The Manchester Variety Of Shigella flexneri 6 Isolated in Kentucky

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THE Manchester variety of Shigella flexneri 6 has been found to be prevalent among the normal population of preschool children in the coal mining region of eastern Kentucky. In diarrheal disease studies conducted by the Cumberland Field Station of the Public Health Service's Communicable Disease Center, this strain was isolated one or more times from 69 children during a 20-month period.

To our knowledge, no previous reports of the identification of the Manchester variety in the United States have appeared in the literature, although several investigators advise us that the strain has been isolated in this country. W. H. Ewing of the International Shigella Center in Atlanta, Ga., has confirmed the identification of this strain, which was isolated by L. F. Ey and C. C. Croft of the Ohio Department of Health during a disease outbreak in Mansfield, Ohio, in July 1949. In this outbreak, 9 infants died and 172 of the estimated 468 residents in an area of substandard homes gave a history of being ill. Ewing has said also that the Manchester variety has been isolated in Louisiana, and W. W. Ferguson of the Michigan Department of Health has reported identification of the strain in his State.

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Dr. W. H. Ewing of the International Shigella Center, Atlanta, Ga., confirmed the identification of 98 Shigella flexneri 6 isolations (5 Manchester and 3 Boyd 88) and 22 other Shigella isolations reported in this paper. The Manchester variety is one of three presently known biotypes of S. flexneri 6. In 1925 Clayton and Warren isolated the first biotype from the feces of a girl suffering from diarrhea in Newcastle, England (1). Later, these workers obtained similar organisms during a small epidemic of diarrhea in a children's home (2). The mannitol negative, aerogenic bacillus described by them, subsequently named the Newcastle dysentery bacillus, was serologically related to other S. flexneri serotypes. Hardy and others (3) have reported that the Newcastle variety is the cause of acute diarrheal disease in England, India, Africa, and South America.

In 1933 Downie, Wade, and Young (4) described organisms which they isolated from five diarrheal cases near Manchester, England, and from a mild case of dysentery in a Nigerian laboratory worker. These organisms were serologically identical with the Newcastle bacillus, but differed biochemically in their ability to ferment mannitol aerogenically. They became known as the Manchester variety of S. flexneri 6.

A third biotype, which has been designated the Boyd 88 strain, was first reported from India by Boyd (5, 6). The organisms he described were aerogenic, fermented mannitol, and were serologically identical to the Newcastle dysentery bacillus. The pathogenicity and recovery of the Boyd 88 biotype have been reported throughout the world.

Scott (7) has shown that the bacilli of Boyd 88, Manchester, and Newcastle are all serologically identical.

Material and Method

One phase of the diarrheal disease studies in the coal mining region of eastern Kentucky is the collection each month of rectal swab specimens from normal preschool children in selected mining camps and rural populations. Specimens are obtained at the homes in the manner described by Hardy and Watt (8), by inserting a sterile cotton swab into the rectum. Immediately after withdrawal from the rectum the swab is used to streak a Shigella-Salmonella (Difco) agar plate and is then placed in a tube of tetrathionate broth. The

inoculated plates are subsequently incubated at 37° C. in the laboratory, and typical colonies are picked to triple sugar iron (TSI) agar at 24 and 48 hours. The swab in tetrathionate broth is incubated at 37° C. for 24 hours and then streaked on brilliant green agar plates, which are then incubated. Colonies typical of Salmonella are picked to TSI agar at 24 and 48 hours. Biochemical and serologic examinations of organisms suspected of being Shigella or Salmonella are performed as described by Edwards and Ewing (9).

Results

During a 20-month period, September 1954 through April 1956, Shiqella organisms were isolated from 272, or 3.3 percent, of 8,392 specimens obtained from normal preschool children. As shown in the tabulation below, the most prevalent type encountered was S. flexneri 6, which represented 43 percent of all Shigella isolations. Of the 118 S. flexneri 6 isolations, 95 percent were Manchester and 5 percent were Boyd 88 biotypes. Shigella sonnei, representing 22 percent of the total Shigella isolations. was the second most prevalent type. Seasonal peaks in Shigella isolations occurred in the fall and early spring, with the proportion of the Manchester variety to all other shigellae remaining fairly constant.

Numb	er of
isola	tions
S. dysenteriae 2	7
S. flexneri 1b	24
S. flexneri 2a	26
S. flexneri 3	5
S. flexneri 4a	33
S. flexneri 6, Manchester variety	112
S. flexneri 6, Boyd 88 variety	6
S. sonnei	59
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Total	272

The 112 positive isolations of the Manchester biotype were obtained from 69 children in 52 families. The organism was isolated once from 48 children, twice from 14 children, 3 times from 1 child, 4 times from 1 child, 5 times from 4 children, and 9 times from 1 child. In 12 children the organism was recovered in 2 consecutive months, and in 2 it was recovered for 3 and 6 successive months respectively. In

seven children the bacillus was recovered twice with a negative culture during the intervening month. From 4 children a second recovery occurred after 2 negative monthly cultures, and from 1 child a second isolation was obtained after 3 successive negative cultures.

Discussion

In view of the few reports of the occurrence of the Manchester biotype in the United States, the high prevalence of the organism in normal populations in Kentucky is striking. The findings may be of particular significance to epidemiological and laboratory workers. It is possible, of course, that the situation in these somewhat isolated communities is not duplicated elsewhere, but it is also possible that the organism is actually more widespread in this country than the paucity of reports would indicate.

Biochemical reactions of the Manchester bacillus are atypical in comparison with the reactions of the other shigellae in that they ferment glucose and mannitol with the production of gas. The Manchester biotypes encountered were nonmotile and did not utilize citrate, produce indol, or hydrolize urea. Lactose, adonitol, and salicin fermentations were negative. The organism consistently fermented glucose and mannitol, with production of gas in both Glucose fermentation is of carbohydrates. particular significance to diagnostic laboratory workers since reactions in TSI agar have been used as one of the principal biochemical screening tests in enteric bacteriology. Gas is formed by the Manchester bacillus in TSI and Kliger's agar slants in small to moderate amounts frequently sufficient to rupture the media. Therefore, cultures showing an alkaline slant and acid butt with gas should not only be considered as possible "paracolons" or non-H₂S-producing salmonellae, but should be checked as this biotype of S. flexneri The Boyd 88, the Manchester, and the Newcastle biotypes cannot be differentiated by serologic tests with absorbed antiserums since they are serologically identical. Final identification and differentiation of the biotype is dependent upon both biochemical reactions and slide agglutination with absorbed antiserums.

Of the 21 children in this study with more than one infection, approximately one-half were members of families in which their preschool siblings became infected, thereby providing ample opportunity for intrafamilial reinfection. For example, M. C. and E. C. are 2- and 9-vear-old sister and brother. M. C. was Manchester positive on specimens taken in July, November, and December 1955, and again in March and April 1956. E. C. was positive in August, September, and October 1955, and again in February and March 1956. Since the Manchester bacillus was found only once in 70 percent of the children infected and since there was ample opportunity for reinfection in children having infections for consecutive months, the duration of infection presumably averaged about 1 month. This period is consistent with previous observations by Watt and his coworkers (10) on the duration of the carrier state for the S. flexneri group.

Summary

Two biotypes of *Shigella flexneri* 6 have been isolated from normal preschool children in eastern Kentucky. Of 272 *Shigella* isolations obtained from specimens taken September 1954 through April 1956, 112 (41 percent) were Manchester bacillus and 6 (2 percent) were the Boyd 88 variety.

Attention is called to the atypical biochemical reactions of the Manchester bacillus in comparison with other *Shigella* and to the possibility that this biotype is more widespread in this country than is currently believed.

The average duration of the carrier state appears to be approximately 1 month, which is

comparable to duration of infection of other S. flexneri types.

REFERENCES

- Clayton, F. H. A., and Warren, S. H.: An unusual bacillus recovered from cases presenting symptoms of dysentery. J. Hyg. 28: 355 (1928).
- (2) Clayton, F. H. A., and Warren, S. H.: A further study of an unusual bacillus recovered from cases presenting symptoms of dysentery. J. Hyg. 29: 191 (1929).
- (3) Hardy, A. V., Watt, J., and DeCapito, T.: Studies of the acute diarrheal diseases. VI. New procedures in bacteriological diagnosis. Pub. Health Rep. 57: 521-524, Apr. 10, 1942.
- (4) Downie, A. W., Wade, E., and Young, J. A.: An organism resembling the Newcastle type of dysentery bacillus associated with cases of dysentery. J. Hyg. 33: 196 (1933).
- (5) Boyd, J. S. K.: New types of dysentery bacilli. In Proceedings of Second International Congress of Microbiology. London, 1936, p. 159.
- (6) Boyd, J. S. K.: Further investigations into the character and classification of the mannitefermenting dysentery bacilli. J. Roy. Army M. Corps. 59: 241, 331, October and November, 1932
- (7) Scott, W. M.: Bacillary dysentery of Newcastle type, with note on bacteriology. Lancet 227: 248 (1934).
- (8) Hardy, A. V., and Watt, J.: Shigellosis (bacillary dysentery). In Communicable diseases, edited by R. L. Pullen. Philadelphia, Lea and Febiger, 1950, p. 835.
- (9) Edwards, P. R., and Ewing, W. H.: Identification of Enterobacteriaceae. Minneapolis, Burgess Publishing Company, 1955.
- (10) Watt, J., Hardy, A. V., and DeCapito, T.:
 Studies of the acute diarrheal diseases. IX
 B. Shigella dysenteriae infections among institutional inmates. Pub. Health Rep.
 57: 1095-1102, July 24, 1942.