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# The hygienic quality of vegetables grown in or imported into the Netherlands: a tentative survey

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SUMMARY

Samples of 61 home grown and 199 imported vegetables of different varieties were examined for *Escherichia coli*, faecal streptococci and, when *E. coli* was present, for salmonellas. Eleven per cent of samples contained $> 10^4$ *E. coli* per 100 g, and 14% $> 10^6$ faecal streptococci per 100 g. Salmonellas were isolated from 23 out of 103 samples examined.

Salmonellas were isolated from 8% of 76 samples with *E. coli* $< 10^4$/100 g, but from 63% of 27 samples with *E. coli* exceeding $10^4$/100 g; from 6% of 65 samples containing $< 10^6$ faecal streptococci/100 g but from 51% of 37 samples containing more than $10^6$/100 g.

*S. typhi* was isolated from one sample of vegetables imported from the tropics. To our knowledge this is the first isolation of *S. typhi* from food in the Netherlands. Products from tropical countries were found to present the highest level of contamination. The hygienic quality of Dutch products is sometimes inferior to that of similar imported products, although the different seasons of sampling may have influenced the result. For the prevention of risk to the consumer of vegetables, good kitchen hygiene would appear to be the most important factor.

## INTRODUCTION

Whilst growing, vegetables may be exposed to many sources of faecal contamination. For example, water from the rivers Rhine and Meuse on entry to the Netherlands is always contaminated with large numbers of salmonellas (Kampelmacher & Van Noorle Jansen, 1973). In addition, salmonellas and other pathogens are derived from effluents from crude and treated sewage (Edel, Guinée, Van Schothorst & Kampelmacher, 1972). If water thus polluted is used for irrigation as artificial rain, which is often practised during long periods of drought as in the summer of 1976 in Western Europe, contamination of field crops will result. Many cases of illness caused by consumption of vegetables treated with surface water have been reported (Geldreich & Bordner, 1971; Ruschke, 1976). Other sources of pollution are birds, rodents, insects, and manuring with farmyard manures.

Although, after contamination as described above, drying may reduce the numbers of bacteria as do also rainfall and sunlight (Geldreich & Bordner, 1971), the vegetables may be recontaminated during or after harvesting. In this respect, the

wetting of vegetables to help keep them fresh, which generally takes place shortly before sale, is of special interest. In the Netherlands the wetting of vegetables with surface water is forbidden when the commodity is likely to be eaten raw, but enforcement of this regulation is difficult even for home produce. Where imported vegetables are concerned, this regulation has of course no effect before import.

Despite large home production, the amount of vegetables imported into the Netherlands is considerable; for example, 125000 tons in 1974. In addition to vegetables grown in the Netherlands (endive, cauliflower, etc.) very exotic products are sometimes concerned. Striking examples of this group are vegetables like 'kouseband' (literally 'garter', resembling a very long butter-bean) and 'kangkoeng' (a plant resembling spinach, often grown in surface water), both imported from the former Dutch overseas territory of Surinam. Shortly before and after the territory became independent in 1975, considerable emigration to the Netherlands took place and in consequence a rise in the import of favoured foods.

The investigation described in this paper was intended to make a tentative survey of the hygienic condition of these products and the danger – if any – to the public health to be expected from this source. In several countries similar investigations have been carried out on home grown produce: United Kingdom (Fraser, Reid & Malcolm, 1956; Tee, 1962), USA (Hall, Brown & Lewis, 1967), Greece (Papavassiliou, Tzannetis, Leka & Michopoulos, 1967), Canada (Duncan & Razzall, 1972), Brazil (De Freitas Leitao, 1973), Spain (Rodriguez-Rebollo, 1974), Western Germany (Käferstein, 1976), Italy (Ercolani, 1976). Some of these investigations refer to an assortment of vegetables, others to only one product. In general, large numbers of Enterobacteriaceae or coliforms (up to or exceeding $10^4$/g) were found – a result which is hardly surprising when the ubiquity in nature of Enterobacteriaceae of non-faecal origin is considered. In this respect, attention may be drawn to the presence of these Enterobacteriaceae *inside* healthy tomatoes, cucumbers, etc. (Samish, Etinger-Tulczynska & Bick, 1963; Meneley & Stanghellini, 1974).

Part of the Enterobacteriaceae or coliform flora is reported to consist of *E. coli* varying from less than one to a high percentage. Direct comparison of the results published in these papers is not possible, however, because of the differences in the vegetables investigated, the methods of examination and the presentation of results (quantitative, presence/absence, unquantified, categorized, or averages). The numbers of faecal streptococci, when determined, show variation both in absolute numbers and in relation to the numbers of *E. coli*.

From the above survey of the literature, salmonella contamination of vegetables would appear to have been investigated only by Ercolani (1976), who found these bacteria in approximately 70% of 209 samples of lettuce and fennel from the Bari area of Italy.

The programme of the present survey is based partly on the experience of the investigators mentioned in the above survey of the literature. In order to investigate as many samples as possible, it appeared desirable to limit the numbers of groups of bacteria determined. Hence in the first instance the numbers of only

*E. coli* and faecal streptococci were determined. Samples in which presumptive *E. coli* were detected were examined for salmonellas. A number of samples from tropical countries were examined for parasites. We intended at first, for obvious reasons, to investigate only products consumed raw. However, the division between products eaten raw and after cooking is difficult to ascertain as personal taste and national habits play an important part in this respect. Indeed when the possibility of cross-contamination is considered, the difference between raw and cooked products seems less relevant. However, in the list of products examined which follows, it can be seen that products intended for consumption raw form the major part.

## MATERIALS AND METHODS

### *The origin of samples*

The survey was carried out mainly in 1976. A total of 260 samples were examined of which 61, mainly lettuce, endive and cabbage, were grown in the Netherlands. Most of the imported products came from France (17 samples, mainly cabbage and 'witloof' chicory), Israel (21 samples, including celery), Italy (36 samples, mainly cabbage, endive and fennel), Morocco (11 samples, mainly courgettes) and Spain (13 samples, including broccoli and celery). Smaller numbers of samples were examined from Belgium, Egypt, Hungary and Jugoslavia. Products from tropical countries came mainly from Surinam (31 samples, including chillies, 'kouseband' and 'kangkoeng'), with smaller numbers of samples from Cuba, Indonesia, Jamaica, Kenya, Senegal and Tanzania. In addition, samples were examined from the U.S.A. (16, mainly radishes and lettuce) with a few samples from the Canary Islands and from South Africa.

### *Nature of samples*

The largest numbers of vegetables examined were endive (24), lettuce (21), celery and paprika ('sweet pepper'), (20 each), cabbage (18, several varieties), chillies (16), fennel (15), aubergine ('egg plant') and cauliflower (13 each), courgettes (11) and radishes (10). Smaller numbers of artichokes, avocado pears, beans, broccoli, mango, spinach, tomatoes, 'kouseband', 'kangkoeng' and other vegetables were examined.

### *Sampling*

Dutch products were bought mainly in shops or markets in Wageningen and other towns in the neighbourhood. Foreign products were obtained mainly via the Food Inspection Departments in Rotterdam and the Hague, from the importers. Surinam vegetables were sometimes bought directly in shops in Amsterdam. The amount of sample was as a rule between 0·5 and 2 kg. The samples were wrapped in sterile plastic bags and transported as soon as possible to the laboratory.

Representative parts of 28 samples from tropical countries were examined for parasites at the Rijks Instituut voor de Volksgezondheid (National Institute of Public Health) at Bilthoven.

*Preparation of the samples*

Leafy vegetables (cabbage, etc.) were placed on a flat stainless-steel surface (ca. 45 × 60 cm), previously treated with 70 % ethanol and flamed. The sample, held in one hand covered with a disposable glove which had been disinfected overnight using 70 % ethanol, was cut with a sterile knife into fine pieces which were spread evenly over the table surface. Solid vegetables (aubergines, avocado, paprika) were peeled, and the peelings treated as described above. Small vegetables (chillies) were not cut, but were spread evenly over the whole surface.

Using a random number table, small quantities of each vegetable up to a total of 50 g were collected from the surface into a sterile mixer beaker, to which 450 ml of buffered peptone water (BPW, I.S.O. 1975) was added and the mixture blended for about 2 min at about 10000 rev./min.

From preliminary experiments it had been concluded that this procedure yielded generally somewhat higher counts than mere rinsing of the vegetables. However, when cauliflower and radishes were blended as above, inhibitory substances from the product appeared to be dispersed into the mixture as, on MPN determination, the lowest dilutions gave negative results whereas higher dilutions gave positive tubes (cf. Abdou, Abou-Zeid, El-Sherbeeny, Abou-el-Gheat, 1972). These products were rinsed, 50 g of vegetable plus 450 ml of diluent being shaken in a sterile 2 l flask for 45 min.

From the primary 1/10 dilutions, further decimal dilutions were prepared in BPW.

Escherichia coli

For determination of the MPN of *E. coli*, a three-tube decimal-dilution method was used. The amounts used were 10 g (100 ml of the primary 1/10 dilution in flasks), 1 g (10 ml of the 1/10 dilution in tubes) and $10^{-1}$, $10^{-2}$, $10^{-3}$ and $10^{-4}$ g (1 ml of the appropriate decimal dilution into tubes containing 10 ml BPW).

The flasks and tubes were incubated at 37 °C for 16–18 h for resuscitation and pre-enrichment. At the end of this time 1 ml volumes were transferred from each flask and tube to tubes of brilliant green-bile-lactose-broth (BBL broth) fitted with Durham's tubes which were incubated in a water bath at 44 ± 0·1 °C.

The tubes were inspected for gas formation after 24 and 48 h incubation. When gas formation was observed, material from the tube was streaked on plates of crystal violet/neutral red/bile/lactose agar (VRBL agar) which were incubated at 30 °C. After 24 h separate colonies were investigated using the Eijkman–McKenzie test. Individual colonies were inoculated into tubes of BBL broth and peptone water containing tryptophane, and were incubated at 44 ± 0·1 °C for 48 h. *E. coli* was characterized by gas formation in BBL broth together with the formation of indole from tryptophane. Indole formation was indicated by the development of a red coloration following the addition of 10 drops of a reagent consisting of 5 g paradimethyl-aminobenzaldehyde, 75 ml of *n*-pentanol and 25 ml of 38 % hydrochloric acid, to the peptone water tubes. From the pattern of positive and negative results, the MPN was determined with the aid of the appropriate MPN table.

The detection of *E. coli* is usually carried out after the sample has been examined for coliforms after enrichment at 30 or 37 °C. As incubation in this temperature range produces, in addition to *E. coli*, many other coliform organisms on VRBL plates, it appeared preferable to incubate the definitive enrichment tubes at 44 °C, as we were interested only in *E. coli*. To determine if the procedure could adversely influence the results, both temperatures of incubation (30 and 44 °C) were applied in parallel to the examination of 52 samples. At both temperatures five samples gave the same MPN. In ten samples the MPN at 44 °C was higher than at 30 °C. The remaining samples had numbers below the limit of detection (3 per 100 g). There were no samples yielding more *E. coli* at 30 °C. As similar results had been obtained in this laboratory with a totally different product (ice cream), incubation was afterwards carried out only at 44 °C.

*Faecal streptococci*

The three-tube decimal dilution method described above was used with direct inoculation into esculin broth (EB). Sample amounts of 1, $10^{-1}$, $10^{-2}$, $10^{-3}$, $10^{-4}$ g were inoculated directly into 10 ml tubes of EB, double-strength EB being used for the tubes containing 1 g. The tubes were incubated at 37 °C and examined after 24 and 48 h incubation for blacking of the medium, when such tubes were subcultured to Steptosel agar. Separate small white colonies were examined by microscopy, and for growth at $45 \pm 0{\cdot}1$ °C in brain–heart infusion, and at 37 °C in 40% bile broth.

The MPN was calculated as indicated for *E. coli*.

*Salmonellas*

Samples were examined for salmonellas if plating on VRBL agar indicated the presence of *E. coli* in numbers approximately 10 or more per 100 g of sample. This procedure of course results in a number of samples being examined for salmonellas in the absence of *E. coli*. In a few samples no examination for salmonellas took place although *E. coli* was present.

To detect salmonellas, samples were incubated for 16–18 h in BPW at 37 °C. One ml volumes were then transferred to tubes of Muller–Kaufmann broth incubated at $42{\cdot}5 \pm 0{\cdot}5$ °C. After 24 and 48 h incubation tubes were subcultured onto brilliant-green/phenol-red agar. Individual characteristic colonies were confirmed as salmonellas using TSI agar, lysine broth, urea agar and serum agglutination (I.S.O. 1975). Strains of *Salmonella* were identified as to serotype and phage type at the Rijks Instituut voor de Volksgezondheid (National Institute of Public Health) at Bilthoven.

*Parasites*

Twenty-eight samples, mainly from Surinam and Indonesia, were examined microscopically for ova and cysts of human pathogenic parasites.

Table 1. E. coli *and faecal streptococci; percentages of samples on a total number of* 260 *in the categories indicated*

| MPN/100 g | Percentage | |
|---|---|---|
| | *E. coli* | Faecal streptococci |
| $< 3$ | 52 | 27 |
| 3–10 | 14 | |
| $11–10^2$ | 12 | |
| $1{\cdot}1 \times 10^2–10^3$ | 4 | 20 |
| $1{\cdot}1 \times 10^3–10^4$ | 7 | 13 |
| $1{\cdot}1 \times 10^4–10^5$ | 4 | 20 |
| $1{\cdot}1 \times 10^5–10^6$ | 3 | 6 |
| $> 10^6$ | 4 | 14 |

## RESULTS

### E. coli *and faecal streptococci*

Table 1 presents the MPN's of *E. coli* (8 categories) and faecal streptococci (6 categories). In 52 % of samples the *E. coli* were below the limit of detection (3/100 g), 26 % samples contained less than $10^2$/100 g. Of the remaining samples 11 % contained between $1{\cdot}1 \times 10^2$ and $10^4$ *E. coli*/100 g, 7 % of samples between $1{\cdot}1 \times 10^4$ and $10^6$ *E. coli* but only 4 % of samples contained $> 10^6$.

The distribution of faecal streptococci differs considerably: 27 % of samples contained between $< 3$ and $10^2$/100 g, 33 % between $1{\cdot}1 \times 10^2$ and $10^3$, and 40 % contained more than $1{\cdot}1 \times 10^4$, of which 14 % contained $> 10^6$ faecal steptococci per 100 g.

### *Salmonellas*

Salmonellas were isolated from 23 out of 103 samples examined. With two exceptions the MPN's were less than 100/100 g. The exceptions were a sample of Dutch lettuce with 240/100 g and a sample of Dutch endive with 24000/100 g. The serotypes of salmonellas isolated were as follows: From Dutch products *S. infantis* 3, *S. typhimurium* phage type I 30, 2; from imported products, *S. bredeney*, *S. california* 3, *S. heidelberg* 2, *S. infantis* 2, *S. java* 4, *S. montevideo* 5, *S. typhi* and one serotype of Group D not further identifiable. All the imported positive samples were from tropical areas.

The five Dutch samples containing salmonellas had been grown in three market gardens in a relatively small area. The serotypes isolated are regularly found in surface water and effluents in the Netherlands.

### *Relation between origin and quality of products*

There were marked differences in the hygienic quality of the products examined. Table 2 presents the results of examination of those products of which more than ten samples were examined. Because of the relatively small number of samples examined percentages have not been calculated. The number of categories has been reduced to four.

Table 2. *Numbers (absolute) of samples containing salmonellas and of samples with MPN's of* E. coli *(upper figure) or faecal streptococci (lower figure in parentheses) in the categories indicated.*

| Vegetable | No. of samples | No. with salmonellae | *E. coli* and faecal streptococci; numbers of samples in the categories (per 100 g) | | | |
|---|---|---|---|---|---|---|
| | | | $< 10^2$ | $1{\cdot}1 \times 10^2$–$10^4$ | $1{\cdot}1 \times 10^4$–$10^6$ | $> 10^6$ |
| Aubergine (egg-plant) | 13 | 2 | 10<br>(3) | 1<br>(5) | 1<br>(2) | 1<br>(3) |
| Cabbage | 18 | 0 | 16<br>(8) | 2<br>(6) | 0<br>(3) | 0<br>(1) |
| Cauliflower | 13 | 1 | 12<br>(9) | 1<br>(2) | 0<br>(2) | 0<br>(0) |
| Celery | 20 | 0 | 15<br>(5) | 4<br>(8) | 1<br>(6) | 0<br>(1) |
| Chilli | 16 | 5 | 3<br>(1) | 8<br>(1) | 2<br>(3) | 3<br>(11) |
| Courgettes | 11 | 0 | 11<br>(1) | 0<br>(2) | 0<br>(5) | 0<br>(3) |
| Endive | 26 | 2 | 21<br>(4) | 2<br>(11) | 2<br>(9) | 1<br>(2) |
| Fennel | 15 | 0 | 11<br>(3) | 4<br>(6) | 0<br>(5) | 0<br>(1) |
| Lettuce* | 28 | 2 | 21<br>(9) | 4<br>(8) | 2<br>(5) | 1<br>(1) |
| Paprika (sweet pepper) | 20 | 0 | 20<br>(17) | 0<br>(3) | 0<br>(0) | 0<br>(0) |

* Some samples of lettuce have not been examined for faecal streptococci.

Paprika, a European product, appears as a very 'clean' product whilst chilli, a typically tropical product, is highly contaminated.

In the same way differences are seen in the hygienic quality of the same product from different countries. It was somewhat surprising that Dutch products did not compare favourably with similar products imported from non-tropical countries. Fig. 1, in which a comparison is shown for Dutch products, corresponding products imported from non-tropical countries and products imported from the tropics, illustrates the point.

In constructing Fig. 1(3), products of which only imported samples have been investigated (mainly paprika, aubergines and fennel) have been omitted, so as to make a more exact comparison. For ease in interpretation the categories of numbers of organisms have been reduced to four.

*Correlations between* E. coli, *faecal streptococci and salmonellas*

Tables 3 and 4 present the number of salmonella positive samples in relation to the MPN's for *E. coli* and faecal streptococci. Both tables show an increase in

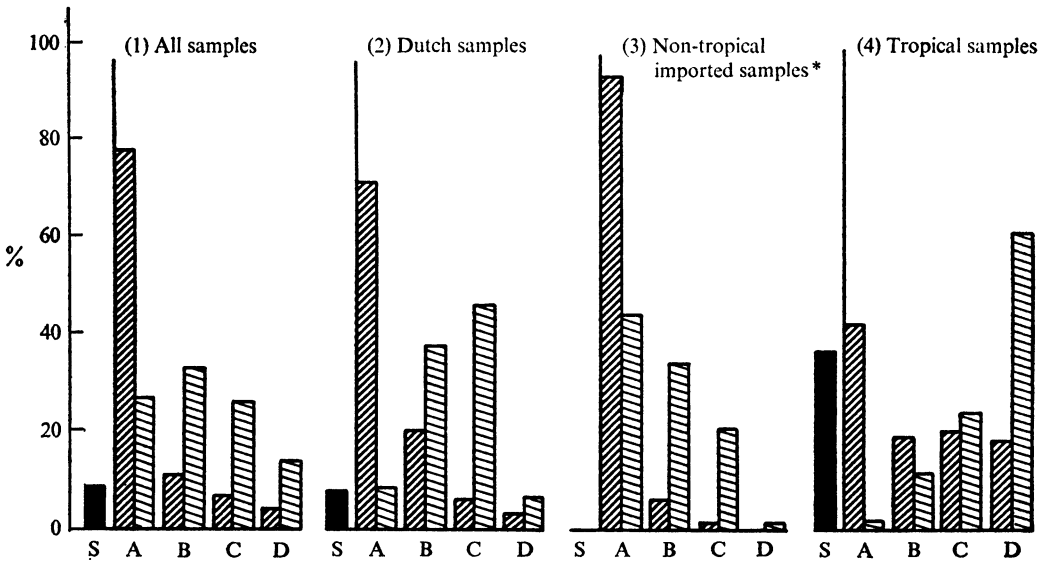


Fig. 1. Vegetables, divided (for each group indicated) into four categories (percentages): MPN's per 100 g. A = $< 10^2$, B = $1{\cdot}1 \times 10^2$–$10^4$, C = $1{\cdot}1 \times 10^4$–$10^6$, D = $> 10^6$, S = % Salmonella.

Table 3. *Correlation of* E. coli *with Salmonella*

| *E. coli* (MPN/100 g) | No. of samples | No. examined for salmonellas | *Salmonella* detected | *Salmonella* detected (%) |
|---|---|---|---|---|
| $< 10^2$ | 204 | 49 | 1 | 2 |
| $1{\cdot}1 \times 10^2$–$10^4$ | 29 | 27 | 5 | 19 |
| $> 10^4$ | 27 | 27 | 17 | 63 |

Table 4. *Correlation of faecal streptococci with* Salmonella

| Faecal streptococci (MPN/100 g) | No. of samples | No. examined for salmonellas | *Salmonella* detected | *Salmonella* detected (%) |
|---|---|---|---|---|
| $< 10^2$ | 75 | 6 | 0 | |
| $1{\cdot}1 \times 10^2$–$10^6$ | 143 | 59 | 4 | 7 |
| $> 10^6$ | 37 | 37 | 19 | 51 |

the number of salmonella positive samples as the number of *E. coli* and faecal streptococci increases which is significant when the $\chi^2$ test is applied. Further subdivision of the categories yields no further information.

From inspection of Tables 3 and 4 it might be expected that samples in which an MPN of *E. coli* $> 10^4$ is combined with an MPN of faecal streptococci $> 10^6$

Table 5. *Correlation of faecal streptococci with* E. coli *for 75 samples, in six categories*

| Log $\frac{\text{faecal streptococci}}{E.\ coli}$ | | | | | |
|---|---|---|---|---|---|
| $< -0{\cdot}5$ | $-0{\cdot}5/+0{\cdot}5$ | $0{\cdot}5/1{\cdot}5$ | $1{\cdot}5/2{\cdot}5$ | $2{\cdot}5/3{\cdot}5$ | $> 3{\cdot}5$ |
| 6 | 6 | 18 | 17 | 19 | 9 |

would yield the greatest percentage of salmonella positive samples. This would appear to be true, as 15 out of 20 such samples (75%) were salmonella-positive: the corresponding figure for the remaining 82 samples examined for salmonella is 8 (10%).

The correlation between *E. coli* and faecal streptococci is presented in Table 5, for those samples in which examination yielded results within the limits of detection. The ratio of faecal streptococci to *E. coli* ranges from < 1 to > 1000 with an average of approximately 100.

*Parasites*

Larvae of the genus Strongyloides were detected in 2 out of 28 samples examined. These parasites infect man usually by active penetration of the skin. No human parasites were found in the remaining samples but many contained plant parasites, mites and/or their ova, and nematode larvae.

## DISCUSSION

The aim of this investigation was to conduct a broad survey for the presence of organisms indicating faecal pollution, and for salmonellas on Dutch and imported vegetables. The data do not allow a fine differentiation regarding the type of product and the country of origin. This would have necessitated far more samples, which were often not available because we were dependent on the supply on the market. In addition, the composition of the sample assortment is almost certainly not representative of the quantitative composition of the assortment of fresh vegetables consumed in the Netherlands and other Western European countries.

Bearing in mind these limitations, nevertheless some general conclusions can be drawn. For example, the presence of salmonellas, and, in view of the faecal contamination, possibly other human pathogens, involves some potential dangers in the consumption or preparation of certain vegetables, in particular the risk of cross contamination in the kitchen.

The risk applies in particular to tropical products. The picture, it is true, is biased by the high proportion of products imported from Surinam, some 60% of this group, but products from Indonesia and some African countries were of unfavourable hygienic quality too. This may have been connected with the conditions under which these products were grown. As mentioned in the Introduction, 'kangkoeng', one of the most contaminated products, may be grown in surface

waters. In addition, other factors may influence contamination, e.g. irrigation, manuring, and periodical inundations as a consequence of monsoon rains.

The isolation of *S. typhi* from one of the samples is particularly disturbing. So far as we know, this micro-organism has never before been isolated from food in the Netherlands. Whilst these tropical products are rarely eaten raw, the possibility of cross contamination of other food remains.

As to the relatively unfavourable picture presented by the Dutch samples, their quality may have been influenced by the season of growth. Cauliflower and endive from other countries are imported mainly during the winter when home production is not possible. Home-grown products are produced and have been sampled mainly in summer and early autumn.

Investigations (Papavassiliou *et al.* 1967) have shown that in Greece (climatically comparable with Italy and Spain, from which the cauliflower and endive examined had been imported) the hygienic quality of vegetables in general may in winter and spring be better than in the warm seasons. It is conceivable, therefore, that conditions during the Mediterranean winter were more favourable than those experienced in North Western Europe during the almost subtropical summer of 1976, during which the long periods of drought may have led to an increase in irrigation with polluted surface water.

The significance of the micro-organisms used as indicators of clean production deserves some comment.

As mentioned above, 52 samples were examined for *E. coli* by incubation at 30 and 44 °C. Subculture of the tubes incubated at 30 °C on VRBL agar allowed an estimate to be made of contamination by coliform organisms. This contamination was often substantial, even in samples from which *E. coli* was isolated only in small numbers or not at all. If *E. coli* is to be regarded as the best indicator of faecal contamination (Geldreich & Bordner 1971, Table 3, above), it may be concluded that the coliform group is not so suitable for the assessment of hygienic quality in these raw products.

The correlation of numbers of faecal streptococci with *E. coli* has already been discussed. In spite of variations in the ratio of these organisms it would seem reasonable, considering the data in Table 4, to use both *E. coli* and faecal streptococci as indicators of cleanness for these products (Fowler & Foster, 1976; Käferstein, 1976).

Faecal pollution of vegetables during growth is difficult to prevent. Thus it is hardly possible to prevent the use of surface water for irrigation applied as artificial rain, especially in dry periods, or the wetting of vegetables after harvest. This last procedure is all the more dangerous because when the commodity reaches the consumer, the pollution is recent and the possibility of sufficient reduction in the number of pathogens having taken place is small.

In view of these considerations, the emphasis in reduction of potential risks must be laid on good kitchen hygiene, particularly in establishments where food is prepared on a large scale. Consumption of uncooked tropical products should of course be discouraged, but more important is the recommendation to rinse vegetables thoroughly even if they are to be cooked. In this respect the thorough

cleaning and disinfection of sinks, etc., is also very important. Prevention of cross-contamination will be enhanced in kitchens where food is prepared on a large scale by observance of the general legal requirements concerning hygiene.

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