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A comparison of the levels of faecal indicator bacteria in water and human faeces in a rural area of a tropical developing country (Sierra Leone)

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SUMMARY

The levels of faecal coliforms (FC), indole-positive FC (presumptive *Escherichia coli*), faecal streptococci (FS), *Streptococcus faecalis* and *Clostridium perfringens* in the natural water sources used by 29 rural settlements in Sierra Leone were investigated. Levels of the same indicators in human faeces were also investigated. The incidence of *Salmonella* spp. in both habitats and the temperature, pH and conductivity of water sources were also recorded. All water sources were contaminated with the indicator bacteria, mean numbers of which occurred in the relationship FC > presumptive *E. coli* ≏ FS ≏ *C. perfringens* > *S. faecalis*. FC were also predominant in human faeces, the relationship of means being FC ≏ presumptive *E. coli* > FS > *S. faecalis* > *C. perfringens*. The need for confirmation of FC counts obtained from water sources was indicated by the large number of positive tubes produced in the FC multiple-tube dilution test from some samples which could not be confirmed as presumptive *E. coli*. *Salmonella* spp. were isolated from 13 water sources and 6% of faecal samples. Mean water temperature was high (26·2 °C), pH low (5·04) and conductivity low (34 μS cm^{-1}). Presumptive *E. coli* was considered the most appropriate indicator of faecal pollution of the types of water investigated.

INTRODUCTION

It is generally considered that natural water sources used by settlements in developing countries are very prone to faecal contamination (Evison & James, 1977; Feachem, 1980). It is also known that the majority of the rural population of these countries is dependent on such sources for its domestic water requirement (W.H.O., 1973). The accurate assessment of faecal contamination of water sources therefore assumes an important role in decision-making during the process of development of water supply in developing countries. In tropical climates, such an assessment will depend on the use of an appropriate faecal indicator which, due mainly to the difference in water temperature, may not be the same as that employed in temperate climates.

In any situation where fully treated water is supplied, it is adequate to assess the presence or absence of the diverse group of coliform organisms as a presumptive

indication of faecal contamination. Standards of acceptability for potable water have been largely based on this criterion (D.H.S.S., 1969; W.H.O., 1971; A.P.H.A., 1981). However, coliforms are not considered to be specifically faecal in origin (Moussa, 1965; Dutka, 1973) and the proportion of non-faecal coliforms in water is thought to increase with water temperature; high levels of saprophytic lactose fermenters have been said to occur in tropical waters, the sanitary significance of such organisms being doubtful (Mara, 1978).

In tropical developing countries, where some degree of faecal contamination of untreated supplies is considered inevitable and where it would not be feasible to condemn such supplies because of the mere presence of coliforms, a quantitative assessment of a more specific indicator is required; differences in pollution relevant to the provision of the best available, if not safe, supply can then be observed. The indicator most commonly advocated for this purpose is the faecal coliform (FC) group, consisting of coliforms with the ability to ferment lactose at 44·0–44·5 °C. However, there are many more coliforms of the Klebsiella and Citrobacter groups which can grow at 44·0–44·5 °C from tropical waters than from temperate waters (Moussa, 1965; Evison & James, 1973). Presumptive *Escherichia coli* represent an even more specific indicator of faecal pollution (particularly by warm-blooded animals), but even this group may not fulfil all the necessary criteria for its acceptance as a reliable indicator of faecal pollution in tropical waters because of the reported capacity for regrowth of *E. coli* when associated with rotting vegetation at elevated temperatures (Taylor, 1972). A faecal streptococcus (FS) count, particularly in conjunction with an FC count (Geldreich & Kenner, 1969; Feachem, 1975), has also been used as a specific indicator of faecal contamination of water. However, FS may well have a high death rate at the water temperatures normally encountered in tropical countries (Evison & James, 1977). *Streptococcus faecalis* has been identified as being particularly associated with human faeces (Mead, 1963) and is, therefore, potentially a more specific indicator of human faecal pollution than the FS group. *Clostridium perfringens* is considered a useful indicator of remote or intermittent faecal pollution (D.H.S.S., 1969).

The appropriateness of the use of any of the above indicators for the accurate quantitative assessment of faecal contamination of untreated tropical waters is questionable, as a result of the lack of data on the relative proportions of these organisms in faeces in tropical developing countries. In India, Rao *et al.* (1968) reported that 20% of faecal samples from subjects tested did not contain *E. coli*. Drasar (1974) presents data suggesting that the level of FS in the faeces of persons living on a predominantly carbohydrate diet in developing countries is higher than in the faeces of persons living on a mixed 'Western' diet. *C. perfringens* occurs in relatively small numbers in faeces, and the survival of *C. perfringens* spores in tropical waters has been questioned (Muhammed & Morrison, 1975).

There is, therefore, a need for a critical assessment of the appropriateness of the commonly used faecal indicator bacteria for water quality testing in tropical developing countries. This investigation was carried out to provide data which would help in such an assessment.

MATERIALS AND METHODS

Water analysis

A water sample of approx. 100 ml was taken aseptically in a sterilized 120 ml bottle from the natural water source used by each of 29 rural settlements in Moyamba District, Southern Province, Sierra Leone over the period March–July 1979. Settlements were selected according to the water source type used, so as to be representative in this respect of the rural population of the entire Southern and Eastern Provinces of Sierra Leone, which had been previously surveyed. The period of study was chosen so that results were obtained over the transition from dry to wet season when, potentially, sources were most susceptible to faecal contamination. All samples were analysed within 6 h of collection.

FC count was estimated by the multiple-tube dilution method using modified Gray's glutamate medium (D.H.S.S., 1969). Inoculated tubes were incubated directly at 44·0 °C to reduce the number of false positives produced (Mara, 1978; Wright, 1982). Positive tubes occurring within 48 h were used to determine the most probable number (MPN) FC count and then subcultured directly to tubes of tryptone water (D.H.S.S., 1969); these were incubated at 44·0 °C for 24 h and tested for indole. Indole-positive tubes were used to determine the MPN presumptive *E. coli* count.

FS count was estimated by membrane filtration of a sample of approx. 15 ml (the exact volume filtered was recorded) through a Millipore 0·45 μm, 47 mm diameter filter and incubation at 45 °C for 48 h on Slanetz and Bartley medium (Slanetz & Bartley, 1957). All red or pink colonies produced were counted as FS and then subcultured to tyrosine-sorbitol-thallous acetate agar (Mead, 1963) and incubated for 3 days at 45 °C; (if the number of FS on any filter exceeded 40, then only 40 randomly selected colonies were subcultured). Red colonies surrounded by a clear zone of tyrosine decomposition were recorded as *S. faecalis*. Counts of FS and *S. faecalis* were calculated as colony-forming units (c.f.u.) dl^{-1}.

C. perfringens count was estimated on a sample which had been heated to 75 °C for 10 min by the multiple-bottle dilution method using differential reinforced clostridial medium (D.H.S.S., 1969), followed by the subculture of positive bottles to tubes of Crossley milk (Oxoid CM 213). Production of a stormy clot in the latter medium was considered indicative of *C. perfringens*, and MPN counts were determined from positive tubes.

The incidence of *Salmonella* spp. was estimated by adding a 10 ml sample to 10 ml double-strength Rappaport broth (D.H.S.S., 1969); this was incubated at 37 °C for 48 h and subcultured to modified brillant green agar (Oxoid CM 329). The Rappaport broth was reincubated at 37 °C. Agar plates were incubated at 37 °C for 24 h and examined for growth of typical colonies of *Salmonella* spp. Subcultures were made to fresh modified brilliant green agar plates of typical or equivocal colonies. Colonies obtained at this stage with an appearance typical of *Salmonella* spp. were subcultured to Dorset egg slopes (Cowan & Steel, 1965) and stored at room temperature for subsequent examination. If no typical colonies were obtained from the primary subculture, the broth was examined again, in a similar

manner, after a total of 7 days incubation. A confirmed positive result was taken to indicate the presence of salmonella organisms in numbers $\geqslant 10\ dl^{-1}$.

The residual water sample was refrigerated after inoculation of media. If counts obtained within 24 h in any of the faecal indicator tests were obviously too high or too low for calculations to be made accurately, repeat determinations were made with a more appropriate volume of sample or diluted sample. Refrigeration for 24 h was not considered to have significantly affected results (Lonsane, Parhad & Rao, 1967).

In addition to the above bacteriological tests, the pH, conductivity and temperature of water sample from each source were recorded at the time of sampling.

Faecal analysis

Fifty-three faecal samples were obtained (samples were requested from one adult male and one adult female inhabitant of each of the 29 settlements which had had their water sources examined: 5 persons were unwilling to give samples). Within 12 h of defaecation, the samples were analysed by vigorously mixing 1·0 g (wet weight) faeces with 99 ml diluent (quarter-strength Ringer's solution) until completely dispersed, then making decimal dilutions to a final dilution of 10^{-10}.

The 10^{-6}–10^{-10} dilutions were examined for FC by the same multiple-tube dilution method used for the analysis of water samples, with subsequent subculture to tryptone water for the detection of indole production.

The 10^{-6} and 10^{-7} dilutions were examined for FS and *S. faecalis* by membrane filtration, incubation and subculture, as described for water samples.

For the examination for *C. perfringens*, the residual 10^{-3} dilution was heated at 75 °C for 10 min and further decimal dilutions than made to 10^{-7}. These dilutions were tested for *C. perfringens* as described for water samples.

The incidence of *Salmonella* spp. was estimated by transferring approx. 0·1 g sample to 20 ml Rappaport broth. This was incubated and subcultured as described for water samples, except that the primary subculture to modified brilliant green agar was made after 24 h incubation.

The residual sample was refrigerated for up to 24 h in case repeat determinations using more appropriate dilutions were required. This was the case with 11 samples for the FS determination and 3 samples for the *C. perfringens* determination. Mean results were therefore not considered to have been significantly affected by any changes in bacterial numbers which may have occurred during refrigeration.

Confirmation of Salmonella *spp.*

Cultures on Dorset egg were tested for purity and then subcultured to urea broth, triple sugar iron agar, XLD agar and bismuth sulphite agar (all Oxoid). Cultures giving reactions typical of *Salmonella* spp. were examined for agglutination with polyvalent 0 and Vi antisera (Fisher Scientific Co., U.S.A.).

Table 1. *Levels of faecal indicator bacteria in* 13 *water sources shown to contain* Salmonella *spp.*

Settlement no./month sampled	Source type*	Faecal indicator count dl^{-1} FC (MPN)	Presumptive *E. coli* (MPN)	FS (c.f.u.)	*S. faecalis* (c.f.u.)	*C. perfringens* (MPN)
2/March	R	15000	15000	3400	500	90
4/March	SW	15000	15000	10000	9200	150
5/April	SW	240000	120000	64000	58000	1500
7/April	SW	11000	640	2000	1800	1500
9/June	St	140	30	180	99	140
13/June	St	40	< 30	60	27	40
14/June	St	40	40	56	56	150
21/June	Sw	230	< 30	750	450	390
24/June	Sp	390	390	340	340	430
26/July	St	430	430	28	17	930
27/July	St	4600	2400	92	85	930
28/July	St	230	230	98	< 7	750
29/July	Sw	90	< 30	7	< 7	150

* See legend to Fig. 1 for key to abbreviations.

RESULTS

Water quality

Water sources used by the 29 settlements under investigation comprised one large river (used by two settlements at different locations), one small river (also used by two settlements at different locations), 9 shallow streams, 3 swamps, 6 shallow wells (situated in dried swamps or stream beds), 2 springs and 5 dug wells. No sources were protected from potential contamination (by surface run-off or nearby laundering and bathing) although the dug wells were reinforced with sticks at ground level to prevent collapse of the walls whilst inhabitants were withdrawing water. Very little sanitation development was evident, the majority of inhabitants in all settlements defaecating 'in the bush'.

The potential for contamination of sources with animal faeces was observed to be low relative to that for contamination with human faeces. Very few domestic animals (there were no cattle; pigs were kept by one household in one settlement only; a small number of goats was kept in some settlements; several chickens and one or two dogs were kept in the majority of settlements) were present in the settlements, fish rather than meat being the usual source of protein in the diet. Wild animals may have contributed significantly to the faecal bacterial pollution load of the two rivers (the large river supported some gallery forest, an ideal habitat for several species of monkey), but other water sources were usually too close (median distance from settlement was 220 m) to human habitation for such a contribution to have been significant.

Fig. 1 depicts the characteristics of water sources examined according to source type. Water temperature was high and relatively constant for all sources tested

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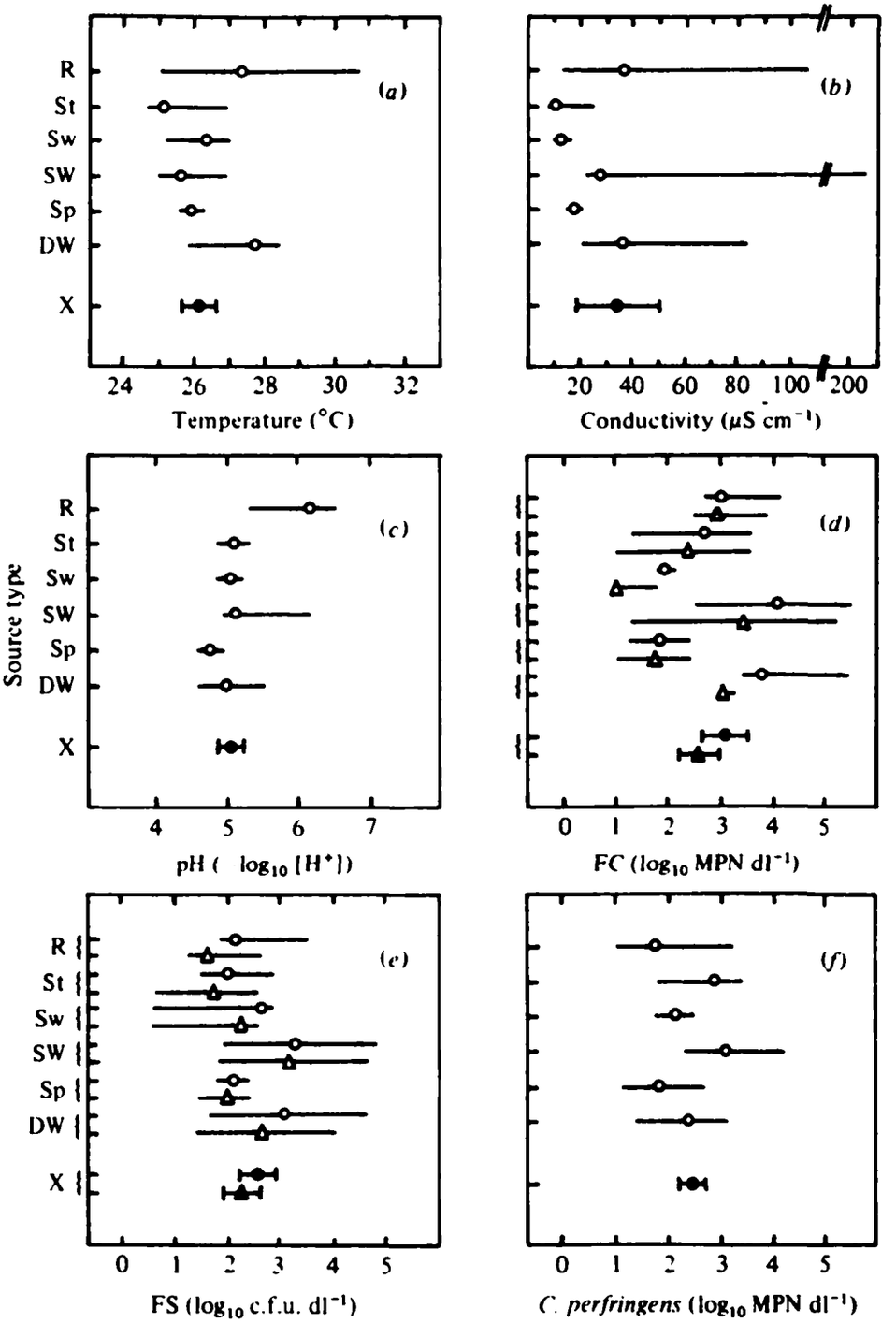

Fig. 1. Water quality characteristics according to source type: river (R), stream (St), swamp (Sw), shallow well (SW), spring (Sp), dug well (DW), all sources (X). Open-ended horizontal bars refer to the range of values, open symbols to median values. Closed symbols and closed horizontal bars refer to the means and 95% confidence limits of the mean values of all sources; means are arithmetic for (*a*) and (*b*) and geometric for (*d*), (*e*) and (*f*); the mean pH value was derived by converting observed pH values to $[H^+]$, taking an arithmetic mean and converting this back to a pH value. In (*d*), circles refer to FC, triangles to indole positive FC. In (*e*), circles refer to FS, triangles to *S. faecalis*.

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Table 2. *Levels of faecal indicator bacteria in human faeces*

Indicator	Log_{10} geometric mean	95% confidence limits	Range
FC (MPN)	8·31	±0·95	5·59–10·04
Presumptive *E. coli* (MPN)	8·28	±0·96	5·59–10·04
FS (c.f.u.)	6·92	±0·99	4·38–8·60
S. faecalis (c.f.u.)	5·94	±1·37	3·68–8·54
C. perfringens spores (MPN)	4·66	±1·31	2·00–7·70

All counts are per g wet weight faeces.

over the period of study. pH was low except for the major river and one shallow well; all sources were acidic, however. Conductivity was variable, from a very low reading of 7·6 μS cm^{-1} to a high of 206 μS cm^{-1}.

Faecal contamination of all sources was evident and 13 of the sources contained salmonella organisms in numbers $\geqslant 10$ dl^{-1}. Six of the 9 streams were positive for *Salmonella* spp.; however, the levels of faecal indicators in these sources were generally lower than in other sources (Table 1). Overall, no significant correlation could be demonstrated between the incidence of *Salmonella* spp. and the levels of any of the faecal indicators tested.

The ratios of FC to FS in the waters tested were, in the majority (18/29) of cases, $> 4:1$ and therefore indicative of human rather than non-human faecal pollution (Geldreich & Kenner, 1969).

The levels of *C. perfringens* encountered were similar to FS levels and higher than those found by Muhammed & Morrison (1975) in similar water sources in East Africa.

Some seasonality of water quality was indicated by the differences observed between shallow wells (exclusively dry-season sources) and streams/swamps (exclusively wet-season sources). From Fig. 1, it can be seen that the dry-season sources were more liable to heavy contamination with faecal bacteria and exhibited a higher conductivity than wet-season sources.

The ratio of geometric mean values for all sources of FC: presumptive *E. coli* : FS : *S. faecalis* : *C. perfringens* was 3·8 : 1·2 : 1·1 : 0·6 : 1.

Indicator bacteria and Salmonella *spp. in faeces*

The levels of faecal indicator bacteria in human faeces are described in Table 2. Three of the 53 samples were positive for *Salmonella* spp. Of the indicator organisms, FC were predominant and > 90% of these were confirmed as presumptive *E. coli*. FS were the next most numerous, the ratio of geometric mean FC count to geometric mean FS count being 25 : 1. However, only approx. 10% of FS were identified as *S. faecalis*; *S. faecalis* may not have been, therefore, the most numerous constituent organism of the FS group in the samples examined.

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C. perfringens spores were present in much smaller numbers than the above indicators; the ratio of geometric mean FC count to geometric mean *C. perfringens* spore count was 4500 : 1.

DISCUSSION

The generally low pH values of water sources were seen to be associated with the low ionic content and corresponding lack of buffering capacity. This is substantiated by the fact that there was a significant ($P < 0{\cdot}01$) linear correlation coefficient ($r = 0{\cdot}581$) between conductivity and pH values. pH readings of low-conductivity waters (especially shallow streams) were also subject to large fluctuations (up to 0·5 units) when the sample was agitated. The survival of faecal micro-organisms in water may be significantly influenced by the combination of high temperature, low conductivity and low pH.

The lack of a demonstrable correlation between faecal indicator levels and the incidence of *Salmonella* spp. casts doubt on the ability to draw conclusions about the acceptability of water sources based on different levels of indicators. It has been suggested that guidelines of acceptability for potable water supplies in developing countries should be set at lower levels than those recommended by W.H.O. (1971), in an effort to encourage an incremental improvement of water quality (Feachem, 1980). However, if the incidence of pathogenic micro-organisms, such as *Salmonella* spp., cannot be related to different levels of faecal indicators in any situation, there is no justification for suggesting, for example, that a water containing 10 FC dl^{-1} is more acceptable than one containing 100 FC dl^{-1}. At the same time it must be recognized that, in many instances, only faecally polluted water sources are available to a settlement and there is no prospect of water treatment. In such cases, the water source with the lowest faecal indicator count (representing an average of sufficient samples) should be preferred (provided the available sources were equivalent in other respects: chemical contamination, distance from settlement and year-round adequacy of volume) until such time as treatment could be provided. This would entail a close monitoring of water quality and increase the importance of the use of an appropriate, specifically faecal indicator. For this reason, more weight should be given to presumptive *E. coli* counts than FC counts (e.g. a water source exhibiting an average FC count of 1000 dl^{-1} and presumptive *E. coli* count of 10 dl^{-1} should be preferred to a source exhibiting an average FC count of 200 dl^{-1} and presumptive *E. coli* count of 200 dl^{-1}).

Insufficient data exist concerning the relative survival of FC and FS in tropical waters to draw sound conclusions from the ratio between the two groups, even using the modified role of this ratio proposed by Feachem (1975). The relationship is further complicated by the fact that FC counts have been obtained from tropical waters where the confirmation rate as *E. coli* has been low (Barrell & Rowland, 1979). In this study, counts of 220000 and 92000 FC dl^{-1} were obtained from two dug wells, but confirmed counts of presumptive *E. coli* were only 790 and 1100 dl^{-1} respectively.

Because all sources were contaminated with FC and FS, the need for a *C. perfringens* determination to be made was not justified. However, the relative

proportion of *C. perfringens* to FC and FS was much higher in water than in faeces, indicating a prolonged survival; the test may prove useful, therefore, for protected springs and wells where recent faecal contamination could not be shown (by the FC or FS test).

The levels of faecal indicator bacteria in faeces were similar to those quoted by Moore & Holdeman (1974) for the faecal flora of 20 Japanese-Hawaiians and by Drasar (1974) for the faecal flora of subjects from Uganda. The suggestion that *E. coli* may occur in much lower proportions in faeces in some tropical developing countries (Evison & James, 1973) has therefore not been substantiated by this study.

Contamination of water with faecal bacteria from non-human sources must influence any observed faecal indicator ratios in the water to some extent. In this study, however, it was felt that human contamination whilst collecting water, the use of many sources for nearby laundering and bathing, and the likely preponderance of human excreta over non-human excreta in the vicinity of most sources, would have resulted in the majority of faecal bacterial pollution being of human origin.

With the data available, presumptive *E. coli* is considered the most appropriate indicator of human faecal pollution in water sources such as those encountered herein. However, the relative survival of *E. coli* to pathogens in typical waters should be investigated, bearing in mind the high proportion of samples found to contain *Salmonella* spp. The term 'faecal coliform' should not be considered synonymous with *E. coli*, and confirmation of all positive tubes occurring in the standard FC multiple-tube dilution test by, at least, the detection of indole production is required. Simplified methods (e.g. Mara, 1978) for the assessment of FC density without confirmation should not be advocated where the incidence of FC other than *E. coli* has been demonstrated to be high. This may be the case in many tropical countries.

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