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Intestinal carriage of *Bacillus cereus*: faecal isolation studies in three population groups

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SUMMARY

The results of examinations of stools for *Bacillus cereus* among three unrelated groups of individuals are presented. The groups consisted of (1) healthy school-children aged 6–11 years in a rural region of South Africa examined during each of the four seasons of the year; (2) 15 healthy volunteers comprising staff of a London microbiology laboratory and their families examined on each of 3 consecutive weeks; (3) 75 unrelated young children, 2 months to 5 years of age, in a second rural region of South Africa examined during a pilot study of 1 week's duration on the aetiology of rural gastroenteritis. The stools of the last group were submitted as being related to present or recent diarrhoea in the respective children.

In group 1, *B. cereus* isolation rates ranged from 24·3% at the autumn visit to 43% at the summer visit with a significantly higher rate of isolation in the summer than at other seasons of the year ($P < 0{\cdot}05$). *B. cereus* was isolated from 40% of group 2 volunteers on week 1, none on week 2 and 20% on week 3. The organism was detected in the 12 positive specimens at levels of approximately 10^2/g and constituted 2·5–30% of the total aerobic spore-forming bacillus population in the stools. In group 3, *B. cereus* was recovered from 18·7% of the stool samples and was isolated consecutively with other pathogens (enteropathogenic *Escherichia coli* and rotavirus) on only five occasions. In groups 1 and 3, < 5% of the stools had '3+' levels of *B. cereus* (> 10 colonies per direct plate culture).

B. cereus was readily isolated from all of 10 food samples, representative of the typical diet of the group 1 individuals, and was present in substantial numbers (10^4 to $5{\cdot}5 \times 10^6$/g) in half of them.

The isolation results, supported by serotyping, indicated that carriage of *B. cereus* in stools is transient and its presence at any one time reflects solely its intake with foods.

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INTRODUCTION

Over the past two decades, *Bacillus cereus* has been recognized as the agent of two distinct types of food poisoning – the 'diarrhoeal' and 'emetic'. Reports of the diarrhoeal type, in the main associated with meats, vegetable dishes, soups, desserts and sauces, were relatively numerous, particularly in eastern Europe, in the 1960s, while the emetic syndrome, mostly associated with Chinese rice dishes but occasionally other foods also, became the subject of frequent reports in the 1970s (Gilbert, 1979; Gilbert *et al.* 1981; Johnson, 1984).

Ideally, epidemiological implication of a bacterium such as *B. cereus* in food poisoning depends on isolation in large numbers of the same strain (as indicated by serotyping, phage typing or other appropriate marker) of the organism from both the incriminated food and stools of the affected patients. For many reasons, such as the unavailability of specimens or time delays in obtaining specimens, these criteria cannot always be met and often the incrimination of a particular aetiological agent is based solely on its isolation from the food and/or stools of some or all the patients. However, *B. cereus* is a common contaminant of many types of cereal-based foods and pulses (Blakey & Priest, 1980) and also of milk (Mostert, Lück & Husmann, 1979; Ahmed, Moustafa & Marth, 1983), and its presence in low numbers in these is unremarkable. Also, although little is known for certain about the likelihood of finding *B. cereus* in normal stools from healthy persons upon random examination, it seems probable that, in most individuals, this depends directly on diet and varies from day to day.

This paper is concerned with increasing the limited information currently available on *B. cereus* carriage in human faeces outside food poisoning situations and presents the results of stool examinations among three unrelated groups of individuals under widely differing circumstances of life style and diet.

MATERIALS AND METHODS

Groups examined

Group 1 consisted of Tswana children aged 6–11 years in a rural school in Boputhatswana (a 'Homeland' approximately 150 km north-west of Johannesburg). Four visits to this school were made at 3-monthly intervals to encompass each of the four seasons of the year. Faecal specimens were collected and cultured with the primary purpose of studying carriage of *Campylobacter jejuni* among healthy rural African children in what was essentially an extension of previous surveys (Bokkenheuser & Richardson, 1960; Richardson *et al.* 1968; Koornhof *et al.* 1979; Richardson, 1984). Advantage was taken of the opportunity to study carriage of *B. cereus* also. The group consisted of 120 children at the outset falling to 112 by the end of the survey due to transfers of 8 of the children to other schools.

Group 2 consisted of 15 volunteers, all either workers in a microbiological laboratory in London or members of their families. Faecal specimens submitted on a weekly basis over 3 consecutive weeks were examined for the presence of *B. cereus* and other *Bacillus* species.

The third group consisted of 75 unrelated infants of ages ranging from 2 months to 5 years from whom stools were collected by local rural clinics or hospitals in

the Elim district of north-eastern Transvaal (300 km north-east of Johannesburg) during a single pilot survey on the aetiology of rural gastroenteritis. The individuals concerned belonged to the Shangaan tribe and were widely dispersed over a geographical area of some 350 km^2 (overall population 150000). These stools were submitted as being related to present or recent diarrhoea in the respective children.

Bacteriology and serology

In group 2, direct counts were made of both the total aerobic spore-forming bacilli and the number of *B. cereus*/g of faeces. For this purpose, 1 g of faecal specimen was homogenized in 9 ml of 0·1 % peptone water. Using a modification (ICMSF, 1978) of the drop count method of Miles & Misra (1938), numbers of vegetative and spore forms of *Bacillus* species in unheated and heated (80 °C for 10 min) portions of this suspension were determined on Columbia-based 5 % horse blood agar (Oxoid CM331) and *B. cereus* selective agar (Oxoid CM617) incubated for 18–20 h at 30 °C.

Presence or absence of *B. cereus* in the stools of groups 1 and 3 individuals were simply based on direct plate culture of loopfuls of faecal sample (50 μl disposable loops inserted a few millimetres into the specimen) spread across plates of 5 % horse blood agar containing 100000 units of polymyxin B per litre. The plates were incubated overnight at 37 °C and, where colonies of *B. cereus* had grown, this was recorded in terms of 1+ (1–5 colonies), 2+ (5–10 colonies) or 3+ (> 10 colonies) growth.

From each 'positive' plate, one to three representative colonies of presumptive *B. cereus* were subcultured on nutrient agar slopes for subsequent confirmation of identity and serotyping in the Food Hygiene Laboratory by the methods described elsewhere (Gilbert, 1979; Kramer *et al.* 1982; Parry, Turnbull & Gibson, 1983).

Food examination

In the case of group 1, arrangements were made at the time of the winter-1982 visit to collect a range of foods typical of the everyday diet of these school-children. As shown in Table 3, maize meal and 'kaffir corn' (*Sorghum vulgaris*) comprise the staple food being supplemented with vegetables and fruits as available.

Ten gram samples of each of the foods listed in Table 3 were weighed into a stomacher bag; 10 ml buffered saline were added and the sample homogenized in a stomacher. Direct plate cultures were made on 5 % horse blood agar and incubated at 37 °C for 20 h. Ninety millilitres of peptone water were then added to the homogenate and the *B. cereus* levels and total viable counts (TVC) determined by the drop count method (ICMSF, 1978; Miles & Misra, 1983) on blood agar plates incubated at 30 °C for 20 h (*B. cereus* counts) and 48 h (TVC). Finally the peptone water suspensions were incubated at 37 °C for 20 h and plated on to blood agar containing 100000 units of polymyxin B. Presumptive *B. cereus* colonies from any of these cultures were transferred to agar slopes and submitted to the Food Hygiene Laboratory for confirmation of identity and serotyping.

Table 1. *Isolation of* Bacillus cereus *from group 1 stools*

Season	All isolations*	Boys*	Girls*	By age (years old)† 6	7	8	9	10	11
Spring (10/81)	33/115 (28·7%)	18/57 (31·6%)	15/58 (25·9%)	7	3	7	7	5	4
Summer (1/82)	43/100 (43·0%)	25/55 (45·4%)	18/45 (40·0%)	7	6	8	8	10	4
Autumn (4/82)	26/107 (24·3%)	17/55 (30·9%)	9/52 (17·3%)	4	7	5	2	3	5
Winter (8/82)	32/108 (29·6%)	14/56 (25·0%)	18/52 (34·6%)	5	10	3	4	6	4
Total positive	134	74	60	23	26	23	21	24	17
Total examined	430	223	207	70	76	69	78	71	66
Percent positive	31·2	33·2	29·0	32·8	34·2	33·3	26·9	33·8	25·8

* Number positive/total examined (percent positive).
† Number positive.

Table 2. *Isolation of* Bacillus cereus *and* Bacillus *species in general from group 2 stools*

Week	Faecal specimens examined	No. positive for *B. cereus* (> 200/g)	No. positive for *Bacillus* species* (> 200/g)
1	15	6 (40%)	15 (100%)
2	15	0	12 (80%)
3	15	3 (20%)†	14 (93%)

* Including *B. cereus*.
† From different individuals to those positive in week 1.

RESULTS

Faecal cultures

The *B. cereus* faecal isolation results for group 1 are summarized in Table 1. The overall isolation rates ranged from 24·3% at the autumn examination to 43% at the summer visit. There were no significant differences between the autumn, winter and spring isolation rates but there was statistical significance ($P < 0{\cdot}05$) in the increased proportion of isolations in the summer. Overall, there were no significant differences in isolation rates from the different sexes or age groups. *C. jejuni* isolation rates were consistently low and ranged from 1 isolation (1·0%) in summer to 7 isolations (6·1%) in spring. *B. cereus* and *C. jejuni* were isolated simultaneously from just 4 of the 12 stools found to be positive for *C. jejuni*.

The findings with group 2, the 15 volunteers – workers in a London laboratory or members of their families – are summarized in Table 2. *B. cereus* was detected in 40% of the group on week 1, in none on week 2 and in 20% on week 3. *Bacillus* species in general were found in the majority of the stool specimens at all three examination times at levels ranging from 2×10^2 to $3{\cdot}5 \times 10^5$/g *B. cereus* counts in the 12 positive specimens ranged from 1×10^2 to 3×10^2/g and constituted 2·5–30% (mean 13·3%) of the total aerobic spore-forming bacillus counts. The total aerobic

Table 3. Bacillus cereus *counts and total viable counts (per gram) in group 1 foods*

Food sample*	Total viable count (30 °C × 48 h)	*B. cereus* count (30 °C × 24 h) (% total count)
1. Unfermented kaffir corn 'porridge'. No salt	15000	15000 (100)
2. Unfermented kaffir corn porridge with roughage	500000	500000 (100)
3. Fermented 'mealiemeal' (maize meal) – 1	5000	5000 (100)
4. Fermented mealimeal – 2	750000	15000 (2)
5. Unfermented mealiemeal with salt	900000	500 (< 1)
6. Fermented kaffir corn and mealiemeal	750000	500 (< 1)
7. Fermented kaffir corn	$3{\cdot}5 \times 10^6$	500 (< 1)
8. Sugar beans	$1{\cdot}25 \times 10^9$	10000 (< 1)
9. 'Morogo' (leaves of bean, tomato, potato and pepper plants with salt)	6000	1000 (17)
10. Ferment of bread + raw malt + brown sugar + yeast (2 h at ambient temperature)	5×10^8	$5{\cdot}5 \times 10^6$ (1·1)

* 'Fermented' generally meant left at ambient temperature for approximately 5 h.

spore-forming bacillus counts were not, however, detectably higher in specimens from which *B. cereus* was isolated than those in which it was not found.

B. cereus was found in the stools of 14 of 75 (18·7 %) of the infants and young children with existing or recent diarrhoea comprising group 3. The isolation rates of other pathogens from these children were as follows: *Salmonella* species 3 (4 %); *C. jejuni* 3 (4 %); enteropathogenic serotypes of *Escherichia coli* 14 (18·7 %); heat-stable enterotoxigenic *E. coli* 2 (2·7 %); rotavirus 12 (16 %), *B. cereus* was only isolated consecutively with enteropathogenic *E. coli* (three stool specimens) and rotavirus (two other specimens). Levels of *B. cereus* in groups 1 and 3 faeces when present were generally low and recorded as 1+ or 2+ (1–10 direct plate colonies); 3+ growth (> 10 colonies) was noted in < 5 % of cases.

B. cereus in group 1 foods

The results of examination of 10 samples of typical daily foods eaten by group 1 children are summarized in Table 3. *B. cereus* was present in all the samples being detectable at low levels (< 500/g) in 3, in moderate numbers (1000–5000/g) in 2 and substantial numbers in the remaining 5. In 3 of the samples, *B. cereus* constituted essentially 100 % of the aerobic microflora. Other growth from the samples consisted of mixed coliform populations.

Serotypes

The range of serotypes found among the *B. cereus* isolates in the different sections of the study are shown in Table 4. Of the 167 South African isolates, 81 (48·5 %) were typable while 7 of the 11 isolates from the London group could be serotyped.

Among group 1 individuals – the largest of the groups studied over the longest period – only in three of the children was the same serotype isolated a second time and only one of these individuals yielded the same serotype on two consecutive examinations. None of the serotypes was isolated more than twice from one individual. Similarly, no serotype was isolated more than once from any of the group 2 individuals.

Table 4. *Distribution of* Bacillus cereus *serotypes according to source of isolation*

'H' Serotype	Group 1 stools	Group 1 foods	Group 2 stools	Group 3 stools
NT	76	14	4	10
17	11	2	1	—
18	10	—	1	—
20	8	1	1	1
16	5	2	—	—
14	5	—	—	—
1	5	1	2	—
C*	5	1	1	1
8	4	—	—	1
6	3	—	—	—
13	3	2	—	1
22	3	—	—	—
G*	3	—	—	—
15	2	2	—	—
2	1	2	—	—
V*	1	—	1	—
19	1			
21	1			
23	1			
A*	1			
E*	1			
H*	1			
iii*	1			
iv*	1			

NT, not typable.
* Experimental serotype designations.

Of the seven different serotypes found in the group 1 food samples collected at the winter 1982 visit, five were also isolated from the faecal specimens examined at the same visit. The remaining two food serotypes were also isolated from stool specimens at other examination times.

DISCUSSION

The higher rates of isolation of *B. cereus* among group 1 individuals at the summer examination as compared with other times of the year parallels findings in previous surveys concerned with salmonellae and shigellae in the same location that the highest isolation rates of these pathogens occurred at the summer collections (Bokkenheuser & Richardson, 1960; Richardson *et al.* 1968). There was no evidence on this occasion, however, to suggest that the *B. cereus* isolations were associated with any problems of gastroenteritis that may have been occurring in the community at the time or that the *B. cereus* isolates should in any way have been regarded as having a pathogenic role.

The terms 'summer', 'winter', 'spring' and 'autumn' in the groups 1 and 3 locations have rather different connotations from the same terms when used in Europe and North America and need some elaboration. These locations are arid and sparsely vegetated to semi-desert in nature; rainfall is generally low the year round and occurs mostly in the hot season or summer (November to March). Day

and night temperatures at this time of year rise and fall approximately between 15 °C and 30 °C. The winter or cool season (May to September) is essentially free of rainfall; night-time temperatures can be as low as 2 °C but the day can still be quite warm (±25 °C). Infantile enteritis is a major problem in these types of communities and is responsible for a significant proportion of infant mortality there. The majority of cases of enteritis occur during the summer months.

No special associations were noted in the group 1 study between isolation rates and age or sex; *B. cereus* was isolated about equally from all six age groups from 6 to 11 years of age and from both boys and girls in the school. Nor was there any obvious association between *C. jejuni* and *B. cereus* isolations; *B. cereus* was isolated from just 4 of the 12 stool specimens that yielded *C. jejuni* during the survey. (The study being designed primarily to investigate the incidence of *C. jejuni* in the group, other enteric bacteria were not looked for.)

A wider range of enteric pathogens was looked for in the group 3 study; *B. cereus* was only isolated consecutively with pathogens in 5 of the 14 specimens in which it was found. The study was carried out over the period of a single week in January 1983 – that is, the peak of summer; the *B. cereus* isolation rate (18·7 %) was, therefore, substantially lower than that found in the group 1 study. There was no obvious explanation for this difference, however.

Group 2 in London was a much smaller group than groups 1 and 3; on the other hand, the group 2 study was, in terms of the methods used for detecting *B. cereus* in the stools, a more intensive examination of faecal carriage. The study took place in March 1983. It is not known whether seasonal differences occur in the incidence of *B. cereus* in the stools of persons living in temperate Western regions though it is doubtful that they do to any significant extent. The week-to-week variation from as high as 40 % of the stools being positive to nil might have been anticipated at any time of the year and probably reflects solely the diet over the previous 24–48 h. It was observed (Turnbull, 1981) in one individual taking a proprietary pharmaceutical product ('Bactisubtil' – Merrell-Touraud, Paris) whose active ingredient was a mutant strain of *B. cereus*, that the organism could be detected in the faeces, but at progressively declining levels, for just 7 days after ingestion of a dose of 8×10^9 spores – more than 1000 × the highest level found in any of the group 1 foods (Table 3).

It was probably true in all three groups that the *B. cereus* isolations represented recent intake of the organism with food. As supported by the results in the group 2 study where stools were examined on a weekly basis, carriage of a particular strain was of short duration only. Extra support for this conclusion was supplied by serotyping results; no serotype was isolated twice from any member of group 2 and, of the 77 isolations from group 1 that were typable, only 3 were from children yielding 1 serotype a second time and only in 1 of these 3 were the isolations of the same serotype made on consecutive visits.

The foods listed in Table 3 are typical components of the traditional everyday diet in the groups 1 and 3 locations. Meat is consumed irregularly and milk only seasonally (Bokkenheuser & Richardson, 1960; Walker, 1966). In that *B. cereus* was present in all the samples, and in moderate to high numbers in 7 of the 10, it is perhaps only surprising that *B. cereus* was not found in closer to 100 % of groups 1 and 3 stool specimens – although this may simply be a reflection of the

insensitivity of the isolation and detection methodology used. The food collection was made at the last (winter) visit; it is possible that even higher *B. cereus* counts might have been encountered if foods had been collected and examined at the summer visit.

It may or may not be relevant that all the serotypes found in the group 1 foods were also represented among the group 1 faecal isolates at one time or another. Conceivably these types represent strains which are predominant in the region though this would be hard to prove. The three most common serotypes found among the group 1 isolates also number among the types most commonly encountered in Britain (Gilbert *et al.* 1981). On the other hand, some of the serotypes found in group 1 faeces and foods (types 2, 6, 13 and 16, for example) are rarely encountered among British isolates.

To some extent, serotyping results revealed the ubiquitous nature of *B. cereus* strains; half of the 153 group 1 faecal isolates, 13 of 27 group 1 food isolates and 4 of the 14 group 3 isolates (in all, 48·5 % of the rural African isolates) proved to be typable with a set of typing sera based largely on strains of *B. cereus* isolated in association with food poisoning incidents in Britain, Canada, the Netherlands and the USA. The typability rates among the African isolates were, however, somewhat lower than that generally anticipated with *B. cereus* strains isolated from sources within Britain.

References to *B. cereus* in faeces of individuals other than in the context of food poisoning, though rare, date back to 1919 (Batchelor, 1919) when it was recorded that *B. cereus* was isolated from approximately two-thirds of 'over fifty' Baltimore children (specifics on the children and the reasons for making the examinations are not given).

In fact, only three other specific references to *B. cereus* in faecal specimens not related to food poisoning were found. The first (Ghosh, 1978), reporting results of a study in the Food Hygiene Laboratory aimed at obtaining data on the presence of *B. cereus* in faeces from healthy persons, recorded isolation of the bacterium from 14 % of 711 specimens using an enrichment technique. Persistence of the organism in the intestinal flora was not studied, however. *B cereus* was also found in 15 % of 400 faecal specimens from healthy individuals in a study in Japan (Itoh *et al.* 1982). In the third report, Prokopova (1981), somewhat surprisingly, was unable to detect *B. cereus* in the faeces of a control group of 284 healthy children in Kiev, although the organism was found at levels of 10^2–10^4/g of faeces in 277 children with various forms of mild enteritis.

Overall though, the many papers and theses on intestinal microflora refer only on the rare occasion to *Bacillus* species as a whole and the picture is then one of intermittent isolation in low numbers. This is perhaps remarkable in the light of the findings reported here. Even in the European group 2, *Bacillus* species were readily detectable in most of the faecal specimens: in less-developed regions of the world where vegetarian-type diets are prevalent, *Bacillus* species, as incidental food contaminants, are undoubtedly ingested in quite substantial numbers. One reason for the rarity of reported isolations, however, may simply relate to the laboratory methods employed: few of the studies, for example, involved examination of heat-treated (65–80 °C for 10–30 min) specimens. In another survey in which *Bacillus* species were found in all faecal specimens collected from groups in

several countries (Crowther, 1971; Hill *et al.* 1971), it was observed that mean aerobic spore-forming bacillus counts were higher in Ugandan, Indian and Japanese groups than in British and US groups.

The indications from the present study are that *Bacillus* species in general are commonly present in normal faeces, that their presence and the presence of *B. cereus* in particular reflects dietary intake and that *B. cereus* does not persist or colonize the intestine following dietary ingestion in the normal healthy individual.

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