# THE OCCURRENCE OF ENTERIC BACTERIA ON LETTUCE LEAVES SOLD IN LOCAL MARKETS IN PENANG, MALAYSIA

S.J. CHAPMAN

## INTRODUCTION

A previous survey of locally grown lettuce in Penang indicated high levels of helminths forming a ready source of infection and resulting from the use of night soil (Khairul Anuar and Ramachandran, 1977). A parallel study also showed the presence of *Entamoeba coli* and other protozoans in many samples (Khairul Anuar, pers. comm.). The aim of this study was to determine the levels of enteric bacteria in such lettuces which could be a source of bacterial infection.

## MATERIALS AND METHODS

Lettuces were obtained from several local markets in Penang and their source determined. Each sample consisted of 300g lettuce (2-3 plants) bought early in the morning to minimize the period since harvesting. A composite subsample of 50g lettuce leaves was blended with 450ml 0.1% peptone water. Serial dilutions were plated out in triplicate using violet red bile agar and incubated 24h at 37 c. Presumptive coliforms were counted as red colonies surrounded by precipitated bile. Typical colonies (6-16) were picked into brilliant green lactose broth (BGLB), 48h at 37 c. Tubes producing growth were subcultured into lauryl sulphate tryptose broth, 24h at 37 c, and then into BGLB, 48h at 44.5 c. Gas production was taken as indicating faecal coliforms.

Two further 25g subsamples of lettuce were enriched in tetrathionate broth and selenite cystine broth, 1-2 days at 37 c, and streaked onto *Salmonella-Shigella* agar and brilliant green agar, 24h at 37 c. Suspected *Salmonella* and *Shigella* colonies were checked for purity on MacConkey agar and inoculated onto phenylalanine agar, Christensen's urea agar and triple sugar iron agar slopes. Isolates giving the typical biochemical reactions were taken as presumptive *Salmonella* or Shigella (Oxoid, 1976).

S.J. Chapman, Ph.D.,
Department of Microbiology,
University of Liverpool,
LIVERPOOL, England.

## RESULTS

The results are shown in Table I. Faecal coliforms varied between $10^4$ - $10^6$ per gram of lettuce and *Salmonella* or *Shigella* were detected in 5 out of 11 samples.

## DISCUSSION

The counts of presumptive coliforms and faecal coliforms obtained were very high in nearly all the lettuce samples. Lim and Jegathesan (1977) reported much lower MPN counts in raw vegetable salads: 92.3 and 23.1% of the samples were positive for coliforms and *Escherichia coli* respectively. However, it is not known whether these vegetables had been pre-washed or had been grown using night soil. Lim and Jegathesan (1978) suggest that the low recovery of faecal *E. coli* from food samples may be due to overgrowth by other coliforms in BGLB tubes whereas in the present study colonies were initially recovered on violet red bile agar plates.

The presence of *Salmonella* and *Shigella* shows only a loose correlation with the level of faecal coliforms. *Salmonella* was isolated from the two most contaminated samples but also from samples with moderate contamination.

The present study shows that bacteria of enteric origin can be found on lettuce grown in areas where night soil is habitually used. Since lettuce is normally eaten raw it is clear that it is a

TABLE I:
LEVELS OF COLIFORMS AND OCCURRENCE OF SALMONELLA AND SHIGELLA IN LETTUCE LEAVES FROM ELEVEN MARKETS IN PENANG

| Market | Locality grown | Presumptive coliforms millions $g^{-1}$ | Fraction of positive tubes in BGLB at 37°C | at 44.5°C | Faecal coliforms millions $g^{-1}$ | Presumptive Salmonella | Presumptive Shigella |
|---|---|---|---|---|---|---|---|
| Bayan Lepas | Ayer Itam | 7.1 | 11/14 | 5/14 | 2.5 | + | — |
| Sungai Dua | Sungai Dua | 5.3 | 5/6 | 1/6 | 0.88 | + | — |
| Fettes Park | Fettes Park | 4.6 | 9/10 | 1/10 | 0.51 | — | — |
| Telok Kumbar | Telok Kumbar | 3.0 | 9/16 | 2/16 | 0.38 | — | — |
| Telok Bahang | N.D. | 0.99 | 12/16 | 7/16 | 0.43 | — | + |
| Balik Pulau | Balik Pulau | 0.89 | 12/16 | 2/16 | 0.11 | — | — |
| Tanjong Bunga | Tanjong Bunga | 0.81 | 2/10 | 0/10 | 0.081 | + | — |
| Jelutong | RIMV | 0.17 | 0/6 | 0/6 | 0.028 | + | + |
| Sungai Nibong | Sungai Nibong | 0.14 | 6/6 | 1/6 | 0.023 | — | — |
| Pulau Tikus | Ayer Itam | 0.056 | N.D. | 4/10 | 0.022 | — | — |
| Glugor | Sungai Ara | 0.032 | 6/6 | N.D. | N.D. | — | — |

N.D. not determined

potential source of bacterial infection resulting in various forms of gastroenteritis. Unpublished results have shown that washing the lettuce with water may only reduce the bacterial levels up to tenfold.

## SUMMARY

A survey of lettuce sold in Penang markets showed them to be heavily contaminated with faecal coliforms and nearly half the samples were positive for *Salmonella* or *Shigella*. The use of night soil on these vegetables is a likely cause of gastroenteritis.

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