Prevalence of Four Enteropathogenic E. Coli Groups In Preschool Children

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THE WORK of Bray (*1*) in 1945, and Bray and Bevan (*2*) in 1948, focused attention on a number of *Escherichia coli* serotypes as causative agents of diarrhea. Since that time, a number of reports in Great Britain (*3-9*) and the United States (*10-16*) have documented specific evidence of the association of certain *E. coli* groups with clinical cases of diarrhea.

While many of these studies included sampling of populations exhibiting no diarrheal symptoms, the cultures were largely obtained from contacts, children reporting to clinics for reasons other than diarrhea, or from children in institutional environments such as orphanages and hospitals. Limited epidemiological information is available, however, on the age-specific prevalence of the enteropathogenic *E. coli* in normal populations, secondary attack rates, duration of carrier state, and case-to-carrier ratios.

In 1956, a limited study of the prevalence of four enteropathogenic *E. coli* groups in healthy preschool age children was conducted in eastern Kentucky by the Cumberland Field Station of the Communicable Disease Center, Public Health Service. The investigations were part of the diarrheal disease studies of the station.

Procedures

Public health nurses and epidemiological aides have visited households in 6 coal mining camps and 5 rural hamlets in eastern Kentucky at monthly intervals since September 1954 to obtain histories of diarrheal disease within entire families. On each visit they also obtained rectal swab cultures from preschool children to determine the prevalence of *Shigella* and *Salmonella* infections. As an adjunct to the determination of diarrheal attack rates and *Shigella-Salmonella* prevalence in the study populations, a concurrent study was begun in February 1956 in four of the study populations to determine the prevalence of four enteropathogenic *E. coli* groups in preschool children. *E. coli* 026 : B6, 055 : B5, 0111 : B4, and 0127 : B8 were selected for study because they had been most frequently associated with outbreaks of infantile diarrhea by previous investigators. Forty-three preschool children in each of 3 coal mining camps and 1 rural hamlet, making a total of 172 children, were selected for the 6-month comparative study. The four areas were selected as representative of the diversity of housing conditions observed in eastern Kentucky and because considerable variations in both morbidity rates and prevalence of *Shigella* had been previously observed in them. The ages of the children in the four areas ranged from 1 month through 5 years, with approximately equal numbers of children in each yearly age group.

At the time the monthly rectal swab cultures were obtained for detection of *Shigella* and *Salmonella* in the preschool children of the four selected areas, one-fourth of a MacConkey agar plate was streaked with the cotton swab for the *E. coli* studies. The inoculated plates were brought to the laboratory within 4 hours, where the streaking was completed with a wire loop. Attempt was made to obtain well-isolated colonies on most of the agar surface. The cultures were incubated for 24 hours, and each of 20 typical *E. coli* colonies was fished to a drop of 0.5 percent saline solution on a smooth slide and tested against pooled antiserums. This polyvalent serum was prepared by mixing equal amounts of *E. coli* 026 : B6, 055 : B5, 0111 : B4, and 0127 : B8 antiserums with 0.5 percent saline solution to obtain a 1 : 10 dilution of each specific antiserum. When a colony was found which gave an agglutination, 20 additional colonies were transferred to tubes of triple-

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sugar-iron agar and heart infusion agar. Final biochemical and serologic identification was carried out according to the recommendations of Edwards and Ewing (*17*). When a child was found to be carrying 1 of these 4 serotypes, an effort was made to obtain additional cultures from family contacts. A total of 1,000 survey cultures and 28 cultures from family contacts were examined between February 6 and August 20, 1956.

Results

Of the 1,000 cultures examined, 13 (1.3 percent) were positive for 1 of the 4 *E. coli* groups, and 59 (5.9 percent) were positive for *Shigella*. *Salmonella* was recovered from three cultures (see table). Because of the limited number of *E. coli* isolations, no seasonal variation in their occurrence was observed.

E. coli 055, isolated from 6 children, was the most common of the enteropathogenic groups encountered. Group 0111 was identified from 4 children and *E. coli* 0127 from 3 others. Dr. W. H. Ewing examined four of the six 055 : B5 cultures and classified them all as serotype 055 : B5 : H6. All the 0111 : B4 strains were 0111 : B4 : H12. Six of the positive children were 2 years old, 4 were in the 1–2-year category, 2 were 3–4 years old, and 1 was less than a year old. Enteropathogenic *E. coli* were not isolated from any of the 13 positive children on subsequent cultures 1 month following the initial isolation. Cultures of 6 familial contacts of 1 positive child resulted in isolation of the same *E. coli* group from 2 siblings. Cultures obtained from 22 siblings of 6 other positive children were negative. None of the positive children gave a history of diarrhea during a period of 4 weeks before or 2 weeks after culturing.

Reported diarrheal attack rates, ranging from 11 to 92 per 1,000 person-months experience, were associated with the range of 2.1 to 10.6 percent in *Shigella* isolations. The percentage of enteropathogenic *E. coli* isolations (1.1 to 1.6 percent) was approximately the same in the four areas.

Discussion

The low percentage of cultures yielding any of the four enteropathogenic *E. coli* groups is typical of the results of earlier studies on cultures from nondiarrheal patients in hospitals and clinics. Giles and associates (*4*) isolated *E. coli* 055 ten times from 324 healthy infants; Stevenson (*7*) recovered *E. coli* 0111 nine times in 1,024 adults; Taylor and Charter (*9*) found four *E. coli* 086 groups among 255 well babies; and Graber and Dunlap (*12*) failed to recover any of 9 enteropathogenic serotypes from 410 pediatric patients in the absence of diarrhea.

Two factors may have contributed to the infrequent isolations of enteropathogenic *E. coli* in the present study. It was necessary to use

Comparison of diarrheal attack rates and isolation rates of E. coli and Shigella in preschool children in eastern Kentucky, February–August 1956

Area	Bacteriological examinations					Reported morbidity (0–5 ages)		
	Number of cultures	Positive for *Shigella*		Positive for *E. coli*		Person-months experience	Number of cases	Rate per 1,000 person-months
		Number	Percent	Number	Percent			
Wheelwright	183	4	2. 2	2	1. 1	183	2	11
Weeksbury [1]	281	6	2. 1	3	1. 1	281	5	18
Wayland	243	18	7. 4	4	1. 6	243	10	41
Jacks Creek [2]	293	31	10. 6	4	1. 4	293	27	92
Total	1, 000	59	5. 6	13	1. 3	1, 000	44	44

[1] 1 *Salmonella paratyphi* B and 1 *Salmonella typhimurium* isolated.
[2] 1 *Salmonella montevideo* isolated.

rectal swabs in the survey in order to handle a larger number of cultures. This method has proved satisfactory for *Shigella* work, but there is still disagreement among workers in the field as to its desirability in *E. coli* studies. Second, because of the large amount of confirmatory work that must be done with the *E. coli* group, it was necessary to use only one MacConkey agar plate for each individual and to limit the number of colonies being screened to 20 per plate. This number of colonies would seem to us to be adequate if 4 or 6 types of *E. coli* are equally distributed on a plate. However, if a particular serotype is present in very small numbers, there is a probability that it will be missed in the procedures employed.

The rate of occurrence of the four *E. coli* serotypes did not correlate with the reported diarrhea morbidity from the study population, in contrast to the correlation of *Shigella* incidence with morbidity (see table). If we may assume that the four enteropathogenic *E. coli* groups were readily recovered by the laboratory tests employed, it is apparent that these particular *E. coli* groups did not contribute appreciably to the diarrheal morbidity in the populations under study. Although the numbers are too small to draw definite conclusions, it is notable that in only 1 of 7 instances was there any evidence of intrafamilial spread. Also of note was the observation that all 13 positive individuals were negative when cultured 1 month later. This low rate of transmission within the family and the apparently short carrier period could possibly be accounted for by the absence of diarrhea. Histories of the positive children indicated that they had no more than one bowel movement in 24 hours. This lessened appreciably the possibility of cross- and auto-infection hazards compared with conditions existing in a household where an ill child has 6 to 12 loose movements during the same period.

Summary

A 6-month survey of the prevalence of *Shigella and Salmonella* and four enteropathogenic *E. coli* groups in 172 healthy preschool children was conducted in eastern Kentucky in four areas having diverse housing and sanitary facilities. Fifty-nine *Shigella*, 3 *Salmonella*, and 13 enteropathogenic *E. coli* were isolated from 1,000 cultures.

Of 13 positive *E. coli* isolations, 6 were group 055, 4 were 0111, and 3 were 0127. *E. coli* 026 was not recovered. None of the 13 positive children gave a history of being ill, and all subsequent monthly cultures were negative. In 28 cultures of siblings of 7 positive children, only one instance of intrafamilial spread was observed.

The four enteropathogenic *E. coli* groups did not contribute appreciably to the diarrheal attack rates in the areas sampled.

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Standard for Enrichment of Milled Rice

A standard for enriching milled rice was published by the Food and Drug Administration on August 27, 1957, specifying vitamins and amounts to be added by rice packers.

The action is the outcome of recommendations by the National Research Council and is based on Food and Drug Administration proposals published December 28, 1956, and on written comments on these proposals.

Under the standard, each pound of milled rice labeled "enriched" must contain 2 to 4 milligrams of thiamine, 1.2 to 2.4 milligrams of riboflavin, 16 to 32 milligrams of niacin, and 13 to 26 milligrams of iron. The cost of these enrichment ingredients would be about 5 cents per 100 pounds of rice. The rice packer who chooses to enrich his product further with vitamin D, or calcium, or both, must add 250 to 1,000 U. S. P. units of vitamin D and 500 to 1,000 milligrams of calcium, to each pound of his product.

At present, no rice on the market fully conforms to the standard. South Carolina's compulsory enrichment regulation matches the new Federal standard in all aspects except that the use of riboflavin is optional.

The standard allows two enrichment processes. In one process, a proportion of the kernels is impregnated with the vitamins. The standard requires packers of this product to apply tests for insuring that the loss in rinsing is kept to a minimum. In another process, all the rice is coated with the enriching ingredients. In this case, the product must be labeled with the caution against rinsing before or draining after cooking.

The standard becomes effective 6 months after publication unless objections are made. Thirty days are given to file objections and to request a public hearing.