ORGANISMS ASSOCIATED WITH INFECTIOUS DIARRHOEA
IN HUMANS: A REAPPRAISAL FOR 1984

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ORGANISMS ASSOCIATED WITH INFECTIOUS DIARRHOEA IN HUMANS:
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1. INTRODUCTION

In an essay of this length I feel it is impossible to cover the large and important topic of human infectious diarrhoea comprehensively. For this reason, it has been necessary to select carefully the information included. Much of the background work is well covered in standard medical and microbiological textbooks and to avoid repetition I have tended to deal with these areas briefly. Instead, I have concentrated on reviewing as fully as possible new fields of understanding; particularly in terms of epidemiology and pathogenesis. Where appropriate, comments are made on the possibilities for further work and the prospects for prevention discussed. For the purposes of this essay I have taken infectious diarrhoea to mean any diarrhoea due to or associated with infection by an organism, and the word organism to mean viruses and protozoa as well as bacteria (Mims 1982).

2. HOW IMPORTANT IS INFECTIOUS DIARRHOEA IN 1984?

Diarrhoeal disease has long been recognized as a major cause of morbidity and mortality, especially in the developing countries (WHO 1964). In 1975 there were 500 million cases of infectious diarrhoea in infants and neonates in Asia, Africa and South America, causing between 15 and 18 million deaths (CIBA 1976). Infectious diarrhoea has been described as "the leading cause of death amongst children in the developing world" (Walsh and Warren 1979) and recently was estimated to cause 7% of deaths under 5 years and 22% of all deaths in South America (Yunes 1981, Guerrant 1983). Even in developed countries infectious diarrhoea presents a major health problem. It is still among the ten leading causes of death for children under 5 years. In the UK, 5% of children develop acute gastroenteritis within the first year of life, and of these about 10% will be ill enough to warrant hospital admission (Tarlow 1984). About 250 million people from developed countries travel internationally each year and it has been estimated that over 100 million of these will suffer 'travellers' diarrhoea' (Nye 1979, DuPont 1981, Gorbach 1981). Between 30 and 40% will be seriously enough affected to alter their travel plans and about 10% will be admitted to hospital. Food-borne illness or 'food poisoning' is another major health problem and is discussed more fully in a later section.
International efforts to combat this worldwide problem include initiation by the World Health Organization (WHO) of the Diarrhoeal Diseases Control Programme (1979). Recently, Snyder and Merson (1982) have reviewed the results of this scheme from a number of selected areas in Africa, South America and Asia. Some of their findings are shown below. See Table 1.

Clearly, even in 1984, infectious diarrhoea continues to be an enormous worldwide problem and it is one that will undoubtedly face us for many years to come.

Table 1
A) Annual Episodes of Diarrhoea per Person by Age Group

<table>
<thead>
<tr>
<th>Country</th>
<th>Age Groups</th>
<th>0-5m</th>
<th>6-11m</th>
<th>&lt;1y</th>
<th>&lt;2y</th>
<th>&lt;3y</th>
<th>&lt;4y</th>
<th>5-14y</th>
<th>&gt;15y</th>
</tr>
</thead>
<tbody>
<tr>
<td>Africa</td>
<td></td>
<td>2.6</td>
<td>4.3</td>
<td>2.3</td>
<td>1.0</td>
<td>0.7</td>
<td>0.4</td>
<td>0.2</td>
<td>-</td>
</tr>
<tr>
<td>Asia</td>
<td></td>
<td>4.4</td>
<td>3.5</td>
<td>3.1</td>
<td>2.3</td>
<td>1.1</td>
<td>0.8</td>
<td>0.6</td>
<td>0.3</td>
</tr>
<tr>
<td>S. America</td>
<td></td>
<td>1.0</td>
<td>2.3</td>
<td>2.0</td>
<td>1.4</td>
<td>1.1</td>
<td>0.5</td>
<td>0.2</td>
<td>0.1</td>
</tr>
</tbody>
</table>

B) Annual Number of Deaths due to Diarrhoea per '000 Population by Age Group

<table>
<thead>
<tr>
<th>Country</th>
<th>Age Groups</th>
<th>&lt;1y</th>
<th>&lt;2y</th>
<th>2-4y</th>
<th>5-9y</th>
<th>&gt;10y</th>
</tr>
</thead>
<tbody>
<tr>
<td>Africa</td>
<td></td>
<td>-</td>
<td>15.6</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Asia</td>
<td></td>
<td>28.5</td>
<td>16.9</td>
<td>5.4</td>
<td>1.3</td>
<td>1.4</td>
</tr>
<tr>
<td>S. America</td>
<td></td>
<td>20.2</td>
<td>34.0</td>
<td>9.2</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
3. ORGANISMS ASSOCIATED WITH INFECTIOUS DIARRHOEA IN HUMANS

Table 2 shows a list of organisms recognized as causes of infectious diarrhoea in humans.

<table>
<thead>
<tr>
<th>(a) Bacteria:</th>
<th>(b) Protozoa:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salmonella spp.</td>
<td>Giardia lamblia</td>
</tr>
<tr>
<td>Shigella spp.</td>
<td>Entamoeba histolytica</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>Cryptosporidium</td>
</tr>
<tr>
<td>Campylobacter spp.</td>
<td>Blastocystis hominis</td>
</tr>
<tr>
<td>Plesiomonas shigelloides</td>
<td>Strongyloides stercoralis</td>
</tr>
<tr>
<td>Vibrio spp.</td>
<td></td>
</tr>
<tr>
<td>Clostridium spp.</td>
<td></td>
</tr>
<tr>
<td>Bacillus cereus</td>
<td></td>
</tr>
<tr>
<td>Aeromonas hydrophila</td>
<td></td>
</tr>
<tr>
<td>Yersinia enterocolitica</td>
<td></td>
</tr>
<tr>
<td>Spirochaetes</td>
<td></td>
</tr>
<tr>
<td>Anaerobospirillum</td>
<td></td>
</tr>
<tr>
<td>Kluyveia spp.</td>
<td></td>
</tr>
</tbody>
</table>

Some of these organisms are familiar whereas others, for example Campylobacter, have only recently been identified as enteropathogens. Recent work has thrown light on many new exciting areas within the field of medical microbiology but, as with any research, problems have been encountered. This is particularly so when trying to elucidate the aetiology of infectious diarrhoea. I think it is well worth considering these difficulties when trying to draw conclusions from the large amount of published work and would like to discuss them briefly here.

Firstly, many workers especially those based in developing countries have found it difficult to identify pathogens in all their cases of presumably infectious diarrhoea. For example, Black et al (1982 a,b,c) found recognized pathogens in only 50% of their study group. There are a number of explanations for this finding:

(a) The other 50% of diarrhoeal cases were not associated with an infectious agent. There are several non-infectious causes of diarrhoea particularly in infants (Tarlow 1984) but it seems unlikely that these relatively uncommon conditions could explain all the 'missing pathogens'.

(b) The remaining cases of diarrhoea could be due to an as yet
unrecognized enteropathogen. For many years, a significant proportion of gastroenteritis was presumed to be viral merely by exclusion. It is only quite recently that positive evidence of viral gastroenteritis has accumulated. This is particularly true of Norwalk-type agents. There may well be many new enteropathogenic organisms to be discovered.

The diagnostic tests used may have been inadequate to detect all the pathogens present.

Whatever the reason for 'missing pathogens', they can clearly introduce large degrees of error into any study. One must be careful to bear this in mind when drawing conclusions as to the relative incidence of various enteropathogens active in any one area. Hopefully, this difficulty will be overcome by improvements in study design and methodology. In particular, better co-operation between workers leading to standardization of study groups, controls, working definitions and diagnostic techniques will allow a more accurate comparison of results. Such co-operation is greatly aided by large, well disciplined, multi-centre programmes such as those initiated by WHO.

Secondly, there are pitfalls to be found when studying pathogenesis. For example, bacterium may well be shown to produce a particular type of toxin but that does not necessarily mean that the toxin is important in actually causing disease. Although in vitro and in vivo techniques such as HeLa cell cytotoxicity and rabbit ileal loop assays are very useful, it must be remembered that the real situation is very much more complex and open to influence from many more variables. These might include the age, immune status and nutrition of the host; the nature of the commensal gut flora; the physiological state of the gut; as well as the infecting dose and routes of transmission. The difficulties are well demonstrated by Escherichia coli which demonstrates a confusing array of potentially pathogenic properties, the relative importance of which may vary between cases and strains. Vibrio cholerae is another example for although one thinks of cholera as being mediated by a cytotoxic toxin, these bacteria express several other properties vital for pathogenicity. Their motility allows them to come in contact with the mucus layer of the gut; their mucinases allow them to penetrate it; and they possess an attachment mechanism which allows them to approximate to the enterocytes long enough for
the toxin to be released and have its effect.

Thirdly, piecing together information to produce a clear epidemiological picture and cycle of infection can be very difficult. New techniques such as 'phage typing, RNA electrophoresis, and DNA hybridization are now being used to make the task easier. Clearly, identifying routes and vectors of transmission within a population is an important step in controlling any disease.

Fourthly, and this is not a problem which obviously springs to mind, there are widely differing views as to what constitutes diarrhoea (Snyder and Merson 1982).

4. THE ROLE OF VIRUSES IN INFECTIOUS DIARRHOEA

4.1 Parvovirus-like Agents (Norwalk Agent)
Parvovirus-like agents have been implicated as the cause of Winter Vomiting Disease, the clinical features of which are shown below. See Table 3.

Table 3
The Clinical Features of Winter Vomiting Disease.
1) Short incubation period of 2 - 3 days.
2) Sudden onset of fever, anorexia, nausea, vomiting and diarrhoea. There may also be vertigo, myalgia and abdominal pain.
3) Recovery occurs within 3 days.
4) Affects mainly older children and adults.
5) Is highly infectious, spreading rapidly by faecal-oral contamination through schools and whole communities.
6) Almost always occurs during winter.

The disease was first described in 1929 by Zahorsky, but it was not until the 1940s that it was shown to be transmissible by ingesting bacteria-free faecal filtrates from sick patients (Zahorsky et al 1945). In 1972 the virus was identified by immune electron microscopy in faeces from a patient in Norwalk, USA. Kapikian et al (1972) saw numerous 27 nm. virus particles which they called Norwalk Agent. Since then a number of similar
particles have been associated with gastroenteritis in several countries (Banatavala 1979). See Table 4.

Table 4
Principal Features of Acute Winter Vomiting from which Parvovirus-like Agents have been Detected.

<table>
<thead>
<tr>
<th>Agent and Location</th>
<th>Date</th>
<th>Community Involved</th>
<th>Proportion Attacked</th>
<th>Size</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Norwalk (Ohio) USA</td>
<td>Oct '67</td>
<td>Primary School</td>
<td>50% Pupils and Staff, 32% Secondary Attack</td>
<td>27-32</td>
<td>Virus detected in 1972 among volunteers fed with faecal filtrates from secondary cases. Related to Montgomery Co. but not Hawaii</td>
</tr>
<tr>
<td>Montgomery Co. (Maryland) USA</td>
<td>Jun '71</td>
<td>Family</td>
<td>Parents and 2 children</td>
<td>27-32</td>
<td></td>
</tr>
<tr>
<td>Hawaii (Honolulu) USA</td>
<td>Mar '71</td>
<td>Family</td>
<td>Parents, child and adult house contact</td>
<td>26-29</td>
<td></td>
</tr>
<tr>
<td>'W' England</td>
<td>Mar '63</td>
<td>Boys' Boarding School</td>
<td>142/800 Pupils, 2 Staff</td>
<td>25-26</td>
<td>Related to Ditchling, but not Cockle. All English strains unrelated to US strain</td>
</tr>
<tr>
<td>Ditchling (Sussex) England</td>
<td>Oct '75</td>
<td>Primary School</td>
<td>30/129 Pupils, 3/6 Staff</td>
<td>21-28</td>
<td>Virus Excretion &gt; 4 weeks</td>
</tr>
<tr>
<td>Cockle S.E. England</td>
<td>Dec '76 to Jan '77</td>
<td>General Community</td>
<td>33 Incidents, 799 Persons</td>
<td>25-26</td>
<td>? Pollution of shellfish by sewage</td>
</tr>
<tr>
<td>Parramatta (NSW) Australia</td>
<td>Aug '77</td>
<td>Primary School</td>
<td>267/381 Pupils, 9/18 Staff</td>
<td>25-26</td>
<td>Virus Excretion &gt; 6 weeks</td>
</tr>
</tbody>
</table>

Seroepidemiological studies have shown that in developed countries antibody to these viruses is acquired gradually and continually through life; beginning slowly in childhood until by the age of 50, about 50% of people are seroconverted (Kapikian et al 1978). Thus most children, regardless of social class or geographical location have not acquired antibody by the age of 5 years. This suggests that Norwalk-type agents are not a common cause of gastroenteritis in young children. However, these agents may be important causes of diarrhoea in older children and adults, although many will have acquired their immunity through subclinical infection (Greenberg et al 1979a).
The importance of these 27 nm. particles as causes of diarrhoea in developing countries is unknown. But, most children in these areas have been shown to acquire antibody within the first few years of life (Greenberg et al 1979b, Blacklow et al 1979, Cukor et al 1980, Black et al 1982a). Again, many of these children must have undergone asymptomatic infection.

The conclusion I have drawn from this somewhat confusing picture is that 27 nm. particles may well be an important cause of infectious diarrhoea that is not fully recognized as yet. They probably affect older children and adults in developed countries whereas in developing countries younger children and neonates may be more at risk.

4.2 Rotaviruses

Rotaviruses were first identified in duodenal biopsies obtained from infants with acute gastroenteritis in Melbourne in 1973 (Bishop et al 1973) and within a short time similar viruses (65 - 75 nm. diameter) were detected by negative staining electron microscopy techniques in the stools of patients in many parts of the world, including a number of developing countries (Bishop et al 1974, Kapikian et al 1974, Flewett et al 1975, Lancet 1975). It was quickly realised that they were morphologically distinct from the Orbiviruses with which they had initially been classed. Due to their wheel-like appearance they were named Rotaviruses and now form a separate group within the family Reoviridae. All the Rotaviruses contain 11 segments of double-stranded RNA (Kalica et al 1976, Schnagl and Holmes 1976), are morphologically identical and share a common group antigen associated with the inner capsid. Antigens associated with the outer capsid are species specific, although there is some evidence to suggest that animal Rotaviruses may cause disease in humans (Blacklow et al 1976, Woode et al 1976, Thouless et al 1977). A variable proportion of particles have been reported to lack the outer capsid layer and these incomplete viruses probably lack infectivity (Bridger and Woode 1976). However, recently, workers have isolated 50 nm. particles which are serologically unrelated to any previous Rotaviruses from patients with acute gastroenteritis in China. They believe that this particle is infectious and is the degraded core of a new fifth sub-type of Rotavirus. Flewett et al (1978) have already described four sub-types. Rotaviruses from different species may also be distinguished by differences in their RNA migration patterns on
gel electrophoresis (Kalica et al 1976).

Rotaviruses are the commonest cause of acute non-bacterial gastroenteritis in infancy and childhood (Tarlow 1984). They account for over 50% of the paediatric hospital admissions for acute diarrhoea in children up to 7 years in Western Europe and the US (Steinhoff 1980). In addition, it has been estimated that they are responsible for between 20 and 40% of all acute diarrhoeas in developing countries (Soenarta et al 1979, Stintzing et al 1981, Hull et al 1982). Many workers consider that illness due to Rotaviruses is more severe than diarrhoea due to other pathogens (Hodes 1980, Black et al 1981, Maki 1981, McCormick 1982). Conservative estimates suggest that 4 - 6 million children die each year from acute diarrhoeal disease and it seems likely that over 1 million of these are due to Rotavirus infection (Snyder and Merson 1982). The clinical features of Rotavirus gastroenteritis are shown below. See Table 5.

Table 5
The Clinical Features of Rotavirus Gastroenteritis.
1) Indistinguishable on purely clinical grounds from other causes of diarrhoea.
2) Short incubation period of 2 - 3 days.
3) Abrupt onset with vomiting often preceding diarrhoea.
4) There is an association with upper respiratory tract infection in a proportion of cases. Some workers have speculated on the importance of droplet as well as faecal-oral spread (Foster et al 1980, Vollet et al 1981).
5) The acute stage of the illness lasts 2 - 3 days but diarrhoeas may continue for up to one week.
6) Any degree of systemic illness may occur and fatal cases have been described particularly in young children and in the elderly.
7) Characteristically affects children less than one year old, but is a significant pathogen up to 6 - 7 years old.
8) Adult infection is less common and is usually milder, as are neonatal infections.
9) Both adult and neonatal infections may be asymptomatic, although this is seldom seen in the more commonly affected age groups.
10) Boys are affected more than girls.
11) The disease is highly infectious and large numbers of viral particles may be excreted for up to one week.
The virus infects and damages the small intestinal mucosa and can be identified in duodenal and jejunal biopsies as well as stools from infected persons.

Rotaviruses are clearly a major cause of severe diarrhoea amongst infants (BMJ 1975, Kapikian et al 1976, Walker-Smith 1978). Rotavirus infection is also common in neonates but is usually milder or even asymptomatic (Christie et al 1975, 1978, Bishop et al 1975, Cameron et al 1976, Totterdell et al 1976, Murphy et al 1977). The reasons for this are not well understood but it is not due to infection by antigenically different or avirulent virus strains, and maternal antibody passively acquired in utero does not seem important. However, antibodies to Rotavirus present in maternal milk which persist for the duration of lactation and, perhaps, other non-specific factors in breast milk may well play an important protective role (Cukor et al 1980, Yolken et al 1978).

Rotavirus infections occur most commonly in temperate climes between the ages of 6 and 24 months (and almost exclusively in winter). Rotaviruses also cause sporadic cases and minor outbreaks of gastroenteritis in older children and adults (Kim et al 1977, Tufvesson et al 1977, von Bonsdorff et al 1978, Wenman et al 1978, Hara et al 1978) but presumably because most people over the age of 7 years have Rotavirus antibodies (Elias 1977) these infections tend to be milder and many are inapparent with the only evidence of infection being positive serology. However, Cubbit and Hazel (1980) have demonstrated serious, even fatal, epidemics of Rotavirus gastroenteritis in long-stay geriatric wards. More recently, a 6 - 10% mortality has been reported in similar outbreaks (Morris et al 1983).

Several authors have demonstrated transmission of Rotavirus infection from children to adults (Wald Haug et al 1978, Wenman et al 1979, Rodriguez et al 1979) and one vice versa (Kim et al 1977). Family studies show that 35 - 50% of adults in contact with children with Rotavirus gastroenteritis have serological evidence of infection and up to 25% may have symptoms (Kapikian et al 1976b, Tufvesson et al 1977). However, outbreaks in adults not in contact with infected children have been reported (von Bonsdorff et al 1976, 1978, Meurman and Lain 1977) and in one case was due to sewage contamination of water supplies (Lycke et al 1978).
It is interesting to speculate whether this contamination was of human or perhaps animal source. The importance of transmission from animals of Rotavirus is unknown.

At present it is difficult to decide what role each of these groups might play in the circulation of virus within the community. This would depend largely on the quantity and duration of viral excretion from ill and recovering patients as well as from asymptomatic 'carriers'. There is only limited information on the amounts of virus required and involved in the spread of the disease (Flewett et al 1975, Davidson et al 1975, Salisbury et al 1980, Nagayoshi et al 1980). Vesikari et al (1981) found Rotavirus shedding in infants to be highest in the first few days of illness with stools containing up to 1 mg ($10^{11}$ particles) of virus per 1 ml. After a week shedding was minimal and in all cases had ceased by one month. Older children shed virus for a longer period and as with adults may shed virus in the absence of symptoms. The stability of the virus, together with the large amounts excreted, make the disease highly infectious and environmental contamination almost inevitable.

In developed countries infant death from Rotavirus infection is uncommon, but more than 30 cases have been described (Davidson et al 1975, Carlson et al 1978). The mean age of the deaths was 11.4 months (range 4 to 36 months) and all occurred within 3 days of symptom onset due to dehydration, hypernatraemia, inhalation of vomit and convulsions. Rotaviruses have also been implicated in Sudden Infant Death Syndrome.

The results of investigations into Rotavirus gastroenteritis in developing countries gives a very variable picture. This may reflect true geographical differences or differences in:

1) The age of the population studied.

2) The season in which the study was carried out.

3) The diagnostic tests used.

Unlike temperate climes, in many hot countries Rotaviruses may be detected throughout the year, although there are seasonal variations, tending to be commoner in the cooler or rainy seasons (Schnagl et al 1977). Age also seems to be important. In Bangladesh, for example, 55% of the children under 2 years old had
Rotavirus diarrhoea but there was no evidence of entero-toxigenic E. coli. In the same area in an older age group 56% of diarrhoas were associated with E. coli but none with Rotavirus (Ryder et al. 1976a). Some of the geographical variations are shown below. See Table 6.

Table 6

The Incidence of Rotavirus as a Cause of Acute Gastroenteritis in Children in Different Tropical Countries.

<table>
<thead>
<tr>
<th>Country</th>
<th>Overall % Rotavirus Positive</th>
<th>Seasonal Prevalence</th>
</tr>
</thead>
<tbody>
<tr>
<td>India: (a) Vellore</td>
<td>26</td>
<td>Cooler (July-Dec)</td>
</tr>
<tr>
<td>(b) Calicutt</td>
<td>66</td>
<td>Rainy (Nov-Dec)</td>
</tr>
<tr>
<td>Australia:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(a) Darwin</td>
<td>24</td>
<td>Rainy (Jan-Mar)</td>
</tr>
<tr>
<td>(b) Alice Springs</td>
<td>54</td>
<td>Cooler (July)</td>
</tr>
<tr>
<td>Guatemala (Rural Village)</td>
<td>14</td>
<td>None</td>
</tr>
<tr>
<td>Costa Rica (Rural and Urban)</td>
<td>27</td>
<td>Low Relative Humidity</td>
</tr>
<tr>
<td>Costa Rica (San Jose)</td>
<td>38</td>
<td>Dry Season (Dec-Jan)</td>
</tr>
<tr>
<td>Venezuela (Caracas)</td>
<td>41</td>
<td>Cooler Months (Nov-Feb)</td>
</tr>
<tr>
<td>Rhodesia (Salisbury)</td>
<td>23</td>
<td>Cooler Months (May-Aug)</td>
</tr>
</tbody>
</table>

A method of controlling Rotavirus disease is urgently required. As has already been said, the difficulties of preventing spread within a community are enormous. One possibility is to develop a live attenuated vaccine. Human Rotavirus adapted for tissue culture (Wyatt et al. 1980, Kutsuzawa et al. 1982, Birch et al. 1983) or reassortments of animal and human Rotaviruses (Greenberg et al. 1981) may prove attenuated enough to form the basis of a vaccine and volunteer trials are underway (Kapikian et al. 1983a,b). As stated already, certain human and animal Rotaviruses are antigenically related and Vesikari et al. (1983) have found an oral live Rotavirus vaccine (of bovine origin) to be immunogenic and safe in young children. Its protective ability is as yet unassessed. So far little is known about protective immunity against Rotaviruses.
and although children rarely have severe Rotavirus gastroenteritis more than once, sequential infections by different serotypes may occur (Rodríguez et al 1979, Simmon et al 1981) and infection by one sub-group may not give protection against another (Fonteyne et al 1978, Yolken et al 1978). Intestinal immunity to Rotavirus may be very short-lived as Lambert et al (1983) have reported re-infection with Rotaviruses of the same sub-group.

4.3 Astroviruses
Astroviruses are also 25 - 28 nm. in diameter but are morphologically distinct from Rotaviruses being star-shaped with 5 - 6 points (Madely and Cosgrove 1975). These viruses may be pathogenic in young babies and have been detected in the stools of patients with acute diarrhoea (Kurtz et al 1977, Lee and Kurtz 1977, Read et al 1978 and Madely 1979).

4.4 Caliciviruses
Caliciviruses are classified as Picornaviruses and are 30 nm. in diameter (Madely and Cosgrove 1976). The association with diarrhoea is rather more tenuous than that of Astroviruses since Caliciviruses are more frequently detected in the stools of asymptomatic children. However, they may be a cause of Winter Vomiting Disease (McSwiggan et al 1978) and have been implicated in a number of cases of acute diarrhoea (Chiba et al 1979, Suzuki et al 1979, Taniguchi et al 1979, Bridger et al 1984).

4.5 Adenoviruses
Adenoviruses may be present in the faeces of healthy children as well as those with diarrhoea. However, a few workers have associated these viruses with sometimes explosive outbreaks of diarrhoea and vomiting (Flewett 1975, Madely et al 1977, Middleton et al 1977, Retter et al 1979, Richmond et al 1979, Brandt et al 1979) which can be fatal (Schnagl et al 1978). Several properties clearly distinguish enteric Adenoviruses from those causing respiratory tract infection (Madely et al 1977). DeJong et al (1983) have recently isolated 200 antigenically related Adenoviruses from cases of infantile gastroenteritis. They were found to have no serological relationship with the 39 human Adenoviruses already discovered. It was proposed that they were 2 new variants with enteropathogenic properties (Kidd et al 1983).
4.6 Coronaviruses
Coronaviruses are important causes of diarrhoea in certain animals but their role in human disease is uncertain. They have been detected in patients with both acute and chronic diarrhoeal disease as well as asymptomatic persons. Prolonged excretion of these viral particles has been reported. Some workers have also speculated about their possible role in tropical sprue (Mathan et al 1975, Schnagl et al 1977).

4.7 Conclusions
There can be little doubt that Norwalk Agents and Rotaviruses cause a significant amount of diarrhoeal disease as:
1) They are seldom found in matched healthy controls.
2) They are excreted during the acute phase of the illness.
3) The infection is associated with a specific immune response.
4) Inoculation of bacteria-free faecal filtrates from sick patients causes similar disease, excretion, and immune response in recipients.

However, the role of the other viruses discussed is more speculative as they do not fulfil the requirements above.

Little is known about the pathogenesis of viral diarrhoea. Histologically the intestinal villi appear blunted and the columnar epithelium is desquamated, being replaced by immature cuboidal cells which have migrated rapidly from the crypts. The lamina propria is infiltrated with lymphocytic cells. Biochemically, adenylate cyclase and cyclic AMP levels are not increased, but D-xylose absorption is impaired and the faecal sodium and chloride concentrations are increased (Tallet et al 1977, Woode et al 1978). Presumably enteropathogenic viruses enter and multiply within mucosal cells causing membrane damage and inhibition of normal protein synthesis leading to cell death and changes in ion fluxes across the mucosa, which in turn leads to malabsorption and secretory diarrhoea.

It is interesting to speculate whether viral infection of the gut can pave the way for secondary bacterial infection. A well-known example of this in the respiratory tract is influenza and measles virus may also do this in the gut. Although in some developed countries elimination of measles may soon be achieved, in developing countries there is a marked association between measles and diarrhoea.
in children. There appear to be two types of diarrhoea linked with measles:

1) 'With-measles' diarrhoea; either pre- or post-rash. This appears to be part of the acute disease and may be directly attributable to viral infection of mucosal cells.

2) 'Post-measles' diarrhoea. It appears that children are susceptible to diarrhoea for a long period (up to several months) after the measles itself has subsided. The aetiology of this diarrhoea is unknown but is often severe and dysenteric. It may well be due to secondary infection with Shigella or Salmonella which gain entry to the gut because of non-specifically depressed immune systems and malnutrition. Clearly measles vaccine may play an important preventative role as it has been estimated that measles is associated with 9% of all diarrhoea in children under 5 years in developing countries and may contribute to 9 - 77% of diarrhoeal deaths (Feachem and Koblinsky 1983).

5. THE ROLE OF PROTOZOA IN INFECTIOUS DIARRHOEA

5.1 Giardia lamblia

Giardiasis is an infection of the upper small intestine caused by the flagellate protozoan *Giardia lamblia*. Although Giardia infection may cause an acute gastro-intestinal upset, most infected people, especially those living where the disease is endemic, excrete the cysts in their stools asymptomatically. A smaller number of people continue to suffer diarrhoea often associated with weight loss and malabsorption. Giardiasis occurs worldwide but is particularly common in the tropics and sub-tropics. It is also a major cause of diarrhoea among travellers returning home after visiting these areas as well as the Soviet Union. In the USA water-borne outbreaks have been reported. Children are most commonly affected and usually have diarrhoea, whereas adults are less commonly affected and may be asymptomatic. Peak prevalence occurs at about 10 years of age after which it declines suggesting the development of specific immunity.

The cycle of infection and the clinical features are shown below. See Fig. 1
Fig. 1
The Cycle of Infection and Clinical Features of Giardiasis

Contaminated food and water → Person to person spread by faecal-oral route particularly in:
mentally-handicapped young children
male homosexuals

Ingestion of cysts
(100 cysts produce infection in 50% of volunteers)

Incubation period
(2 weeks)

Excretion of cysts in stools
(often for many months after patient has become asymptomatic)

Cysts become the motile binucleate trophozoite.
Found in upper small intestine attached to microvillous border by ventral sucker

Clinically producing:
prolonged diarrhoea
pale and offensive stools
malabsorption
weight loss
nausea

Fever, rigors and dysentery are uncommon.
Sigmoidoscopic appearances normal

Microvilli are damaged causing malabsorption and the accumulation of actively osmotic particles in the gut leading to diarrhoea
5.2 Entamoeba histolytica

Amoebiasis is caused by the protozoan *Entamoeba histolytica*. It occurs worldwide and in endemic areas may affect up to 10% of the population. In many cases it is a commensal, with individuals excreting the cysts in their stools quite asymptptomatically. In some cases, however, the protozoan invades the gut wall causing bloody diarrhoea. The reason why certain individuals and communities are susceptible to disease is unknown. Recently, however, Simjee (1984) has shown that only 7 of the 18 serotypes of *Entamoeba histolytica* are pathogenic.

Amoebiasis occurs mainly in adults especially 20 - 30 years old and occasionally in children, particularly those under 2 years. It is also a growing problem amongst practising male homosexuals in developed countries. The cycle of infection and clinical features are shown below. See Fig. 2

**Fig. 2**
The Cycle of Infection and Clinical Features of Amoebiasis

<table>
<thead>
<tr>
<th>Prolonged excretion of cysts in stools with or without symptoms</th>
<th>Asymptomatic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ingestion of cysts</td>
<td>Cysts release trophozoites which colonize the large bowel</td>
</tr>
<tr>
<td>Incubation period (variable, depending partly on size of challenge)</td>
<td>Invasion of mucosa causing masses of granulation tissue called amoebomas</td>
</tr>
<tr>
<td>Contamination of food and water</td>
<td>Fulminating dysentery with fever, abdominal pain, blood and mucus. Perforation of the bowel may lead to faecal and amoebic peritonitis</td>
</tr>
<tr>
<td>Person to person spread</td>
<td>Extra-intestinal complications: hepatic amoebiasis amoebic abscess of liver, pleura lung and pericardium necrotic, ulcerating skin lesions</td>
</tr>
</tbody>
</table>
5.3 Cryptosporidiosis

Cryptosporidiosis is an infection of the intestinal mucosa by a protozoan of the enteric Coccidia group (Tzipori et al. 1983) and has been recognized as a cause of diarrhoea in animals since the beginning of this century. However, until recently the evidence for them having a pathogenic role in humans was circumstantial and cryptosporidiosis was considered a rare and opportunistic infection in man (Kenneth et al. 1983). The disease was first described in humans in 1976 and since then 9 cases have been described, 5 of which were in immunocompromised patients (2 cases fatal). Although cryptosporidium causes chronic and severe diarrhoea in homosexual patients with the acquired immunodeficiency syndrome (Weinstein et al. 1981, CDC 1982b) and in other immunodeficient patients (Meisel et al. 1976, Lasser et al. 1979, Weisburger et al. 1979, Stemmerman et al. 1980, Sloper et al. 1982) it has also been shown to cause self-limiting episodes of diarrhoea in healthy laboratory workers who have ingested oocysts (Reese et al. 1982, CDC 1982a).

The source of infection is unknown but cryptosporidium oocysts are infective in freshly passed stools (Tzipori et al. 1981) and have also been isolated from the stools of well and diarrhoecal cats (Kenneth et al. 1983).

Cryptosporidium may be an unrecognized cause of diarrhoea in many persons because it is missed by routine bowel biopsy (Sloper et al. 1982) and isolation techniques (Reese et al. 1982). Although much more needs to be understood, cryptosporidiosis should be considered in the differential diagnosis of any patient (particularly if immunocompromised) with unexplained acute or chronic diarrhoea. Clearly, as in other areas of medical microbiology, with the increasing numbers of immunosuppressed patients one has to recognize that previously unimportant micro-organisms may become significant causes of opportunistic infectious diarrhoea.

However, cryptosporidium may be important in healthy patients as well as Hojlyng et al. (1984) have described this organism as being the cause of 5 - 10% of diarrhoea in otherwise healthy children in Liberia.
5.4 Blastocystosis

*Blastocystis hominis* has been classified both as a yeast and a protozoan. Although it is usually a harmless commensal of the gut, large numbers of this organism have been associated with diarrhoea in animals and man (Philips and Zierdt 1976). Recently 2 cases of mild but persistent diarrhoea associated with this organism have been reported (Ricci et al 1984).

5.5 Strongyloidiasis

*Strongyloides stercoralis* infection of the gut may provoke severe gastroenteritis in countries where it is endemic. The worm can be found in the stools, duodenal aspirate and in biopsies from infected patients.

6. THE ROLE OF BACTERIA IN INFECTIOUS DIARRHOEA

6.1 Food Poisoning

Food poisoning, that is gastroenteritis caused by ingesting enteropathogenic organisms or their toxic products in food (or in milk) has been described as "the second largest cause of morbidity in Europe ranking after respiratory infections" (WHO 1978). There are also reports of increasing incidence of food-borne disease in the USA, Australasia and several developing countries (Turnbull 1979). The exact size of the problem is difficult to assess but it is clearly worldwide and affects even the rich countries with generally high levels of hygiene and sanitation. The vast majority of food-borne disease is due to bacteria (see Table 7) presumably because unlike viruses they are able to multiply in food. Viruses are only involved where raw meats (particularly sea-food) are eaten and the precise viral agents are unidentified.

Table 7.../
Table 7
Characteristics of the Principal Food Poisoning Bacteria

<table>
<thead>
<tr>
<th>Agent</th>
<th>Type of Food Poisoning</th>
<th>Incubation Period</th>
<th>Duration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salmonella</td>
<td>Infection. Enterotoxin possibly also involved</td>
<td>6 - 8 hours (usually 12 to 24 hours)</td>
<td>1 - 7 days if uncomplicated</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>Intoxication. Toxin preformed in food</td>
<td>2 - 6 hours</td>
<td>6 - 24 hours</td>
</tr>
<tr>
<td>Clostridium perfringens</td>
<td>Intermediate</td>
<td>8 - 22 hours</td>
<td>24 - 48 hours</td>
</tr>
<tr>
<td>Clostridium botulinum</td>
<td>Intoxication. Toxin preformed in food</td>
<td>12 - 96 hours (usually 18 - 36 hours)</td>
<td>Death 1 to 8 days or slow recovery over 6 to 8 months</td>
</tr>
<tr>
<td>Vibrio parahaemolyticus</td>
<td>Infection. Enterotoxin also involved</td>
<td>2 - 48 hours (usually 12 to 18 hours)</td>
<td>2 to 5 days</td>
</tr>
<tr>
<td>Bacillus cereus (vomiting-type)</td>
<td>Intoxication. Toxin preformed in food</td>
<td>1 - 5 hours</td>
<td>12 to 24 hours</td>
</tr>
<tr>
<td>Bacillus cereus (diarrhoeal-type)</td>
<td>Unknown</td>
<td>8 - 20 hours</td>
<td>c12 hours</td>
</tr>
</tbody>
</table>

6.2 Salmonellosis and the Enteric Fevers

Salmonella species can be divided into three groups on the basis of their host predilection. See Table 8

Table 8
Classification of Salmonella Species

| Salmonella primarily adapted to man | S.typhi | S.paratyphi A,B,C | These organisms cause the enteric fevers. They are found almost exclusively in man although S.paratyphi has been isolated from certain animals. These animals are never ill and there is no evidence of transmission from animals to man. |
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Salmonellosis is characterized by an incubation period of 12 - 24 hours followed by the abrupt onset of headache, malaise, nausea, vomiting and diarrhoea which may contain blood and mucus. Abdominal pain may be mild or severe and the systemic illness variable. The acute stage lasts for about 48 hours although the patient may not feel fully well for several days afterwards.

All Salmonella are potentially able to invade the intestinal mucosa leading to septicaemia and a 'typhoid-like' illness. Certain serotypes seem particularly able to cause this severer form of disease.

Salmonellosis continues to be a significant health problem (Turnbull 1979). For example, Centres for Disease Control estimate that "Salmonella infects more than 2 million Americans each year resulting in 500,000 hospitalizations, thousands of deaths, and an annual cost of $1,5 billion in medical expenses". Apart from the medical considerations the disease weighs a considerable economic toll, both in terms of medical expenses and losses in salaries and production. Some of the reasons why Salmonellosis remains the problem that it is even in high developed countries are shown below. See Table 9

Table 8 (continued)

<table>
<thead>
<tr>
<th>Unadapted Salmonella</th>
<th>The remainder of the genus (over 1700 serotypes) have no host preference. They are all potential causes of human infection but in practice the problem of Salmonellosis is limited to a few serotypes.</th>
</tr>
</thead>
<tbody>
<tr>
<td>The epidemiology with respect to man involves a number of animal reservoirs. Transmission to man occurs through contamination by these animals of food and milk in which the bacteria may multiply. Person to person spread by the faecal-oral route also occurs.</td>
<td></td>
</tr>
</tbody>
</table>

Table 9 .../
Table 9

Some Reasons for the Persistence of Salmonellosis as a Major Health Problem Today in Developed Countries.

1) Many foods are traded internationally with many possible sites of contamination.
2) The techniques of mass production mean that a single contamination is spread to a much greater number of people.
3) The cycle of animal-to-animal and animal-to-man infection is very difficult to break.
4) More and more individuals are eating in commercial canteens and restaurants practising bulk food preparation.
5) Symptomless excreters, particularly those who are food handlers, are important sources of infection and difficult to identify.

Although almost 2,000 different Salmonella serotypes exist, only about 5% of these are associated with human disease with any frequency. Within serotypes there is the possibility for 'phage typing which has proved very useful for epidemiological tracing. Salmonella cause a variable clinical spectrum from diarrhoea with severe constitutional upset, particularly in the young and the old, to mild disease and even asymptomatic excretion. The reasons for this are not clearly understood. Factors such as the infecting dose, the immune status of the host and the particular strain of Salmonella involved are probably important.

Despite intensive investigation the pathogenesis of Salmonellosis is still unclear. The disease appears to be due to the release of a cholera-like cytotoxic enterotoxin, while at the same time there appears to be invasion of the mucosa. Some observations are shown in Table 10 (Molina and Peterson 1980, Jiwa 1981, Caprioli et al 1982).

Table 10

Some Observations on the Pathogenesis of Salmonellosis

1) Salmonella have been shown to invade the intestinal mucosa cells.
Table 10 (continued)

2) Bacteria also enter the lamina propria causing an acute inflammatory response and may also penetrate blood vessels.

3) They are also phagocytosed by macrophages, where they resist intra-cellular killing mechanisms, and are taken to local lymph tissue such as lymph nodes and Peyers' patches.

4) Salmonella also release an enterotoxin which activates membrane adenylate cyclase causing a secretory diarrhoea.

5) They also produce a cytotoxin which can break down microvilli, destroying membranes and possibly allowing penetration of mucosal cells.

6) Recently it has been shown that Salmonella release a toxin which can inhibit protein synthesis and cause extensive cell death in vitro (Koo et al 1984).

The enteric fevers, that is Typhoid and Paratyphoid fevers are systemic illnesses caused by *S. typhi* and *S. paratyphi* A,B,C. As with the other Salmonella the route of infection is by ingestion of the organism but unlike the non-enteric Salmonella animal transmission is not important and the organisms depend on human to human transfer for their continued existence.

Typhoid is endemic in many parts of the world where inadequate water supplies and poor standards of hygiene provide ample opportunity for the spread of the organism. The bacteria may survive many weeks in sewage, although in fresh water more than 90% will die in less than 4 weeks. In the Western world typhoid fever is uncommon. For example, there are about 200 cases of typhoid reported in England and Wales each year, 90% of which occur in returning travellers mostly from the Indian subcontinent. The organism is present in stools and often in urine during the acute illness, but the chronic, asymptomatic human carrier is mainly responsible for the persistence of the disease. Typhoid may occur sporadically or in epidemics. For example, in times of war or earthquake contamination of drinking water by raw sewage results in epidemic spread. Epidemics may also occur through the ingestion of contaminated food or milk.

Typhoid is a severe systemic illness only one feature of which is a watery 'pea soup' stool. The faeces may also contain blood and the commonest complication (7 - 8% of patients) is bowel
haemorrhage following invasion and erosion of the Payers' patches. Again the pathogenesis is poorly understood but the observations made for the non-enteric organisms probably still hold true. Typhoid is primarily an invasive disease but the role of other toxins cannot be discounted.

6.3 Escherichia coli

E.coli is a normal component of the commensal gut flora but may be pathogenic. I would like to consider this organisms under three headings:

Enteropathogenic E.coli (EPEC). EPEC are significant pathogens in children under 2 years old and particularly in the first few months of life. Classical EPEC do not correspond well to toxin producing strains and the relationship between invasiveness, adherence and toxin production is unclear (Matthewson et al 1983). Pathogenicity of some strains may be related to their ability to attach to the mucosa and colonize the small gut. Focal attachment with resultant damage to the absorptive epithelium has been seen in some children with protracted diarrhoea. With few exceptions EPEC do not produce known toxins nor do they invade the mucosa. However, ultra-structural studies have shown E.coli closely associated with mucosal cells (Baldini et al 1983a,b). This property appears to be plasmid mediated. Other strains have been shown to destroy the microvillous border and the apical cytoplasm causing mucosal sloughing and inflammation of the lamina propria (Cantney and Blake 1977, Takeuchi et al 1978, Rothbaum et al 1982). These E.coli were called cytotoxic strains and O'Brien et al (1977) postulated that they might release a Shiga-like toxin. However, it is not clear whether these strains belong to the classical EPEC or are just entero-invasive E.coli (O'Brien et al 1983b).

Enteroinvasive E.coli (EIEC). Kanswalshuk et al (1977) first reported that certain strains of E.coli produced a heat labile cytotoxin (VT) which destroyed Vero cells and was distinct from ST and LT. Such strains, like Shigella, can penetrate and multiply within mucosal cells and have been isolated from infants with diarrhoea and cases of haemorrhagic colitis (DuPont et al 1971, Guerrant et al 1975, Jobson et al 1983, O'Brien et al 1983b). There is also good evidence to suggest that some cases proceed relentlessly to post-infective tropical sprue (Cook 1980).
It is interesting that other coliform bacteria (*Klebsiella pneumoniae, Enterobacter cloacae*) also isolated from tropical sprue patients also produce structural changes in the mucosa but without invasion (Klipstein and Schenk 1975).

O'Brien et al (1983b) had postulated for some time that VT was related to Shiga toxin and recently (O'Brien et al 1984) have reported that one strain of EIEC (O26) produces a toxin which is biologically indistinguishable from Shiga toxin (O'Brien et al 1982, 1983a,b, Wade et al 1979).

EIEC comprise a limited number of strains and are much less common than classical EPEC. However, recently several outbreaks of illness have been ascribed to a strain of *E.coli* (O157:H7) not previously recognized as a human pathogen. The symptoms were cramping abdominal pain followed by bloody diarrhoea. Thus another bacterium has been added to the list causing presumably invasive haemorrhagic colitis (Lambert 1984).

Enterotoxigenic *E.coli* (ETEC). ETEC are able to produce heat-stable (ST) toxin or heat-labile (LT) toxin or both. Both toxins are coded for on plasmids and thus presumably transmissible between strains and even species, although this is still controversial. Takeda and Murphy (1978) have demonstrated, however, that for at least one strain of ETEC, LT is coded for on a bacteriophage.

LT is a heat-labile protein complex (Gyles et al 1974) consisting of a single A subunit (28 K Daltons) and 5 identical B subunits (11.5 K Daltons each). The B subunits bind to the mucosal epithelium by GM₁ ganglioside or glycoprotein receptor. Then, the A subunit penetrates the membrane and activates adenylate cyclase by catalysing the transfer of ADP-ribose from NAD⁺ to the regulatory subunit of the enzyme. The change in levels of cAMP cause an alteration in the ion and therefore fluid flux across the membrane leading to a secretory diarrhoea.

*V.cholerae* produces a heat-labile toxin which is now recognized as the prototype for a growing number of enterotoxins. This includes LT and recently it has been shown that DNA coding for Choleragen and LT are 78% homologous. However, whereas LT is plasmid mediated, Choleragen seems exclusively chromosomally; although there have been reports of LT being chromosomally coded in some ETEC.
ST is a low molecular weight, heat-stable, non-antigenic polypeptide, which does not act via adenylyl cyclase. Two groups of activity have been identified:

1) STa which has biological activity in mice and piglets.
2) STb which has activity only in piglets.

STa contains 18 or 19 amino acids and acts via the intra-cellular activation of particulate guanylate cyclase. It has also been shown to bind to rat basophils, causing histamine release. STa binds to a non-ganglioside specific high affinity receptor, and can be blocked by inhibitors of calcium ionophores and prostaglandin metabolism. This has led to speculation as to whether STa acts primarily by affecting the production of prostaglandins from arachidonic acid. The mechanism and nature of STb is unknown.

It appears that LT is the main toxin causing diarrhoea in humans but the role of ST is not yet known (Frantz et al 1984).

ETEC are important causes of diarrhoea in developing countries. They have been associated with serious infantile diarrhoea (Stintzing et al 1982) as well as adults with milder disease (particularly 'travellers' diarrhoea') and asymptomatic individuals (Echeverria et al 1982). However, the incidence figures for ETEC diarrhoea vary considerably between countries (Stintzing et al 1981). This may reflect true geographical differences or other variables such as study population and seasonal variations. Thus the precise epidemiology and clinical relevance of ETEC infection is poorly understood in many areas. For this reason ETEC are one of the research priorities suggested by the WHO within their Diarrhoeal Control Programme (WHO Scientific Working Group 1980). Most studies indicate that ETEC are not numerically important in infantile diarrhoea in developed countries and their incidence in adult diarrhoea is unclear. Echeverria et al (1984) have developed a typing method for ETEC involving DNA hybridization which should prove very useful for epidemiological studies.

ETEC even from widely separated geographical areas belong to a relatively small number of serotypes (Echeverria et al 1982). These strains usually produce both LT and ST (or just LT), express certain common surface antigens (Orskov and Orskov 1977, Evans et al 1977, Back et al 1980) and have been shown to lose their toxin-coding plasmids less easily than other serotypes.
(Henriquetta et al 1982). Some ETEC express surface antigens giving them an ability to adhere to mucosal cells and, presumably, colonize the gut. Diarrhoea caused by these ETEC may well be clinically worse (Levine 1981).

6.4 Campylobacter

Campylobacter species were well known as veterinary pathogens for many years, but it was not until 1972 that they were isolated in the stools of patients with diarrhoea (Deheyser et al 1972, Butzler and Skirrow 1979). They were initially described as Vibrios but were re-named Campylobacter in 1963 (Smith and Taylor 1919, Sebald and Vernon 1963). The genus is divided into two groups:

1) Catalase-negative organisms; not known to be pathogenic in man.

2) Catalase-positive organisms; which consist of C.fetus and a closely related set of organisms.

These 'related Campylobacters' (King 1957) are the organisms associated with acute enteritis in man. Smibert (1975) lists them as C.fetus sub-species jejuni in Bergey's Manual whereas Veron and Chatelaine (1973) see them as two separate species, C.jejuni and C.coli.

Whatever one chooses to call them, their main distinguishing feature is that they are relatively thermophilic, that is, they grow at 42°C but not at 25°C (whereas C.fetus grows at 25°C but not at 42°C). C.fetus only causes disease in already compromised patients and has no association with diarrhoea. There are more than 50 serotypes of 'related Campylobacter' which is useful for epidemiological purposes (Kosunen et al 1982). C.coli and C.jejuni do not always cause disease, and when they do the severity varies greatly from mild to even fatal illness. Although the latter is mainly in the elderly and debilitated, they may cause severe disease even in healthy young people. The clinical features of Campylobacter gastroenteritis are shown below. See Table 11.
Table 11
The Clinical Features of Campylobacter Gastroenteritis

1) Incubation period of 3 - 5 days, longer than most other enteric infections.
2) Prodromal period of malaise is followed by severe abdominal pain which heralds the onset of diarrhoea.
3) The temperature is frequently raised above 40°C.
4) The stools often contain blood and mucus.
5) Nausea is common but vomiting is rare.
6) The diarrhoea lasts 2 - 3 days but the abdominal pain and malaise may persist for up to 2 weeks.
7) Stools from patients not treated with antibiotics will often remain positive for Campylobacter up to 5 weeks.
8) Children are less severely affected than adults but fresh blood is more common (90%) and in a few cases abdominal pain may continue for up to 6 weeks.
9) Complications include:
   i. False 'acute abdomen' or intussusception, often leading to laparotomy.
   iii. Pancreatitis in 6% of patients admitted to hospital.
   iv. Occasional septicaemia with purulent arthritis and meningitis.
   v. Haemorrhagic colitis.

The main site of infection is the jejunum and ileum but knowledge of the pathology is limited (Evans and Dodswell 1967, Cadranel et al 1973). The colon may be involved later in the disease and clinically, as well as on colonoscopy, appearances have been confused with ulcerative colitis (Lambert et al 1979). Antibody responses appear by the fifth day, rise quickly to a maximum, and then gradually decline over a few months. After this period re-infection is possible but the disease tends to be milder (Butzler 1978).

The pathogenesis of Campylobacter diarrhoea is not well understood but there may be several mechanisms at work. See Table 12.
Table 12

The Pathogenesis of Campylobacter Diarrhoea

1) Naess and Hofstad (1982) have suggested that Campylobacter lipopolysaccharide may be important in virulence. This LPS may be particularly involved in causing the systemic upset as well as the extra-intestinal complications.

2) Campylobacter appears to produce a cholera-like toxin which also acts on adenylate cyclase (Lee et al. 1983, Ruiz-Palacios et al. 1983). This toxin appears to have a similar A subunit to Choleragen but different B subunits, and therefore presumably a different receptor. Olsvik et al. (1984) using DNA hybridization techniques have concluded that the gene coding for this toxin is Campylobacter is not closely homologous to the genes coding for either Choleragen or ETEC LT.

3) Johnson and Lior (1984) have isolated a heat-labile cytotoxin from C. jejuni but the existence of such a toxin has not been confirmed by others (Waldstrom et al. 1983, McConnell and Madden 1984).

4) However, the clinical significance of these three activities has not yet been elucidated.

In Europe and North America, Campylobacter is now widely recognized as an important cause of diarrhoea, associated with severe gastrointestinal illness, travellers' diarrhoea and outbreaks in hospitals and communities (butzler 1973, Skirrow 1977, Bruce et al. 1977, Brunton and Heggie 1977, Dale 1977, Lindquist et al. 1978, Steel and McDermott 1978, Carmali and Fleming 1979, Svedham and Kaiser 1980, Kendall and Tanner 1982). However, Campylobacter have received less attention in the tropical and developing countries. Initial studies in Bangladesh (Blaser et al. 1980) suggested that the epidemiology and clinical manifestations of Campylobacter infections were different from those in developed countries. For example, Campylobacter was frequently isolated from asymptomatic children and was more often associated with watery diarrhoea than dysentery, and primarily affected neonates and infants rather than adults (Glass et al. 1983). Similar patterns have been found in the low income groups of other countries; for example, South Africa (Bokkenhauser et al. 1979),
The Gambia (Billingham 1981), Zaire (deMol et al 1983), India (Rojan and Mathan 1982), Australia (Berry et al 1981), Indonesia (Ringertz et al 1980) and China. In developed countries, Campylobacter infection is almost always associated with disease and young children are not at the greatest risk (Blaser and Retter 1981). The reasons for these differences are not known but it is not due to a geographical variation in the virulence or serotypes of the organisms involved (Lauwer et al 1978, Speelman et al 1983).

The epidemiology of Campylobacter is poorly understood but it appears to involve a wide range of animal reservoirs, as well as person to person spread and contamination of food, milk and water. See Fig. 3

Fig. 3
Epidemiology of Campylobacter Infection

Humans - organisms excreted in faeces for up to 5 weeks. Infectivity seems low and although outbreaks have been reported, these are usually in children or in closed communities such as hospitals

Animals - although several pathways from animals to man have been shown to exist (Hastings 1980, Duffell and Skirrow 1978), we do not know what proportion of cases are due to animal infection. Animals involved include chickens, dogs, pigs and sheep

Food - there is some circumstantial evidence (Hayek and Cruikshank 1977, Peel and McIntosh 1978).

Water - transmission via water through sewage contamination of drinking supplies is probably an important route of transmission, particularly in developing countries. Contamination may be of human or animal source (Pearson et al 1977, Knill et al 1978)

Milk - there is a definite association between unpasteurized cow's milk. Campylobacter are probably introduced through faecal contamination (Robinson et al 1979)
6.5 Shigella and Plesiomonas shigelloides

The bacillary and amoebic forms of dysentery were first distinguished in 1898 by Kyoshi Shiga who described the prototype shigella strain, S. dysenteriae type 1 during a severe epidemic in Japan (mortality 25%). The Shigellae are thin, non-motile, non-sporing, Gram-negative bacilli closely related to E. coli. There are now 4 recognized species; S. dysenteriae, S. flexneri, S. boydii, and S. sonnei.

The Shigellae only naturally infect man and a few primates. In almost all cases infection is spread by the faecal-oral route (Christie 1968). Very few Shigella organisms (as few as 10 - 100 for S. dysenteriae) are needed to produce disease (Levine et al 1973). Because of this, Shigella spread very well by direct contact without the aid of a vector such as food, water or milk. However, the likelihood of transmission is enhanced wherever sanitation is poor and food and water can become contaminated. In these cases, widespread food- and water-borne epidemics may occur (Donadio and Gangarosa 1969, DuPont et al 1970). Shigella can also be spread venereally, particularly in male homosexuals (Mildran et al 1977).

During this century, there has been an as yet unexplained change in the species' prevalence. Until about 1930, most isolates were of S. dysenteriae type 1, but between the wars, S. sonnei came to predominate (Shiga 1936, Retter et al 1969). More recently, however, the numbers of S. dysenteriae cases had been increasing and perhaps we are about to witness the re-emergence of this particularly virulent species (Mata et al 1970).

When the organisms are ingested they colonize and then invade the mucosa of the colon and, sometimes, the proximal small bowel (Levine et al 1973). Shigellae release a toxin which is heat-labile and has a molecular weight of 72,000 Daltons (McIver et al 1975). This toxin was originally described as having neurotoxic, enterotoxic, and cytotoxic effects. Although this toxin causes fluid accumulation in ileal loop assays, the main characteristic of the disease is bloody diarrhoea caused by invasion of the mucosa. Work on mutant
strains lacking either toxic or invasive properties show that an invasive capacity is the one important in producing disease. Toxigenic but non-invasive bacteria can apparently colonize the colon but are unable to cause dysentery. It has been shown that the ability to penetrate and multiply within enterocytes is associated with a plasmid but the precise mechanism is at present unknown. Shigellosis appears to be a two-stage disease. Firstly, a small bowel secretory diarrhoea and then an acute ulcerating colitis (Keusch 1978). Sequential involvement of the organs in this way will explain the change from diarrhoea to dysentery often seen as part of the clinical picture. The clinical features vary considerably between patients and between infecting species. _S._sonnei often only causes mild diarrhoea whereas _S._dysenteriae infection is usually accompanied by severe bloody diarrhoea, cramp abdominal pain and severe systemic upset. Haematological, ocular, skeletal and urinary tract complications are also common. This variation may reflect a spectrum of different toxin production and other virulence factors present between the species. In summary, it seems that Shigellae may release two types of toxin:

1) an enterotoxin that causes a secretory diarrhoea perhaps by stimulating adenylate cyclase.
2) a cytotoxin which allows mucosal cell invasion.

Work has shown in studies on the cytotoxic properties of Shigella toxin preparations on HeLa cells that:

1) patients with symptomatic Shigellosis develop an IgM toxin-neutralizing antibody response.
2) toxin binds to a cell membrane receptor which appears to contain Bl-4 linked N-acetyl glucosamine.
3) the toxin appears to be taken into the cell by endocytosis.
4) the toxin is released into the cytoplasm.
5) the toxin inhibits protein synthesis, eventually causing cell death.

Apart from toxin production, there are probably other surface properties of Shigella including adherence and enzyme production which allow them to penetrate the intestinal mucosa. LPS may also be important.
Another Shigella-like organism, Plesiomonas shigelloides has been found to cause acute diarrhoea and colitis in several countries. Contaminated oysters, and other foods, were the sources of these outbreaks (Lambert 1984).

6.6 Vibrios

1) 01 Vibrio cholerae (classical and El Tor strains).
2) Non-01 Vibrio cholerae (non-agglutinating or non-cholera Vibrios).
3) Vibrio parahaemolyticus.
4) Vibrio fluvialis.
5) Vibrio mimicus.
6) Vibrio hollisae.

Cholera is caused by two biotypes of V.cholerae (classical and El Tor) and man is their only host. The El Tor variant has spread widely since its appearance in 1961; from the historic source in the Ganges delta, across Asia, through the whole of Africa, into the Mediterranean, and even as far as the Gulf Coast of the USA. Although this biotype causes less severe disease, it produces a higher carrier-to-case ratio than the classical variety. Cholera is a water-borne, and occasionally, food-borne (Holmberg et al 1984), disease spread by the faecal-oral route. The organism extensively colonizes the bowel but produces no inflammation and does not invade the mucosa. The bacteria produce a toxin which alters fluid and electrolyte balance in the small bowel, causing a massive outpouring of up to 15 - 20 litres a day.

V.parahaemolyticus is a common inhabitant of coastal waters in many parts of the world and some strains are pathogenic to man. It readily contaminates sea-food, especially shellfish. Most cases have been reported in Japan but outbreaks have also occurred in other countries, as well as on cruise ships and aeroplanes. The incubation period varies from a few hours to a few days. The main symptom is profuse watery diarrhoea but a proportion of patients get dysentery and systemic illness. Thus, unlike V.cholerae, the illness combines both toxigenic and invasive
Non-cholera Vibrios cause less severe diarrhoea than that found in cholera but also seem to resemble V.parahaemolyticus in that there is often an inflammatory colitis.

The pathogenesis of Vibrio diarrhoea has proved an interesting and rewarding field of research. Cholera enterotoxin (See Fig. 4) is responsible for the severe diarrhoea associated with O1 V.cholerae, but except for a few non-O1 V.cholerae, most enteropathogenic Vibrios do not make, or have the genetic potential to make, this toxin (Blake et al 1980, Kaper et al 1981). Thus, the mechanisms by which these other Vibrios cause diarrhoea have not yet been established. However, it seems that human isolates of non-O1 V.cholerae and V.parahaemolyticus can produce enterotoxins and cytotoxins that are distinct from Choleragen (Carruthers 1975, Sanyal et al 1983, Madden et al 1983, Nisibuchi et al 1983). It has also been found that some O1 V.cholerae make these toxins and so Choleragen may not be the only factor responsible for cholera diarrhoea. O'Brien et al (1984) have proposed that at least one of these Vibrio cytotoxins resembles Shiga toxin produced by S.dysenteriae type 1. At the moment, however, the exact role played in vivo by any of these toxins is unknown.

Fig. 4 .../
The Nature and Mechanism of Action of Choleragen

Chromosomally Coded

A subunit synthesis (28,000 Daltons)

Split by bacterial proteases between two Cysteine residues to form $A^1$ and $A^2$ joined by di-sulphide bridge

$A^1$ (21,000 Daltons) $A^2$ (7,000 Daltons)

B subunits bind to a specific $GM_1$ ganglioside receptor in the mucosal cell membrane. This causes a conformational change in the B subunits, exposing hydrophobic regions allowing insertion into the lipid bilayer. The A subunit then passes into the cell, probably via a channel formed by the 5 B subunits.

Within the cell the A subunit is reduced to form separate $A^2$ and the active $A^1$ components.

$A^1$ catalyses

\[ NAD \rightarrow \text{nicotinamide} + \text{ADP-ribose} \]  
(ADPR)

ADPR binds to and inhibits GTPase in the membrane GTPase converts active adenylate cyclase (E-GTP) to inactive (E-GDP).

\[ \text{E-GDP} \rightarrow \text{E-GTP} \]

\[ \text{ATP} \rightarrow \text{cAMP} \]

There is consequently an increase in cyclic AMP cAMP inhibits Na$^+$ Cl$^-$ transport into the cell The result is an outpouring of salt followed by water leading to diarrhoea.
6.7 Clostridium

Clostridium difficile is well known to be the primary cause of antibiotic-related Pseudomembranous Colitis (PMC) (Larsen et al 1978, Bartlett et al 1978, George et al 1978b, Cudmore et al 1982) George et al (1982) however, claimed that it was not responsible for at least 60% of antibiotic-related diarrhoea in which PMC is not documented (Teasley et al 1983, Boriello et al 1984). It has also been suggested that C. difficile may cause diarrhoea without prior exposure to antimicrobials (Wald et al 1980, Harvard et al 1980) and that other enteric organisms may predispose an individual to colonization and infection with C. difficile (Falsen et al 1980). The organism is not usually found in the faeces of healthy adults (3 - 14%) but is present in up to 40% of healthy neonates (Falsen et al 1980, Gilligan et al 1981, Keighley et al 1982, Brettle et al 1982, Riley et al 1983).

Clusters of C. difficile PMC have been reported in hospitals (Mogg et al 1977, Milligan and Kelly 1979, Greenfield et al 1981, Rodgers et al 1981, Pearce et al 1982) and recently Tabaqchali et al (1984) have provided strong evidence that PMC is infectious and that the C. difficile responsible is initially acquired from the hospital environment.

C. difficile produces a cytotoxin but its role in the pathogenesis of PMC is unclear. For example, although 40% of healthy neonates have C. difficile and its cytotoxin in their faeces they suffer no symptoms (Keighley et al 1982). Similarly, George et al (1982) have found little correlation between disease and the detection of toxin in the faeces of diarrhoeal patients.

It has also been suggested that C. difficile is responsible for acute diarrhoeal relapses in inflammatory bowel disease (Keighley et al 1978, Lamont and Trika 1980, Bolton et al 1980) but other workers have refuted this (Blaser et al 1984).
After Salmonella, *C. perfringens* is the commonest cause of food poisoning and is associated with improperly re-heating cooked meat. The incubation period is about 12 hours and the symptoms are diarrhoea, abdominal pain and rarely vomiting. Bacteria contaminating food sporulate on warming and release a heat-labile enterotoxin (Duncan 1973, Craven 1980, Craven et al 1981, Granum and Skjelkvale 1977). Some toxin may also be pre-formed in food and ingested cold. Although this type of food poisoning is best associated with *C. perfringens* type A, types C and D have also been shown to produce a similar enterotoxin (Skjelkvale and Duncan 1975, Uemura and Skjelkvale 1976). The mechanism by which the toxin acts is unclear but binds to specific cell receptors and causes severe membrane damage (Jarmund and Telle 1982).

Boriello et al (1984) have also implicated *C. perfringens* as a possible cause of antibiotic-associated diarrhoea.

6.8 Bacillus cereus

*Bacillus cereus* causes two distinct illnesses:

1) An acute illness characterized by vomiting with an incubation period of 1 - 5 hours following ingestion of inadequately re-heated rice.

2) A mainly diarrhoeal illness with an incubation period of 8 - 14 hours associated with various contaminated foodstuffs.

The pathogenesis and the nature and action of any toxins involved are unknown. Two other species *B. licheniformis* and *B. subtilis* have also been implicated in some cases of food related diarrhoeal illness (Lambert 1984).

6.9 Aeromonas hydrophila

This bacterium is a common inhabitant of water especially in warmer climates. Some strains produce an enterotoxin, and although the evidence is not conclusive, it has been associated with diarrhoea both in children and in adults (Pitarangsi et al 1982, Gracey et al 1982). Most cases so far have been in Western Australia, Indonesia and Thailand. Here the condition
usually presents with watery diarrhoea, fever and vomiting, but sometimes it resembles ulcerative colitis. About one-third of patients have diarrhoea lasting more than 2 weeks and occasionally it can last much longer. In the USA the bacterium has also been isolated from patients with mild self-limiting diarrhoea as well as some with more severe prolonged illness. On the Indian subcontinent A. hydrophila has been implicated in a cholera-like illness.

6.10 Yersinia enterocolitica

The genus Yersinia includes several human pathogens, the most important of which is Y. enterocolitica (Langeland 1984, Lancet 1984). Although nowhere very prevalent, it is being increasingly isolated from humans with diarrhoea, and seems responsible for appreciable morbidity in the cooler regions of Europe and North America. In Germany and Canada, for example, it rivals Salmonella and surpasses Shigella as a cause of acute gastroenteritis (Marks et al 1980, WHO 1983).

The organism is found in wild and domestic animals, shellfish, water, and unpasteurized milk. Most infections are sporadic and their source is unknown but family outbreaks and large community outbreaks from a common source of contaminated food have been reported. There is also some suggestion of person to person spread.

This diarrhoeal illness is most common in young children although adults may also be affected. The infection usually presents with abdominal pain and diarrhoea, and about half are febrile. The stools are pale, watery and often contain mucus; but blood is uncommon. The diarrhoea usually lasts for 2 weeks but may persist for months. The commonest form of Yersinia infection is acute terminal ileitis which is easily confused with appendicitis. This is associated with a thickened inflamed ileum and a mesenteric adenitis.

Pathogenicity is associated with a 40 - 50 MegaDalton plasmid which conveys the ability to invade the mucosa (Zink et al 1980, Jones et al 1982, Sansonetti et al 1982, Harris et al 1982) as well as other properties perhaps including mucosal adherence and toxin production (Martínez 1979, Pai and DeStephano 1982).
However, the precise mechanism by which the plasmid governs virulence is unknown. The role of an enterotoxin produced by most clinical isolates and which is physico-chemically, biologically and antigenically similar to the ST of ETEC is uncertain but may be important in some cases of Yersinia food poisoning (Brenner et al 1980, Francis et al 1980).

6.11 Spirochaetosis

Spiral bacteria have been reported in patients with cholera, infantile diarrhoea, appendicitis, ulcerative colitis, protracted diarrhoea in adults, and in healthy individuals (Shera 1962, Douglas and Krucioli 1981, Lancet 1984). Recently, Hovind-Haugen et al (1982) have described an organism, Brachyspira aalborgi, which may be associated with diarrhoea and abdominal pain (Horland and Lee 1967). Its precise role in the pathogenesis of colorectal disease remains unclear (Nielsen et al 1983) although it may be an important organism in the polymicrobial diarrhoea of homosexual men (Quinn et al 1983) and has been shown to cause subtotal villous atrophy of enterocytes (McMillan and Lee 1981).

Anaerobospirillum species have also been implicated as possible causes of human diarrhoea (Malnick et al 1983).

6.12 Kluyveia

The pathogenicity of Kluyveia species has not yet been fully established. Although normally regarded as saprophytes, they have been isolated from blood, bile, and sputum in humans. Fainstein et al (1982) have isolated Kluyveia species from the stools of 5 cancer patients and 1 healthy individual with diarrhoea. They may also be a cause of food-borne diarrhoea.

7. Conclusions ...
7. CONCLUSIONS

Although our understanding of the aetiology and pathogenesis of many types of infectious diarrhoea has been greatly furthered in recent years, many questions still remain unanswered in 1984. Infectious diarrhoea is still a major cause of morbidity and mortality in many areas of the world. Even in developed countries, food-borne disease and travellers' diarrhoea are still common. With the increasing numbers of immuno-compromised patients presenting in medical circles we may find many new opportunistic pathogens are capable of causing diarrhoeal disease. The real question is whether we can do anything to control the major problems that face us. The World Health Organization has initiated several important schemes and Peachem et al (1982) has recently put forward a number of strategies. See Table 13.

Table 13
Possible Interventions for the Control of Infectious Diarrhoea

1) By case management:
   (a) Oral rehydration - at home, hospital or primary care centre (PCC)
   (b) Parenteral rehydration
   (c) Appropriate feeding
   (d) Appropriate chemotherapy

2) By increasing host resistance:
   (a) Improving maternal nutrition. Pre-natally to increase birth-weight and post-natally to increase breast milk production
   (b) Improving child nutrition. By promoting exclusively breast-feeding up to 6 months of age. By implementing supplementary feeding up to the age of 5 years. By using standard growth-weight-height charts
   (c) Immunization. Implementing programmes for vaccines already available (Cholera and Measles). Developing new vaccines, for example, Rotavirus.
   (d) Appropriate chemoprophylaxis for those at high risk

3) .../
3) By reducing transmission:
   (a) Improving water supplies and sewage disposal
   (b) Promoting personal and domestic hygiene
   (c) Encouraging and legislating for controls on food hygiene
   (d) Identifying and controlling animal reservoirs
   (e) Insect control

4) By preventing and controlling epidemics
   (a) Epidemic surveillance
   (b) Epidemiological research

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