# GASTROINTESTINAL INFECTION IN A NEW ZEALAND COMMUNITY: A ONE YEAR STUDY.

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# ABSTRACT

Diagnostic medical microbiology laboratories detect and identify pathogens in submitted specimens. The techniques used should maximise the detection of pathogens (sensitivity) while minimising the number of tests for their detection (efficiency). To achieve the best compromise between sensitivity and efficiency, it is necessary to have information on both the relative prevalence and clinical importance of various pathogens within the relevant community, and the relative efficiency of various detection techniques.

This investigation had three primary objectives: to establish what pathogens were associated with community-acquired gastrointestinal symptoms in the Eastern Bay of Plenty, and the incidence and relative importance of each; to compare the merits of various methods for detecting these pathogens (in those cases where more than one method was available); and to collect data from patients so as to identify potential sources and/or risk factors for infection.

997 faecal specimens from 716 episodes of illness were tested over a one year period. Patients completed a questionnaire on symptoms, and food and environmental exposures. Using one or more standard techniques, the specimens were tested for bacteria and parasites which may cause gastroenteritis. Specimens from young children were also tested for the presence of rotavirus.

The incidence rates of the various pathogens, expressed as a rate per 100 000 persons per year, were as follows: *Blastocystis hominis*, 358; *Campylobacter species*, 208; *Giardia lamblia*, 158; *Yersinia species*, 87; *Cryptosporidium parvum*, 67; *Salmonella species*, 62; *Aeromonas species*, 62; *Dientamoeba fragilis*, 29; *Plesiomonas shigelloides*, 21; *Escherichia coli* (*E coli*) O157, 4; *Vibrio cholerae* non-O1, non-O139, 4; and *Shigella species* < 4.

Faecal specimen macroscopic form, microscopic findings, season, and patient age showed little correlation with the presence of specific pathogens. Consequently the tests selected for the detection of pathogens in faeces should not be based on any of the above parameters. Furthermore, the symptoms associated with parasitic and bacterial infections were similar, so it is not possible to select the appropriate tests on this basis. The presence of rotavirus in patients older than five years was not investigated so incidence in the general population can not be calculated. A study of all age groups for the

presence of this organism would be appropriate.

From the above findings, and an evaluation of the literature, it is recommended that all specimens should be examined for the following organisms and, on the basis of our observations, the most cost-effective method is shown in brackets: *Salmonella* (selenite enrichment subcultured to xylose lysine desoxycholate agar); *Shigella* (none were detected, so a cost-effective medium could not be determined), *Campylobacter* (5% sheep blood agar supplemented with 32 mg/l cefoperazone); *Yersinia* (*Yersinia* selective agar (YSA), plus selenite enrichment subcultured to YSA); *Giardia lamblia* (detection of antigen); *Cryptosporidium parvum* (detection of antigen).

While routine testing for *E coli* O157 is not recommended, laboratories should have the capability to test for this pathogen if a patient presents with haemolytic uraemic syndrome, thrombotic thrombocytopenic purpura or unexplained bloody diarrhoea. Likewise, routine culture for *Vibrio species* is not recommended; however, laboratories should test specimens using thiosulphate citrate bilesalt sucrose agar if the requesting clinician suspects cholera, or the patient has a recent history of shellfish consumption. A trichome stain for *Dientamoeba fragilis* is recommended for patients with chronic gastrointestinal symptoms who are to be investigated for neoplastic and other non-infectious conditions. Pathogenic parasites other than those noted above were not detected. However, since such organisms are isolated in New Zealand, usually in association with overseas travel or institutionalisation, it is recommended that a trichrome stain and a faecal concentration technique should be performed on specimens from all cases of gastroenteritis who have recently travelled overseas or who are institutionalised. Close liaison between the laboratory and the clinician is essential to ensure appropriate selective testing for these less common pathogens.

The presence of *Blastocystis hominis* and Aeromonads should be reported, but the report should note that their pathogenicity is uncertain. *Dientamoeba fragilis* and *Plesiomonas shigelloides* are probably pathogenic, but further work is needed to clarify this point.

Correlation of data from the questionnaires and the laboratory findings identified the following risk factors: (the relative risk, 95% confidence interval and p-value are shown in the brackets). *Campylobacter species*: consumption of unpasteurised milk (4.67, 2.39 - 9.11, p = <0.001); *Salmonella species*: overseas travel (7.20, 1.67 - 20.9, p = 0.040), eating a barbecued meal (4.55, 1.37 - 15.12, p = 0.026), eating shellfish (3.80, 1.18 - 12.21, p = 0.032); *Yersinia species*: consumption of water from a home supply (3.46, 1.32

- 9.10, p = 0.016), handling cattle (4.88, 1.73 - 13.76, p = 0.008), handling sheep (14.80, 4.93 - 44.46, p = 0.001); *Giardia lamblia*: consumption of unpasteurised milk (3.93, 1.63 - 9.46, p = 0.011), attendance at a day care centre (2.70, 1.17 - 6.27, p = 0.033), handling cattle (3.39, 1.59 - 7.22, p = 0.005), handling horses (5.27, 1.85 - 14.97, p = 0.002); *Cryptosporidium parvum:* consumption of water from a home supply (5.08, 1.88 - 13.71, p = 0.002), consumption of unboiled water from a natural waterway (3.97, 1.29 - 12.24, p = 0.031), attendance at a day care centre (3.30, 1.06 - 10.22, p = 0.054), handling cattle (5.41, 1.88 - 15.58, p = 0.006), owning a cat (4.50, 1.02 - 19.91, p = 0.029); *Plesiomonas shigelloides:* eating shellfish (13.67, 1.44 - 130.13, p = 0.020); and *Dientamoeba fragilis*: consumption of unboiled water from a natural waterway (7.46, 1.71 - 32.48, p = 0.019).

The risk factors suggest the value of the following precautions to prevent gastrointestinal infection: maintaining a high standard of both personal hygiene (particularly in the rural environment) and environmental hygiene in areas that food is prepared; avoiding consumption of untreated water or unpasteurised milk; cooking animal-derived food thoroughly - especially barbecued food and shellfish; and washing hands thoroughly after animal contact. Persons with diarrhoeal symptoms should take particular care with personal hygiene. Those travelling overseas should be conscious of the risk associated with the consumption of food and water which is not properly cooked or treated.

These findings should assist New Zealand laboratories to optimise their approach to the detection of faecal pathogens and should also assist in formulating policy for prevention of infection by enteric pathogens.

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# ABBREVIATIONS

| | |
|---|---|
| ACE | acetamide |
| ADH | arginine dihydrolase |
| ADO | adonitol |
| *Aeromonas* | *Aeromonas species* |
| ARA | arabinose |
| ARG | arginine |
| ASM | *Aeromonas* selective agar |
| *Blastocystis* | *Blastocystis hominis* |
| CAMP | 5% sheep blood agar plus 32 mg/ L cefoperazone |
| *Campylobacter* | *Campylobacter species* |
| CCDA | *Campylobacter* blood-free selective agar |
| CEL | cellibiose |
| CET | cetrimide |
| CIT | citrate |
| CMT | coumarate |
| COL | colistin |
| CON | arginine control |
| CWP | concentrated wet preparation |
| *Cryptosporidium* | *Cryptosporidium parvum* |
| DCA | desoxycholate citrate agar |
| *Dientamoeba* | *Dientamoeba fragilis* |
| DNA | deoxyribonucleic acid |
| ds | double stranded |
| DWP | direct wet preparation |
| EBOP | Eastern Bay of Plenty |
| *E coli* | *Escherichia coli* |
| EIA | enzyme immuno-assay |
| ESC | esculin |
| FER | fermentation control |
| FITC | fluoroscein isothiocyanate |
| g | gram |
| GAL | α-galactosidase |
| GAT | galacturonate |
| GN | Gram negative enrichment broth |
| GP | general practitioner |
| GRT | glucuronate |
| GSA | *Giardia* specific antigen |
| Hek | hektoen enteric agar |

| | |
|---|---|
| $H_2S$ | hydrogen sulphide |
| ID01, 02, 03 | commercial bacterial identification kit: Identify trays 1, 2 and 3 |
| IDP | alkaline phosphatase |
| IF | immunofluorescence |
| IND | indole |
| INO | inositol |
| KOH | potassium hydroxide |
| l | litre |
| LDC | lysine decarboxylase |
| Mac | MacConkey agar |
| MAL | malonate (primary identification kit) |
| MAL | maltose (secondary identification kit) |
| MAN | mannitol (primary identification kit) |
| MAN | mannose (secondary identification kit) |
| MEL | melibiose |
| MLT | maltose |
| MNT | malonate (secondary identification kit) |
| µl | microlitre |
| ml | millilitre |
| mm | millimetre |
| nm | nanometre |
| ODC | ornithine decarboxylase |
| O/F | oxidation/fermentation test |
| ONAG | ß-D-glucosaminidase |
| ONPG | ß-galactosidase |
| PD | phenylalanine deaminase |
| PLE | palatinose |
| *Plesiomonas* | *Plesiomonas shigelloides* |
| PNPG | ß-galactosidase |
| PPA | phenylalanine |
| PSS | permanent stained smear |
| PVA | polyvinyl alcohol fixative |
| RAF | raffinose |
| RBC | red blood cells |
| RHA | rhamnose |
| RNA | ribonucleic acid |
| SAC | sucrose (secondary identification kit) |
| SAL | salicin |
| *Salmonella* | *Salmonella species* |
| SBA | 5% sheep blood agar |
| Sel | selenite enrichment broth |
| *Shigella* | *Shigella species* |
| SMAC | sorbitol MacConkey agar |

| | |
|---|---|
| SOR | sorbitol |
| SS | *Salmonella/Shigella* agar |
| SUC | sucrose (primary identification kit) |
| TCBS | thiosulphate citrate bilesalt sucrose agar |
| TRE | trehalose |
| TTR | tetrathionate reductase |
| URE | urease |
| VP | Voges Proskauer test |
| XLD | xylose lysine desoxycholate agar |
| WBC | white blood cells |
| *Yersinia* | *Yersinia species* |
| YSA | *Yersinia* selective agar |
| ZN | modified Kinyoun stain |
| 5KG | 5-ketogluconate |

# 1 INTRODUCTION

It has been estimated that on a global basis over four million people die annually from diarrhoeal illness (Snyder and Merson, 1982), however, the majority of deaths occur in the developing world because poor hygiene and a high population density facilitate the spread of infection in populations which include many individuals who are immunocompromised through malnutrition.

Deaths from infectious diarrhoea are comparatively rare in the industrialised world, nevertheless this syndrome is estimated to be a leading cause of morbidity: thus Glass *et al.,* (1991) estimated that American children, on average, experience one diarrhoeic episode requiring a visit to a physician each year, for the first five years of life. It is also recognised that diarrhoeal illness in the industrialised world is economically significant in terms of medical care and decreased productivity (Todd, 1984). However, it is not usually possible to identify the causative agent in any infection on the basis of clinical presentation alone, hence it is desirable to have efficient laboratory procedures in place so as to rapidly make, or confirm a diagnosis.

Establishment of generally agreed procedures is complicated because the range of enteric pathogens is very large, and although many of them have a worldwide distribution, the relative importance of each pathogen varies from country-to-country and from place-to-place. The reasons for such variations are multitudinous but the level of hygiene, the density of population, the climate, food preferences and food preparation techniques, and behaviour differences related to culture are likely to be critical. Consequently, while much information is available concerning the causes of infectious diarrhoea in the United States and Europe, these data may not be applicable to New Zealand.

Efficiency requires diagnostic laboratories to attempt to maximise the detection of pathogens while, at the same time, minimising the number of tests and time required to detect these pathogens. To achieve the best compromise between minimum tests and maximum pathogen detection, it is necessary to have information on the relative prevalence of various pathogens within the surrounding community and the most sensitive methods for detecting them.

This investigation was undertaken with three primary objectives in view: to establish what pathogens were present and the relative importance of each; to compare the merits of various methods for detecting these pathogens (in those cases where more than one method is readily available); and to collect data on the lifestyle of patients so as to identify potential sources or risk factors for infection.

This study should help New Zealand laboratories to optimise their approach to the detection of faecal pathogens and the data relating to the epidemiology of infectious diarrhoea in one New Zealand community may assist in formulating policy for control of enteric pathogens.

# 2 LITERATURE REVIEW OF METHODS AND APPROACHES FOR THE DETECTION OF PATHOGENS IN FAECES

## 2.1 Definition and significance of infectious gastroenteritis

Infectious gastroenteritis is a communicable disease initiated by microorganisms which infect the gastrointestinal tract. It is characterised by gastrointestinal symptoms including nausea, vomiting, diarrhoea, abdominal pain and/or cramping.

Snyder and Merson (1982) estimated that in excess of four million people die annually from diarrhoeal illness. Ten years later, Bern *et al.*, (1992) suggested that the death rate had declined, but concluded that 3.3 million children die each year of diarrhoea. In the developed world, infectious gastroenteritis causes much morbidity but the mortality is low: thus Murray and Lopez (1994) estimated that only 6 900 deaths were attributable to diarrhoeal disease in the developed world in 1990.

In the United States infectious gastroenteritis accounts for 10.6% of all hospitalisations in children under 5 years (Glass *et al.*, 1991). The actual incidence of food and waterborne illness in the United States is approximated at 1.4 - 3.4 million cases per year and it has been suggested that for every culture-confirmed case of *Salmonella*, 29.5 cases occur (Hauschild and Bryan, 1980). Many cases of infectious diarrhoea are unrecognised because only 1% - 5% seek medical help (Archer and Kvenberg, 1985). Because of this under-recognition, it is difficult to estimate the costs associated with infectious gastroenteritis. Cost factors include direct medical and diagnostic costs, lost wages and productivity, and industry loss through embargo, voluntary destruction and recall if a food source is implicated.

In the United States this cost has been estimated at between US$1 to $10 billion per year (Todd, 1984). Based on this American estimate, a 1988 New Zealand Audit Office report estimated the economic loss due to foodborne illness in New Zealand in 1985 to be $34 million. The cost of the 371 hospital admissions for foodborne illness in the same year was estimated at $942 000. In 1994, the Public Health Commission's advice to the Minister of Health on food safety re-estimated the cost of foodborne illness in New

Zealand in the 1990/91 year at $59 million. The number of cases of foodborne illnesses reported in New Zealand in 1985 was between 4, 000 and 4, 500. In 1994 the number of notified cases of gastrointestinal infection nationwide was 10 000 (Galloway Y. 1995. Information analyst, ESR:Health Communicable Disease Centre, personal communication).

## 2.2 Epidemiology and transmission of infectious gastroenteritis

The agents capable of initiating gastrointestinal infections are numerous and multi-sourced. However, the pathogenicity factors invariably fall within three mechanisms: adhesion, invasion and toxin production (Ashkenazi and Pickering, 1989). The portal of entry is nearly always via ingestion (Guerrant, 1990), and the mode of transmission may be direct contact - that is touching a contaminated substance and then ingesting the organisms by placing the fingers in the mouth, or by consumption of contaminated food or water. Food may be contaminated naturally; during preparation by an infected person; by contact with other contaminated food; or, by vectors such as flies or cockroaches.

Host defence mechanisms include the acidity of the stomach; the peristaltic action of the intestine which inhibits attachment of organisms to the intestine; secretory IgA; phagocytic cells and eosinophils; and the normal intestinal flora. An alteration in any of these mechanisms may predispose the host to gastrointestinal infection (Finegold and Baron, 1986; Cantey, 1985).

Control of infection may be effected by improving personal hygiene, the quality of food and water, and food preparation and storage. But to maximise this form of control it is desirable to know which foods and factors need to be targeted.

Abbott and Janda (1992) categorised bacteria which may cause gastroenteritis into three groups: those unquestionably associated with infection (Group 1); those reputed to be enteropathogens and strongly linked with symptoms (Group 2) and bowel commensals which have been reported as infrequent causes of intestinal symptoms (Group 3).

Organisms in Group 1 include: (bacteria) *Salmonella*, *Shigella*, *Campylobacter jejuni* and *Campylobacter coli*, certain strains of *Escherichia coli*, *Vibrio cholerae* and *Vibrio parahaemolyticus*, *Yersinia enterocolitica* biotypes 1B - 5 and *Yersinia pseudotuberculosis*, toxin producing strains of *Bacillus cereus*, *Staphylococcus aureus*, *Clostridium perfringens*, and *Clostridium difficile*; (parasites) *Entamoeba histolytica*, *Giardia lamblia*,

*Cryptosporidium parvum*, various nematodes, cestodes and trematodes; (viruses) rotavirus, adenovirus (particularly types 41 and 42), caliciviruses such as Norwalk virus, astrovirus and other small round structured viruses.

Organisms in Group 2 (reputed enteropathogens) include: (bacteria) *Aeromonas species*, *Plesiomonas shigelloides*, *Edwardsiella tarda*, other *Vibrio species*, other *Campylobacter species*, and other *Yersinia species*; (parasites) *Dientamoeba fragilis* and *Blastocystis hominis*; (viruses) coronavirus.

The third group of organisms includes: (bacteria) *Bacteroides fragilis*, *Citrobacter freundii*, *Klebsiella pneumoniae* and *Klebsiella oxytoca*, *Providencia alcafaciens*, and *Hafnia alvei;* (parasites) *Entamoeba coli, Endolimax nana*, and *Iodamoeba butschlii*; (virus) parvovirus. Abbott and Janda, 1992; (Mims *et al.*, 1993, Sherlock *et al.*, 1989, Garcia 1994.)

The aetiology and epidemiology of Group 1 and Group 2 organisms are outlined in Table 1.

## 2.3 Diagnosis

Although some symptoms are considered typical of various gastrointestinal pathogens, definitive diagnosis requires isolation and identification of the agent.

The isolation of pathogens from a stool specimen is complicated by the presence of a large number and variety of commensal bacteria, in the order of $10^{11}$ - $10^{12}$ bacteria per gram (Sack *et al.*, 1980). In comparison, the number of pathogens may be small, so a selective method is necessary to detect, or reliably exclude the presence of, specific pathogens in a stool specimen. Nearly every pathogen requires a different selective technique thus the detection of a range of pathogens requires numerous tests on each specimen. The methods and/or culture media available for pathogen detection in stools are summarised in Table 2. As the frequency of detection of many pathogens is low in comparison to the effort involved, investigation of every stool for all pathogens is financially prohibitive. Consequently some rationalisation is necessary (Nolte, 1994).

**Table 1. Epidemiological features of selected microorganisms responsible for symptomatic gastrointestinal infections**

| Organism | Disease form | Reservoirs/sources (Predisposing conditions) | Pathogenic mechanism(s) | Infective dose (colony forming units) | Incubation period | Symptoms | Complications or sequelae |
|---|---|---|---|---|---|---|---|
| **BACTERIA** | | | | | | | |
| *Aeromonas species* | | water | enterotoxin, cytotoxin | unknown | unknown | diarrhoea | |
| *Bacillus cereus* | diarrhoeal | meat/vegetables | enterotoxin | | 6-24 hours | abdominal pain, diarrhoea | |
| | emetic | fried rice | preformed enterotoxin | | 1-6 hours | nausea/vomiting | |
| *Campylobacter jejuni* and *coli* | | water, animals (including cows, poultry, cats,dogs, pigs, birds), raw milk | invasion enterotoxin cytotoxin | < 500 | 3-11 days | diarrhoea abdominal pain, fever, malaise, nausea, vomiting | reactive arthritis, Guillain Barré syndrome |
| *Clostridium difficile* | | antimicrobial therapy | cytotoxin | | 4-9 days | diarrhoea | pseudo-membranous colitis |
| *Clostridium perfringens* | | meat products | enterotoxin | $10^9$-$10^{10}$ | 8-16 hours | diarrhoea, nausea, duodenal pain | |
| *Edwardsiella tarda* | | ? reptiles (overseas travel) | invasion ? cytotoxin ? | | | mild to severe diarrhoea | |
| *Escherichia coli* | toxigenic | food/water (overseas travel) | Heat stable and heat labile enterotoxins | $10^6$-$10^8$ | 4-24 hours | fluid diarrhoea | |
| | invasive | food (overseas travel) | invasion | | 8-24 hours | diarrhoea with blood & mucous | |
| | verocytotoxigenic | meat/milk | verocytotoxin | | 3-5 days | bloody diarrhoea | haemolytic uraemic syndrome, thrombotic thrombocytopenic purpura |
| | adherent | man | attachment and effacement | | | acute and chronic diarrhoea | |
| *Plesiomonas shigelloides* | | water/shellfish | invasion, enterotoxin cytotoxin | | 1-2 days | diarrhoea | |
| non-typhoidal *Salmonella* | | man, animals, food: eggs, milk, meat products | invasion, enterotoxin cytotoxin | $10^2$-$10^8$ | 6-72 hours | headache, abdominal pain, nausea, diarrhoea, vomiting | extra-intestinal infection |
| *S.* Typhi & Paratyphi | | man, food | invasion | | 1-3 weeks | systemic infection | |
| *Shigella* | | man, food | invasion, cytotoxin | 10-200 | 8 hours-5 days | diarrhoea, fever, nausea, abdominal cramps | toxic megacolon, haemolytic uraemic syndrome |

**Table 1. Epidemiological features of selected microorganisms responsible for symptomatic gastrointestinal infections, continued**

| Organism | Reservoirs/sources (Predisposing conditions) | Pathogenic mechanism(s) | Infective dose (colony forming units) | Incubation period | Symptoms | Complications or sequelae |
|---|---|---|---|---|---|---|
| *Staphylococcus aureus* | meat, dairy products | preformed enterotoxin | | 1-6 hours | nausea, vomiting, diarrhoea | |
| *Vibrio cholerae* 01 and 0139 | water, shellfish | enterotoxin | $10^8$ | 1-5 days | profuse watery diarrhoea: cholera | |
| Other *Vibrio cholerae* and *Vibrio parahaemolyticus* | shellfish | enterotoxin, cytotoxin, invasion | $10^6$-$10^8$ | 15-24 hours | watery diarrhoea, abdominal cramps, nausea, vomiting | |
| *Yersinia enterocolitica* and *pseudotuberculosis* | food, water, animals | invasion, enterotoxin | | 16 hours-7 days | nausea, vomiting, fever, abdominal pain, pseudoappendicular syndrome | erythema nodosum reactive arthritis, extra- intestinal infection |
| **PARASITES**<br>*Entamoeba histolytica* | man, food, water | invasion | | | | extra-intestinal infection |
| *Giardia lamblia* | man, animals, food, water | villus atrophy? absorption interference? | < 100 cysts | 5-25 days | abdominal pain, bloating, loose pale stools weight loss, profuse watery diarrhoea | |
| *Cryptosporidium parvum* | man, cattle, water (AIDS) | | | 1-12 days | | |
| *Dientamoeba fragilis* | ? man via various nematodes | | | | abdominal pain, diarrhoea, nausea, vomiting, flatulence, fatigue | |
| *Blastocystis hominis* | man and animals | | | | abdominal pain, diarrhoea, nausea, vomiting, flatulence, fatigue | |
| **VIRUSES**<br>Rotavirus | man, animals | cell damage due to viral replication in intestinal epithelium | | 1-5 days | fever, vomiting, watery diarrhoea, respiratory symptoms | |
| Norwalk virus<br>Adenovirus 40/41<br>Astrovirus<br>Calicivirus<br>Coronavirus | man, shellfish, water | | | 10-50 hours<br>8-10 days | nausea, vomiting, diarrhoea, abdominal pain, low grade fever, headache, malaise | |

*Reference:* Abbott and Janda (1992), Abbott and Janda (1992a), Ashkenazi and Pickering (1989), Benenson (1990), Cantey (1985), Cover and Aber (1989), Dupont and Sullivan (1986), Gilligan *et al.*, (1992), Hazen and Guerrant (1991), Mims *et al.*, 1993, Sherlock *et al.*, (1989), Skirrow (1990), Wright (1991).

**Table 2. Organism characteristics and diagnostic techniques for selected Group 1 and Group 2 gastrointestinal pathogens**

| Organism | Characteristics | An indication of the variety of the selection and detection methods to identify organisms in stool specimens |
|---|---|---|
| **BACTERIA** | | |
| *Aeromonas* | Oxidase positive, Gram negative bacilli, facultative, glucose fermenting, resistant to vibriostatic agent O/129 | Non-selective medium : 5% sheep blood agar (SBA)*<br>Selective media : SBA + 10 µg/ml ampicillin*<br>: Bile salt - irgasan - Brilliant green agar* |
| *Bacillus cereus* | Aerobic Gram positive spore forming bacilli, producing enterotoxin | Serial dilution of stool to determine if significant numbers are present and if significant numbers are detected, testing isolates for enterotoxin. |
| *Campylobacter* | Oxidase positive, Gram negative, curved bacillus, microaerophile | 1) Skirrows lysed horse blood agar + trimethoprim, vancomycin and polymyxin B*.<br>2) Blasers brucella agar base + sheep blood + trimethoprim, vancomycin, polymyxin B and amphotericin*.<br>3) Butzlers thioglycollate agar plus sheep blood, bacitracin, novobiocin, cycloheximide, cefazolin*.<br>4) Preston's charcoal and blood medium with polymyxin B, rifampin, trimethoprim and cycloheximide*.<br>5) Preston's blood-free medium, with cefoperazone* plus others. |
| *Clostridium difficile* | Anaerobic Gram positive spore forming bacilli, producing cytotoxin | Detection of cytotoxin by cell culture, latex agglutination*, or enzyme* immunoassay.<br>Culture. |
| *Clostridium perfringens* | Anaerobic Gram positive spore forming bacilli, producing enterotoxin | Serial dilution of stool to determine if significant numbers are present and if significant numbers are detected, testing isolates for enterotoxin. |
| *Escherichia coli* | Oxidase negative Gram negative bacilli, facultative, glucose fermenting | |
| | - Enterotoxin producing | Detection of toxin - latex (LA) agglutination, enzyme immunoassay (EIA), cell culture, animal studies, molecular techniques. |
| | - Invasive | Detection of invasin - animal studies, molecular techniques. |
| | - Verocytotoxin producing | Sorbitol MacConkey agar* to detect non-sorbitol fermenting *E. coli* O157<br>Detection of toxin - latex agglutination, enzyme immunoassay, cell culture, molecular techniques. |
| | - Adherent | Cell culture, molecular techniques. |
| *Plesiomonas shigelloides* | Oxidase positive, Gram negative bacilli, facultative, glucose fermenting, sensitive to 150 µg O/129, non-haemolytic | Inositol brilliant green bilesalts agar (selective)<br>MacConkey (Mac)*, hektoen (Hek)*, or xylose lysine desoxycholate (XLD)* agar (may be selective).<br>SBA* (non selective). |
| *Salmonella* | Oxidase negative, Gram negative bacilli, facultative, glucose fermenting, usually produce hydrogen sulphide and do not ferment lactose | Mac*, Eosinmethylene blue* (EMB) (differential).<br>Hek*, XLD*, Salmonella-Shigella agar (SS)*, desoxycholate citrate agar (DCA)* (moderately selective).<br>Brilliant green *, Bismuth sulphite* (highly selective)<br>Enrichment: Gram negative broth (GN)*, selenite F broth (Sel)*, Tetrathionate broth*, Rappaport broth*. |

**Table 2. Organism characteristics and diagnostic techniques for selected Group 1 and Group 2 gastrointestinal pathogens, continued**

| Organism | Characteristics | An indication of the variety of the selection and detection methods to identify organisms in stool specimens |
|---|---|---|
| *Shigella species* | Oxidase negative, Gram negative bacilli, facultative, glucose fermenting. Do not produce hydrogen sulphide and do not ferment lactose | Mac*, EMB*, Hek*, XLD*, SS*, DCA* - primary isolation medium.<br>GN broth*, Sel* - enrichment. |
| *Staphylococcus aureus* | Catalase positive, facultative, coagulase positive. Gram positive cocci, produce enterotoxin | Enterotoxin detection. |
| *Yersinia species* | Oxidase negative, Gram negative bacilli, facultative, glucose fermenting. Do not produce hydrogen sulphide and do not ferment lactose, motile at 27°C | Mac*<br>*Yersinia* selective agar*<br>Cold enrichment for 3 weeks in phosphate buffered saline* |
| **PARASITES** | | |
| *Entamoeba histolytica* | 12-60 µm protozoan, trophozoite :<br>1 nucleus, central nuclear karyosome, peripheral nuclear chromatin, may contain ingested red blood cells,<br>cyst - 2-4 nuclei | Permanent stained smear such as trichrome stain (PSS)*.<br>Direct wet preparation (DWP)*.<br>Concentrated wet preparation (CWP)*.<br>Serum serology. |
| *Giardia lamblia* | trophozoite : pear shaped 8-20 µm<br>: 2 nuclei<br>: 4 pairs of flagella<br>cyst : oval 8-19 µm<br>: 4 nuclei | PSS*, DWP*, CWP*, direct antigen detection* by EIA*, fluorescent microscopy*. |
| *Dientamoeba fragilis* | trophozoite : 5-15 µm<br>: 1 or 2 nuclei<br>: Karyosome comprises 4-8 granules<br>no cysts | PSS*. |
| Various trematodes, cestodes - nematodes | Distinctively shaped and structured ova, larvae, worms or proglottides | CWP*, various other selection and concentration techniques. |
| *Cryptosporidium parvum* | Oocysts round, 4-6 µm when mature contains 4 sporozoites | Sugar flotation, acid fast stain*, EIA*, fluorescent microscopy*. |
| *Blastocystis hominis* | Round, 6-40 µm large central body | PSS*, DWP*, CWP*. |
| *Isospora species* | Ellipsoidal oocyst 20-30 µm x 10-19 µm, when mature contains 2 sporocysts | Acid fast stain*. |

**Table 2. Organism characteristics and diagnostic techniques for selected Group 1 and Group 2 gastrointestinal pathogens, continued**

| Organism | Characteristics | An indication of the variety of the selection and detection methods to identify organisms in stool specimens |
|---|---|---|
| **VIRUSES** Rotavirus | 70 nm ds RNA virus resembling a spoked wheel with a large hub cap | EIA*, LA*, EM (Electron microscopy) molecular techniques. |
| Adenovirus 40/41 | 80 nm DNA icosahedral virus | EM, culture in Graham 293 cells, EIA*, LA*, molecular techniques. |
| Norwalk and Norwalk-like | 30 nm, cubic symmetry, non enveloped RNA virus | EM, molecular techniques. |
| Astrovirus | 30 nm non enveloped RNA virus with cubic symmetry and the appearance of a 5 or 6 pointed star | EM, molecular techniques. |
| Calicivirus | 30-35 nm icosahedral, non enveloped RNA viruses with cup-like indentations on the particle surface | EM, molecular techniques. |
| Coronavirus | 60-220 nm pleomorphic RNA virus with widely spaced petal like projections | EM, molecular techniques. |

* readily available for diagnostic laboratory use in New Zealand

*References:* Difco Technical Information, (1986), Dolin (1990a), Dolin 1990 (b), Finegold and Baron (1986a), Finegold and Baron (1986b), Garcia (1986), Gilligan *et al.*, (1992), Grasmick (1992), Marsh and Ratram 1986, Pedler and Orr (1990), Sherlock *et al.*, 1989, Smith and Scotland (1993).

Historically literature advising on general stool testing protocols have focused on bacterial pathogens. As methods have not been so readily available for the detection of parasites and viruses, these organisms have not been discussed in detail and their detection has been advocated only if symptoms persist and a bacterial agent has not been detected.

Gilligan (1986) recognised the need for rationalisation in diagnostic procedures for the investigation of gastroenteritis and advocated the use of such selective criteria as patient age, history, and local epidemiology. This worker's recommendations are summarised as follows:

* routine faecal investigation should comprise culture for *Salmonella*, *Shigella* and *Campylobacter* as well as parasite investigation
* during winter, children under three years should be tested for rotavirus only
* specimens from children should only be processed by "routine faecal investigation" during summer or if the rotavirus test is negative
* *Cryptosporidium* testing should be performed only if the patient has acquired immune deficiency syndrome (AIDS), or the symptoms are persistent
* *Clostridium difficile* testing should be performed only if the patient has recently received antimicrobial or immunosuppressant therapy
* testing for *Vibrio* and *E coli* should be undertaken only if there is an indicative travel or food history or the patient has bloody diarrhoea.

Gilligan (1986) suggested that *Yersinia* selective media is unnecessary if investigators adequately examine differential culture plates and the minimum ability of laboratories is recommended to be: ability to culture/select and recognise *Campylobacter*, *Shigella*, *Salmonella*, *Yersinia* and parasites including *Cryptosporidium*, and the ability to detect *Clostridium difficile* cytotoxin.

Guerrant *et al.*, (1985) reported that stool culture is costly and ineffective with the cost per positive result being approximately US$900 - US$1000. These workers advocated:

* culturing only for invasive organisms and doing so only if leucocytes are noted on stool microscopy
* culturing methods should include: direct culture for *Salmonella*, *Shigella* and *Campylobacter* and enrichment culture for *Salmonella*
* investigating for *Yersinia* only if there is a history of persistent abdominal pain or fever

* investigating for *Giardia* only if there are prolonged symptoms or weight loss
* investigating for *Vibrio* only if the patient reports a history of seafood ingestion
* investigating for *Cryptosporidium* only if the patient is immuno-compromised

Guerrant *et al.*, (1985) based these criteria on the rationale that definitive identification is only necessary when the result is likely to indicate the need for specific therapy.

Doern (1989) noted that it is not often possible for a clinician to predetermine the aetiology of diarrhoeal illness unless the case presents in the midst of an outbreak of known aetiology or as a "classic" presentation. He advocated:

* microscopic screening of specimens and culturing those with white blood cells for *Salmonella*, *Shigella* and *Campylobacter*
* close liaison between the laboratory and the clinician as this interaction is essential to an efficient diagnostic process
* if the local prevalence of a pathogen exceeds the local prevalence of either *Salmonella*, *Shigella* or *Campylobacter*, then a laboratory should consider routinely screening for that pathogen
* to determine what pathogens are prevalent it may be appropriate for a laboratory to perform routine surveillance for a specific organism for a specific period of time

As well as advocating a processing protocol, Doern (1989) also indicated which detection methods should be used: a differential Gram negative medium, such as MacConkey agar or eosin methylene blue agar, and a semi-selective Gram negative agar, such as hektoen enteric agar (Hek) or xylose lysine desoxycholate agar (XLD) for *Salmonella* and *Shigella*; and a *Campylobacter* selective medium. Doern (1989) was ambivalent concerning the value of an enrichment broth for Gram negative bacilli.

Guerrant and Bobak (1991) stated that clinical history coupled with the stool microscopy should be used to guide what laboratory investigation is undertaken. They advocated:

* examining a stool only if the patient has been ill for more than one day
* culturing a stool for *Salmonella*, *Shigella* and *Campylobacter* if white blood cells are seen on the stool microscopy
* testing for *Clostridium difficile* if white blood cells are seen on the stool microscopy and the patient has a history of antimicrobial or immunosuppressive

therapy

* not culturing a stool if no white blood cells are seen, and the patient has been ill less than 10 days
* performing a parasite screen and stool culture if illness has lasted longer than 10 days, regardless of the findings of the stool microscopy

This rationale assumes that as invasive and cytotoxic intestinal pathogens provoke an inflammatory response they will be indicated by the presence of white blood cells; and that as organisms which are pathogenic by way of enterotoxins cause a secretory diarrhoea, white blood cells will not excreted.

Guerrant and Bobak (1991) based this protocol on three factors: the need to decrease the cost of stool cultures; the assumption that white blood cells are a reliable indicator of the agent, and that the majority of diarrhoeal illnesses are treated with oral rehydration and therefore identification of the agent is not necessary.

The use of microscopic screening as a basis for testing decisions has not been endorsed by all workers. Tarr *et al.*, (1992) responding to the recommendations of Guerrant and Bobak (1991) reported that white blood cells were seen in only 188 of 353 stools in which pathogens were found and concluded that investigating only stools in which white blood cells are found will result in under-diagnosis of infection with invasive and cytotoxic organisms such as *Campylobacter* and *Salmonella*.

Pedler and Orr (1990) did not recommend routine microscopy of stools, but did advocate:

* routine culture for *Salmonella*, *Shigella* and *Campylobacter*
* culture for *Yersinia* using specific *Yersinia* selective media in many clinical instances
* use of selective media for *Vibrio*, *E coli*, *Aeromonas* and *Plesiomonas* in selected instances based on clinical data

The methods these workers recommended include: the use of either desoxycholate citrate agar (DCA), XLD, Hek or *Salmonella-Shigella* agar (SS) as a primary medium to detect *Salmonella* and *Shigella* and selenite broth as an enrichment medium for these organisms. No one *Campylobacter* medium was specifically recommended.

Abbott and Janda (1992a) advocated:

* routine screening for *Salmonella*, *Shigella* and *Campylobacter*
* additional testing for other bacteria based on patient age, duration of symptoms, pre-existing conditions, recent medications, symptoms, and food and travel history
* the use of appropriate selective techniques
* the need for a confirmatory test for all organisms of public health significance; this should be undertaken at a reference laboratory

Abbott and Janda (1992a) note that pathogens may express more than one virulence factor and that traditionally expected presentations and microscopic findings can not be relied upon to indicate which pathogens should be sought. These workers are also among the few to include the bacterium *Edwardsiella tarda* in their discussions (as a Group 2 organism).

Huicho *et al.*, (1993) reported the results of a two-year study in which the microscopic and culture results for 446 children were compared. These workers found that presence of occult blood or microscopic white blood cells did not correlate with the presence of bacteria of known invasive or cytotoxic ability. Conversely, they found white blood cells in stools from patients who were infected with viruses and enterotoxigenic bacteria. These workers concluded that if the criteria of Guerrant and Bobak (1991) had been applied to their population, significant numbers of cases of "invasive infection" would have been missed.

Nolte (1994) advocated:

* the need for testing all bloody stools for *E coli* O157
* using toxin detection rather than culture as a means to confirm *Clostridium difficile* infection
* rationalisation of faecal screening of hospitalised patients by establishing when symptoms onset occurred in relation to admission - if the symptoms commence more than three days after hospitalisation then the stool should be investigated for *Clostridium difficile* only. If symptoms commence prior to, or within three days of admission then culture is indicated
* infant specimens should be tested only for Rotavirus in winter
* full parasite screen, including testing for *Cryptosporidium* should be performed on all AIDS patients

Persaud and Eykyn (1994) assessed the relative abilities of DCA, Hek and XLD as both primary and secondary media (selenite broth was used as the enrichment) to detect *Salmonella* and *Shigella* and also surveyed British laboratories as to current methods and rationales. They concluded that DCA was a superior isolation medium compared with XLD and Hek and that personal convictions, often unsubstantiated, were the basis for most laboratory rationales and methods.

No complete New Zealand data are available on prevalence rates determined under controlled conditions. Okell and Wright (1989) reported that the prevalence of *Giardia* in the Eastern Bay of Plenty was greater than that of *Salmonella*. McCarthy and Fenwick (1991) reported that the prevalence of *Yersinia* in Auckland was greater than that of *Shigella*. Carter (1986) examined the prevalence of *Cryptosporidium* in the Taranaki area in 1986. Incidence of *Campylobacter*, *Salmonella* and *Shigella* are reported regularly in the New Zealand Public Health Report as these organisms are notifiable in New Zealand.

In Australia Pryor *et al.*, (1987) reported a prospective study of 74 adults who presented with acute diarrhoea at a hospital in Sydney. A total of 34 pathogens were detected in stools from 32 patients as follows: *Campylobacter* (15), rotavirus (7), *Clostridium difficile* (5), *Salmonella* (2), *Aeromonas* (1), *Vibrio parahaemolyticus* (1), adenovirus (1), small round viruses (1), *Giardia* (1). The investigators reported that clinical, epidemiological and microscopic features were not good predictors of the pathogen involved and advocated the need for specific diagnosis if symptoms are severe or protracted. Nine unspecified selective media were used in this study. The parasite examination performed did not include a permanent stained smear but did include a specific stain for *Cryptosporidium*.

Thus it is generally agreed that stool specimens should be cultured for *Salmonella*, *Shigella* and *Campylobacter*; and, that the routine use of media for the isolation of other bacterial agents should vary depending on the local prevalence rates and the availability of technical equipment and reagents, geographic location and patient presentation.

# 3 MATERIALS AND METHODS

## 3.1 Culture media

### 3.1.1 5% sheep blood agar (SBA)

This medium was reported by Ellner *et al.*, (1966) and contains the following ingredients per litre: peptone 23 g, starch 1 g, sodium chloride 5 g, and agar 10 g with the final pH being 7.3 +/- 0.2 (Oxoid CM331, Anonymous, 1990). After autoclave sterilisation, the medium was supplemented with 50 ml per litre defibrinated sheep's blood (supplied by Life Technologies, P.O. Box 12-502, Penrose, Auckland). This is a non-selective medium on which organisms may be differentiated by their ability to haemolyse blood cells.

### 3.1.2 5% sheep blood agar plus cefoperazone (CAMP)

5% sheep blood agar, as described in 3.1.1, was supplemented with 32 mg/l cefoperazone (Sigma 4292, supplied by Biolab Scientific, 39 Woodside Avenue, Northcote, Auckland, New Zealand) at the time of adding the sheep's blood. The addition of cefoperazone selects for *Campylobacter* by inhibiting most members of the *Enterobacteriaceae*.

### 3.1.3 MacConkey agar with crystal violet (Mac)

Based on a medium initially described by MacConkey (1905), this medium contains the following ingredients per litre: peptone 20 g, lactose 10 g, bile salts 1.5 g, sodium chloride 5 g, neutral red 0.03 g, crystal violet 0.001 g, and agar 13.5 g with the final pH being 7.2 +/- 0.2 (Anonymous, 1984). The medium was sterilised by autoclaving.

The principle of the selection and differentiation properties of this medium is that bile salts and crystal violet select non-fastidious Gram negative bacilli which are differentiated on the basis of lactose fermentation. Fermenters such as *E coli* appear pink and non-fermenters, such as *Salmonella* and *Shigella*, appear colourless.

### 3.1.4 Sorbitol MacConkey agar (SMAC)

Based on the formulation described by Rappaport and Henig (1952), this medium contains the following ingredients per litre: peptone 20 g, sorbitol 10 g, bile salts 1.5 g, sodium chloride 5 g, neutral red 0.03 g, crystal violet, 0.001 g and agar 15 g, the final pH being 7.1 +/- 0.2 (Oxoid CM813, Anonymous, 1990). The medium was sterilised by autoclaving.

The principle of the selection and differentiation properties of this medium is that bile salts select non-fastidious Gram negative bacilli which are differentiated on the basis of sorbitol fermentation.

### 3.1.5 Xylose lysine desoxycholate agar (XLD)

First described by Taylor (1965) this medium contains yeast extract 3 g, L-Lysine 5 g, xylose 3.75 g, lactose 7.5 g, sucrose 7.5 g, sodium desoxycholate 2.5 g, sodium chloride 5 g, sodium thiosulphate 6.8 g, ferric ammonium citrate 0.8 g, phenol red 0.08 g, and agar 15 g, the final pH being 7.4 +/- 0.2 (Anonymous, 1984). The medium was heated to boiling but not autoclaved.

The principle of the selection and differentiation properties of this medium is that sodium desoxycholate selects non-fastidious Gram negative bacilli which are differentiated by carbohydrate fermentation and production of hydrogen sulphide. Non-fermenters of the three carbohydrates, such as *Shigella*, appear as pink colonies. Lactose and sucrose fermenters appear as yellow colonies. Although fermenters of xylose, *Salmonella* also appear pink on this medium as they deplete this substrate during incubation and continue to grow by utilising lysine. Decarboxylation of lysine results in the alkaline product, cadervine. Organisms capable of hydrogen sulphide production utilise the sulphur from sodium thiosulphate. The hydrogen sulphide produced reacts with ferric ammonium citrate to produce a black precipitate.

### 3.1.6 Hektoen enteric agar (Hek)

Developed by King and Metzger (1968) this medium contains per litre: proteose peptone 12 g, yeast extract 3 g, lactose 12 g, sucrose 12 g, salicin 2 g, bile salts 9 g, sodium chloride 5 g, sodium thiosulphate 5 g, ferric ammonium citrate 1.5 g, acid fuchsin 0.1 g, bromothymol blue 0.065 g, and agar 14 g, the final pH being 7.5 +/- 0.2 (Anonymous, 1984).

The principle of the selection and differentiation properties of this medium is that bile-salts select non-fastidious Gram negative bacilli which are differentiated by their action on the three fermentable carbohydrates in the medium. Hydrogen sulphide production is detected via the inclusion of sodium thiosulphate and ferric ammonium citrate (See medium 3.1.5). Non-fermenters of the three carbohydrates, such as *Shigella*, appear as green colonies and *Salmonella* appear as green-blue colonies with or without black centres.

### 3.1.7 Thiosulphate citrate bilesalt sucrose agar (TCBS)

Developed by Kobayashi *et al.*, (1963), this medium contains yeast extract 5 g, proteose peptone 10 g, sodium thiosulphate 10 g, sodium citrate 10 g, ox bile 8 g, sucrose 20 g, sodium chloride 10 g, ferric citrate 1 g, bromothymol blue 0.04 g, thymol blue 0.04 g, and agar 15 g, the final pH being 8.6 +/- 0.2 (Anonymous, 1984).

The principle of the selection and differentiation properties of this medium is that the high sodium chloride concentration and pH as well as the inclusion of ox bile renders the medium highly selective for Vibrio species, which are differentiated by their ability to ferment sucrose. Fermenters such as *Vibrio cholerae* appear as yellow colonies and non-fermenters such as *Vibrio parahaemolyticus* as green or blue-green. Hydrogen sulphide production is detected via the inclusion of sodium thiosulphate and ferric citrate (as described in 3.1.5).

### 3.1.8 *Yersinia* selective agar (YSA)

Initially developed by Schiemann (1979), the basal medium contains peptone 20 g, yeast extract 2 g, mannitol 20 g, sodium pyruvate 2 g, sodium chloride 1 g, magnesium sulphate 0.01 g, sodium desoxycholate 0.5 g, sodium cholate 0.5 g, neutral red 0.03 g, crystal violet 0.001 g, irgasan 0.004 g, and agar 13.5 g (Anonymous, 1984). After autoclaving to sterilise, the medium was supplemented with cefsulodin 4 mg and novobiocin 2.5 mg with the final pH being 7.4 +/-0.2.

The principle of the selection and enrichment properties of this medium is that sodium desoxycholate and crystal violet select non-fastidious Gram negative bacilli. The selective supplement further selects *Yersinia* whilst inhibiting both Gram positive and Gram negative faecal flora. Growth is differentiated by mannitol fermentation, the mannitol fermenting *Yersinia* appearing pink.

### 3.1.9 *Aeromonas* selective agar (ASM)

This medium contains per litre: beef extract 5 g, proteose peptone 5 g, xylose 10 g, bile salts 8.5 g, sodium thiosulphate 5.44 g, irgasan 0.005 g, brilliant green 0.005 g, neutral red 0.025 g, and agar 11.5 g, the final pH being 7.0 +/- 0.2 (Difco Technical Information (medium 1864), 1986).

The principle of the selection and differentiation properties of this medium is that the majority of faecal organisms are inhibited by the selective agents: bile salts, irgasan, and brilliant green. Aeromonads are able to grow, but do not ferment xylose and thus appear as clear colonies.

### 3.1.10 *Campylobacter* blood-free selective agar (CCDA)

Based on the formulation of Bolton *et al.*, (1984), the basal medium contains nutrient broth 25 g, bacteriological charcoal 4 g, casein hydrolysate 3 g, sodium desoxycholate 1 g, ferrous sulphate 0.25 g, sodium pyruvate 0.25 g, and agar 12 g, the final pH being 7.4 +/- 0.2 (Oxoid CM739, Anonymous, 1990). After autoclave sterilisation, this basal medium was supplemented with 32 mg/l cefoperazone (Oxoid SR125, Anonymous, 1990). Cefoperazone inhibits members of the *Enterobacteriaceae*, thus selecting *Campylobacter*.

### 3.1.11 Selenite broth (Sel)

Based on the formulation of Leifson (1936), the medium contains per litre: tryptone 5 g, lactose 4 g, sodium selenite 4 g, and sodium phosphate 10 g, the final pH being 7.0 +/- 0.2 (Anonymous, 1984). The medium was heated to boiling but was not autoclaved.

The principle of this medium is that at near neutral pH, sodium selenite is toxic to most *Enterobacteriaceae* but not *Salmonellae*. Neutral pH is maintained by the inclusion of a buffer (sodium phosphate). Care must be exercised when using and preparing this medium as it is toxic and teratogenic.

### 3.1.12 Gram Negative broth (GN)

Based on the formulation of Hajna (1955), the medium contains tryptone 20 g, dextrose 1 g, D-mannitol 2 g, sodium citrate 5 g, sodium desoxycholate 0.5 g, dipotassium phosphate 4 g, monopotassium phosphate 1.5 g, and sodium chloride 5 g, the final pH being 7.0 +/- 0.2 (Anonymous, 1984).

The principle of this medium is that sodium citrate and sodium desoxycholate are inhibitory to most Gram positive organisms and members of the *Enterobacteriaceae* excluding *Salmonella* and *Shigella*. The increased concentration of mannitol over dextrose inhibits *Proteus species* and accelerates the growth of mannitol fermenting *Salmonella* and *Shigella*.

### 3.1.13 Glucose oxidation/fermentation (O/F) medium

The glucose oxidation/fermentation medium is based on the medium of Hugh and Leifson (1953). The medium contains per litre: tryptone 2 g, sodium chloride 5.0 g, dipotassium phosphate 0.3 g, bromothymol blue 0.08 g, agar 2 g, and glucose 10 g, the final pH being 6.8 +/- 0.2 (Oxoid CM883, Anonymous, 1990). The medium was dispensed into tubes and autoclaved to sterilise.

The principle of this medium is that organisms which utilise glucose oxidatively produce yellowing of the medium only in the presence of air. Organisms which utilise glucose fermentatively cause yellowing of the medium both in the presence of, and in the absence

of air. Organisms which do not act on glucose will either result in no colour change in either tube or a blue colour in the open tube (due to utilisation of the peptones in the medium).

### 3.1.14 Christensen's urea agar

Based on the medium of Christensen (1946), the medium contains the following ingredients per litre: peptone 1 g, glucose 1 g, sodium chloride 5 g, potassium hydrogen phosphate 2 g, phenol red 0.012 g, 5 ml 40% urea solution, and agar 15 g (Anonymous 1984).

The principle of this medium is that organisms which produce urease cleave the urea in the medium to produce ammonia. Being alkaline, ammonia results in pinkening of the medium.

Media 3.1.1, 3.1.2, 3.1.3, 3.1.5, 3.1.11, and 3.1.13 were prepared in-house. The remaining media were supplied pre-made by Fort Richard Laboratories, P. O. Box 22-172, Otahuhu.

## 3.2 Bacterial identification tests

### 3.2.1 Gram Stain

The Gram stain used in this study was as follows:

A heat fixed smear was covered with crystal violet (1%) for 30 seconds and then washed in tap water and flooded with iodine solution (0.66% iodine, 1.2% potassium iodide) for one minute. The slide was washed in tap water and flooded with acetone to de-stain for no more than 10 seconds before being washed with tap water and flooded with basic fuchsin (0.1%), to counterstain, for 30 seconds. The slide was then washed in tap water and air dried.

The principle of this test is based on the difference in cell wall composition of Gram positive and Gram negative organisms. Gram positive organisms are impermeable to acetone and do not decolorise from blue/black. Gram negative organisms are decolorised and appear red.

### 3.2.2 Oxidase test

Based on the findings of Kovacs (1956), the solution was prepared by dissolving 1 g of tetra-methyl-para-phenylene-diamine-dihydrochloride in 100 ml of 95% ethanol. The solution was then applied to blotting paper which, when dry, was cut into strips and stored in the dark at room temperature.

The test was performed by applying a strip of oxidase paper to a colony and then observing the strip for the appearance of a purple colour within five seconds of application to the colony.

The principle of the test is that organisms possessing the enzyme cytochrome oxidase reduce the reagent to a purple coloured product. The test is not appropriate for growth on most selective media and was only performed on growth on 5% sheep blood agar, *Aeromonas* selective agar and the two *Campylobacter* isolation media.

### 3.2.3 Glucose utilisation

The test organism was stab inoculated to the bottom of each of two 3 ml tubes of glucose oxidation/fermentation medium, and then streaked to a 5% sheep blood agar/MacConkey agar split plate, for a purity check. The medium in one tube was overlaid with sterile paraffin oil and both tubes and the purity check were then incubated at 37°C, in air, overnight.

### 3.2.4 O/129 sensitivity

Based on the findings of Shewan and Hodgkiss (1954), 2, 4-diamino-6, 7-di-iso-propylpteridine phosphate (O/129) was used to differentiate *Vibrio species* from other organisms and from each other.

The organism under test was streaked to SBA and a 150 μg O/129 disc (Oxoid DD15; supplied in New Zealand by Intermed Scientific, P.O. Box 102-253, North Shore Mail Centre, Auckland) placed on the main inoculum. The plate was then incubated at 37°C, in air, overnight. A zone of growth inhibition around the disc was considered sensitive.

*Vibrio species* and *Plesiomonas shigelloides* are usually sensitive to 150 µg O/129, whereas Aeromonads are resistant.

### 3.2.5 Urease test

The organism under test was streaked down a slope of Christensen's urea agar which was then incubated at 37°C, in air, for up to four hours. The slope was then examined for colour change.

### 3.2.6 Confirmation of microaerophile status and growth temperature.

As other organisms may grow under microaerophilic conditions, all oxidase positive organisms growing on *Campylobacter*-selective media were subcultured from the primary isolation medium to each of three 5% sheep blood plates. One plate was incubated microaerophilically at 42°C, one microaerophilically at 37°C and one in air at 37°C overnight. Growth in air indicated the organism was not a *Campylobacter species*.

### 3.2.7 Hippurate hydrolysis

Based on the rapid hippurate hydrolysis method of Mugg (1983), the hippurate solution consisted of equal volumes of 1% aqueous sodium hippurate and ninhydrin solution (3.5 g ninhydrin, 50 ml acetone, 50 ml butanol). Care was taken when preparing and using the reagent as ninhydrin is carcinogenic. Once prepared, the hippurate solution was applied to blotting paper which, when dry, was cut into strips and stored in the dark at room temperature.

A hippurate strip was applied to the colony and the strip was then observed for the appearance of purple colouring on the imprint of the colony within five minutes of strip application.

The principle of the test is that hydrolysis of hippurate results in the product glycinate, which reacts with ninhydrin to give a purple colour.

### 3.2.8 Sensitivity to nalidixic acid and cephalothin

Commercially available antibiotic discs were used to determine the sensitivity of *Campylobacter* to nalidixic acid 30 µg (Difco 6793-89-2; supplied in New Zealand by Fort Richard Laboratories, P.O. Box 22-172, Otahuhu) and cephalothin 30 µg (Difco 6393-90-3).

The discs were stored at 4°C and warmed to room temperature prior to use. The *Campylobacter species* under test was inoculated to a 5% sheep blood agar and streaked for single colonies. A nalidixic acid and a cephalothin disc were each applied to the main inoculum, at a distance of 2.5 cm. After overnight incubation at 37°C microaerophilically, the plate was examined for the presence of a zone of growth inhibition around each of the discs.

### 3.2.9 *Escherichia coli* O157 latex agglutination test

The commercial *E coli* O157 latex test (Code DR620, Anonymous, 1990; supplied by Intermed Scientific, P.O. Box 102-253, North Shore Mail Centre, Auckland) was stored at 4°C and warmed to room temperature prior to use. Growth from a 5% sheep blood agar purity plate was emulsified in 3 ml sterile 0.85% saline to give a very turbid suspension. A drop of this suspension was applied to each of two reaction circles on the card supplied with the kit. One drop of test latex reagent was applied to one circle and one drop of control latex reagent was applied to the other. Each organism/latex suspension was mixed with a wooden stick, then the reaction card was rocked gently for 60 seconds and examined macroscopically for agglutination.

The principle of the test is that antibodies to the *E coli* somatic antigen O157 are coated onto the test latex reagent. This reagent will show macroscopic agglutination in the presence of the O157 antigen. No agglutination with the test latex and appropriate reactions with the positive and negative controls which were tested each time were considered to exclude *E coli* O157.

### 3.2.10 *Salmonella* antigen identification

The following range of commercial *Salmonella* somatic antisera (Murex, supplied by Murex Diagnostics, New Zealand, P.O. Box 22-305, Otahuhu) was used: polyvalent A-G, O group A, O group B, O group C, O group D and O group E. Organisms biochemically reacting as *Salmonellae* were further tested for somatic antigens using the slide agglutination method of Kauffmann (1966). For each antiserum under test, a drop of 0.85% saline was placed on a microscope slide and a drop of antiserum placed adjacent to it. A loopful of organism was taken from a 5% sheep blood agar plate and emulsified in the saline drop. The emulsification was then drawn into the antiserum drop. The slide was then rocked gently for 30 seconds and observed macroscopically for agglutination. Each organism was also tested in saline only, to ensure that auto-agglutination (agglutination independent of antiserum) was not occurring.

## 3.3 Commercial kit identification of Enterobacteriaceae and Vibrionaceae

### 3.3.1 Primary identification system

#### 3.3.1.1 The Identify system

The commercial identification system, Identify, was used as the primary biochemical identification system for potential *Vibrionaceae* and *Enterobacteriaceae*. The system is marketed in New Zealand by SCIANZ, P.O. Box 6848, Auckland, and was comprehensively evaluated by McCarthy (1985). The system comprises three separate transparent trays. Trays one and two are used for the identification of oxidase negative organisms; and tray three, for oxidase positive bacteria. Although the supplier states the system can be read after four hours incubation, all reactions were incubated overnight prior to reading. The trays each contain 10 biochemical wells, covered by a sliding plastic lid. Tray one includes the following biochemical tests: ß-galactosidase (ONPG), arginine dihydrolase (ADH), lysine decarboxylase (LDC), ornithine decarboxylase (ODC), hydrogen sulphide production ($H_2S$), urease (URE), Voges Proskauer Test (VP), phenylalanine dehydrogenase (PD), indole (IND), citrate utilisation (CIT). Tray two includes the following biochemical tests: Malonate utilisation (MAL); fermentation of: rhamnose (RHA), adonitol (ADO), salicin (SAL), arabinose (ARA), inositol (INO),

sorbitol (SOR), sucrose (SUC), mannitol (MAN), raffinose (RAF). Tray three includes the following biochemical tests: growth in cetrimide (CET); utilisation of: acetamide (ACE), malonate (MAL), and citrate (CIT); fermentation of maltose (MAL); hydrolysis of esculin (ESC), arginine dihydrolase (ARG), arginine control (CON), urease (URE), indole (IND). The principle and interpretation of each test is as follows:

ONPG: ß-galactosidase hydrolyses σ-nitrophenol-ß-d-galactopyranoside (colourless) to galactose and σ-nitrophenol (yellow).

ADH: Arginine dihydrolase transforms arginine into citrulline and ammonia, resulting in a pH increase and a colour change from yellow to purple.

LDC: Lysine decarboxylase transforms lysine into cadervine resulting in a pH increase and a colour change from yellow to purple.

ODC: Ornithine decarboxylase transforms ornithine into putrescine resulting in a pH increase and a colour change from yellow to purple.

$H_2S$: Hydrogen sulphide is produced by the enzymatic hydrolysis of thiosulphate which reacts with ferric citrate to give a black precipitate.

URE: Enzymatic hydrolysis of urea by urease results in the production of ammonia which causes an indicator change from yellow to pink.

VP: Production of acetoin, an intermediary glucose metabolite, is determined by incubating the organism in glucose and then adding the reagents potassium hydroxide and alpha naphthol. Acetoin complexes with these chemicals to produce a red colour.

PD: Deamination of phenylalanine produces phenyl pyruvic acid which produces a green colour in the presence of ferric chloride.

IND: Tryptophan breakdown results in the production of indole which forms a pink complex with Kovacs' reagent.

CIT, MAL, ACE: Utilisation of citrate (or malonate, or acetamide) as the sole carbon source produces alkalinisation of the medium. The resultant rise in pH changes the indicator from green to blue.

RHA, ADO, SAL, ARA, INO, SOR, SUC, MAN, RAF: Utilisation of a carbohydrate to an acid end product is indicated by a colour change from green to yellow.

CET: Tolerance to cetrimide results in growth, indicated by turbidity.

MAL: Utilisation of a maltose to an acid end product is indicated by a colour change from red to yellow.

ESC: The hydrolysis of esculin is detected by ferric ammonium citrate which forms a black precipitate.

ARG: Arginine dihydrolase transforms arginine to citrulline and ammonia. This causes a pH rise and the indicator changes from yellow to red. (Although the reaction is the same as tested for in ADH, the indicator system in this well is different.)

CON: This tube contains basal medium only and the colour in this tube is compared to that of the ARG tube, if the ARG tube is pinker, the ARG test is deemed positive.

#### 3.3.1.2 Use of the Identify system

Oxidase negative organisms (potential *Salmonella*, *Shigella* (urease negative, non-lactose fermenting organisms), *Yersinia* and *E coli* O157):

The organism under test was inoculated into 3 ml of sterile water and then the inoculating loop streaked to a SBA/Mac split plate for purity checking. The inoculum water was then mixed well before 1 ml was delivered to the rear of tray one (ID01). The tray was then tilted left and right to ensure even distribution of the inoculum fluid prior to tilting the tray forward to fill the wells. Two drops of sterile paraffin oil were then added to each of the following wells: ADH, LDC, ODC, URE, $H_2S$; before the tray was covered and incubated along with the purity check overnight at 37°C in air.

After overnight incubation, the purity checks were reviewed and if they appeared pure, reading proceeded, if not, the tests were repeated.

Reagents were added to three test wells as follows:
PD: 10% ferric chloride, one drop. Presence or absence of green coloration was recorded immediately on addition of reagent.

VP: 6% alpha naphthol, one drop; 40% KOH, 1 drop. The presence or absence of red colour was noted 15 minutes after the reagent was added.

IND: one drop of 5% para dimethyl amino benzaldehyde in 75% isoamyl alcohol, 25% concentrated hydrochloric acid (Kovacs' reagent), and the presence or absence of a red colour noted after one minute.

Reaction results for all wells were recorded and a numerical code calculated by dividing the substrates into groups of three. The first well of the triplet was given a value of 1 if positive; the second well, a value of 2 if positive; and the third a value of 4 if positive. The calculated code was then compared with the comprehensive data base provided by the supplier to determine the probable identification of the isolate. For example, for an organism reacting as follows:

| | | |
|---|---|---|
| ONPG + | 1 | |
| ADH - | 0 | |
| LDC + | 4 | |
| | | 5 |
| ODC + | 1 | |
| H2S - | 0 | |
| URE - | 0 | |
| | | 1 |
| VP - | 0 | |
| PD - | 0 | |
| IND + | 4 | |
| | | 4 |
| CIT - | 1 | |
| | | 0 |

the organism's code is 5140, the most common profile for an *E coli.*

The second tray (ID02) was inoculated and incubated in the same way as the ID01 except that no wells required paraffin oil overlay and no wells required the addition of reagents.

When the two trays are used, the first two reactions of tray two combine with the last reaction of tray one to give a substrate triplet. The last two reactions of tray 2 are calculated as a doublet.

Oxidase positive organisms:

The organism under test was inoculated into 3 ml of sterile 0.85% saline and then the inoculating loop was streaked to a SBA/Mac split plate for purity checking. The inoculum saline was then mixed well before 1 ml was delivered to the rear of tray three (ID03). The tray was then tilted left and right to ensure even distribution of the inoculum fluid prior to tilting the tray forward to fill the wells. Two drops of sterile paraffin oil were then added to each of the following wells: ARG, CON, URE; before the tray was covered and incubated, along with the purity check, overnight at 37°C in air.

After overnight incubation the purity checks were reviewed and, if they appeared pure, reading proceeded; if not, the tests were repeated.

Kovacs' reagent was added to the IND well as per the ID01 method and the reaction profile determined. The profile consists of only three digits as only nine reactions are detected with this tray; the CON well being only for comparative purposes with the ARG.

### 3.3.2 Secondary Identification system

The API32E strip (API-biomerieux SA, La Balme-les-Grottes, France) was used as the secondary identification system for both non-conforming oxidase positive and negative organisms. This identification system is marketed in New Zealand by Med-Bio Enterprises Ltd, P.O. Box 11016, Sockburn, Christchurch and was evaluated by Freney *et al.*, (1991).

The system comprises a plastic strip containing two rows of 16 wells. Each well contains a dehydrated substrate. Reactions tested for include:

Four enzymatic reactions with chromogenic substrates: α-galactosidase (GAL); ß-galactosidase (PNPG); ß-d-glucosaminidase (ONAG); and alkaline phosphatase (IDP).

Eight conventional tests: lysine decarboxylase (LDC), ornithine decarboxylase (ODC),

phenylalanine deaminase (PPA), tetrathionate reductase (TTR), indole production (IND), malonate utilisation (MNT), urease production (URE), esculin hydrolysis (ESC), and oxidase test.

Sixteen carbon substrate fermentation tests: arabinose (ARA), mannitol (MAN), cellibiose (CEL), glucuronate (GRT), mannose (MAN), sorbitol (SOR), maltose (MAL), trehalose (TRE), 5-ketogluconate (5KG), palatinose (PLE), galacturonate (GAT), raffinose (RAF), sucrose (SAC), rhamnose (RHA), melibiose (MEL), and adonitol (ADO).

Two inhibition tests: colistin (COL), and coumarate (CMT).

One fermentation control well (FER).

The organism under test was inoculated into 3 ml of sterile 0.85% saline to a dense turbidity.

Using a sterile pasteur pipette, two drops of inoculum were delivered into each well and the three top wells on the left of the strip (URE, LDC and ODC) were each overlaid with one drop of sterile paraffin oil. The plastic cover supplied with the kit was placed over the reaction strip and the strip was incubated at 37°C in air for between four and five hours.

After incubation, one drop of Kovacs' reagent was added to the IND well and then all the reactions were read and results recorded as positive or negative as follows:

Top row, left to right:

URE: Enzymatic hydrolysis of urea by urease result in the production of ammonia which causes an indicator change from yellow to pink.

LDC: Lysine decarboxylase transforms lysine into cadervine an alkaline product causing an indicator change from yellow to purple.

ODC: Ornithine decarboxylase transforms ornithine into putrescine, an alkaline product causing an indicator change from yellow to purple.

ESC: The hydrolysis of esculin is detected by ferric ammonium citrate which forms a

black precipitate.

FER, ARA, ADO, RHA, MAN, SOR, CEL, MEL, GRT, MNE, MAL, TRE: Fermentation of the carbon substrate results in an acid end product which is indicated by a colour change from red to orange-yellow.

Bottom row, left to right:

IND: Tryptophan breakdown results in the production of indole which forms a pink complex with Kovacs' reagent.

MNT: Utilisation of malonate as the sole carbon source produces alkalinisation of the medium. The resultant rise in pH changes the indicator from yellow to blue-green.

PPA: Deamination of phenylalanine produces phenyl pyruvic acid which results in a colour change from colourless to orange-brown.

SAC, 5KG, PLE, GAT, COL, CMT: positivity is indicated by a colour change from red to orange-yellow.

TTR: tetrathionate reduction is indicated by a colour change from blue-green to yellow.

ONAG, PNPG, GAL: hydrolysis of the substrate results in an indicator change from colourless to yellow

IDP: reduction of indoxyl phosphate results in an indicator change from colourless or pale blue to deep blue.

RAF: Fermentation of the carbon substrate results in an acid end product which is indicated by a colour change from red to orange-yellow.

Oxidase: this test was performed outside of the test strip as described in 3.2.2.

Reaction results for all wells were recorded and a numerical code calculated by dividing the substrates into groups of three. The first well of the triplet was given a value of 1 if positive, the second well, a value of 2 if positive and the third a value of four if positive. The sum of each triplet becomes a digit in the code which incorporates, in order: the top

12 wells, beginning at URE; the bottom 12 rows beginning at IND; the triplet beginning with GRT; the triplet beginning GAL; the doublet comprising TRE and the oxidase reaction. The first eight digits of the code were then compared with the data base provided by the supplier to determine the probable identification of the isolate. If the code was inconclusive or not listed in the index, the supplier was contacted and the full code investigated in the supplier's more comprehensive database.

## 3.4 Rotavirus testing

The *Rotalex* rotavirus detection kit (Orion, distributed in New Zealand by SCIANZ, P.O. Box 6848, Auckland) was used to detect rotavirus in faeces. The principle of the test is that latex particles coated with anti-rotavirus antibodies are agglutinated by stool specimens containing rotavirus. A control latex, which is not coated with antibodies is included to detect non-specific agglutination.

Approximately 0.1 g of stool was emulsified in 1 ml of buffer, and then centrifuged for 10 minutes at 1000 rpm. 50 µl of stool supernatant was applied to each of two reaction areas on the card supplied with the kit. One drop of control latex reagent was added to one reaction area and one drop of test latex reagent was added to the other. The reagent and stool were then mixed within the reaction area and the card then gently rocked for one minute.

A positive reaction is denoted by macroscopic agglutination with the test latex only, a negative reaction, by no agglutination in the either area, and an equivocal reaction by agglutination in both areas.

## 3.5 Parasite detection methods

### 3.5.1 Direct wet preparation

The swab used for inoculating stool into the selenite broth (3.6.1) was swilled in the selenite broth and then touched to a clean microscope slide. The resultant drop was covered with a 22 x 22 mm glass coverslip and examined by light microscopy at a magnification of 400 times for a minimum of one minute. The presence of white blood

cells, red blood cells and protozoa was noted and semi-quantitatively scored at + (less than five per field), ++ (between five and twenty per field) or +++ (more than twenty per field). If no cells or protozoa were seen, the specimen was recorded as "No abnormality detected". Protozoa were identified by comparing a structure's size, shape, internal and peripheral appearance with the text of Ash and Orihel, (1980).

### 3.5.2 Polyvinyl alcohol fixation

Based on the formulation of Brooke and Goldman (1949), the polyvinyl alcohol fixative (PVA) was prepared as follows: 50 g PVA (Dupont 85-60 grade) was mixed well with 15 ml glycerol to ensure all PVA was glycerol-coated; 625 ml of distilled water was added and the mixture (which had the consistency of cottage cheese) was left at room temperature overnight. Schaudinns fixative was prepared as follows: 19 g mercuric chloride was dissolved in 125 ml 100 % ethanol and 250 ml of distilled water was then added. Precautions were to taken to reduce contact with the mercuric chloride because of its toxicity. The PVA mixture was heated to 75°C for one hour prior to the addition of the Schaudinns fixative. The PVA fixative was kept at 75°C for a further hour, during which time the mixture clarified. Once prepared, PVA fixative was stored at room temperature.

On the day of receipt, approximately 4 g of each stool specimen was mixed with 15 ml of PVA fixative and stored at room temperature until tested. If less than 4 g of stool were received, a drop of PVA fixative was placed on a microscope slide and a small amount of stool specimen was mixed in the drop. A wooden stick was then used to roll the drop over the slide to produce a thin film.

### 3.5.3 Formalin fixation

Formalin fixative was prepared by diluting 40% formaldehyde 1:10 in distilled water. On the day of receipt, approximately 0.5 g of each faeces specimen was mixed with 4.5 ml of formalin fixative and stored at room temperature.

### 3.5.4 Trichrome staining

The trichrome stain of Wheatley (1951) was used as a permanent stain for the recognition and identification of protozoa. The stain was prepared as described by Kaplan (1992) as follows: chromotrope 2R 0.6 g, light green SF 0.3 g, phosphotungstic acid 0.7 g, glacial acetic acid 1.0 ml, were mixed together and stood 30 minutes at room temperature. 100 ml of distilled water was added, the solution mixed and stored at room temperature.
The staining procedure was performed as follows: a few drops of thoroughly mixed PVA/stool mixture was applied to an absorbent tissue and left for three minutes. After this time, the stool was gently removed from the tissue with a wooden stick which was then rolled to a produce a thin smear of stool on a glass microscope slide. The smear was air dried and stained as follows: 70% ethanol, five minutes; 70% ethanol + sufficient Gram's iodine added to give a port wine colour, five minutes; 70% ethanol, five minutes; trichrome stain, 10 minutes; 90% ethanol/1% acetic acid, one second; 100% ethanol, one second; 100% ethanol, five minutes; xylol, five minutes; xylol, five minutes. The stained smear was then mounted with a 40 mm by 22 mm glass coverslip and a drop of mounting medium. Once dry, the mounted smear was observed for five minutes using 1000 times oil immersion light microscopy. Protozoa were identified by comparing a structure's size, shape, internal and peripheral appearance with the text of Ash and Orihel (1980).

### 3.5.5 Formalin-ethyl acetate concentration

The method for the concentration of faeces was based on that described by Crede (1992). The principle is that ova, cysts and larvae are concentrated by centrifugation whilst faecal matter is removed by filtration and ethyl acetate extraction. The method was performed by filtering 4.5 ml of formalin fixed specimen through double layer gauze into a stoppered tube which was then centrifuged at 1500 rpm for two minutes. The supernatant was then discarded and the deposit resuspended in 5 ml saline, to which 2 ml ethyl acetate was then added. The tube was re-stoppered and shaken for 30 seconds before being centrifuged at 1500 rpm for two minutes. The upper "plug" was disturbed with the wooden end of a cotton swab stick and the fluid discarded, leaving a small amount of sediment. The cotton end of the swab was used to clean the inside of the tube. The tube was then re-stoppered.

The concentrate was examined by mixing one drop of concentrate with one drop of Gram's iodine on a glass microscope slide and covering with a 22 mm x 22 mm glass coverslip. The preparation was examined by light microscopy at 100 times magnification.

Parasite identity was confirmed at 400 times magnification.

### 3.5.6 Modified Kinyoun acid fast stain for *Cryptosporidia*

The stain as described by Shimizu (1992) was prepared as two solutions which were then combined. Solution A (Kinyoun carbol fuchsin) comprised 4 g basic fuchsin and 20 ml 95% ethanol, and solution B comprised 8 g of phenol crystals in 100 ml distilled water. The counterstain comprised 0.3 g of methylene blue in 95% ethanol. All reagents were stored at room temperature.

The principle of the stain is that the cell wall of the Cryptosporidial oocyst is permeable to the Kinyoun carbol fuchsin, but impermeable to 1% sulphuric acid. Thus the oocyst takes up the stain and resists decolorisation, appearing red against a blue background.

The stain was performed on a drop of concentrated sediment (3.5.5) which had been smeared onto a clean glass microscope slide to give a thin film. After air drying, the smear was fixed in absolute methanol for one minute and then flooded with Kinyoun carbol fuchsin for five minutes. The smear was then briefly rinsed in 50% ethanol and then decolorised with 1% sulphuric acid for two minutes before being counterstained with methylene blue. The slide was washed with water and air-dried and examined by oil immersion light microscopy at 1000 times magnification.

### 3.5.7 *Giardia/Cryptosporidium* Direct Immunofluorescence

The kit used was manufactured by Merifluor (Catalog #250050, Meridian Diagnostics, Inc., 471 Hills Drive, Cincinnati OHIO 45244, USA) and is supplied in New Zealand by SCIANZ, P.O. Box 6848, Auckland. The principle of the method is that the detection reagent contains a mixture of fluoroscein isothiocyanate (FITC) labelled monoclonal antibodies to cell wall antigens of *Giardia lamblia* cysts and *Cryptosporidium parvum* oocysts. In the presence of the target antigen, FITC remains bound to the antigen which appears green when viewed under fluorescent microscopy.

The method was performed in accordance with the manufacturer's instructions: one drop of concentrated sediment (3.5.5) was spread over the treated slide well supplied with the kit and allowed to air dry. One drop of detection reagent was applied to the well, followed by one drop of counterstain. The reagents were mixed with an orange stick taking care not to scratch the treated well surface. The slide was then incubated in the dark for 30 minutes, at room temperature. The concentrated wash buffer supplied with the kit was diluted 1:20 in distilled water and the dilute buffer used to gently rinse the slide. Excess buffer was dried from the slide, but the slide was not dried completely prior to the addition of a drop of mounting medium to the well and the application of a 22 x 22 mm glass coverslip. Each well was screened in its entirety using a fluorescent microscope and 100 x magnification. Confirmation of a positive result was made at 400 x magnification.

### 3.5.8 *Giardia* antigen enzyme immunoassay.

The ProSpect™ Giardia EZ Microplate Assay™ kit used is manufactured by Alexon, Inc., 1190 Borregas Avenue, Sunnyvale California, 94089-1302 USA; and is supplied in New Zealand by Ngaio Diagnostics Ltd., P.O. Box 4015, Nelson South. The principle of the test is that antibody to the Giardia-specific antigen, 65 (GSA 65), is conjugated to an enzyme and will bind to GSA 65, resisting a washing process. The enzyme then catalyses a chromogenic reaction on addition of substrate. The method was performed in accordance with the manufacturer's instructions as follows: 0.5 ml of formalin fixed specimen was prediluted with 1 ml of specimen dilution buffer. Two drops of enzyme conjugate and 100 µl of the diluted stool suspension were added to the reaction well, and incubated at room temperature for one hour. The concentrated wash buffer was diluted 1:10 in distilled water and the dilute buffer used to wash the well five times. Four drops of colour substrate were added to the well and, following incubation for 10 minutes at room temperature, one drop of stop solution was added to the well. A positive result was signified by a yellow colour, negative tests appeared colourless.

## 3.6 Laboratory Processing Methods

### 3.6.1 Culture processing: inoculation and incubation

On the day of receipt, specimens for which consent (3.9) had been given were processed in the following manner:

The macroscopic appearance of the stool specimen was noted and a sterile wooden applicator swab was used to apply the specimen to one quarter of each agar plate. A heat sterilised loop was used to streak each plate to obtain single colonies. Media inoculated included XLD/Mac split plate, SBA, CAMP, and Sel; these media comprising the routine laboratory method and being selective for the detection of *Salmonella*, *Shigella*, *Campylobacter*.

Additional media inoculated included Hek/SMAC split plate, TCBS half plate, ASM/YSA split plate, CCDA, and GN.

A direct wet preparation examination was performed at the time of primary culture inoculation.

All media excluding CAMP, CCDA, and ASM/YSA were incubated at 37°C in air overnight. ASM/YSA were incubated at 30°C for a total of two days. CAMP and CCDA media were incubated at 42°C in microaerophilic conditions for two days. Microaerophilic conditions were achieved by placing the culture plates, a palladium catalyst and a microaerophilic generating kit (Oxoid product BR60, supplied by Intermed Scientific, P.O. Box 102-253, North Shore Mail Centre, Auckland) in a 2.5 litre anaerobic jar (Oxoid product HP11, supplied by Intermed Scientific). Tap water (10 ml) was added to the generating kit and the jar sealed and incubated. The principle of the microaerophilic generating system is that in the presence of a palladium catalyst, the sachet contents (sodium borohydride, tartaric acid and sodium bicarbonate) react with water to form hydrogen gas, carbon dioxide and water, reducing the oxygen concentration within the jar to approximately 5%.

After overnight incubation, the enrichment broths were subcultured using a sterile loop and a streaking technique: Sel to XLD/Mac, YSA and Hek; and GN to XLD/Mac, YSA, Hek and TCBS. The subcultures were incubated and examined in the same way as their primary culture counterparts.

### 3.6.2 Culture processing: reading of cultures

#### 3.6.2.1 XLD/Mac/Hek

Pink colonies on XLD, clear colonies on Mac and green, blue/green colonies on Hek (non-lactose fermenters) were considered to be potential *Salmonella* or *Shigella*. In addition, black (hydrogen sulphide producing) colonies on XLD and Hek were considered to be potential *Salmonella*. Tiny colonies on Mac were considered to be potential *Yersinia*. Representative colonies with any of these appearances were further investigated.

If typical *Proteus species* were noted on the SBA (large colonies with a spreading edge,) non-lactose fermenting colonies from the XLD and Mac plate were screened initially with a urease test. Urease positive colonies, were considered to be *Proteus species* and were not further investigated.

Colonies negative in the urease test after four hours (no colour change) and colonies for which a urease test was not indicated were identified using the ID01.

If the ID01 reaction profile was a high likelihood (for example profile 4301, *Salmonella*) somatic antigen slide serology was performed from the blood agar purity check plate.

If the ID01 reaction profile indicated a high likelihood of *Yersinia* - eg 0500 - and the typical small colony appearance was noted on the purity plates, then the identification was accepted as *Yersinia*.

If the ID01 profile showed no likelihood of *Salmonella*, *Shigella* or *Yersinia*, the organism was not further investigated.

If the ID01 code was inconclusive or not listed in the index, the ID01 and purity check were repeated and an ID02 also set up.

If the profile remained inconclusive (*Salmonella, Yersinia* or *Shigella* not confirmed nor excluded), an API32E identification panel was inoculated.

#### 3.6.2.2 SMAC

Non-sorbitol fermenting colonies were considered to be potential *E coli* O157 and were identified using the Identify system. Organisms confirmed as *E coli* were then tested for O157 (see 3.2.9).

#### 3.6.2.3 SBA/ASM

The oxidase reactions of both the main inoculum and of single colonies on the final streaking were determined. If the result was positive, and individual colonies could be accessed, the positive colonies were tested for glucose oxidation/fermentation (O/F). If individual colonies could not be accessed, a sterile loop was used to lift the positive organism from the oxidase paper and this organism was then streaked to SBA for O/F testing once individual colonies were accessible.

Glucose-fermenting, oxidase positive organisms were considered to be probable *Vibrionaceae* and were inoculated into an ID03 and the O/129 test was performed.

If reactions were inconclusive, the organism was inoculated into an API32E identification panel.

#### 3.6.2.4 TCBS

If growth was noted, representative colonies were plated to SBA for oxidase testing. Oxidase positive organisms were tested for O/F glucose reaction. Non-fermenters were discarded; glucose-fermenting, oxidase positive organisms were considered to be probable *Vibrionaceae* and were inoculated into an ID03 and the O/129 test was performed. If reactions were inconclusive, the organism was inoculated into an API32E identification panel.

#### 3.6.2.5 CAMP/CCDA

All growth on either CAMP or CCDA was considered to be potential *Campylobacter*. Growth was initially tested for oxidase reaction. Organisms which tested oxidase positive were then Gram stained to confirm the characteristic Gram negative, curved bacilli or "sea-gulls" of *Campylobacter species*. Confirmation of genus and species was made by confirming microaerophilic requirement and hippurate reaction. In addition, isolates from the CCDA medium were tested for susceptibility to nalidixic acid and cephalothin. Based on the criteria of Penner (1988) organisms were identified as follows: hippurate positive, microaerophilic Gram negative, curved bacilli which were resistant to cephalothin and sensitive to nalidixic acid were reported as *Campylobacter jejuni*. Hippurate negative, microaerophilic Gram negative curved bacilli which were sensitive to both cephalothin and nalidixic acid were reported as *Campylobacter upsaliensis*.

#### 3.6.2.6 Organism Confirmation and Typing

Full *Campylobacter* identification was performed in-house. *Salmonella*, Aeromonads, Plesiomonads, and *Vibrio* were referred to the Enteric Reference Laboratory at ESR:Health Communicable Disease Centre for full identification. *Yersinia* were referred to the Diagnostic Laboratory, Auckland for confirmation, speciation and serotyping.

### 3.6.3 Rotavirus testing

Rotavirus testing was performed on the day of specimen receipt if a faeces was from a child aged younger than five years, or if testing was specifically requested by a clinician.

### 3.6.4 Parasite screening

On the day of receipt, a portion of each stool specimen was fixed in PVA a second portion fixed in formalin fixative. Parasite methods as detailed in 3.5 were set up in weekly batches.

## 3.7 Study community

The area chosen for study was the Eastern Bay of Plenty (EBOP), a region comprising a population of approximately 49 000. Approximately 65% of the population are based in the towns of Whakatane/Ohope (17 000), Kawerau (8 300), Opotiki (3 500) and Edgecumbe (1 900), with the remaining population living in small towns (<1 000) or on rural properties. Approximately 80% of the EBOP population is serviced by water supplies which are monitored and treated by local authorities. The laboratory work was performed at the Whakatane Hospital laboratory which, at the time of the study, performed all faecal testing for the study population.

## 3.8 Ethical approval

Prior to the commencement of the study, ethical permission was sought, and gained, from the Bay of Plenty Area Health Board ethics committee.

## 3.9 Patient Selection and Specimen/Data Collection

Prior to the commencement of the study, all clinicians were visited to discuss the intent of the study and the practicalities involved. Feedback was sought and, as a result, specimen collection kits were used to minimise the clinicians' time involvement in the study. These kits were distributed to all general practitioner (GP) surgeries, and all hospital wards and patient departments. The collection kits contained: a written explanation of the study; a consent form; written step-by step instructions for specimen collection and despatch; a questionnaire on patient demographics, symptoms and possible risk factors (Appendix 1); a collection receptacle (500 ml plastic container); a wooden spatular (to transfer the specimen); a 60 ml commercial specimen container; and a commercial biohazard transport bag.

Before commencement, the questionnaire was reviewed by Alistair Stewart, Department of Community Health, University of Auckland.

GP's were requested to pass over a collection kit to each patient from whom a faeces specimen was requested. Local radio and newspapers gave coverage of the study to help publicise the intent to the EBOP population to assist in gaining patient co-operation.

Hospital wards and departments were requested to enrol patients in the study if gastrointestinal symptoms developed within three days of hospitalisation and these symptoms were considered to be unrelated to post-hospitalisation events.

Most study participants completed the questionnaires unassisted. Project staff completed a small number of questionnaires at the direction of hospitalised patients.

A specimen was included in the project if: the specimen was accompanied by a signed consent form or/ the specimen was from a patient who had previously signed a consent form or/ the patient did not sign a consent form but gave a clear verbal indication of agreement to participate.

## 3.10 Data Analysis

Questionnaire and specimen result data were single entered into EpiInfo 5.01 software (Dean *et al.*, 1990). Each entry was then individually checked. Data were analysed using the EpiInfo 5.01 Analysis Program. If an option such as "contact with farm animals" showed a significant association with a disease, then a variable was created for each animal listed in the free text response field and these new variables also analysed. A risk was considered significant if the relative risk was greater than 1.0, the 95% confidence limits did not include 1.0, and the p-value was $\leq 0.05$.

The costs in consumables per organism investigation was calculated as follows: for each pathogen, the price of the selective isolation media and the various identification procedures used to confirm, or exclude, the pathogen in each specimen were totalled. When an organism was confirmed on several different media, the cost of the first confirmation only was noted. Costs for investigating each specimen for each pathogen were recorded in EpiInfo 5.01 and the average cost per pathogen per specimen was then extracted. The total cost of isolation media and confirmation/exclusion testing was divided by the number of positive isolates of the specific pathogen to give the cost per positive isolate.

The average cost to confirm or exclude a pathogen in one specimen (d) $= \frac{a + b}{c}$

where a = total price of isolation medium selective for that pathogen; b = total cost of additional testing to confirm or exclude the pathogen; and c = the total number of specimens.

The cost per positive isolate of each pathogen across the study population (f) $= \frac{a + b}{e}$

where e = the total number of specimens positive for that pathogen.

Rates per 100 000 were calculated by multiplying the number of cases by 2.04 (100 000 divided by 49 000; the population of the study catchment area) and the total number of stool cultures received, divided by the number of stools which were eligible for inclusion in the study.

Thus the rate per 100 000 $= \frac{2.04 \times n}{s}$

where n = the total number of stool specimens received for culture at the Whakatane Hospital Laboratory during the study year and s = the total number of specimens included in the study.

The project year commenced 19th July 1993 and concluded 18th July 1994.

# 4 RESULTS

## 4.1 Case and Organism data

### 4.1.1 General Case and organism data

A total of 2033 faecal specimens were received for culture during the project year. Based on patient consent, 997 (49%) of the 2033 were included in this study.

The 997 specimens were received from 716 episodes of gastrointestinal illness. 337 (34%) specimens from 247 episodes of illness yielded a total of 391 Group 1 and/or Group 2 organisms.

The age range of patients submitting specimens was two months to 89 years with a mean of 28 years and a median of 26 years. 370 patients (51.6%) were female. A completed questionnaire was returned for 603 episodes, including 233 from which either a Group 1 or Group 2 organism was detected. In addition, some partially completed questionnaires were received.

The total number of organisms detected in the 997 specimens, the number of cases these represent and the estimated rates of infection per 100 000 in the Eastern Bay of Plenty population are presented in Table 3.

### 4.1.2 Group 1 case and organism data additional to that shown in Table 3

***Campylobacter***

Sixty-five (6.5%) specimens yielded *Campylobacter*, 64 isolates (49 cases) were confirmed as *Campylobacter jejuni*, and one isolate (one case) was identified as presumptive *Campylobacter upsaliensis*.

***Salmonella***

Thirty-eight (3.8%) specimens yielded *Salmonella*, 29 isolates were confirmed as *S* Typhimurium (11 cases, four of whom were household contacts), five as *S* Paratyphi A

## Table 3.

**Total number of positive tests, cases and incidence rates per 100 000 noted during a one year study in the Eastern Bay of Plenty**

| | Total number detected | Total number of cases | Eastern Bay of Plenty Incidence rate/100 000 | Notification rate for the same time period[1] |
|---|---|---|---|---|
| **Group 1 organisms** | | | | |
| *Campylobacter* | 65 | 50 | 208 | 181.9 |
| *Salmonella* | 38 | 15 | 62 | 47 |
| *Shigella* | 0 | 0 | <4 | 0 |
| *E coli* O157 | 3 | 2* | 4 | |
| *Yersinia* | 30 | 21 | 87 | |
| *Vibrio cholerae* non-01, non O139 | 2 | 1 | 4 | |
| Rotavirus | 38 | 34 | 141 | |
| *Giardia* | 52 | 38* | 158 | |
| *Cryptosporidium* | 20 | 16 | 67 | |
| **Group 2 organisms** | | | | |
| *Aeromonas* | 17 | 15 | 62 | |
| *Plesiomonas* | 6 | 5 | 21 | |
| *Dientamoeba* | 9 | 7 | 29 | |
| *Blastocystis* | 111 | 86 | 358 | |

[1]The New Zealand Public Health Report August 1994, the data published in this report are based on six months of notification data, seasonally adjusted.

* The specimen from one case was referred from outside the study area and is not included in the incidence rate.

(from two cases who were household contacts), three as *S* Enteritidis (one case) and one as *S* Hadar.

***Yersinia***

Thirty (3%) specimens yielded *Yersiniae*, 23 (15 cases) isolates were identified as *Y enterocolitica* serotype O:3, four as *Y enterocolitica* biotype 1A (three cases), one as *Y enterocolitica* serotype O:5,27, one as *Y frederiksenii*, and one as *Y kristensenii*.

***E coli* O157**

Three specimens from two male patients yielded *E coli* O157. One case was aged one year and the other, two years. Both presented with bloody diarrhoea and haemolytic uraemic syndrome. One specimen was fluid and the other two were unformed. One specimen from each patient showed both WBC and RBC, the remaining specimen showed no microscopic abnormalities.

***Vibrio***

Two specimens yielded *Vibrio cholerae* non-O1 non-O139. Both isolates were from the one patient who presented with abdominal pain, diarrhoea, vomiting, nausea, lethargy, and foul smelling stools. Both specimens were unformed, one showed protozoa on microscopic examination and the other, no abnormality. The patient had eaten shellfish within a week of becoming ill.

**Rotavirus**

A total of 172 specimens were tested for rotavirus. Of the specimens tested, 170 were from patients aged less than five years. Two older cases were tested on physician request. Thirty-eight specimens (21.5%) from 34 patients, including both of the patients aged more than five years, gave a positive result for rotavirus.

**Table 4.**

**The sex and age distribution of cases of gastrointestinal infection with Group 1 or Group 2 gastrointestinal pathogens detected in a one year study of 716 episodes of gastrointestinal illness in the Eastern Bay of Plenty**

| Organism | Number of cases | % female | < 1 year Number | (%) | 1 - 15 years Number | (%) | >15 years Number | (%) | Age range detecting all isolates | Age mean* | Age median* |
|---|---|---|---|---|---|---|---|---|---|---|---|
| *Campylobacter* | 50 | 36 | 3 | (6) | 15 | (30) | 32 | (64) | <1 - 78 | 24 | 21 |
| *Salmonella* | 15 | 33 | 1 | (7) | 3 | (20) | 11 | (73) | <1 - 74 | 31 | 36 |
| *Yersinia* | 21 | 43 | 1 | (4.5) | 2 | (9.5) | 18 | (86) | <1 - 60 | 36 | 40 |
| *E coli* O157 | 2 | 0 | 1 | (50) | 1 | (50) | | | <1 - <3 | | |
| *Vibrio* | 1 | 100 | 0 | | 0 | | 1 | (100) | 18 | | |
| *Giardia* | 38 | 66 | 0 | | 13 | (34) | 25 | (66) | <2 - 65 | 23 | 24 |
| *Cryptosporidium* | 16 | 31 | 1 | (6) | 11 | (69) | 4 | (25) | <1 - 68 | 12 | 2 |
| *Aeromonas* | 15 | 33 | 1 | (7) | 5 | (33) | 9 | (60) | <1 - 80 | 27 | 17 |
| *Plesiomonas* | 5 | 100 | 0 | | 0 | | 5 | (100) | 18 - 59 | 33 | 30 |
| *Dientamoeba* | 7 | 0 | 0 | | 2 | (29) | 5 | (71) | 3 - 73 | 39 | 39 |
| *Blastocystis* | 86 | 55 | 0 | | 17 | (20) | 69 | (80) | <2 - 82 | 31 | 27 |

* The mean and median ages are shown for each organism for which there were more than four cases. As specimens from all age groups were not routinely screened for Rotavirus, data for cases with this organism have not been in in this Table.

1 With the exception of Plesiomonas, Giardia and Blastocystis, all organisms for which there were more than two cases were more frequently seen in males. Overall 47% of positive cases were female, yet 51.6% of the cases re specimens were female.

2 All organisms were noted over a wide age range. *Cryptosporidium* shows a predominance in children when compared to infants or adults, other organisms show a predominance in adults.

**NOTE: The age distribution is such that limiting the tests for any organism to a selected age group would lead to missing many positive diagnoses.**

### 4.1.3 Group 2 case and organism data additional to that shown in Table 3

***Aeromonas***

Seventeen (1.7%) specimens yielded Aeromonads. Nine isolates were confirmed as *Aeromonas hydrophila* (seven cases), six (six cases) as *Aeromonas caviae*, and two (two cases) as *Aeromonas veronii biovar sobria.*

### 4.1.4 Case demographics and organism seasonality

The sex and age distribution for cases from which Group 1 and Group 2 organisms were isolated is shown in Table 4. The seasonal distribution of all cases of infection with Group 1 and Group 2 organisms are shown in Table 5.

## 4.2 Specimen findings

### 4.2.1 Specimen form and microscopy

Table 6 shows the macroscopic and microscopic appearances of specimens from which selected Group 1 and Group 2 organisms were isolated.

### 4.2.2 Specimens/episodes yielding multiple organisms

Of the 247 episodes from which a Group 1 or Group 2 organism was detected, 46 specimens (4.6%) from 37 episodes yielded multiple organisms (excluding the Group 3 protozoa *Endolimax nana* and *Entamoeba coli),* as follows:

Combination of Group 1 organisms

- *Campylobacter* + *Salmonella* (three specimens/ two episodes)
- *Campylobacter* + *Cryptosporidium* (two specimens/ two episodes)
- *Campylobacter* + *Giardia*, (two specimens/ two episodes)
- *Giardia* + *Yersinia enterocolitica* (two specimens/ two episodes)
- *Giardia* + *E coli* O157: H7 (one specimen/episode)

**Table 5.**

**Seasonality of episodes of infection with Group 1 and Group 2 gastrointestinal pathogens detected in a one year study of 716 episodes of gastrointestinal illness in the Eastern Bay of Plenty**

| Organism | Total | Spring (Sept - Nov) | | Summer (Dec - Feb) | | Autumn (March - May) | | Winter (June - Aug) | |
|---|---|---|---|---|---|---|---|---|---|
| | | Number | (%) | Number | (%) | Number | (%) | Number | (%) |
| *Campylobacter* | 50 | 20 | (40)* | 14 | (28) | 5 | (10) | 11 | (22) |
| *Salmonella* | 15 | 4 | (27) | 9 | (60)* | 2 | (13) | 0 | |
| *Yersinia* | 21 | 10 | (48)* | 6 | (29) | 2 | (9) | 3 | (14) |
| *E coli* O157 | 2 | 1 | (50) | 1 | (50) | 0 | | 0 | |
| *Vibrio* | 1 | 0 | | 1 | (100) | 0 | | 0 | |
| *Rotavirus* | 34 | 8 | (23) | 2 | (6) | 4 | (12) | 20 | (59)* |
| *Giardia* | 38 | 9 | (24) | 11 | (29) | 3 | (8) | 15 | (39)* |
| *Cryptosporidium* | 16 | 9 | (56)* | 1 | (6) | 3 | (19) | 3 | (19) |
| *Aeromonas* | 15 | 2 | (13.5) | 8 | (53)* | 3 | (20) | 2 | (13.5) |
| *Plesiomonas* | 5 | 0 | | 4 | (80)* | 0 | | 1 | (20) |
| *Dientamoeba* | 7 | 1 | (14) | 1 | (14) | 0 | | 5 | (72)* |
| *Blastocystis* | 86 | 18 | (21) | 20 | (23) | 16 | (19) | 32 | (37)* |

* Denotes the season during which the most cases were detected.

1 For organisms for which there were fewer than five isolates, no conclusions can be drawn.

2 For other organisms, each showed a season during which the rate of detection increased: *Giardia*, *Dientamoeba*, *Blastocystis* and rotavirus showed a winter increase; *Campylobacter*, *Yersinia* and *Cryptosporidium* showed a spring increase; and *Salmonella*, *Aeromonas* and *Plesiomonas* showed a summer increase.

3 Despite showing a seasonality, all organisms for which there were more than four isolates were noted in at least two seasons. All except *Plesiomonas*, *Salmonella*, and *Dientamoeba* were detected throughout all seasons.

4 The numbers of *Salmonella*, *Plesiomonas* and *Dientamoeba* isolates are insufficient to determine if testing in certain seasons could be discontinued

**Note: If testing for any organism was restricted to only the season during which incidence was greatest, significant under-diagnosis would occur.**

Combination of Group 1 and Group 2 organisms

- *Campylobacter* + *Blastocystis* (five specimens/ three episodes)
- *Giardia* + *Blastocystis* (seven specimens/ five episodes)
- *Cryptosporidium* + *Blastocystis* (two specimens/two episodes)
- *Yersinia enterocolitica* + *Blastocystis* (two specimens/ two episodes)
- *Giardia* + *Blastocystis* + *Dientamoeba* (one specimen/ episode)
- *Salmonella* + *Blastocystis* (one specimen/episode)
- *Cryptosporidium* + *Aeromonas* + *Yersinia frederiksenii* (one specimen/ episode)
- *Campylobacter* + rotavirus + *Blastocystis* (one specimen/ episode)
- *Campylobacter* + rotavirus + *Yersinia kristensenii* (one specimen/ episode)
- *Salmonella* + *Aeromonas* (two specimens/ one episode)
- *Salmonella* + *Aeromonas* + *Giardia* (one specimen/ episode)
- *Giardia* + *Aeromonas* + *Blastocystis* (one specimen/ episode)
- *Vibrio cholerae* non-O1 non-O139 + Plesiomonas (two specimens/ one episode)

Combination of Group 2 organisms

- *Blastocystis* + *Aeromonas* (two specimens/ two episodes)
- *Blastocystis* + *Dientamoeba* (five specimens/ three episodes)
- *Blastocystis* + *Plesiomonas* (one specimen/episode)
- *Aeromonas* + *Plesiomonas* (one specimen/ episode)

Thus in 15% of organism-positive episodes, more than one potential pathogen was detected.

### 4.2.3 Multiple specimens

For 553 episodes of illness, a single specimen was submitted. For 163 episodes of illness between two and seven specimens were submitted. Of these 163 episodes, 81 (49.6%) yielded Group 1 or Group 2 organisms. For 69 (85.1%) of these 81 episodes, all organisms were detected in the first specimen submitted. The remaining infections were detected on subsequent specimens as follows:

**Table 6.**

**Macroscopic form and microscopic findings for stool specimens positive for Group 1 and Group 2 gastrointestinal pathogens, found during a one year study in the Eastern Bay of Plenty and for which this information was record**

| | **Macroscopy** | | | | | | **Microscopy*** | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Organism (number of specimens) | Formed Number | (%) | Semiformed Number | (%) | Unformed Number | (%) | Fluid Number | (%) | NAD Number | (%) | WBC Number | (%) | RBC Number | (%) | Protozoa Number | (%) |
| *Campylobacter* (59) | 4 | (7) | 4 | (7) | 36 | (61) | 15 | (25) | 35 | (55) | 22 | (35) | 15 | (24) | 5 | (8) |
| *Salmonella* (32) | 5 | (16) | 2 | (6) | 16 | (50) | 9 | (28) | 18 | (72) | 6 | (24) | 4 | (16) | 0 | |
| *Yersinia* (23) | 4 | (17) | 2 | (9) | 16 | (70) | 1 | (4) | 16 | (70) | 4 | (17) | 2 | (9) | 3 | (13) |
| *Giardia* (52) | 9 | (17.5) | 10 | (19) | 26 | (50) | 7 | (13.5) | 20 | (39) | 8 | (16) | 6 | (12) | 25 | (49) |
| *Cryptosporidium* (19) | 2 | (10.5) | 2 | (10.5) | 11 | (58) | 4 | (21) | 15 | (79) | 1 | (5) | 0 | | 3 | (16) |
| *Rotavirus* (36) | 2 | (5.5) | 5 | (14) | 26 | (72) | 3 | (8.5) | 32 | (86) | 5 | (14) | 2 | (5) | 0 | |
| *Aeromonas* (16) | 0 | | 2 | (12.5) | 13 | (81) | 1 | (6.5) | 12 | (80) | 3 | (20) | 2 | (13) | 0 | |
| *Plesiomonas* (6) | 0 | | 0 | | 5 | (83.3) | 1 | (16.7) | 3 | (50) | 2 | (33) | 1 | (16.6) | 1 | (16.6) |
| *Dientamoeba* (9) | 0 | | 0 | | 9 | (100) | 0 | | 3 | (33) | 0 | | 0 | | 6 | (67) |
| *Blastocystis* (106) | 17 | (16) | 21 | (20) | 60 | (56.5) | 8 | (7.5) | 56 | (53) | 5 | (5) | 3 | (3) | 45 | (43) |

\# the information was not recorded for all specimens, see text (4.3.1) for explanation

* NAD, no microscopic abnormalities detected; WBC, white blood cells; RBC, red blood cells. The microscopic findings: WBC, RBC and Protozoa are not mutually exclusive, thus the percentages for an organism may exceed 1

1 More than 50% of specimens which were positive for any organism were macroscopically "unformed".

2 More than 50% of specimens which were positive for any organism, except *Giardia* and *Dientamoeba*, showed NAD on microscopy

**Note: There is no obvious correlation between specimen form or microscopic appearance and pathogen type. Although some organisms such as *Plesiomonas* and *Dientamoeba* were detected in only some specimens types, the nu of isolates of these organisms are too few to draw conclusions.**

- Two cases of *Aeromonas* infection where three specimens were submitted on the one day and only one specimen yielded the organism
- One case submitted three specimens, the first two yielded *Giardia* and *Blastocystis*, the third, *Giardia*, *Blastocystis* and *Aeromonas species*
- Two cases of *Yersinia enterocolitica* detected only on the second specimen
- One case of *Salmonella* plus *Campylobacter*, from which the *Campylobacter* was only detected in the second specimen
- One case of *Campylobacter* plus *Cryptosporidium* from which the *Campylobacter* was only detected in the second specimen
- One case of *Salmonella* plus *Aeromonas* plus *Giardia* (detected by EIA only) where only the *Salmonella* was isolated from the first specimen
- Two cases of *Campylobacter* where multiple specimens were submitted on the same day and not all specimens yielded the organism
- One case of *Blastocystis* where the organism was not detected in the first of three specimens
- One case of *Salmonella* plus *Blastocystis* plus *Campylobacter*, where the *Salmonella* was detected in three of three specimens, the *Campylobacter* was detected in the second specimen only and the *Blastocystis* was detected in the third specimen only.

Thus the following episodes of infection with specific organisms would not have been detected had multiple specimens not been submitted: *Aeromonas*, 4/16 (25%); *Campylobacter*, 5/50, (10%); *Blastocystis*, 2/86, (2.3%); *Giardia*, 1/38 ( 2.6%); *Yersinia*, 2/21 (9.5%).

## 4.3 Method comparison

### 4.3.1 Sensitivity, specificity and costs associated with various detection methods.

The method found to be the most effective for the detection of each organism is shown in Table 7a. The information from which these conclusions were derived is shown in Table 7b, including: the sensitivity of each medium/method for the detection of each organism; the specificities of selected methods for specific organisms; the costs of materials used for selected detection methods; and the amount spent in selecting each positive isolate.

## Table 7a.

## Methods found to be the most effective for the laboratory investigation of faeces for specific pathogens# (based on the results shown in Table 7b)

| Organism | Primary medium | Enrichment medium | Secondary medium | Other test(s) | % positive by this method in our study |
|---|---|---|---|---|---|
| *Campylobacter* | 5% sheep blood agar + cefoperazone (CAMP) | | | | 98.4 |
| | CCDA | | | | 87.7 |
| *Salmonella* | | Selenite | Xylose lysine desoxycholate | | 97.2 |
| *Yersinia* | Yersinia selective agar (YSA) | Selenite | YSA | | 100 |
| *E coli* O157 | Sorbitol MacConkey | | | | 100 |
| *Vibrio* | 5% sheep blood agar | | | | 100 |
| | Thiosulphate citrate bilesalt sucrose agar | | | | 100 |
| *Giardia* | | | | Immunofluorescent (IF) antigen test | 78.8 (87.2)* |
| | | | | Enzyme immunoassay antigen test | 96.1 (95.7)* |
| *Cryptosporidium* | | | | IF antigen test | 100 |
| *Aeromonas* | Aeromonas selective medium | | | | 94.1 |
| *Dientamoeba* | | | | Trichrome stain | 100 |
| *Blastocystis* | | | | Trichrome stain | 84.7 |

\# As no *Shigella* were detected, no medium tested in this study can be recommended as cost-effective, this point is further elaborated in the discussion

* See text (4.3.2) for explanation

NOTE: **The information from the results suggests that Group 1 organisms should be investigated for routinely in all specimens from all ages at all times of year.**

**Routine investigation for Group 2 organisms may not be appropriate until the role of these organisms is better established.**

**Table 7b.**

**Comparative positive yields, sensitivity, specificity and costs in materials for detection methods for Group 1 and Group 2 gastrointestinal pathogens used in a the study of 997 faecal specimens over a one year period in the Eastern Bay of Plenty**

| Organism | Method/ Medium* | detection ratio# | percent sensitivity | visual accuracy ratio+ | percent specificity | cost/ specimen ($) | cost/ isolate ($) |
|---|---|---|---|---|---|---|---|
| *Campylobacter* | 1 | 61/62 | 98.4 | 61/63 | 97 | 0.42 | 6.74 |
| | 2 | 57/65 | 87.7 | 57/65 | 88 | 0.61 | 10.67 |
| *Salmonella* | 3 | 6/26 | 23.1 | | | | |
| | 4 | 23/36 | 63.9 | | | | |
| | 5 | 17/38 | 44.7 | | | | |
| | a3 | 12/17 | 70.6 | | | | |
| | a4 | 35/36 | 97.2 | | | | |
| | a5 | 33/38 | 86.8 | | | | |
| | b3 | 15/38 | 39.5 | | | | |
| | b4 | 23/38 | 60.5 | | | | |
| | b5 | 18/38 | 47.4 | | | | |
| | 3/4/a3/a4 | 35/36 | 97.2 | 33/155 | 23 | 1.33 | 37.7 |
| | 5/b3/b4/b5/a5 | 37/38 | 97.3 | 37/193 | 19 | 2.74 | 73.83 |
| | 4/a4 | 35/36 | 97.2 | 35/138 | 25 | 1.22 | 34.26 |
| *Shigella* | 3/4/a3/a4 | | | 0/390 | 0 | 2.13 | >2118 |
| | 5/b3/b4/b5/a5 | | | 0/605 | 0 | 3.95 | >3943 |
| *Yersinia* | 3 | 3/30 | 10 | | | | |
| | 4 | 1/30 | 3.3 | | | | |
| | 6 | 26/30 | 86.7 | | | | |
| | a3 | 14/30 | 46.7 | | | | |
| | a4 | 2/30 | 6.7 | | | | |
| | a6 | 26/30 | 86.7 | | | | |
| | b3 | 0/30 | 0 | | | | |
| | b4 | 0/30 | 0 | | | | |
| | b6 | 17/30 | 56.7 | | | | |
| | 3/4/a3/a4 | 14/30 | 46.7 | 14/203 | 7 | 1.57 | 110.35 |
| | 6/a6/b6 | 30/30 | 100 | 30/325 | 9 | 3.79 | 125.95 |
| | 6/a6 | 30/30 | 100 | 30/309 | 10 | 2.28 | 75.77 |
| *E coli* O157 | 7 | 3/3 | 100 | 3/309 | 1 | 1.20 | 398.80 |
| *Vibrio|* | 8 | 2/2 | 100 | 7/55 | 13 | 0.44 | 107.91 |
| | 9 | 2/2 | 100 | 2/474 | 0.4 | 0.79 | 393.42 |
| *Aeromonas|^* | 8 | 4/15 | 26.7 | | | | |
| | 10 | 16/17 | 94.1 | 17/166 | 10 | 0.81 | 50.47 |
| *Plesiomonas|^* | 8 | 2/6 | 33.3 | | | | |
| | b4 | 5/6 | 83.3 | | | | |

**Table 7b, continued.**

**Comparative positive yields, sensitivity, specificity and costs in materials for detection methods for Group 1 and Group 2 gastrointestinal pathogens used in a the study of 997 faecal specimens over a one year period in the Eastern Bay of Plenty**

| Organism | Method/ Medium* | detection ratio# | percent sensitivity | visual accuracy ratio+ | percent specificity | cost/ specimen ($) | cost/ isolate ($) |
|---|---|---|---|---|---|---|---|
| *Giardia* | 11 | 25/52 (25/47) | 48.1 (53.2) | | | | |
| | 12 | 39/52 (39/47) | 75.0 (83) | | | | |
| | 13 | 34/52 (34/47) | 65.4 (72) | | | | |
| | 14 | 50/52 (45/47) | 96.1 (95.7) | | | 6.04** | 120.43 (133.82) |
| | 15 | 41/52 (41/47) | 78.8 (87.2) | | | 5.74** | 93.82*** |
| *Dientamoeba* | 11 | 5/9 | 55.6 | | | | |
| | 12 | 9/9 | 100 | | | | |
| *Cryptosporidium* | | | | | | | |
| | 16 | 11/20 | 55 | | | | |
| | 17 | 20/20 | 100 | | | | *** |
| *Blastocystis* | 11 | 47/109 | 42.3 | | | | |
| | 12 | 94/111 | 84.7 | | | | |
| | 13 | 38/111 | 34.2 | | | | |

* 1, 5% sheep blood agar plus cefoperazone (CAMP); 2, blood-free *Campylobacter* medium (CCDA); 3, MacConkey agar (Mac); 4, xylose lysine desoxycholate agar (XLD); 5, hektoen enteric agar (Hek); 6, *Yersinia* selective agar (YSA); 7, Sorbitol MacConkey agar (SMAC); 8, 5% sheep blood agar (SBA); 9, Thiosulphate citrate bilesalts sucrose agar (TCBS); 10, *Aeromonas* selective agar (ASM); 11, direct wet preparation; 12, trichrome stain; 13, concentration; 14, *Giardia* enzyme immunoassay; 15, *Giardia* immunofluorescence; 16, modified Kinyoun stain; 17, *Cryptosporidium* immunofluorescence; a, selenite (Sel) enrichment and subsequent subculture; b, Gram negative broth (GN) enrichment and subsequent subculture.

\# number positive by this method/number positive by all methods

\+ number positive by this method/number deemed by colonial observation to potentially be the organism sought

| 5% sheep blood agar was used as a selective medium for all Vibrionaceae, thus the number positive (7) represents Vibrio, Aeromonas and Plesiomonas detected using this medium

^ one isolate was detected on a medium other than that specified

** the cost of performing the antigen testing was calculated using the cost of the kit at the time of the study, and included the cost of two controls in each weekly batch.

*** the cost per positive isolate included both *Giardia* and *Cryptosporidium* (total 61 detected by this method) as the method detected both of these parasites.

The number of isolates reported for each medium do not always match the organism total. This is for a variety of reasons including:

- The laboratory routine procedure did not specifically evaluate each agar. If *Salmonella* or *Yersinia* were isolated on primary media, these organisms were not further sought on subculture media.

- Laboratory staff inadvertently discarded nine laboratory reports at some time during the study period and these results are not included.

- The routine laboratory continued its procedure of investigating subsequent specimens from known cases for only the known organism - thus *Salmonella* follow up specimens were processed only for *Salmonella*.

### 4.3.2 Commentary on the detection methods for various microorganisms

***Campylobacter***

CAMP cost $0.35 per plate and CCDA, $0.55. CAMP failed to detect the one *Campylobacter* which was not *Campylobacter jejuni*. CCDA failed to detect eight *Campylobacter jejuni*.

***Salmonella***

The cost calculations are based on selection for further testing: hydrogen sulphide producing ($H_2S$ +) colonies on XLD and Hek; and non-lactose fermenting colonies on Mac when $H_2S$ + colonies had been noted on other media for the specimen.

Organisms not identified as *Salmonella* were predominantly *Citrobacter species and Proteus species*.

***Shigella***

The cost calculations were based on selection of non-lactose fermenting colonies for further testing, in the absence of hydrogen sulphide producing colonies on other media. Organisms were subsequently identified as *Escherichia coli, Hafnia alvei*, or other members of the *Enterobacteriaceae.*

***Escherichia coli* O157**

Both isolates were detected on the sorbitol MacConkey agar. Organisms not identifying as *Escherichia coli* O157 were predominantly other *Escherichia coli.*

***Yersinia***

The *Yersinia* were all isolated on either YSA, or the Sel subculture to YSA. Organisms not identifying as *Yersinia* from YSA were predominantly *Citrobacter species*. This organism grew well on the selective medium, resulting in a low specificity.

***Vibrio***

Organisms growing on thiosulphate citrate bile-salt sucrose medium (TCBS) were first plated onto SBA in order to perform an oxidase test. The majority of organisms which grew were detected only after incubation for two days and were predominantly *Staphylococcus species and Enterococcus species.* The *Vibrio* were readily detected after 24 hours, as were some members of the *Enterobacteriaceae.*

***Giardia***

The specificity of all methods, except the enzyme immunoassay (EIA), was considered to be 100% as the organism was visualised and confirmed as conforming to the typical morphology.

On nine occasions the EIA was the only test positive for *Giardia*. Two of the nine were positive for *Giardia* by other methods in other specimens from the illness episode, and were considered to be true positives. Two were from specimens from one episode of illness and were also considered to be true positives. The remaining five positive EIA

specimens were either the only specimen from an episode of illness, or were the only positive specimen from a multiple specimen episode of illness. Thus these five results were not confirmed as positive. The sensitivities of the *Giardia* methods are shown with both all the EIA positive specimens being included (total 52) and with the five unsubstantiated positive results excluded (total 47).

One positive result was detected by trichrome stain only and another by immunofluorescence only.

***Cryptosporidium***

The specificity of each method, was considered to be 100% as the organism was visualised and confirmed as conforming to the typical morphology.

The immunofluorescent method detected organisms in nine specimens which were not detected by a five minute screen using the modified Kinyoun (ZN) stain. To confirm that the immunofluorescent results were true positives, each corresponding ZN stain was re-examined and further ZN-stained films prepared. In all cases, acid fast organisms resembling *Cryptosporidia* were eventually noted, but often in very low numbers. As these acid fast organisms were not detected within the limits set for the method, the ZN result was recorded as negative.

***Aeromonas***

The majority of organisms growing on ASM and being selected for further testing were Pseudomonads. One of the *Aeromonas* isolates was found only on the TCBS medium.

***Plesiomonas***

None of the media included in this study was considered to be selective for Plesiomonas, thus sensitivity, specificity and costs have not been calculated for this organism

***Dientamoeba***

The specificity of the trichome stain was considered to be 100% as the organism was visualised and confirmed as conforming to the typical morphology. The wet preparation allowed recognition of protozoal structures but did not allow confirmation of species.

***Blastocystis***

The specificity of the trichome stain was considered to be 100% as the organism was visualised and confirmed as conforming to the typical morphology. The wet preparation allowed recognition of protozoal structures but did not allow confirmation of species.

## 4.4 Symptoms and consequences of infection

Of the 716 cases of illness, 109 (15.2%) were hospitalised and a further 13 (1.8%) patients were referred to hospital specialists. Symptom duration was less than a week for 241 (40%) patients. 236 patients (39%) noted that symptoms had been occurring for between one week and one month and 131 (21%) had been symptomatic for greater than one month. Of the 281 who attended either work or school, 182 (65%) had taken sick leave because of their symptoms. 156 cases (26%) reported that other householders were ill.

The symptoms and consequences of infection with each of the Group 1 and group 2 organisms of which there were more than four cases are shown in Table 8.

## 4.5 Risk factor analysis

Of the water supplies included in the questionnaire, all but Ruatoki were noted by more than four respondents, thus all but Ruatoki were included in the analysis. In addition, 111 respondents listed a home water supply as being consumed in the month prior to illness and this was also included in the analysis.

Analysis of the risk factor information showed that 97 people had handled farm animals in the month prior to illness. Of the animals listed by respondents, cows, pigs, sheep and horses were each noted by more than four cases and were therefore included as risks in the analysis.

Table 9 shows the risk activities for each organism for which analysis showed either a significant relative risk and/or and a significant chi-square p-value. The full set of risk analysis tables may be obtained from the author.

**Table 8.**

**Symptoms and consequences reported by all cases of illness, and cases of infection with Group 1 and Group 2 gastrointestinal pathogens (except *E coli* O157 and *Vibrio*), who submitted faeces specimens for a one year study in the Eastern Bay of Plenty**

| | | Cases | | | | | |
|---|---|---|---|---|---|---|---|
| **Symptoms** | **All** | ***Campylobacter*** | ***Salmonella*** | ***Yersinia*** | **Rotavirus** | ***Giardia*** | ***Cryptosporidium*** |
| Abdominal cramping | 212/611 (35%) | 20/43 (46%) | 5/11 (46%) | 6/16 (38%) | 1/24 (4%) | 16/31 (52%) | 4/15 (27%) |
| Abdominal pain | 333/611 (54%) | 30/43 (68%) | 6/11 (55%) | 11/17 (65%) | 4/24 (17%) | 17/31 (55%) | 6/15 (40%) |
| Vomiting | 186/611 (30%) | 9/43 (21%) | 6/12 (50%) | 0/16 (0%) | 24/27 (89%) | 10/31 (32%) | 6/15 (40%) |
| Diarrhoea | 535/655 (82%) | 45/46 (98%) | 11/12 (92%) | 13/16 (81%) | 29/32 (91%) | 32/34 (89%) | 14/16 (88%) |
| Constipation | 41/607 (7%) | 0/42 (0%) | 0/11 (0%) | 0/16 (0%) | 0/34 (0%) | 4/31 (13%) | 0/15 (0%) |
| Nausea | 206/609 (34%) | 14/42 (33%) | 5/11 (46%) | 4/16 (25%) | 1/24 (4%) | 21/33 (64%) | 4/15 (27%) |
| Lethargy | 221/607 (36%) | 16/42 (38%) | 6/11 (55%) | 10/16 (63%) | 8/24 (33%) | 12/31 (39%) | 3/15 (20%) |
| Foul smelling stools | 270/610 (44%) | 22/42 (52%) | 6/11 (55%) | 7/16 (44%) | 16/25 (64%) | 17/31 (55%) | 11/16 (69%) |
| Increased flatulence | 233/608 (38%) | 11/42 (26%) | 4/11 (34%) | 8/16 (50%) | 2/25 (8%) | 14/31 (45%) | 4/15 (27%) |
| Bloody stools | 53/608 (9%) | 8/42 (19%) | 1/11 (9%) | 2/16 (13%) | 1/24 (4%) | 5/33 (15%) | 0/15 (0%) |
| Increased belching | 101/606 (17%) | 4/42 (10%) | 1/11 (9%) | 3/16 (19%) | 2/24 (8%) | 5/31 (16%) | 0/15 (0%) |
| **Duration** | | | | | | | |
| Less than 1 week | 241/604 (40%) | 24/42 (57%) | 7/10 (70%) | 5/16 (32%) | 20/27 (74%) | 6/31 (19%) | 10/15 (67%) |
| Between 1 week and 1 month | 236/604 (39%) | 15/42 (35%) | 3/10 (30%) | 10/16 (63%) | 5/27 (19%) | 17/31 (55%) | 5/15 (33%) |
| More than one month | 131/604 (21%) | 3/42 (8%) | 0/10 (0%) | 1/16 (6%) | 2/27 (7%) | 8/31 (26%) | 0/15 (0%) |
| **Consequences** | | | | | | | |
| Hospitalised | 109/653 (18%) | 4/45 (10%) | 5/12 (42%) | 1/16 (6%) | 13/31 (42%) | 2/34 (6%) | 1/15 (7%) |
| Required leave from work/school | 182/281 (65%) | 23/26 (89%) | 5/5 (100%) | 11/16 (69%) | 8/8 (100%) | 9/12 (75%) | 5/5 (100%) |
| Other householders ill | 156/605 (26%) | 4/42 (10%) | 3/11 (27%) | 3/15 (20%) | 5/25 (20%) | 17/34 (50%) | 6/14 (43%) |

**Table 8, continued**

**Symptoms and consequences reported by all cases of illness, and cases of infection with Group 1 and Group 2 gastrointestinal pathogens (except *E coli* O157 and *Vibrio*), who submitted faeces specimens for a one year study in the Eastern Bay of Plenty**

| | Cases | | | |
|---|---|---|---|---|
| | ***Plesiomonas*** | ***Aeromonas*** | ***Dientamoeba*** | ***Blastocystis*** |
| **Symptoms** | | | | |
| Abdominal cramping | 3/5 (60%) | 1/11 (9%) | 2/7 (29%) | 32/68 (47%) |
| Abdominal pain | 2/5 (40%) | 4/11 (36%) | 1/7 (14%) | 40/69 (58%) |
| Vomiting | 3/5 (60%) | 2/11 (18%) | 0/7 (0%) | 22/69 (32%) |
| Diarrhoea | 5/5 (100%) | 8/12 (67%) | 6/7 (86%) | 53/71 (75%) |
| Constipation | 1/5 (20%) | 2/11 (18%) | 0/7 (0%) | 6/68 (9%) |
| Nausea | 3/5 (60%) | 5/11 (46%) | 1/7 (14%) | 32/69 (46%) |
| Lethargy | 2/5 (40%) | 3/11 (27%) | 4/7 (57%) | 25/68 (37%) |
| Foul smelling stools | 4/5 (80%) | 8/11 (73%) | 4/7 (57%) | 32/68 (47%) |
| Increased flatulence | 2/5 (40%) | 3/11 (27%) | 3/7 (43%) | 33/68 (49%) |
| Bloody stools | 1/5 (20%) | 0/11 (0%) | 0/7 (0%) | 3/68 (4%) |
| Increased belching | 2/5 (40%) | 1/11 (9%) | 2/7 (29%) | 14/68 (21%) |
| **Duration** | | | | |
| Less than 1 week | 5/5 (100%) | 2/11 (18%) | 0/7 (0%) | 22/69 (32%) |
| Between 1 week and 1 month | 0/5 (0%) | 5/11 (45%) | 5/7 (71%) | 29/69 (42%) |
| More than one month | 0/5 (0%) | 4/11 (36%) | 2/7 (29%) | 18/69 (26%) |
| **Consequences** | | | | |
| Hospitalised | 3/5 (60%) | 1/12 (8%) | 0/7 (0%) | 6/69 (9%) |
| Required leave from work/school | 1/1 (100%) | 1/4 (25%) | 0/4 (0%) | 22/37 (59%) |
| Other householders ill | 2/4 (50%) | 2/8 (25%) | 4/7 (57%) | 15/70 (21%) |

**NOTE: There are no obvious correlations between symptoms and pathogens, this point is further elaborated in the discussion**

**Table 9.**

**Significant associations with infection with specific Group 1 and Group 2 organisms compared with all other cases from whom the organism was not detected (non-cases) demonstrated by univariate analysis of exposures and risks of infection**

***Campylobacter species***

| Exposure | No. of Cases Exposed | No. of Cases | No. of Non-cases Exposed | No. of Non-cases | Relative Risk | 95% Confidence Interval | chi square[@@] P-value |
|---|---|---|---|---|---|---|---|
| Consumption of water from the Opotiki supply# | 13 | 43 | 86 | 561 | *2.21* | 1.20 - 4.08 | 0.011MH |
| Consumption of unpasteurised milk: | | | | | | | |
| within a month of onset | 8 | 43 | 20 | 557 | *4.67* | 2.39 - 9.11 | <0.001F |
| within a week of onset | 7 | 43 | 11 | 557 | *6.29* | 3.25 - 12.16 | <0.001F |

**# Note: this implicates presence in the Opotiki area as a risk factor, rather than actually consuming water from the town supply (see discussion).**

***Salmonella species***

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Overseas travel within a week of onset | 2 | 11 | 16 | 590 | *7.20* | 1.67 - 20.95 | 0.040F |
| Eaten a barbecued meal within a week of onset | 4 | 11 | 63 | 589 | *4.55* | 1.37 - 15.12 | 0.026F |
| Eaten shellfish within a week of onset | 5 | 11 | 103 | 589 | *3.80* | 1.18 - 12.21 | 0.032F |

[@@] F = Fisher exacted 2-tailed P-value
MH = Mantel-Haenszel P-value
* = Statistically significant at 95% level ($P \leq 0.05$)

**Table 9, continued.**

**Significant associations with infection with specific Group 1 and Group 2 organisms compared with all other cases from whom the organism was not detected (non-cases) demonstrated by univariate analysis of exposures and risks of infection**

***Yersinia species***

| Exposure | No. of Cases Exposed | No. of Cases | No. of Non-cases Exposed | No. of Non-cases | Relative Risk | 95% Confidence Interval | chi square** P-value |
|---|---|---|---|---|---|---|---|
| Consumption of water from a home supply | 7 | 16 | 104 | 589 | *3.46* | 1.32 - 9.10 | 0.016F |
| Handled farm animals: | | | | | | | |
| within a month of onset | 8 | 15 | 89 | 587 | *5.95* | 2.21 - 16.03 | <0.001F |
| within a week of onset | 6 | 15 | 68 | 585 | *4.74* | 1.74 - 12.93 | 0.006F |
| Handled: | | | | | | | |
| cows within a month of onset | 5 | 15 | 51 | 587 | *4.88* | 1.73 - 13.76 | 0.008F |
| sheep within a month of onset | 3 | 15 | 7 | 587 | *14.80* | 4.93 - 44.46 | 0.001F |

** F = Fisher exacted 2-tailed P-value
MH = Mantel-Haenszel P-value
* = Statistically significant at 95% level ($P \leq 0.05$)

**Table 9, continued.**

**Significant associations with infection with specific Group 1 and Group 2 organisms compared with all other cases from whom the organism was not detected (non-cases) demonstrated by univariate analysis of exposures and risks of infection**

***Giardia lamblia***

| Exposure | No. of Cases Exposed | No. of Cases | No. of Non-cases Exposed | No. of Non-cases | Relative Risk | 95% Confidence Interval | chi square** P-value |
|---|---|---|---|---|---|---|---|
| Consumption of water from the: | | | | | | | |
| Matata supply# | 2 | 31 | 9 | 572 | 3.71 | 1.01 - 13.66 | 0.105F |
| Waimana supply# | 2 | 31 | 5 | 572 | *5.87* | 1.73 - 19.97 | 0.046F |
| Consumption of unpasteurised milk: | | | | | | | |
| within a month of onset | 5 | 31 | 23 | 569 | *3.93* | 1.63 - 9.46 | 0.011F |
| within a week of onset | 3 | 31 | 15 | 569 | 3.46 | 1.16 - 10.35 | 0.060F |
| Attendance at a day care centre: | | | | | | | |
| within a week of onset | 6 | 31 | 43 | 570 | *2.70* | 1.17 - 6.27 | 0.033F |
| Handled farm animals: | | | | | | | |
| within a month of onset | 11 | 31 | 86 | 571 | *2.86* | 1.42 - 5.78 | 0.009F |
| within a week of onset | 9 | 31 | 65 | 569 | *2.91* | 1.39 - 6.07 | 0.009F |
| Handled: | | | | | | | |
| cows within a month of onset | 8 | 31 | 48 | 571 | *3.39* | 1.59 - 7.22 | 0.005F |
| horses within a month of onset | 3 | 31 | 9 | 571 | *5.27* | 1.85 - 14.97 | 0.002F |

**# Note: this implicates presence in these areas as a risk factor, rather than actually consuming water from the town supply (see discussion).**

** F = Fisher exacted 2-tailed P-value
MH = Mantel-Haenszel P-value
* = Statistically significant at 95% level ($P \leq 0.05$)

**Table 9, continued.**

**Significant associations with infection with specific Group 1 and Group 2 organisms compared with all other cases from whom the organism was not detected (non-cases) demonstrated by univariate analysis of exposures and risks of infection**

***Cryptosporidium***

| Exposure | No. of Cases Exposed | No. of Cases | No. of Non-cases Exposed | No. of Non-cases | Relative Risk | 95% Confidence Interval | chi square** P-value |
|---|---|---|---|---|---|---|---|
| Consumption of water from a home supply | 8 | 15 | 103 | 589 | *5.08* | 1.88 - 13.71 | 0.002F |
| Consumption of unboiled water from a natural waterway within a month of onset | 4 | 14 | 51 | 587 | *3.97* | 1.29 - 12.24 | 0.031F |
| Attendance at a day care centre within a month of onset | 4 | 14 | 61 | 587 | 3.30 | 1.06 - 10.22 | 0.054F |
| Handled farm animals within a month of onset | 6 | 14 | 91 | 587 | *3.90* | 1.38 - 10.98 | 0.015F |
| Handled cows within a month of onset | 5 | 14 | 51 | 587 | *5.41* | 1.88 - 15.58 | 0.006F |
| Cat owner | 12 | 14 | 331 | 586 | *4.50* | 1.02 - 19.91 | 0.029MH |

** F = Fisher exacted 2-tailed P-value
MH = Mantel-Haenszel P-value
* = Statistically significant at 95% level ($P \leq 0.05$)

**Table 9, continued.**

**Significant associations with infection with specific Group 1 and Group 2 organisms compared with all other cases from whom the organism was not detected (non-cases) demonstrated by univariate analysis of exposures and risks of infection**

***Plesiomonas shigelloides***

| Exposure | No. of Cases Exposed | No. of Cases | No. of Non-cases Exposed | No. of Non-cases | Relative Risk | 95% Confidence Interval | chi square** P-value |
|---|---|---|---|---|---|---|---|
| Eaten a barbecued meal within a week of onset | 2 | 4 | 65 | 596 | 7.96 | 1.14 - 55.55 | 0.064F |
| Eaten shellfish within a week of onset | 3 | 4 | 105 | 596 | *13.67* | 1.44 - 130.13 | 0.020F |

***Aeromonas species***

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Overseas travel: | | | | | | | |
| within a month of onset | 2 | 8 | 36 | 595 | 4.96 | 1.03 - 23.73 | 0.085F |
| within a week of onset | 2 | 8 | 16 | 593 | *10.80* | 2.34 - 49.86 | 0.021F |
| Handled pigs within a month of onset | 1 | 8 | 5 | 594 | 14.19 | 2.05 - 98.24 | 0.077F |

***Dientamoeba fragilis***

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Overseas travel within a month of onset | 2 | 7 | 36 | 596 | 5.95 | 1.19 - 29.65 | 0.066F |
| Consumption of unboiled water from a natural waterway within a month of onset | 3 | 7 | 52 | 595 | *7.46* | 1.71 - 32.48 | 0.019F |
| Handled sheep within a month of onset | 1 | 7 | 9 | 595 | 9.87 | 1.31 - 74.58 | 0.111F |

** F = Fisher exacted 2-tailed P-value
MH = Mantel-Haenszel P-value
* = Statistically significant at 95% level ($P \leq 0.05$)

**Table 9, continued.**

**Significant associations with infection with specific Group 1 and Group 2 organisms compared with all other cases from whom the organism was not detected (non-cases) demonstrated by univariate analysis of exposures and risks of infection**

***Blastocystis hominis***

| Exposure | No. of Cases Exposed | No. of Cases | No. of Non-cases Exposed | No. of Non-cases | Relative Risk | 95% Confidence Interval | chi square[**] P-value |
|---|---|---|---|---|---|---|---|
| Consumption of water from the: | | | | | | | |
| Kawerau supply# | 11 | 70 | 160 | 533 | **0.47** | 0.25 - 0.87 | 0.012MH |
| Waimana supply# | 3 | 70 | 4 | 533 | *3.81* | 1.57 - 9.23 | 0.037F |
| Consumption of unboiled water from a natural waterway: | | | | | | | |
| within a month of onset | 14 | 70 | 41 | 532 | *2.49* | 1.48 - 4.16 | <0.001MH |
| within a week of onset | 8 | 70 | 28 | 531 | 2.03 | 1.05 - 3.90 | 0.056 |
| Handled: | | | | | | | |
| sheep within a month of onset | 3 | 70 | 7 | 532 | 2.65 | 1.00 - 7.02 | 0.099F |
| horses within a month of onset | 4 | 70 | 8 | 532 | *2.98* | 1.30 - 6.85 | 0.040F |
| Eaten a takeaway meal within a week of onset | 30 | 70 | 157 | 530 | *1.66* | 1.07 - 2.57 | 0.025MH |

** **A protective risk, that is the activity appears to protect against infection.**

# **Note: this implicates presence in the Opotiki area as a risk factor, rather than actually consuming water from the town supply (see discussion).**

[**] F = Fisher exacted 2-tailed P-value
MH = Mantel-Haenszel P-value
* = Statistically significant at 95% level ($P \leq 0.05$)

# 5 DISCUSSION

## 5.1 Incidence of infection with organisms capable of causing gastroenteritis

The incidence of notifiable organisms was higher in our study than the notified rates for the EBOP for the same time period. However, the published notification data was based on six months which was annualised. This may account for the discrepancy. Under-notification occurs, for example, Lane and Baker (1993) noted that of 784 cases of campylobacteriosis reported by laboratories from the Wellington region, only 660 (84%) were notified by practitioners. Presumably this applies to all notifiable organisms; so, although the incidences found in our study are higher than the notified rates, they can be used for comparing relative incidence of organisms in our study population.

It may be questioned as to whether our study measures incidence or prevalence. Incidence is defined as the number of instances of illness commencing during a given period in a specified population, whereas prevalence indicates the number of instances of illness in a specified population at a point in time without distinction between old and new cases (Anonymous, 1966). Of the 233 cases who completed a questionnaire and from whom either a Group 1 or Group 2 organism was isolated, 195 (86%) reported being ill for less than one month at the time of submitting a sample. Most of the 38 who were ill for longer than one month submitted their specimens more than one month after the study commenced. Thus, a few of the cases we detected may have become ill prior to the time the study commenced; but overall, our results indicate incidence.

The incidence rates calculated here are an underestimate of the level of infection in the EBOP community. It is recognised that not all cases are symptomatic; not all symptomatic cases seek medical assistance; practitioners' requesting habits vary and not all practitioners request specimens routinely from diarrhoeal cases; and not all cases who are requested to submit a faecal specimen do so. In order to ascertain the true incidence of infection within a community, a more comprehensive study would be necessary.

Doern (1989) suggested that if an organism is more prevalent than *Salmonella*, *Shigella* or *Campylobacter*, it should be sought routinely in stool culture. If this rationale is applied to our population, all specimens would be routinely screened for all the organisms sought in this study as all were more prevalent than *Shigella*, which was not detected.

## 5.2 Specimen form and microscopy

As shown in Table 6, the specimen form was not a useful predictor of which pathogen is likely to be present as >50% of all specimens from which a pathogen was isolated were unformed. Although no Aeromonads, *Dientamoeba* or Plesiomonads were isolated from formed specimens, it would be inappropriate to exclude such specimens from investigation for these organisms as the sample number is too small to draw conclusions on which to base such recommendations.

Guerrant and Bobak (1991) advocated screening specimens initially for white blood cells (WBC) and culturing only those in which WBC are seen. But, as seen in Table 6, the majority of specimens in which pathogens were detected showed no abnormality on microscopy. For example, 65% of *Campylobacter*-positive specimens and 76% of *Salmonella*-positive specimens did not show WBC's on microscopy.

## 5.3 Age of patient

Janda and Abbott (1992a) advocated that selection of specimens for testing for bacteria other than *Salmonella*, *Shigella* or *Campylobacter* could be based on patient age. Table 4 shows that, of the organisms which were sought in all specimens, all but *Cryptosporidium* were more frequently seen in adults than in children or infants. No organism was associated with only one age group. Therefore any attempt to select faeces for testing on the basis of the age of the patient will result in under-diagnosis.

Rotavirus has been considered an infection of young children only and several investigators have advocated testing for this organism only if the patient is less than three years of age. Resource constraints meant that a similar rationale was applied in our study and testing was undertaken only on specimens from patients over five years when this was specifically requested by a physician. On both of the occasions when this was done, the result was positive. The results of Pryor *et al.*, (1987) in a prospective study of 74 adults with acute symptoms showed that seven (9.5%) were rotavirus positive. This indicates that this traditional infant pathogen may be a more common cause of adult infection than is currently accepted and its significance in people of all ages in New Zealand needs to be established.

## 5.4 Correlation of season with the prevalence of pathogen

The numbers of cases of infection with *Vibrio* (1), *E coli* O157 (2), *Plesiomonas* (5) and *Dientamoeba* (7) were insufficient to draw conclusions about their seasonality.

Table 5 shows that some of the remaining organisms exhibited seasonal variation. *Campylobacter* and *Cryptosporidium* exhibited spring peaks (August - October), an internationally recognised characteristic of both organisms. The *Campylobacter* peak is also in accordance with the annual notification peak for campylobacteriosis in New Zealand. *Campylobacter* incidence remained relatively high through the summer months, but only one case of *Cryptosporidium* (6%) in the period December - February.

*Salmonella* exhibited a summer peak (February), which mirrors the national notification peak. We detected no cases of salmonellosis during the winter months. However, the national notification data show that although infection with this organism peaks in the summer months, infection occurs throughout the year. Thus if the time period during which this organism was sought were restricted, under-diagnosis would result.

Rotavirus is considered to be more prevalent in winter. In our study a peak was noted in winter, but a second peak occurred in November. So, if testing had been performed only in the winter months (April - August), 32% of cases would not have been detected.

The remaining organisms each showed a seasonal peak prevalence but were detected throughout all seasons. The data here coupled with national notification data indicate that investigation for *Campylobacter*, *Salmonella*, *Yersinia, Giardia, Blastocystis, Aeromonas* should be performed throughout the year.

Our data for *Cryptosporidium* suggest that there are some months when the organism is not present in the study population and therefore testing could be restricted to certain time periods. However, our case number is small and a previous New Zealand study of 54 cases found that although cases peaked in the spring and early summer, they were detectable throughout the year (Carter, 1986). Thus it is probable that restricting testing to certain seasons would compromise detection of cases.

## 5.5 Mixed infections

Mixed infections occur and if all specimens are not investigated for all organisms, under-diagnosis will occur. Many workers recommend investigating the stools of young children for rotavirus only and testing for other organisms only if this test is negative. In this study, two cases (4%) of *Campylobacter* would have gone undetected if this rationale had been followed. Other investigators recommend screening for parasites only if bacterial culture is negative. Had this rationale been applied seven cases of giardiasis (18%) would have gone undetected and, more importantly, untreated. Likewise five cases (31%) of cryptosporidiosis would not have been detected. The data also show the importance of testing for both bacteria and parasites simultaneously when a Group 2 parasite is detected. *Blastocystis hominis* was detected on 16 occasions when a Group 1 bacteria or parasite was also present. Had the testing for Group 1 organisms not been undertaken, the *Blastocystis* would have been presumed to be the cause of the symptoms.

## 5.6 Multiple Specimens

Approximately 15% of organisms causing infections were not detected in the first specimen submitted, which indicates that more than one faecal test is necessary to exclude an infectious cause for symptoms. If a pathogen is found in the first specimen, it may not be the sole pathogen involved in the disease process.

## 5.7 Comparison of Methods of Organism detection

### 5.7.1 *Campylobacter*

Of all the organisms sought in this study, *Campylobacter* was the most cost effective to both isolate and to identify.

Of the two media used to isolate it, the blood-based medium (CAMP) was more sensitive and cheaper than the charcoal-based CCDA medium. However, the CCDA medium was inoculated later in the day, and this could have resulted in loss of organism culturability. The multiple specimen data show that 10% of *Campylobacter* was not detected in the first

specimen submitted. This suggests that loss of viability may occur following specimen collection, which could affect detection of this organism.

It is interesting to note that the isolate presumptively identified as *Campylobacter upsaliensis* was isolated only on CCDA. This species is susceptible to the first generation cephalosporin, cephalothin. Both CAMP and CCDA contain 32 mg/l of the third generation cephalosporin, cefoperazone. The other constituents of the CCDA may have assisted the organism to grow. Alternatively, the charcoal in the CCDA may have reduced the potency of the cefoperazone by partial adsorption. This phenomenon has been noted in resistotyping tests by other workers (Wareing D. 1995. Microbiologist, Royal Preston Hospital, England, personal communication).

Endtz *et al.,* (1991) evaluated six *Campylobacter* isolation media including CCDA. Although two blood-based media were incorporated in their study, 5% sheep blood agar with 32 mg/l cefoperazone was not. The most sensitive medium in the study of Endtz *et al.,* (1991) was a charcoal-based medium (CSM), supplemented with haemin and made selective by the incorporation of vancomycin, cycloheximide and cefoperazone; CCDA was the second most sensitive medium, with a sensitivity of 83%; a sensitivity comparable with our finding for CCDA medium of 88%.

Endtz *et al.,* (1991) also found that no one method detected all isolates of *Campylobacter* but that the CSM and the CCDA were more selective than the other media tested. They did not evaluate the importance of multiple specimens, but found that incubation for a total of 72 hours increased the number of isolates.

Two recent documents which detail methods for detection of *Campylobacter* (Gilligan *et al.,* 1992; Grasmick, 1992) do not specifically recommend any one medium because no one medium detects all isolates. Our results indicate that CAMP is a cost effective medium to detect the majority of *Campylobacter* cases; but, to detect the maximum number of cases, multiple specimens and the use of more than one detection medium are necessary.

### 5.7.2 *Salmonella*

Of the three solid media evaluated, XLD was the most sensitive, detecting 63.9% of the salmonella isolates. When combined with selenite broth enrichment, XLD had a sensitivity of 97.2%. When the results of the Hek and Mac cultures were included, the sensitivity did not increase (Table 7b). Gilligan *et al.*, (1992) and Grasmick (1992) recommend the use of an enrichment broth coupled with both a differential medium (Mac or Hek) and a selective medium such as XLD for the detection of *Salmonella*. Our results suggest that a differential medium does not increase the isolation rate and, since it increases the cost, its use is not justified. However, some strains of *Salmonella* ferment sucrose, a reaction which inhibits production of hydrogen sulphide, Bulmash and Fulton (1964). Such strains appear as yellow colonies on XLD and will be recognised only if a lactose-based medium, such as Mac is inoculated (on which they will appear colourless).

Enrichment with GN failed to increase the detection rate of *Salmonella*. This agrees with Doern (1989) who stated that routine use of GN may not result in significantly increased rates of enteric pathogen detection. Doern (1989) also questioned the value of enrichment broths as few laboratories are set up to subculture them at the appropriate time. However, our results show that selenite enrichment increased the detection rate on XLD from 63.9% to 97.2 %.

XLD detected both hydrogen sulphide-producing *Salmonella* and non-$H_2S$-producing Paratyphi A strains. However, to exclude or confirm *Salmonella* considerable expense was necessary. Organisms which gave similar colonial appearance and which could only be excluded by biochemical testing included *Hafnia alvei*, *Citrobacter species* and members of the tribe *Proteeae*. The novel *Salmonella* selective media, Rambach (Rambach 1990), is now available, but this was not used in our study as the price was prohibitive. The value of Rambach medium appears to be that it allows the differentiation of most *Salmonella* (red) from *Proteus species* (colourless) based on colony colour. However, *Salmonella* Typhi and Paratyphi A are indistinguishable from some members of the tribe *Proteeae*. Thus to exclude these serotypes, all colourless colonies have to be investigated which increases the cost and effort involved. It appears that the greatest potential for this medium is in the veterinary field where the serotypes Typhi and Paratyphi A are not likely to be encountered.

A limitation of our findings is that although 38 positive specimens were evaluated, they represented 15 cases, six of whom were from two family groups, thus it is probable that

only 11 strains were evaluated.

Of the media combinations used, the most cost effective method for *Salmonella* detection was the use of XLD and selenite broth. However, it must be noted that selenite is teratogenic and this must be emphasised to those using it. Pregnant women should not prepare this medium, nor should they inoculate it, or subculture from it.

### 5.7.3 *Shigella*

To detect *Shigella* Gilligan *et al.,* (1992) and Grasmick (1992) recommend the use of the use of a differential and a selective medium from the following list: Hek, Mac, XLD, *Salmonella-Shigella* agar, or desoxycholate agar. The prevalence of shigellosis in the Eastern Bay of Plenty is very low and the media used to test for *Shigella* in this study were not very selective. This made it expensive to exclude *Shigella*.

Our data might be used to suggest that the incidence of shigellosis is so low, that testing is uneconomic. However, perusal of the international literature leaves no doubt that the organism should be screened for routinely. Furthermore, national notification data show that *Shigella* are endemic in New Zealand and isolates have been previously reported from the Eastern Bay of Plenty (Okell and Wright, 1990).

A more cost-effective medium/method for detection of *Shigella* is necessary to contain costs. One possible medium is desoxycholate citrate. While it is not widely advocated in the United States, it is widely used in the United Kingdom (Pedlar and Orr, 1990) and should be evaluated in New Zealand.

### 5.7.4 *Yersinia*

Of the media evaluated, the *Yersinia* selective medium (YSA) was the most sensitive and when coupled with selenite enrichment, detected all the isolates detected in the study. Although the combination was sensitive, it was not selective. Only 10% of the isolates on the YSA were confirmed as *Yersinia*. This is mainly because *Citrobacter species* grew well on the medium and produced colonies indistinguishable from *Yersinia*. MacConkey agar was not a suitable selective medium, detecting only 46.7% of strains when used in combination with selenite broth. Thus the findings here do not support Gilligan (1986)

who suggested that if the differential medium (Mac) was examined properly, a yersinia-selective medium was not necessary. The findings do agree with McCarthy and Fenwick (1991) who reported that selenite broth plus YSA was a suitable media combination to detect *Yersinia*. The problem of *Citrobacter species* appearing indistinguishable from *Yersinia* on YSA and the subsequent costs in excluding or confirming *Yersinia* could be overcome by incorporating an indicator in the medium to differentiate between the two organisms. Such an indicator could be a mechanism to detect hydrogen sulphide production - a characteristic of up to 80% of *Citrobacter species* but of 0% *Yersinia*.

Speciation of *Yersinia* is essential, as is biotyping of *Yersinia enterocolitica*. The significance of *Yersinia species*, other than *Yersinia enterocolitica* biotypes 1B - 5 and *Yersinia pseudotuberculosis*, in stool samples is uncertain. It is recommended that other *Yersinia* should be reported, but with a comment noting their uncertain status.

### 5.7.5 *Vibrio*

TCBS is expensive and in this study detected only two isolates (one case) of *Vibrio*. Both were also detected on SBA. TCBS frequently grew organisms other than *Vibrio* so subculture for oxidase testing was necessary to exclude *Vibrio*. These other organisms were usually detectable only after two days incubation and appeared as small colonies when compared with *Vibrio* strains. Thus the selectivity of the medium could be increased by incubating for 24 hours only.

The incidence of *Vibrio* infection was low in the population tested, so the routine use of TCBS is not justified. SBA appears to offer a cost-effective alternative to TCBS, but as the medium is not selective, some cases of infection with *Vibrio* may not be identified with SBA. To improve cost-effectiveness patient history may be used to selectively test for this low-frequency pathogen. Specimens from patients who have a history of recent overseas travel, and who present with fluid diarrhoea, should be cultured on TCBS to ensure that cases of cholera are identified. Shellfish are known to be a reservoir of other *Vibrio* (Table 1), so specimens should be inoculated onto TCBS if a patient has a history of recent shellfish consumption. The case detected in this study had consumed shellfish within seven days of becoming ill.

### 5.7.6 *Escherichia coli* O157

Sorbitol MacConkey agar (SMAC) was effective in detecting the first and third cases of *E coli* O157 in New Zealand (Wright *et al.*, 1993). However, during the course of the study many non-sorbitol-fermenting isolates were investigated and found not to be O157. These results differ from those of March and Ratnam (1986) who found SMAC to be efficient for the selection of *E coli* O157. To save resources, our study used the SMAC medium as a half plate, rather than a whole petri dish, per specimen. This resulted in crowded colonies some of which falsely appeared non sorbitol fermenting. It is therefore recommended that the SMAC agar be used as a whole plate when testing for *E coli* O157.

A modified SMAC medium made selective by the addition of rhamnose and cefixime was introduced by Chapman *et al.*, (1991). This medium should be further evaluated in New Zealand to assess its cost effectiveness.

Smith and Scotland (1993) outlined the numerous methods now available for the detection of verocytotoxin producing *E coli* (VTEC) O157 and other VTEC. Tests for verocytotoxin (VT) detection include: vero cell testing, polymerase chain reaction, gene probe testing, enzyme immunoassay, latex agglutination. However, some of these tests require specialised equipment and the remainder require expensive reagents. The modified SMAC medium of Chapman *et al.*, (1991) appears to be a suitable medium for the detection of *E coli* O157, but the limitation of any SMAC medium is that it differentiates strains on the basis of sorbitol fermentation, not VT production. Thus screening specimens by this medium will fail to detect sorbitol fermenting strains of VTEC.

VTEC is apparently a relatively new pathogen in New Zealand, so more work is necessary to determine the appropriate testing protocol for the detection of this potentially fatal organism. However, patient history may be used to selectively test for VTEC. This organism is associated with bloody diarrhoea, haemolytic uraemic syndrome, and thrombotic thrombocytopenic purpura (Table 1), so faeces from patients presenting with any of these syndromes should be inoculated onto SMAC. Both cases detected in this study presented with haemolytic uraemic syndrome.

### 5.7.7 *Giardia*

In our study the direct wet preparation detected only 48% of cases so it is not an efficient diagnostic test for giardiasis. This finding is in accordance with Watson *et al.,* (1988), who found direct wet preparations to be less than half as sensitive as other methods.

The concentration of parasites from faeces using the formalin ethyl acetate method was not as sensitive as the trichrome stain for the detection of *Giardia*. This concurs with Shetty and Prabhu (1988) who state that whilst a combination of concentration and permanent stained smear is preferable, as a single method, the permanent stained smear is the most effective for detection. The stained smear has the advantage that it can be performed either directly from the stool on the day of receipt or from a preserved aliquot at a later time. The stained smears may be stored indefinitely.

Neither the trichrome stain nor the concentration methods are as sensitive as the newer antigen detection techniques. However, the value of the traditional methods is that a number of different parasites may be detected in the one specimen using a single method.

The enzyme immunoassay (EIA) test does not require specialised equipment and is easy to perform. It detects an antigenic component of both trophozoites and cysts (GSA 65). The technique does not require intact particles to give a positive result and is more sensitive than traditional methods. Because of this, it is difficult to assess the validity of a positive result when a single specimen is positive by this method only. Rosoff *et al.,* (1989) evaluated this EIA method and found that the EIA method detected almost 30% more cases than traditional methods and did not give positive reactions in the presence of parasites other than *Giardia*. They concluded that the patients in their study who were positive only in the EIA test were genuine giardiasis cases. However, the fact that other parasites do not carry GSA 65 does not preclude a similar antigen being present in other matter, thus EIA positives which are not substantiated either by other methods, or epidemiological data, must be interpreted with caution.

The immunofluorescence (IF) technique is relatively easy to perform, but does require specialised equipment (fluorescent microscope) and a greater level of technical expertise than the EIA method. The IF test detects an antigen found in the cyst wall and not the trophozoite and thus requires intact cysts to give a positive signal. False negatives will occur if the case is excreting only trophozoites or if no intact cysts are present in the specimen. The advantages of the IF method include the ability to visualise the cysts and

thus use morphology to confirm a positive result and (using two antibodies) the ability to detect *Cryptosporidium* within the same test.

The cost of either antigen detection method is high when compared with culture for a specific bacterium. The sensitivity of the EIA test is such that it could be performed on a single specimen thus limiting the cost to one test per illness episode (instead of the recommended minimum of three tests if traditional parasite detection methods are being used). However, the specificity of the EIA test needs to be first be established.

### 5.7.8 *Cryptosporidium*

The modified Kinyoun stain (ZN) detected only 50% of the positive results when a single smear was examined for five minutes. This implies that for the ZN stain to detect a greater number of positives, more time must be invested in scanning smears. Other disadvantages of the ZN stain include that the stain contains phenol and that acid fast staining of some yeasts and other background material can result in interpretation difficulties. Small numbers of *Cryptosporidium* were seen with relative ease in the IF test, indicating that the test is more sensitive than the ZN stain.

Our findings agree with those of Tee *et al.*, (1993) who compared four different methods for their sensitivity in detecting *Cryptosporidium*. They found that direct immunofluorescent antibody testing detected the greatest number of organisms in seeded specimens, and that the sensitivity of ZN staining was 29.7%. They also found that the IF test detected four positives from 20 random specimens whereas the ZN method detected only one. Tee *et al.*, (1993) and MacPherson and McQueen (1993) both found that the non-specific immunofluorescent stain auramine-rhodamine gave comparable results to commercially available IF antibody methods. This non-specific stain should be further evaluated in New Zealand to determine its cost-effectiveness.

EIA tests have now become available for the detection of *Cryptosporidium* (Rosenblatt and Sloan 1993) but require evaluation in New Zealand.

### 5.7.9 *Aeromonas*

The *Aeromonas* selective medium (ASM) detected more Aeromonads than the 5% sheep blood agar; but the ability of Pseudomonads to grow on the selective medium added to the cost of isolating Aeromonads using this medium. The medium used in our study is similar to that of Hunt *et al.,* (1987) who found Aeromonads in 51 of 2009 (2.53%) specimens using ASM as the primary medium. These workers increased the number isolated to 89 (4.43%) by using alkaline peptone enrichment in conjunction with ASM. It is apparent that use of a selective medium increases the yield of Aeromonads from faecal specimens, and that the yield can be further increased by the use of an enrichment broth. However, as the significance of this genus in gastrointestinal disease is not yet confirmed, the appropriateness of any specific investigation for this organism is uncertain.

### 5.7.10 *Plesiomonas*

The Plesiomonads were inadvertently detected in our study as no specific selective medium was used. The organisms were detected on xylose lysine desoxycholate agar (XLD) following selenite enrichment (SEL) and the specificity of this technique is uncertain. A *Plesiomonas*-selective medium has been described by Schubert (1984). It incorporates brilliant green and bile salts as selective agents and inositol as a differentiating agent. This medium should be evaluated in New Zealand to determine the incidence of *Plesiomonas* infection and to determine if the use of such a selective medium is appropriate to the New Zealand situation.

### 5.7.11 *Dientamoeba*

The direct wet preparation detected only 55% of cases so it is an inefficient diagnostic test for dientamoebiasis. This parasite does not have a cyst phase and is not detectable by concentration. So we concur with the recommendation of Garcia (1994) that the method of choice for the detection of *Dientamoeba* is the permanent stained smear.

Our results for *Giardia* showed that trichrome staining was not as sensitive as antigen testing so it is probable that the same would apply for *Dientamoeba*. If so, our findings probably under-represent the true incidence of this organism in our study population. Sawangjaroen *et al.,* (1993) reported on a culture method for *Dientamoeba* and noted that

this method was more sensitive than microscopy and should be used for prevalence studies. Such a method should be applied in New Zealand to determine true incidence, but would be laborious for routine use. An indirect immunofluorescence detection method was reported by Chan *et al.,* (1993). These workers noted that the method was specific which implies that antigen detection tests can be applied to this organism. Such an antigen test would be less labour intensive than currently available detection methods.

### 5.7.12 *Blastocystis*

The direct wet preparation detected only 42% of cases so it is not an efficient diagnostic test for *Blastocystis*. The trichome stain detected the majority of the isolates (84.7%), but 17 (15.3%) isolates were detected only by concentration. Thus to detect all the isolates both a permanent stained smear and a concentration procedure are necessary. As the significance of this parasite remains undetermined, a method for routine testing for its presence is not advocated here.

## 5.8 Infection risks, signs, symptoms and consequences

The following section discusses only those organisms of which there were more than four cases of infection detected.

### 5.8.1 *Campylobacter*

Our results show that campylobacteriosis is a debilitating illness (89% of cases who attended work or school required leave because of their symptoms and 10% were hospitalised). Cases usually presented with diarrhoea (98%) but also exhibited a variety of other symptoms (only constipation was not reported by any cases).

Although campylobacteriosis is considered to be an acute infection, 35% of cases were ill for more than a week and 8% for more than one month at the time of laboratory investigation thus showing it to be a disease of variable duration. These results indicate that a specific clinical presentation is not apparent for this organism.

*Campylobacter* was the most prevalent organism detected in our study. This is not surprising because nationally *Campylobacter* is the most prevalent gastrointestinal pathogen, and its incidence has increased in recent years, Lane and Baker (1993).

McNicholas *et al.*, (1995) found that changes in laboratory methods which have occurred in New Zealand during the last five years were insufficient to have effected the increase in national statistics reported by Lane and Baker (1993). These reports of increased incidence prompted a national multi-centre case control study on risk factors for campylobacteriosis (known as the MAGIC study). The results of this study are as yet only preliminary, but the consumption of poorly cooked chicken was identified as the predominant risk factor when the activities of the 621 cases were compared with controls which were matched by age, sex and geographical area. Other risk factors identified included: overseas travel; consumption of non-city water (outside of home-supply) or rain water supply; puppy ownership; ownership of three or more cage birds; contact with animal carcases, particularly calf or pig; and consumption of unpasteurised dairy products or prepackaged salad or food prepared away from the home, Eberhart-Phillips *et al.*, (1995). A smaller case control study was carried out in Christchurch during the summer of 1992/1993. This study of 100 case control pairs also identified consumption of undercooked chicken, consumption of chicken prepared away from the home and consumption of water from a non-urban supply as significant risk factors. It also identified barbecued chicken and chicken bought fresh rather than frozen as risk factors (Ikram *et al.*, 1994).

The above-mentioned case control studies included a larger number of cases than detected in our study. They also included a greater number of questions and were personally administered, either face-to face or via the telephone and for these reasons are not directly comparable with our study. We did not identify consumption of under-cooked chicken as a significant risk factor for campylobacteriosis. However, we did identify consumption of unpasteurised milk as a risk factor, a factor which was also identified in the MAGIC study and by Benenson (1990). The above-mentioned New Zealand studies were also both case control studies where symptomatic bacteriologically proven cases of campylobacteriosis were compared with asymptomatic age, sex and geographically matched controls. In our study, the cases of campylobacteriosis were compared with symptomatic cases of gastrointestinal illness who may or may not have been positive for other pathogens but who were negative for *Campylobacter*. Thus it is to be expected that our results may differ from those found in the above-mentioned case control studies.

Our study also suggested that consumption of water from the Opotiki area was a significant risk activity. But, because of the limitations of the study, the water supply risk can only be equated as a risk of being in the Opotoki area, which includes drinking from that water supply.

### 5.8.2 *Salmonella*

Our results show that salmonellosis is a debilitating illness with 100% of cases who attended work or school requiring leave because of their symptoms and 42% of cases being hospitalised. Cases usually presented with diarrhoea (92%) but also exhibited a variety of other symptoms (only constipation was not reported by any cases). Although salmonellosis is considered an acute infection, 30% of cases were ill for more than a week at the time of laboratory investigation thus showing it to be a disease of variable duration. These results indicate that a specific clinical presentation is not apparent for this organism.

*Salmonella* is a well known enteric pathogen with a widespread distribution, so it is surprising that it was detected less frequently in this study than were *Giardia*, *Cryptosporidium* or *Yersinia*. Although salmonellosis is notifiable in New Zealand, Fraser (1993) reported that epidemiological information on human salmonellosis in New Zealand is incomplete and a national case control study has not been undertaken for salmonellosis. Small, localised studies may be initiated in the event of an outbreak and these studies may or may not elucidate the causative food or activity. Delicatessen chicken was implicated in one outbreak (Lane *et al.*, 1993) and hangi food in another (Mansoor *et al.*, 1993). Our questionnaire did not seek information on recent consumption of hangi food. In 1975, Begg reported a cluster of patients with salmonellosis related to mussel consumption in Otago and *Salmonella* have been isolated from mussels elsewhere in New Zealand (Wong *et al.*, 1985).

We identified three risk factors for the acquisition of *Salmonella* infection: overseas travel, consumption of barbecued food and consumption of shellfish. The food available in some overseas countries may not be prepared in a hygienic manner, thus putting travellers at risk. Likewise, barbecued meat and eggs may not be thoroughly cooked. Raw and partially cooked shellfish (steamed until the shells open) are a risk food which is not so well recognised in New Zealand and warrants further public education.

### 5.8.3 *Yersinia*

Our results show that yersiniosis is a debilitating illness with 69% of cases who attended work or school requiring leave because of their symptoms. Cases usually presented with diarrhoea (81%) but also exhibited a variety of other symptoms (only constipation was not reported by any cases). As 63% of cases were ill for more than a week and 6% for more than one month at the time of laboratory investigation it would appear that yersiniosis symptoms are tolerable for a short period of time but as symptoms persist, medical assistance is sought. These results indicate that a specific clinical presentation is not apparent for this organism.

McCarthy and Fenwick (1991) reported *Yersinia enterocolitica* to be the third most common bacterial enteric pathogen in Auckland and Wright *et al.,* (1995) reported that *Yersinia* was the second most common pathogenic enteric bacterium detected in Auckland in 1994.

Fifteen of the cases detected in our study were due to *Yersinia enterocolitica* serotype 3. Wright *et al.,* (1995) reported that this type is present in New Zealand pigs at a relatively high level, as it is in many other pig producing countries (Cover and Aber, 1989).

Lee *et al.*, (1990) implicated pork in a yersiniosis outbreak in America, and Ostroff *et al.,* (1994) found that in Norway consumption of pork and pork products was a significant risk factor for *Yersinia* infection as was consumption of untreated water.

Our results also indicate consumption of untreated water to be a significant risk factor for yersiniosis as well as contact with farm animals, particularly cows and sheep. Contact with pigs was not identified as a risk factor. We also did not identify consumption of undercooked pork as a risk factor. Our results suggest that the potential role of domestic farm animals other than pigs, in the transmission of yersiniosis, should be further evaluated in New Zealand.

McNicholas *et al.*, (1995) found that only 77% of New Zealand diagnostic laboratories routinely culture for *Yersinia* whereas all culture for *Salmonella* routinely. They reported that some laboratories culture selectively based on patient age whereas others culture on physician request only. Our results show that there is little basis for such selectivity given that yersiniosis affects all age groups, and that symptoms associated with yersiniosis are non-specific and clinically indistinguishable from infections with other organisms.

### 5.8.4 Rotavirus

No risk factors were identified for infection with rotavirus. However, our results can not be considered complete as adults were not routinely tested and many of the cases were too young to verbalise their symptoms, thus the symptoms recorded were usually actually signs noted by the care-giver. Our results indicate that rotavirus infection is a debilitating illness with 42% of cases being hospitalised and 100% of those attending work or school requiring leave. A variety of symptoms were reported by cases/care-givers, the predominant symptom being diarrhoea (91%). The only symptom not noted was constipation.

### 5.8.5 *Giardia*

Our results show that giardiasis is a debilitating illness with 75% of cases who attended work or school requiring leave because of their symptoms and 6% being hospitalised. Cases usually presented with diarrhoea (89%) but also exhibited a variety of other symptoms; each of the other symptoms were reported by at least four patients. As 19% of cases were ill for less than one week at the time of laboratory testing at least this number of cases (six) would have gone undiagnosed had the criteria of Guerrant and Bobak (1991) been applied (testing for parasites only if the case is symptomatic for more than 10 days). These results indicate that a specific clinical presentation is not apparent for this organism. 50% of cases reported that other householders had a similar illness, thus implying either a common source of infection or that person-to-person transmission was occurring.

Giardiasis is not notifiable in New Zealand, but laboratory-based surveillance of this organism has indicated that the national incidence is at least 85/100 000 (van Duivenboden and Walker, 1993). Okell and Wright (1990) reported that *Giardia* was endemic in the Eastern Bay of Plenty, second in prevalence to *Campylobacter*. Our results confirm their findings.

Benenson (1990) noted that asymptomatic carriage of *Giardia* is high so the incidence of giardiasis in New Zealand can not be determined without a community study of both symptomatic and asymptomatic people.

The modes of transmission of *Giardia* are known to include person-to-person, particularly in day-care centres, and consumption of contaminated food or water (Benenson, 1990). In New Zealand the organism has been isolated from many water sources (Waugh, 1991) and many animal species including cats and dogs (Tonks *et al.*, 1991); sheep, cows, horses, goats, deer and hens (Brown *et al.*, 1991); possums, house mice and ship rats (Marino *et al.*, 1992). An outbreak associated with a conference venue in Auckland in 1993 resulted in a case control study which identified several potential risk foods including a water source, subsequently shown to contain *Giardia* cysts (Thornton *et al*, 1993).

We identified several risk factors for giardiasis, including consumption of water from two supplies. Because of the limitations of our study, the water-supply risk can only be equated as a risk associated with being in the area of the supply, which, perhaps only coincidentally, includes drinking water supplies in rural areas. As noted above, the risk of attendance at a day care centre is an internationally recognised risk for giardiasis. The other risk factors we identified are all associated with the rural environment. These included consumption of raw milk, and contact with farm animals specifically cows and horses. Our findings coupled with those of Brown *et al.*, (1991) suggest that the role of domestic animals in the transmission of giardiasis should be further investigated.

Although specific antimicrobial treatment is not recommended for most enteric pathogens, such treatment is recommended for giardiasis. Of the anti-protozoal drugs which have been advocated for giardiasis (Farthing, 1994) metronidazole and tinidazole are available in New Zealand. Failure to test for giardiasis can result in cases undergoing expensive investigations for neoplastic and other conditions. Associated with such testing is the trauma of possibly having a "dread" disease. We conclude that routine specific investigation of stools for *Giardia* is justified.

### 5.8.6 *Cryptosporidium*

Our results show that cryptosporidiosis is a debilitating illness with 100% of cases who attended work or school requiring leave because of their symptoms. Cases usually presented with diarrhoea (88%) but also exhibited a variety of other symptoms, with only bloody stools and constipation not being reported. As 67% of cases were ill for less than one week at the time of testing at least this number of cases (10) would have gone undiagnosed had the criteria of Guerrant and Bobak (1991) been applied (testing for

parasites only if the case is symptomatic for more than 10 days). These results indicate that a specific clinical presentation is not apparent for this organism. 43% of cases reported that other householders had a similar illness, thus implying either a common source of infection or that person-to-person transmission was occurring.

Cryptosporidiosis is not notifiable in New Zealand. Van Duivenboden (1995) assessed laboratory data collected during 1993 and determined a national incidence of 11.2/ 100 000, but concluded that laboratory selection criteria and testing techniques were such that this figure was not a reliable indication of the true national incidence. He found that factors influencing the decision to test included patient age, clinical presentation, specimen form and clinician request. Our results indicate that selection based on any of these parameters will result in under-diagnosis thus confirming van Duivenboden's conclusion that the rate of 11.2/100 000 under-represents the true national incidence.

Our findings agree with those of Carter (1986) who undertook a 12 month study of cryptosporidiosis in the Taranaki region and found that 74% of cases were detected in the spring and early summer. He found that the majority of cases were aged between one and 15 years and he also concluded that direct contact with farm animals was the probable source of infection in the majority of cases.

Benenson (1990) notes that the reservoirs for *Cryptosporidium* include man and cattle, and modes of transmission include person-to-person, animal-to-person and waterborne. Benenson (1990) cites the following groups as having an increased risk of infection: children over two years, animal handlers, travellers, homosexual men, and those with close personal contact such as day care workers. We identified risk factors for cryptosporidiosis which concur with this author: consumption of home-supply water, consumption of raw water from a natural waterway, contact with farm animals, specifically cows, and attendance at a day care centre. In addition, we identified owning a cat as a significant risk for cryptosporidiosis.

Cryptosporidiosis can be life-threatening in immuno-compromised people. Specific treatment is not yet available but the aminoglycoside paromomycin has been reported to give encouraging results (Bissuel *et al.*, 1994).

### 5.8.7 *Aeromonas*

Our results show that Aeromonas infection is not as debilitating as infections with other organisms as only 25% of cases who attended work or school required leave because of their symptoms. Cases usually presented with diarrhoea (67%) and reported foul smelling stools (73%) but also exhibited a variety of other symptoms, with only bloody stools not being reported by any case. However, red blood cells were noted in two stools which were subsequently positive for Aeromonads. As 81% of cases were ill for more than one week at the time of laboratory testing it appears that the symptoms may be tolerable for a short period but intolerable over a longer period of time. Our results indicate that a specific clinical presentation is not apparent for this organism.

Aeromonads are fresh water organisms of wide distribution, with the majority of isolates being detected in the spring and summer (Khardori and Fainstein, 1988). This seasonality concurs with our findings but we did not identify consumption of untreated water as a risk factor for infection with Aeromonads. The two risk factors we identified were overseas travel and contact with pigs. However, as only one of eight cases had a history of contact with pigs this data must be interpreted with caution and a study of a larger population undertaken to determine if our results can be substantiated.

Seven of 15 cases were positive for other organisms, four of which were Group 1 organisms. Thus the role of the Aeromonads in the disease process in at least four cases is uncertain. It is possible that two Group 2 organisms may act in synergy to enhance each others pathogenic potential. This hypothesis requires further investigation.

Janda (1991) notes that although the role of Aeromonads as gastrointestinal pathogens is still controversial, mounting evidence indicates that at least some strains are involved in diarrhoeal disease. Experiments using this organism have not fulfilled Koch's postulates and no outbreaks have been well documented. However, some cases have been shown to have mounted an immune response to the strain isolated from their stools and various potential virulence factors have been detected in a number of strains.

It is possible that in the future specific testing for a virulence factor rather than culturing for organisms of the genus *Aeromonas* may be the norm. In the interim, as laboratories strive for cost-effectiveness, it is difficult to justify routine examination of stools for an organism of unknown significance.

### 5.8.8 *Plesiomonas*

The results for *Plesiomonas* must be interpreted with caution as a specific medium was not used to detect this organism.

Our results show that *Plesiomonas* infection is debilitating as the single case who attended work or school required leave because of her symptoms and three of the five cases were hospitalised. All cases presented with diarrhoea but also exhibited a variety of other symptoms. All cases presented for testing within a week of symptom onset. These results suggest that infection with *Plesiomonas* is a severe, acute illness without a specific clinical presentation.

Janda (1991a) reports that gastroenteritis is the most common illness associated with *Plesiomonas* infection, but its role as an enteric pathogen has not been proven. Janda (1991a) notes that seafoods, particularly raw oysters have been implicated as sources of infection. This concurs with our finding that consumption of shellfish was a significant risk factor for infection. The relative risk for consumption of a barbecued meal was also significant (7.96, 95 % confidence limit, 1.14 - 55.55) but the p value ($p = 0.064$) reflects the small number of cases and limits the reliability of the findings.

Sack *et al.*, (1994) postulated that infection with *Plesiomonas* provides immunity against shigellosis as the two genera share some antigens. It is possible that the low level of shigellosis in our study population could in part be attributable to immunity developed following *Plesiomonas* infection. This potential association requires further investigation as does the severity of illness and the link with shellfish. A study of a larger population utilising the selective medium of Schubert (1984) should be undertaken to gain more information on the role of this organism in New Zealand.

### 5.8.9 *Dientamoeba*

Our results show that *Dientamoeba* infection is not as debilitating as infections with other gastrointestinal pathogens as no cases who attended work or school required leave because of symptoms, nor were any cases hospitalised. Cases usually presented with diarrhoea (86%) but also exhibited a variety of other symptoms not including constipation or bloody stools. All cases presented for testing after one week of symptoms, including 29% after one month of illness, indicating that dientamoebiasis is a chronic disease and is otherwise

without a specific clinical presentation. However, Spencer *et al.*, (1982) reported that of 45 patients with dientamoebiasis, 16 presented within seven days of symptom onset.

Little is known of the epidemiology of this organism but it has been postulated that *Dientamoeba* (which has no cyst phase) is transmitted via nematode vectors with both *Enterobius vermicularis* (Yang and Scholten, 1977) and *Ascaris lumbricoides* (Sukanahaketu, 1977) being implicated. Risk factors identified in our study included: overseas travel, consumption of untreated water and contact with sheep. In each of these situations the case could also have been exposed to various nematodes.

*Dientamoeba* is an under-recognised potential pathogen with just eight articles recorded under this heading on the Ovid CD Rom Medline system for the period January 1991 to December 1995. Specific treatment using tetracycline is advocated (Wright, 1991) and paromomycin has been shown to be active against *Dientamoeba* in vitro (Chan *et al.*, 1994).

The significance of this organism needs to be further evaluated in New Zealand and testing should be performed on symptomatic patients prior to any investigations for neoplastic and other physiological conditions which can cause chronic gastrointestinal symptoms.

### 5.8.10 *Blastocystis*

Our results show that *Blastocystis* infection may be debilitating as 59% of cases who attended work or school required leave because of symptoms, and 9% of cases were hospitalised. However, these results must be interpreted with caution as in 26% (22/86) of cases, *Blastocystis* was detected in conjunction with other gastrointestinal pathogens. Cases usually presented with diarrhoea (75%) but also exhibited a variety of other symptoms. 68% of cases presented for testing after one week of illness, suggesting that infection with *Blastocystis* may be a chronic condition, without a specific clinical presentation.

Boreham and Stenzel (1993) stated that nowhere was knowledge of *Blastocystis* more lacking than its epidemiology. Doyle *et al.*, (1990) evaluated 130 cases and noted that infection was more prevalent in adults (mean age 37), a slight increased prevalence in females, and risk was associated with animal exposure and overseas travel. Our findings

support all of those of Doyle except that we did not find overseas travel to be a risk. Four of the risks we identified were associated with the rural environment: consumption of untreated water, consumption from the Waimana water supply (indicative of being in the Waimana area, a rural locality), and contact with sheep and horses. Boreham and Stenzel (1993) note that *Blastocystis* is widespread throughout the animal kingdom. They also note that several studies found that infection was more prevalent during hot weather, particularly hot, wet weather. Our findings show that infection was more prevalent during the winter months.

It is interesting, if puzzling, to note that consumption of water from the Kawerau supply was identified as a protective factor for infection with *Blastocystis*.

*Blastocystis* was the most frequently detected organism in our study (86 cases), but in 22 cases other Group 1 and/or Group 2 pathogens were found. In the remaining cases a viral or non-infectious cause of symptoms was not excluded.

Prevalence does not automatically imply significance and the role of *Blastocystis* in gastrointestinal infection continues to be debated. After a prospective Nepalese case control study, Shlim *et al.,* (1995), concluded that despite detecting a high prevalence of *Blastocystis*, their results suggested that *Blastocystis* does not cause diarrhoea. They also concluded that the previously held maxim that high organism numbers equate to symptoms was a misconception. Experiments with this organism have not fulfilled Koch's postulates and studies which have concluded it is pathogenic have usually not considered the possibility of a viral or non-infectious cause (Boreham and Stenzel, 1993). Markell (1995) concludes that whilst a minor role for *Blastocystis* in gastroenteritis can not be excluded, there is no reason to treat cases, and cases should be investigated further to exclude other causes.

Of the organisms sought in our study, *Blastocystis* was the most prevalent and was also relatively easily detected, however the uncertain clinical relevance of this organism means that routine investigation and reporting may be more misleading than helpful so we do not advocate it.

## 5.9 Control and Prevention of Infection

Control of infectious diarrhoeal disease may be general or specific. For either approach, a knowledge of the organisms and the mode of infection is desirable.

General control is based on a knowledge of the range and relative importance of organisms causing illness in a specific community and translating this knowledge into community education on safe practices to reduce the risk of exposure. For example, if an agent is known to be transmitted in raw meat, general control measures would relate to education on thorough cooking of meats and appropriate storage of meats in order to prevent raw meat contaminating food which will not be, or has already been, cooked. In addition, the meat industry would be required to implement measures to minimise contamination and organism dissemination.

Specific control is reliant on recognising individual cases of disease, knowing the aetiological agent involved and instituting intervention and control measures specific for that case and that agent. If a patient with salmonellosis is employed in food preparation, specific control measures would include: removing the person from work until microbiological clearance of infection; investigating potential sources of infection and implementing specific containment measures.

It may be argued that if general control measures were adhered to then specific measures would be unnecessary, and testing of faecal specimens would also be unnecessary. However, this is not advocated for two reasons: we do not know all the risks associated with gastrointestinal infection and can only come to establish all the causes and modes of transmission if we continue to investigate individual cases. Specific treatment is not recommended for most gastrointestinal organisms, so laboratory investigation of stool specimens is principally for the benefit of the public health rather than individual health. However, as the organisms which do require specific treatment result in symptoms which are generally indistinguishable from those associated with the other organisms, routine laboratory investigation also benefits the individual.

# 6 CONCLUSIONS

Faecal specimen macroscopic form and microscopic findings, time of year and patient age are not specific for any one pathogen so selective criteria for faecal testing should not be based on any of the above parameters. Parasitic and bacterial causes of gastrointestinal symptoms do not generally present differently and therefore both should be sought routinely.

Detection of one pathogen does not exclude the presence of others and investigations should not stop when a pathogen has been found. A single specimen will detect most pathogens but to reliably exclude a pathogenic cause a minimum of two specimens are advocated.

Our findings indicate that, as a minimum, all specimens should be examined routinely for the following organisms (for which the most cost-effective method was shown to be): *Salmonella* (selenite enrichment subcultured to xylose lysine desoxycholate agar); *Shigella* (none were detected, so a cost effective medium could not be determined); Campylobacter (5% sheep blood agar plus cefoperazone); *Yersinia* (selenite enrichment subcultured to *Yersinia* selective agar); *Giardia* (detection of antigen); and *Cryptosporidium* (detection of antigen).

While routine testing for *E coli* O157 is not recommended, laboratories should have the capability to test for this pathogen if a patient presents with haemolytic uraemic syndrome, thrombotic thrombocytopenic purpura or unexplained bloody diarrhoea. Likewise, routine culture for *Vibrio species* is not recommended; however, laboratories should test specimens using thiosulphate citrate bilesalt sucrose agar if the requesting clinician suspects the patient has cholera, or the patient has recently eaten shellfish. A trichrome stain should be performed on symptomatic patients prior to any investigations for neoplastic and other physiological conditions which can cause chronic gastrointestinal symptoms in order to exclude *Dientamoeba*.

Pathogenic parasites other than those noted above were not detected in our study. Such parasites are detected from patients in New Zealand, but usually in association with overseas travel or institutionalisation (Paltridge G., Parasitologist, Canterbury Health Laboratories, personal communication, 1996). It is therefore recommended that a faecal

concentration method and a trichrome stain should be performed on all cases of gastroenteritis who have a history of overseas travel or who are institutionalised.

Close liaison between the clinician and the laboratory is essential. This will ensure that the laboratory is provided with adequate patient information on which to base selective testing for the less common pathogens.

*Blastocystis* when detected, should always be reported cautiously. Likewise if laboratories choose to test for *Aeromonas*, positive findings should be reported with a comment noting that its role as a pathogen is uncertain. Of Group 2 organisms sought in our study, *Dientamoeba* and *Plesiomonas* appear to be the more likely potential pathogens and further work on the role of these organism in disease in New Zealand is desirable.

On the basis of correlating data collected from the questionnaire and our laboratory findings, suggestions to reduce the risk of infection are as follows:

* maintain a high standard of personal hygiene. This especially applies to those with diarrhoea and those in the rural environment.

* do not drink untreated water or unpasteurised milk

* cook all animal derived food thoroughly - including barbecued food and shellfish

* wash hands thoroughly before preparing or consuming food

* prepare food in a hygienic environment, avoid recontamination after cooking and store appropriately

* take particular care over food and water consumption when travelling overseas

It is recognised that due to resource constraints this study did not cover a number of agents identified in Table 1. It is recommended that these also be evaluated in the New Zealand context to determine incidence and value of testing.

Our study identified a number of areas for further study, these are listed in Appendix 2.

# 7 APPENDICES

## 7.1 Appendix 1. Information, consent form and questionnaire included in the specimen collection kit

Eastbay Health
Te Whatumauri Hauora

EASTBAY HEALTH LTD
STEWART STREET
PO BOX 241
WHAKATANE
TELEPHONE 0-7-307 8999
FACSIMILE 0-7-307 0451

**EASTERN BAY OF PLENTY GASTROINTESTINAL PATHOGENS PROJECT**

CONSENT FORM

We are doing a one year study on the causes of diarrhoea and we would like you to take part in this study.

If you agree (by signing this form) your specimen will be tested by a technician who is skilled in research methods. She will examine your specimen by many more methods than are routinely used in order to find out if a micro-organism is causing you discomfit.

What we learn through this project will be used to ensure that future specimens will be routinely tested by methods which will best detect disease causing organisms.

We would appreciate you taking the time to fill in the accompanying questionnaire. We will use this information to help us find out from where you may have caught your infection and this may help us prevent similar illnesses in the future.

Your results and the information you give us will be treated as confidential.

Thank you.

Jackie Wright
Project Leader
Laboratory, Whakatane Hospital
Eastbay Health.
phone 3078767.

I understand that my specimen will be used for research purposes and I give my consent for this.

_____ Your signature

_____ Date

## HOW TO COLLECT YOUR SAMPLE.

You have been supplied with:

* a plastic tub

* a wooden stick

* a yellow topped specimen container

* a specimen transport bag

1 - Pass your bowel motion into the plastic tub - or use a child's potty if you prefer.

2 - Do Not pass urine into this container

3 - Use the wooden stick to transfer some bowel motion to the specimen container.

- Please add sufficient specimen to 1/4 - 1/2 fill the container.

- If you have passed bloody or mucoid portions please ensure they are added to the specimen container

4 - Screw the lid tightly on to the specimen container

5 - Label this container clearly with your name, the time and the date the specimen was collected.

6 - Wrap the plastic tub and wooden stick in newspaper and discard into secured rubbish bag.

7 - Place the specimen container into the plastic transport bag provided and seal the bag.

8 - Wash your hands thoroughly

9 - Place your completed questionnaire, your request form and your signed consent form into the outer sleeve of the transport bag.

10- Deliver your specimen on the day collected to any of the Eastbay Health's laboratory collection centres (Whakatane hospital, Opotiki hospital or Kawerau Clinic) or return it to your doctor's surgery for collection by our courier.

Eastbay Health
Te Whatumauri Hauora

STEWART STREET
PO BOX 241
WHAKATANE
TELEPHONE 0-7-307 8999
FACSIMILE 0-7-307 0451

# EASTERN BAY OF PLENTY GASTROINTESTINAL PATHOGENS PROJECT

## QUESTIONNAIRE

Name_____ Age_____

Sex__ Occupation_____

Tick in the box beside appropriate answer(s). For questions 1, 6 & 7 you may need to tick several boxes.

1) Which of the following are you suffering from:
- ☐ Stomach cramps
- ☐ Stomach pain
- ☐ Vomiting
- ☐ Diarrhoea
- ☐ Constipation
- ☐ Nausea
- ☐ Lethargy
- ☐ Foul smelling bowel motions
- ☐ Increased passage of wind
- ☐ Blood in bowel motions
- ☐ Excessive belching
- ☐ Other - explain_____

2) Have these symptoms been
- ☐ continuous OR/
- ☐ on and off

3) How long have you felt unwell
- ☐ less than 1 week
- ☐ 1 week - 1 month
- ☐ greater than 1 month

4 Has your illness resulted in you having to take time off from work or school?
- ☐ Yes
- ☐ No
- ☐ Not applicable

5 Has any one else in your household had a similar illness?
- ☐ Yes
- ☐ No
- ☐ Not applicable

If Yes, did they feel unwell before or after you?
- ☐ Before
- ☐ After
- ☐ At the same time

Were their symptoms more or less severe than yours?
- ☐ More
- ☐ Less
- ☐ The same

Please turn over .....

6) What water supplies have you drunk from in the last month?

- ☐ Braemar / Te teko
- ☐ Kawerau Town
- ☐ Opotiki Town
- ☐ Ruatoki
- ☐ Matata Town
- ☐ Taneatua Town
- ☐ Waimana town
- ☐ Whakatane / Ohope
- ☐ Other - specify_____

7) In the **month** before you began to feel unwell did you do any of the following

a ☐ Travel overseas - Where to_____
b ☐ Drink unpasteurised milk
c ☐ Drink unboiled water from a natural waterway Eg. lake, river or stream.
d ☐ Attend a day-care centre
e ☐ Handle any farm animals - what _____
f ☐ Eat undercooked poultry
g ☐ Eat " pork
h ☐ Eat " red meat
i ☐ Eat a take-away meal
j ☐ Eat a barbecued meal
k ☐ Eat shellfish

Did you do any of the above in the **week** before you felt unwell?

- ☐ Yes
- ☐ No

If yes, which? _____
(Respond a,b,c as appropriate)

Do you own a cat

- ☐ Yes
- ☐ No

Do you own a dog

- ☐ Yes
- ☐ No

Any other comments _____

Thank you for agreeing to participate in our study and for taking the time to complete this questionnaire.

If you have any questions about this study, please contact:

Jackie Wright
Laboratory, Whakatane Hospital
Phone 3078767

## 7.2 Appendix 2. Areas identified as requiring further investigation.

* use of a selective method on all specimens from a specified population to detect the true incidence of *Plesiomonas*, rotavirus, Norwalk and other small round viruses, the various *Escherichiae* associated with gastroenteritis, and toxin producing strains of *Clostridium difficile*, *Bacillus cereus* and *Clostridium perfringens*

* evaluation of the medium desoxycholate citrate agar for *Shigella* isolation in New Zealand

* evaluation of sorbitol MacConkey plus rhamnose and cefixime for the detection of New Zealand strains of *E coli* O157

* development of a *Yersinia* selective medium which differentiates *Yersinia* from *Citrobacter species*

* evaluation of the auramine-rhodamine stain for the detection of New Zealand strains of *Cryptosporidium*

* development of a cost effective antigen test for *Dientamoeba*

* further evaluate the role of *Dientamoeba* in New Zealand using culture techniques

* further evaluate the role of domestic animals in the transmission of *Yersinia*, *Cryptosporidium* and *Giardia*

* evaluation of the number of asymptomatic giardiasis cases in New Zealand

* further investigation of the possibility of *Plesiomonas* providing immunity against shigellosis

* investigation of the risk factors for *Aeromonas* infection in a larger population

* further investigation of the hypothesis that two Group 2 organisms may act synergistically to enhance each other's pathogenic potential

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