 72 (approximately 66.6 per cent) were isolated from children between the ages of 0 and 2, 29 (approximately 26.8 per cent) from children in the age group from 2 to 6 and 7 (approximately 6.4 per cent) from older children between 6 and 14 years of age, (fig. 5). Before leaping to a conclusion

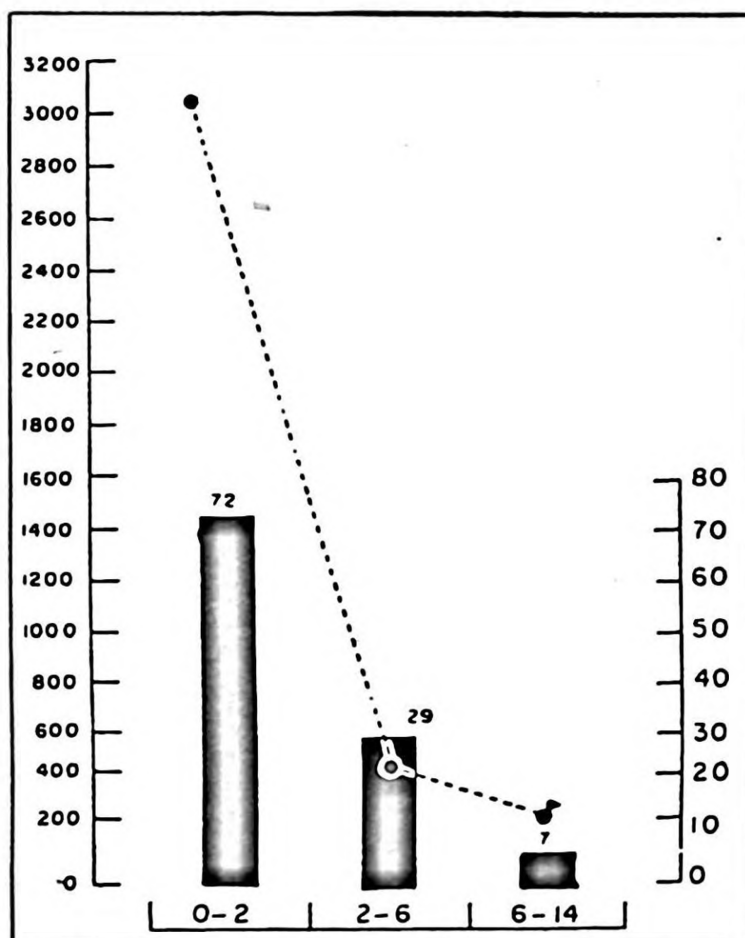


Fig. 5. — A comparison of the numbers of feces cultures and shigella isolations from children in three different age groups, both being three year totals. The left hand scale indicates the total number of feces cultures and the scale on the right indicates the number of strains isolated. The blocks represent the total number of strains isolated and the dots connected by the line reflect the total number of feces specimens cultured in the three age groups.

that bacillary dysentery is more frequently seen among children in the age group of 0-2, we thought it would be worth while to compare the total numbers of feces cultures performed for each of the three age groups with the numbers of shigella strains isolated. Many other factors also might have played a role in producing such a result, but this comparison alone proves that there is simply a direct correlation between the number of feces specimens cultured

and the number of bacteria isolated. The greater the number of feces cultures, the greater are the number of isolations, (fig. 5).

It is apparent, then, that on the basis of the present data no conclusions regarding variations in susceptibility in various age groups can be drawn, since it seems likely that the larger number of strains isolated from the younger age groups merely reflects the fact that many more specimens were obtained from those age groups.

Discussion

The real number of shigellosis cases among children during the past three years in Ankara, was, it seems obvious, much greater than would be indicated by the number of stool cultures which were positive for shigella. The discrepancy between the number of probable clinical cases and the strains actually isolated may be due to any of the following factors:

1. Most of the cases were not observed in hospitals. (Sometimes this was because of difficulties in transportation, sometimes because of self treatment with broad spectrum antibiotics. Undoubtedly there were children with severe and rapidly progressive disease who died before they could be taken to a hospital. In addition, physicians in private practice, who are most concerned to treat their cases quickly, frequently make no effort to determine the etiologic agent in seemingly ordinary cases of gastroenteritis.)

2. Some of the patients were observed at such a point in the course of the illness that the chances, if any, for isolation of the etiologic agent were extremely low. Onul¹⁰ mentions that the chance for shigella isolations is about 70 per cent at the beginning of the illness, while it decreases to 40 per cent at the end of the first week. By the end of the second week the bacilli have usually completely disappeared from the stool.

3. As in any other laboratory, technical failures are undoubtedly a limiting factor; 100 per cent success in isolation is impossible.

Our findings that most of the strains isolated from children were *Sh. flexneri* (81.48 per cent of the total), coincides with the findings reported before in this country. For example, İrdelp¹ with his two co-workers observed that 95 per cent of the shigella strains isolated in Istanbul and Thrace were *Sh. flexneri* and 5 per cent of the total were *Sh. dysenteriae* in the year 1912. The same author (1944) reports that in a shigellosis epidemic in Istanbul, there were 12 *Sh. flexneri* isolations from 100 clinical cases. Alantar and Tümay⁷ reported that out of 19 shigella strains isolated from children in the years

1949-1950 in the Children's Clinic of Istanbul University, 14 were *Sh. flexneri* and 5 were *Sh. shigae* (*Sh. dysenteriae*). Simmons and his co-workers¹¹ write that 60 per cent of the shigella strains isolated between 1941 and 1950 in Turkey, were *Sh. flexneri*, 20 per cent *Sh. dysenteriae* and the remainder *Sh. sonnei* and *Sh. schmitzii*.

In a report by the author in collaboration with Drs. Aksoykan and Berkman, presented before the Ninth National Microbiology Congress (Sept. 21-24, 1960, Istanbul) the strains isolated both from children and from adults in Turkey were described, and it was observed that the incidence rates of the different types of shigella strains were: group B 50.3 per cent, group D 36.3 per cent, group A 6.6 per cent and group C 6.6 per cent. Again it is seen that the rates are of the same order as those presented here for children.

On the basis of bacteriological surveys made among the inhabitants of this country, we may conclude that the most prevalent strain in shigellosis in Turkey is *Sh. flexneri*. It is interesting to observe that the incidence rates for different strains of shigella encountered among the children from another country differ from the rates which we found in Turkish children. Dr. Berkman,¹² of the United States Air Force Hospital in Ankara, has found that among 32 shigella strains which he isolated from American children living in Turkey between July 1959 and August 1960, there were 17 *Sh. sonnei*, 11 *Sh. flexneri*, 2 *Sh. boydii* and 2 *Sh. ambigua* (*Sh. dysenteriae*). There were more *Sh. sonnei* than *Sh. flexneri* in comparison with the findings in Turkish children. Which factors produced such a difference we do not know exactly. One of the factors, probably, is that the children from the two countries were infected from different sources, since many Americans do not use city water, or else boil it, and eat little food from public markets. There is no significant difference in the media and methods used in shigella isolations in this institution and in the United States Air Force Hospital in Ankara.

As far as we know there is no difference among the people of the two countries in susceptibility to any strain of shigella.

Summary

In the Hacettepe Children's Hospital in Ankara, within the last three years, 108 strains of shigella were isolated from children between 0 and 14 years of age. Of these 81.48 per cent were *Sh. flexneri*, 12.96 per cent *Sh. sonnei*, 4.63 per cent *Sh. dysenteriae* and only one *Sh. boydii*. These results coincide well with the results published by other workers in Turkey.

The most prevalent type of *Sh. flexneri* is Type 2b; out of 51 strains of *Sh. flexneri*, 35 (or 68.6 per cent) proved to be of this type. Berkman, who is the microbiologist of the United States Air Force Hospital in Ankara, informed us that the most prevalent type among 32 strains of shigella he isolated from American children was *Sh. sonnei* with *Sh. flexneri* occupying the second place in bacillary dysentery. The cause of this difference is not known.

Most of the strains were isolated during the summer months. The chance of isolation increases after May and again decreases after September. There is no correlation between the isolations and the age groups of children as far as we could determine.

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