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

A thesis presented in fulfilment of the requirements
for the degree of Master of Science in Microbiology
at Massey University, Palmerston North.

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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 cannot 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 bile salt 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.
ACKNOWLEDGEMENTS

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Once I started work at ESR, I would slither out early on Fridays for my weekends in Whakatane and arrive back Mondays, weary. Thank you Carolyn, Helen and Dave for allowing this to happen.

Thank you to my supervisor, Professor John Clarke, who has been so helpful in pulling it all together.

Finally, thanks are extended to the Eastern Bay of Plenty clinicians for their support and to the patients who participated, without whom the study could not have happened.

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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
citrate
coumarate
colistin
arginine control
concentrated wet preparation
Cryptosporidium Cryptosporidium parvum
desoxycholate citrate agar
Dientamoeba Dientamoeba fragilis
deoxyribonucleic acid
double stranded
direct wet preparation
Eastern Bay of Plenty
Escherichia coli Escherichia coli
enzyme immuno-assay
esculin
fermentation control
fluorescein isothiocyanate
gram
α-galactosidase
galacturonate
Gram negative enrichment broth
general practitioner
glucuronate
Giardia specific antigen
hektoen enteric agar
H₂S  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  β-D-glucosaminidase
ONPG  β-D-galactosidase
PD  phenylalanine deaminase
PLE  palatinose
Plesiomonas  Plesiomonas shigelloides
PNPG  β-D-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
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