on January 16, 1950. She stated that since the age of 10 she had often been infested with tapeworms. She had noticed worm segments in her stools on one occasion a year previously, and again in September, 1949. For a fortnight before coming to hospital she had suffered from postural dizziness, a symptom she associated with the presence of worms. Apart from slight abdominal discomfort, she had no other symptoms of note. On examination there were no abnormal physical signs. Her blood count was normal. *Diphyllobothrium* ova were present in the stools. Disinfestation has not yet been carried out.

We are grateful to Sir Arnold Stott for permission to publish this case. We are also indebted to Dr. A. J. Shinnie, medical officer of health for the City of Westminster, and Professor J. J. C. Buckley, of the London School of Tropical Medicine and Hygiene, for help and advice. We wish to thank Professor N. F. MacLagan and the staff of the Biochemical Department, Westminster Hospital, for performing the fractional test meals.

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A SPECIFIC SEROLOGICAL TYPE OF BACT. COLI FOUND IN INFANTS' HOME IN ABSENCE OF EPIDEMIC DIARRHOEA

BY

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During the past few years attention has been drawn to the association of a particular serological type of *Bact. coli* with outbreaks of infantile diarrhoea and vomiting and with intercurrent attacks in hospital wards (Bray, 1945; Bray and Beavan, 1948; Giles and Sangster, 1948; Giles *et al.*, 1949; Holzel *et al.*, 1949; Taylor *et al.*, 1949). The symptoms of cases excreting this organism have often been severe, and a number of outbreaks have shown a high mortality rate.

In contrast to the frequency with which these observers isolated the organism from infants with symptoms of gastro-enteritis, a very low incidence has been reported among control groups of symptomless children. These control observations to determine the incidence of the organism among healthy children have been carried out on single samples from hospital patients suffering from other conditions and from babies attending welfare clinics and in residential homes.

The present investigation developed from the isolation in a residential babies' home of a strain of *Bact. coli*

reported by Dr. Joan Taylor as serologically identical with *Bact. coli* D 433 (Taylor *et al.*, 1949), and the organism will be described as such in this paper. In view of the control observations made by previous workers on a single sample, it was decided to examine rectal swabs for *Bact. coli* D 433 from all babies in the home at frequent intervals for an extended period. Throughout this time, a period of one year, the clinical condition of the babies was kept under constant observation by one of us (A. M. M. P.).

The purpose of this paper is to give an account of the incidence of *Bact. coli* D 433 during this survey, and to relate its occurrence in babies to the presence of gastrointestinal symptoms.

Previous Infection in the Home

The home is a private residential one with a training school for nursery nurses. It takes up to 18 infants at a time, the ages ranging from a week or two to 2 years. Most children are from a few weeks to a year old. They are usually admitted singly at an average rate of about one a week. The majority are admitted from the London boroughs, having been abandoned, for adoption, or because of the illness of the parents. Some are "private" residents admitted during the illness or absence of the parents. There is no distinction between the two groups in the home. The home is well run and the children are happy and well cared for. Almost all infants are bottle-fed.

In the winter of 1943-4 there was a sporadic outbreak of infantile diarrhoea and vomiting with five deaths spread over a period of four months. Ever since then there have been frequent attacks of diarrhoea with offensive motions, sore buttocks, vomiting, and loss of weight lasting about a week. Almost every child has been affected more or less, usually soon after admission and sometimes more than once. In June, 1948, two children (twins) were seriously ill, and this was the immediate cause of a request to this laboratory for assistance.

Present Investigation

The home was first visited on June 23, 1948. It was found that one infant (T. H.) was ill with diarrhoea and vomiting of moderate severity, and, since it appeared likely that she might need intravenous therapy, her admission was arranged to the Hospital for Sick Children, Great Ormond Street. Her twin sister (M. H.) was convalescent from a moderate attack of diarrhoea and vomiting. No other children had had significant symptoms during the previous month. Rectal swabs were taken on June 23 from all infants and from the three permanent staff. Including both T. H. and M. H., five out of sixteen infants were found to be excreting *Bact. coli* D 433. The organism was also isolated from T. H.'s vomit. None of the other three children had suggestive symptoms, but one of them (L. M.) developed symptoms on June 24 compatible with a mild attack of infantile diarrhoea and vomiting. The other two had no previous history suggestive of infantile diarrhoea and vomiting, and remained well. These four children were isolated, with separate staff and equipment. No further children were found to be excreting *Bact. coli* D 433 or had developed symptoms. Rectal swabs taken on July 5 showed that one of the two symptomless infants and M. H. were then free from the organism, and on July 15 all swabs were negative. On her return from hospital on July 24 a swab from T. H. was also negative.

Since then all infants have had rectal swabs examined for the presence of *Bact. coli* D 433 on admission and at intervals of two to four weeks up to the end of December,

1948. During January, 1949, few swabs were taken owing to shortage of agglutinating serum, but from February to June, 1949, swabs were taken from all children at intervals of one to two weeks. Records were kept of all gastro-intestinal symptoms. These varied from mild feeding difficulties to attacks of infantile diarrhoea and vomiting. The symptoms have been divided into two groups: (1) intestinal symptoms associated with feeding difficulties, and (2) infantile diarrhoea and vomiting. In some cases it was difficult to decide into which category the symptoms should be placed. The criteria adopted were as follows:

Feeding Difficulties.—A mild disturbance associated with fretfulness; colicky pains; slight looseness of the motions, not more than three or four daily, which might be tinged with green and contain curds or undigested fat with traces of slime; failure to gain weight, or the loss of up to about 4 oz. (113 g.); no temperature; excoriation of buttocks not prominent; no dehydration; responding in a few days to reduction in strength of feeds.

Infantile Diarrhoea and Vomiting.—A definite illness, usually of sudden onset, with frequent loose motions with or without slime, frequently green and offensive, and often associated with vomiting; loss of weight, usually more than 4 oz., with or without obvious dehydration; excoriation of the buttocks; reluctance to feed; some fretfulness when disturbed, but as a rule lethargy; temperature sometimes raised, especially at the onset; colicky pain not marked.

Diarrhoea associated with parenteral infection is excluded, but this did not occur in the presence of *Bact. coli* D 433.

Bacteriological Investigation

Since Bray (1945) first reported the occurrence of serologically homogeneous strains of *Bact. coli* var. *neapolitanum* among infants suffering from "summer diarrhoea" a varying nomenclature has been adopted by subsequent workers for the same serological strain of *Bact. coli*. The position has recently become more confused by the isolation of a new type of *Bact. coli*, also frequently associated with infants suffering from gastro-enteritis and called the β -type (Giles *et al.*, 1949; Smith, 1949). This type differs from the original or α -type antigenically and in certain of its cultural characteristics. Both types were also recognized among the cases studied by Holzel *et al.* (1949).

A summary of the names by which the α -type of *Bact. coli* has been previously described is given in Table I. A

TABLE I.—Nomenclature Used by Previous Authors for Strains of *Bact. coli* Serologically Identical

Author	Nomenclature
Bray (1945)	<i>Bact. coli</i> var. <i>neapolitanum</i>
Bray and Beavan (1948)	..
Giles and Sangster (1948)	..
Giles <i>et al.</i> (1949)	.. <i>Bact. coli</i> (later referred to as α -type)
Holzel <i>et al.</i> (1949)	.. <i>Bact. coli</i> var. <i>neapolitanum</i>
Rogers <i>et al.</i> (1949)	.. <i>Bact. coli</i> B.G.T. (Bray-Giles-Taylor)
Smith (1949)	.. <i>Bact. coli</i> α -type
Taylor <i>et al.</i> (1949)	.. <i>Bact. coli</i> D 433

study of the literature reveals that all these strains are, in fact, antigenically identical (though different from the β -type), and they are the same as that described in this paper. It must be emphasized that the identity and similarity of the strains depend primarily on serological behaviour, though they can be regarded as a serologically homogeneous group of coliform organisms usually having the biochemical reactions ascribed to *Bact. coli* var. *neapolitanum*.

Methods

During the period of 12 months that this investigation was being undertaken 337 rectal swabs from babies were examined. In the absence of symptoms, each swab was inoculated on to a layered horse-blood/digest-agar plate

and a MacConkey plate, both of which were incubated aerobically at 37° C. Swabs from babies with symptoms of gastro-enteritis were also inoculated on to desoxycholate citrate agar and on to Wilson and Blair's medium, both directly and after enrichment in Kauffmann's tetrathionate broth and in selenite F. Non-lactose fermenters and suspicious colonies on Wilson and Blair's medium were further investigated.

The first five strains of *Bact. coli* D 433 isolated were identified from blood-agar plates sent to Dr. Taylor for investigation. The remaining strains were recognized in this laboratory as a result of slide agglutination of at least four colonies from the primary blood-agar or MacConkey plate. Except in two instances, when first isolated from a baby each positive strain was inoculated into a set of "sugars" and also sent to Dr. Taylor for serological confirmation. In subsequent isolations the identification of *Bact. coli* D 433 was made by slide agglutination only.

Results

Bact. coli D 433 was isolated from 40 of the 337 rectal swabs examined. It was often present on the primary blood plate as a practically pure growth, and in 24 of the 40 cases 75% or more of the colonies tested gave a positive agglutination result. The 40 positive swabs were obtained from 17 of the 60 babies investigated. Strains from 15 of these 17 babies were examined biochemically and showed similar reactions.

All these strains were non-motile when grown in broth at 37° C., were indole-positive, and failed to produce H₂S. All fermented lactose, glucose, mannitol, and maltose with acid and gas production after overnight incubation at 37° C. except one strain which did not ferment maltose till the third day. Fermentation of sucrose and salicin was usually delayed for 48 hours and fermentation of dulcitol for four days. Rarely sucrose, salicin, or dulcitol was not fermented. The strains were M.R.-positive, V.P.- and citrate-negative, and gave acid and gas in MacConkey broth at 44° C. All strains were agglutinated to titre by *Bact. coli* D 433 K and O sera. No member of the *Shigella* or *Salmonella* group was isolated from any of the babies with symptoms of gastro-enteritis.

Use of MacConkey Agar

During most of the course of this investigation MacConkey agar was used in parallel with blood agar. It was hoped that on those plates where *Bact. coli* D 433 formed only a small proportion of the total growth the colonies might be more readily recognized on MacConkey agar than on blood agar. It was found, however, that the relatively small colonies of *Bact. coli* D 433 were usually overgrown or suppressed by the heavier growth of other intestinal organisms.

There was reasonably good correlation between the results on blood agar and on MacConkey agar. Of 28 instances in which a positive result was obtained, both media were positive in 24, blood agar only in three, and MacConkey agar only in one. The blood-agar plate on this occasion was overgrown by a spreading *Proteus*. When a *Proteus* strain rendered the blood-agar plate unsuitable for examination, slide agglutination was done on colonies from the MacConkey plate.

In view of the occasional failure to isolate the organism from MacConkey agar when the blood agar was positive, it is possible that slightly better results might have been obtained by using chloral hydrate blood agar as an alternative medium. The proportion of blood-agar plates

contaminated by spreading *Proteus* strains was not large—24 out of 337. A few babies consistently produced *Proteus* organisms, and the 24 strains isolated were, in fact, all obtained from 12 of the 60 babies examined.

Epidemiological Findings

The epidemiological results are summarized in Tables II, III, IV, and V.

TABLE II.—Incidence of *Bact. coli* D 433 Related to the Number of Examinations Made

No. of Examinations per Infant	No. of Infants Examined	No. of Infants Excreting <i>Bact. coli</i> D 433 on One or More Occasions
1-2	23	0
3-11	25	6
12 and over	12	11
Total	60	17

TABLE III.—Incidence of *Bact. coli* D 433 and Associated Symptoms Among 17 Babies in Age Groups

Age in Weeks when <i>Bact. coli</i> D 433 First Isolated	Infantile Diarrhoea and Vomiting	Intestinal Symptoms Associated with Feeding Difficulties	No Symptoms	Total
0-5	0	0	2	2
6-11	2	1	2	5
12-23	3	1	5	8
24 and over	0	0	2	2
Total	5	1	11	17

TABLE IV.—Duration of Excretion of *Bact. coli* D 433 and Associated Symptoms

Duration of Excretion of <i>Bact. coli</i> D 433 in Weeks	Infantile Diarrhoea and Vomiting	Intestinal Symptoms Associated with Feeding Difficulties	No Symptoms	Total
0-2	1	0	6	7
3 and over	4	1	5	10
Total	5	1	11	17

TABLE V.—Incidence of *Bact. coli* D 433, February-June, 1949

	Date in Months and Weeks											
	Feb.		March				April		May		June	
	3	4	1	2	3	4	5	2	4	1	3	4
No. of infants examined ..	14	15	15	15	13	14	17	15	15	16	16	15
No. of positive <i>Bact. coli</i> D 433 ..	4	6	8	7	2	1	0	0	1	0	4*	0
No. of new positives ..	4	2	3	1	0	0	0	0	1	0	1	0
No. of cases of infantile diarrhoea and vomiting ..	0	2	2	0	0	0	0	0	0	0	0	0
No. of new cases ..	0	2	0	0	0	0	0	0	0	0	0	0

* Three of these had been positive in February-March.

During the course of the investigation rectal swabs were examined from 60 infants, and it was found that 17 of these excreted *Bact. coli* D 433 on one or more occasions. The number of examinations made on each infant varied considerably, and Table II shows the association between this and the incidence of *Bact. coli* D 433. The organism was isolated from as many as 11 of the 12 infants examined on twelve or more occasions, but from none of the 23 infants examined once or twice only.

Symptoms of infantile diarrhoea and vomiting were observed among only 5 of the 17 infants with positive swabs and 11 infants had no symptoms at all while excret-

ing *Bact. coli* D 433. Four of these 11 infants were under 3 months old (Table III) and five infants continued to excrete the organism for at least three weeks (Table IV). The frequency of symptoms among those infants who were not excreting *Bact. coli* D 433 at the time is not shown in the tables. In the course of the year six of these developed infantile diarrhoea and vomiting, and symptoms associated with feeding difficulties occurred on 13 occasions.

When *Bact. coli* D 433 was first recognized in the home in June, 1948, it was isolated from five babies. The organism was not detected in any of these babies after the beginning of July and, in fact, was not again isolated in the home until the third week of February, 1949. It then spread rapidly through the home, and during the next four weeks was isolated from 10 of the 15 infants at risk, though only two of these developed symptoms of infantile diarrhoea and vomiting (Table V).

Weight charts were kept for all infants throughout the year, and it was thought that irregularity of the charts might provide a more sensitive indication of the effect of carriage of *Bact. coli* D 433 than the presence of intestinal symptoms. It was found, however, that all 11 infants who did not develop symptoms while excreting *Bact. coli* D 433 continued to gain weight steadily.

Discussion

It can be seen from the findings of some previous observers (Table VI) that the incidence of this serological type of *Bact. coli* among the healthy infants forming the

TABLE VI.—Relationship Between a Specific Serological Type of *Bact. coli* and Symptoms

Reference	Infantile Diarrhoea and Vomiting		Diarrhoea—Unclassified, Doubtful, or Due to Other Causes ¹		Controls (Infants Only). No Gastro-Intestinal Symptoms	
	No. Examined	% Pos.	No. Examined	% Pos.	No. Examined	% Pos.
Bray (1945) ..	50	95	—	—	100*	4
Bray and Beavan (1948)	40	87.5	—	—	80*	4
Giles <i>et al.</i> (1949)	207	94.7	208	9	271*	2.6
Holzel <i>et al.</i> (1949)	79	63	—	—	—	—
Taylor <i>et al.</i> (1949)	54†	96	52‡	21	208§	0

* Mainly hospital patients, including "healthy" contacts. † Includes groups A to E (*loc. cit.*). ‡ Includes groups F, G, and H (*loc. cit.*). § Infants in residential and day nurseries.

control groups is low. These control infants were taken from a number of different environments, and the results are based on single examinations of different infants.

Recent findings by Smith (1949) and Rogers *et al.* (1949) cannot be tabulated in the same way, but both confirm the frequent association between a specific serological type of *Bact. coli* and infantile diarrhoea and vomiting. Smith also describes the occurrence of a different serological type of *Bact. coli*, known as type β , in cases of diarrhoea and vomiting. This type was not looked for in the present investigation. Rogers *et al.* (1949) found that 25 out of 86 infants excreting *Bact. coli* B.G.T. (see Table I) did not develop symptoms. The remaining 61 developed gastrointestinal disturbances which varied from severe gastro-enteritis to a mild diarrhoea with or without vomiting. It is worthy of note that 82 of these infants acquired the organism after admission to hospital.

In the present investigation the infants were selected by the fact that they were in the home, and not because of any symptoms. Therefore the best estimate of the general

incidence of *Bact. coli* D 433 must include all positive results irrespective of the presence of symptoms. Sixty babies were examined and 17 of them were found to be excreting *Bact. coli* D 433 on one or more occasions in the course of a year. It appears that the frequency of isolation of *Bact. coli* D 433 is related to the number of times the infants were examined (Table II). However, the relatively high incidence among babies examined on twelve or more occasions may equally well be due to the length of their stay in the same semi-closed community resulting in an increased risk of exposure.

In many intestinal infections some susceptible children may excrete the causative organism for a short period without developing symptoms. Prolonged excretion without symptoms is, however, unusual. In the course of this investigation five infants were found to be excreting *Bact. coli* D 433 in large numbers for three weeks or more and yet did not develop symptoms. One further infant had very mild symptoms due to feeding difficulties during a long period of excretion. The ages of these six infants at the time were 4, 8, 8, 12, 16, and 20 weeks. Adequate past histories were obtained for all these children and only one had had infantile diarrhoea and vomiting. She was under observation in the home at the time and was not excreting *Bact. coli* D 433. Breast-feeding is widely accepted as affording a degree of protection from infantile diarrhoea and vomiting, but none of these six infants was breast-fed at the time of excreting the organism and four of them had never been breast-fed. Eleven infants developed infantile diarrhoea and vomiting during the year, but only five of them were excreting *Bact. coli* D 433.

These findings, and the fact that 11 of the 17 children excreting the organism did not develop symptoms at the time, do not support the hypothesis that there is an aetiological connexion between *Bact. coli* D 433 and infantile diarrhoea and vomiting, though they are compatible with such a theory. However, the figures of previous workers summarized in Table VI strongly suggest some such connexion unless an alternative explanation of the very high incidence among ill children is found. It has already been suggested that the incidence among healthy babies may be higher than earlier results have shown. The present methods of isolating the organism cannot be considered satisfactory, and in the absence of selective media it is probable that scanty infections might be missed.

In this survey the organism was not isolated from any infant for more than four weeks at a time. The strain then died out or was present in the faeces in insufficient numbers to be detected. In the latter case it is possible that the appearance of the organism in the home in May was not due to a fresh introduction from outside, but to its persistence in small numbers in the stools of one or two children. In this event some unknown factor may have caused rapid proliferation of the organism. The fact that three of the four babies excreting the organism in May are known to have previously excreted it in February and March might be regarded as supporting this suggestion. No evidence of the possible nature of this factor was found. It is not necessary for the stools to be loose for the organism to flourish. Further work is necessary to determine whether such a factor exists.

The part played by *Bact. coli* D 433 in the aetiology of infantile diarrhoea and vomiting remains uncertain, but it has been shown that an outbreak of infantile diarrhoea and vomiting did not follow the spread of the organism among a group of infants, the majority of whom were presumed to have been susceptible to the disease.

Summary

A clinical and bacteriological investigation of infants in a residential home was carried out for a period of one year. Sixty infants came under observation.

A specific serological type of *Bact. coli* identified as D 433 (Taylor *et al.*) was isolated from 17 infants on one or more occasions. Eleven of these infants had no symptoms, one had slight symptoms associated with feeding difficulties, and five had infantile diarrhoea and vomiting. Six infants developed infantile diarrhoea and vomiting in the absence of *Bact. coli* D 433.

At one time as many as 10 out of the 15 infants in the home were excreting *Bact. coli* D 433, but no outbreak of infantile diarrhoea and vomiting occurred.

The aetiological relationship between *Bact. coli* D 433 and infantile diarrhoea and vomiting is discussed.

We are indebted to Dr. Joan Taylor for identifying the first five strains of *Bact. coli* D 433 reported in this paper, for confirming the identity of strains isolated subsequently, and for the supply of agglutinating serum. Our thanks are due to the matron and staff of the home for their whole-hearted co-operation.

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BACT. COLI D 433 IN CASES OF DIARRHOEA IN ADULTS

BY

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Recent investigations concerning the causal factors of diarrhoea and vomiting in infants have indicated that the disease is very often associated with the presence in the intestine of a particular variety of *Bact. coli*. An organism of this nature (*Bact. coli* var. *neapolitanum*) was isolated by Bray (1945) from over 90% of cases of summer diarrhoea; Giles and Sangster (1948) isolated a similar organism from over 90% of cases occurring in an outbreak in 1947. Taylor *et al.* (1949) described the isolation of a non-motile variant of *Bact. coli* (D 433) from a large number of cases occurring in eight outbreaks of infantile diarrhoea and vomiting, and identified this organism with that previously isolated by Giles and Sangster.

In order to investigate strains of *Bact. coli* encountered in this laboratory from cases of infantile diarrhoea and vomiting, D 433 antiserum was kindly supplied by Dr. Joan Taylor. At the time the investigation was begun, however (April, 1949), there were few cases of infantile gastroenteritis, and it was decided in the first place to confine attention to adults and establish a routine method of procedure which could be used in the summer months if and when outbreaks developed.

Procedure

Specimens of faeces arriving at the laboratory for routine investigation were inoculated on to blood agar, MacConkey, and Difco "bacto S.S." agar. The plates were inoculated