

Organisms Associated With Infectious Diarrhoea-

A Reappraisal For 1984.

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The Problem of Infectious Diarrhoea

In the under developed countries of the world acute diarrhoea causes 3-5 billion episodes of illness a year, resulting in 5-10 million deaths (Kapikian et al., 1980) In the developed countries diarrhoea is the second most common cause of illness affecting the community. It is especially severe in children under 5 years: 5% of children in the U.K. develop diarrhoea in the first year of life; 10% of these require hospitalisation (Tarlow, 1984).

The differential diagnosis of diarrhoea should include consideration of such conditions as inflammatory bowel disease, food intolerances (including gluten hypersensitivity or lactose intolerance), diverticulitis, colonic carcinoma and drugs such as purgatives or digoxin. However infectious agents are responsible for the majority of cases of diarrhoea.

In this essay infectious diarrhoea is taken to mean diarrhoea caused by a microorganism. This includes those illnesses caused by an organism elaborating a preformed toxin outside the host, and also those caused by localisation and multiplication of an organism in the gut. Diarrhoea can result from both systemic and local infections. In young children diarrhoea is a common accompaniment of meningitis, septicaemia, upper respiratory and urinary tract infections. Here attention is focused on those organisms that are primarily pathogens of the gastrointestinal tract.

A piece of this length cannot attempt to be comprehensive. This essay surveys those organisms associated with infectious diarrhoea, and where appropriate examines features of particular interest to us in 1984.

Infectious Diarrhoea in 1984

The interaction between man and microorganism is a complex equation that is affected by many variables. It is convenient to differentiate these into three areas: the host, the environment and the pathogen. What factors are particularly relevant to infectious diarrhoea in 1984?

The Host

An individual's lifestyle and habits will bring him into contact with a particular set of pathogens. The outcome on challenge by such pathogens will, in part, be influenced by the degree of host immunity.

In 1984 intercontinental travel has never been so simple. This exposes an individual to pathogens not normally met in his native country. He may therefore have a lower level of innate immunity to such pathogens than the inhabitants of the foreign country. In addition the speed of air travel greatly facilitates the spread of microorganisms; where previously we had epidemics now we have pandemics.

Other features of a person's lifestyle may be significant. In Japan the consumption of large amounts of raw seafood is associated with diarrhoea due to Vibrio parahaemolyticus. In Britain it has been suggested that the increase in incidence of gastroenteritis caused by Bacillus cereus is linked to an increasing number of Chinese restaurants serving insufficiently cooked rice.¹

World-wide one of the most significant features affecting a person's immunity is his nutritional status. Malnutrition is still prevalent in much of the third world. Poorly

¹ It still remains to be seen whether the Chancellor of the Exchequer's recent levy of 15% Value-added Tax on carry out food will decrease the incidence of food poisoning in Britain due to B. cereus!

nourished individuals have defective cell mediated and antibody responses. In addition the activity of phagocytes and the integrity of skin and mucous membranes are impaired. Infections are both more severe and more frequent in such people.

Developed countries also have a population of immunocompromised patients. 1984 sees the results of improvements in therapy: patients who would normally have perished as infants can survive into adulthood, yet still have compromised defences (sufferers from cystic fibrosis for example). Use of cytotoxic and immunosuppressive drugs, antibacterial drugs disturbing the commensal flora and the many invasive procedures necessary in modern medical practice result in a compromised patient population. In addition the ever increasing population of elderly residents in Britain provides a group of people whose immune functions are less active than they once were. Fungal overgrowth and parasitical infections are a troublesome cause of diarrhoea particularly in compromised hosts.

The Environment

Many of the organisms associated with infectious diarrhoea are transmitted in contaminated water. The prevalence of infection is partly determined by the standard of sanitation and supply of clean water. In 1984 the standard of sanitation in developed countries is arguably as high as it has ever been. This contrasts with the situation in the third world: it has been estimated that 30,000 people die every day as a result of poor water or sanitation. In 1980 the United Nations set up "The Decade", the aim of which was to provide safe water and sanitation for all by 1990. However a shortage of money and lack of interest by many third world governments has resulted in lack of progress.¹ Already the World

¹ The World Bank estimated that it would require an annual expenditure of 30 billion dollars. In the first year total expenditure was only 10 billion. Only 26 countries have set firm targets for The Decade, 39 are still considering it. (Economist, 1983).

Health Organisation (W.H.O.) has had to lower its objectives which are now to provide clean water in 95% of towns and 85% of rural areas, with sanitation in 80% of towns. Actions to improve the environment can lead to unforeseen side-effects. In the tropics the building of dams and development of irrigation schemes has led to an increase in both schistosomiasis and malaria (by increasing the number of sites available to their hosts for breeding). Paradoxically contaminated water can help protect against disease: frequent exposure to an organism in an area endemic for that disease can build up natural immunity in the population. Piecemeal provision of clean water might leave some groups at risk in endemic areas. This is well seen with polio, and could occur with some diarrhoeal disease, cholera for example.

The Pathogen

Some organisms have long been known to cause diarrhoeal disease: Vibrio cholerae, Salmonella, Shigella, Entamoeba histolytica for example. A knowledge of the epidemiology and partial understanding of the pathogenesis of these infections often enables rational approaches to therapy and control to be made.

In contrast however, are organisms only recently established as pathogens. Our understanding of rotavirus infection reflects the fact that only within the last decade has this virus been accepted as a pathogen. Even more uncertain are those entities such as the "small round viruses" whose very existence, let alone their association with diarrhoea, is questionable.

Are the pathogens changing in any way? Of enormous importance to medicine is the development of drug resistance. Antibiotics frequently are not indicated in the treatment of infectious diarrhoea. However infections such as systemic salmonellosis or severe bacillary dysentery do justify the institution of appropriate chemotherapy. However the organisms are now frequently resistant to a wide range of drugs. Plasmid mediated transferable

drug resistance is particularly common amongst the enteric pathogens. In many parts of the world liberal and injudicious use of antibiotics has led to^a relatively resistant bacterial population in 1984.

The pathogens may be changing in other ways. Vibrio cholerae appears to be becoming less virulent for man. It has been suggested that it may be evolving towards a state of "balanced pathogenicity" (Mims, 1982).

Certain enteropathogenic strains of Escherichia coli have become less common in the United Kingdom and the United States. This essay suggests reasons for this changing epidemiology that may be related to the bacterial genetics.

Bacterial Associated Diarrhoea

Many features of bacterial infections causing diarrhoea are well understood. The occurrence, transmission and clinical pictures of such infections are often well defined. Less advanced is our understanding of the pathogenesis of these diseases; a fuller definition of the processes involved could lead to new methods of combating disease. Progress is being made; it is appropriate then ^{to} reappraise the current state of knowledge in these areas.

Vibrio cholerae

Arguably more is known about the pathogenesis of cholera than any other diarrhoeal disease. It is said that all manifestations of disease are mediated by the effects of a single toxin. The mode of action of the toxin has been elucidated as far as the molecular level. Space precludes a detailed examination of the toxin's action. Briefly however, the toxin causes an accumulation of cyclic adenosine monophosphate (cAMP) in small intestine mucosal cells by activating adenyl cyclase. This prevents villous absorption of sodium and chloride from the lumen, whilst stimulating secretion of these ions by intestinal crypt cells. The effect is a net secretion of water and electrolytes that overwhelms the colon's reabsorptive capacity, resulting in effortless diarrhoea. The rate of fluid loss may be as high as 1 litre per hour (Carpenter, 1982).

However other factors must operate in the pathogenesis of cholera. The role of adhesion is poorly understood. Most models of cholera postulate that the vibrios adhere to the mucosa where they elaborate toxin. Adhesion may involve the binding of bacterial "adhesins" to cellular "receptors". The adhesin may be a lectin (a molecule having specificity for certain carbohydrate residues); the receptors may be fucose and mannose (Jones, 1980). The intestinal surface is covered by

a mucous gel: to mediate disease the vibrios apparently would have to penetrate this. Motility and chemotaxis are thought to be involved (Freter, 1981). However to what extent does the mucous gel impede the vibrios? Freter has suggested that penetration may not be essential. Vibrios may adhere to the mucus (rather than the cell) and elaborate toxin that diffuses to reach the cell. A better understanding of the processes involved in adhesion may suggest new approaches to preventing disease: immunisation against bacterial adhesins for example. Several extra-cellular products of V. cholerae have been described that are distinct from cholera toxin. These include a haemolysin (Honda and Finkelstein, 1979), a lecithinase (Magnusson and Gulasekham, 1965), and a phospholipase (Rodrick et al., 1982). It is not known whether they play a role in pathogenesis, or are incidental products of bacterial metabolism. Other areas of uncertainty include the question of why some individuals should become asymptomatic carriers, while others manifest the most severe form of cholera? This may be related to the infecting dose. It is known that vibrios grow to large numbers in the gut (10^6 - 10^8 vibrios/ml). (Carpenter, 1982). But how many bacteria does it take to establish infection? What role do specific and non-specific host defences play?

Balanced Pathogenicity?

Mims (1982) suggests that cholera is evolving towards a state of balanced pathogenicity with man. What this means is that the organism inflicts minimal damage, compatible with its need to enter, multiply and exit the host. Classical cholera is characteristically a short yet severe illness, often lethal within 24 hours. The vibrios are fairly sensitive to inactivation when outside the host, being excreted in the faeces for several weeks. The El Tor biotype originated in 1961 and has spread across Asia, throughout Africa and into Mediterranean Europe, replacing the classical strain. This new variety is less virulent but more

infectious.¹ Symptomless carriers occur more frequently and the organisms are fairly resistant to inactivation. Indeed the El Tor strain may not require water for its transmission and can be passed from person to person by simple contact. (Swain, 1978).

What are the implications of this in 1984? A high carrier to case ratio together with the premise that contaminated water is not necessarily needed for transmission may restrict opportunity to control the disease. Indeed we might expect to see an increasing occurrence in previously unaffected areas of the world, with asymptomatic carriers bringing in the disease.

Hence the need for effective therapy and control.

Fluid and electrolyte replacement is a highly effective form of treatment. Newer methods have tried using chlorpromazine to reverse the secretory processes once toxin has bound to the intestinal cells. (Rabbani et al., 1979). Tetracycline may be used prophylactically during epidemics and also may decrease the volume and duration of diarrhoea. However this is limited by the rapid development and spread of drug resistance (Towner et al., 1980). The present vaccine, a killed whole cell type, gives a maximum of 50-60% protection for 6 months (Bull. WHO, 1973). This is inadequate for either prophylaxis or control. Future vaccines may involve cholera toxoid or live attenuated strains. The observation that in areas endemic for cholera adults do have some immunity (presumably as a result of repeated low grade exposure to the organism), does suggest that immunisation may be a practical possibility given the correct approach.

1 Nevertheless cholera can still be a lethal disease when caused by the El Tor biotype. Obviously V. cholerae is still some distance from the true state of balanced pathogenicity!

Vibrio parahaemolyticus

Vibrio parahaemolyticus is also associated with diarrhoeal disease. The incidence varies throughout the world. This reflects both the organisms distribution and the activities of the local human population. In Japan the organism has been reported to cause 70% of all cases of gastroenteritis- the organism lives in coastal waters contaminating much seafood (Rodrick et al., 1982). Outbreaks in other parts of the world are less common, although some cases in the U.S.A. have also been associated with contaminated seafood (Lancet 1974). Once again the pathogenesis is poorly understood. A heat stable toxin has been isolated that will cause fluid secretion in ileal loops. Adhesion and invasion have also been demonstrated (Carruthers 1977).

Vibrio cholerae non O group 1

V. cholerae non O group 1 (nonagglutinating vibrios NAGs) are an important cause of diarrhoea in certain parts of the world. In areas endemic for cholera, it has been estimated that 5% of acute diarrhoeal illness may be due to NAGs. At least 50 serotypes have been identified.

The virulence of these organisms is poorly understood. A toxin resembling, yet distinct, from cholera toxin has been found in some strains (Zen-yoji et al., 1965). Escherichia coli -like ST and LT toxins may also be involved (Thorsteinsson et al., 1974). Some strains are invasive (Robins-Browne et al., 1977).

The epidemiology of NAGs is only partially defined: they would appear to be widespread in aquatic-associated environments (Rodrick et al., 1982).

We are left with many questions about the NAGs: do all the serotypes cause disease? Do they utilise the same virulence factors? Do human carriers exist? What is the relationship between the NAGs and classical

and El Tor strains of V. cholerae?

Group F (EF 6) Vibrios

The relationship of Group F vibrios and infectious diarrhoea has not been proved. These have been found in marine environments in the U.K. (Lee et al., 1978), and in patients with diarrhoea in Bangladesh, Bahrain and Jordan (Rodrick et al., 1982). Little is known about these organisms' pathogenicity.

Other vibrio species have been described- V. metschnikovii, V. vulnificus, V. alginolyticus. None appear to cause diarrhoeal infections.

Shigella

Bacillary dysentery was known in the fourth century B.C. and has been of great importance throughout history often putting entire armies out of action (Davis et al., 1980). In 1896 Shiga first described Shigella dysenteriae. It is now known that three other species also cause bacillary dysentery: S. flexneri, S. sonnei and S. boydii. With the exception of S. sonnei, disease is much commoner in tropical countries than temperate climates.

The pathogenesis of Shigella infections is partially understood: the bacteria invade epithelial cells in the terminal ileum and colon, generally being confined to the mucosa. Certain virulence factors have been defined:

Kopecks et al., (1980) have shown that the lipopolysaccharide (LPS) of S. sonnei occurs in two phases. Change from phase 1 (smooth) to phase 2 (rough) is associated with loss of virulence, and occurs with loss of a 120 megadalton plasmid.

Another virulence determinant is the ability to invade epithelial cells. Non-invasive variants of S. dysenteriae do not cause disease (Levine et al., 1973). Shigella appear to promote endocytosis in normally non-phagocytic intestinal cells by the elaboration of a heat labile extra-cellular product (Levine 1982).

A toxin has also been shown to be elaborated by S. dysenteriae. This toxin induces fluid secretion in ileal loops and is also cytotoxic (Keusch et al., 1972)¹. Cell death may be due to toxin inhibiting protein synthesis (Brown et al., 1980). The toxin is synthesised as a proenzyme; how it is activated in vivo has not been shown (Brown et al., 1980). Some evidence exists suggesting that the other

1 Ligated ileal loops are used as a model of the small intestine when testing toxins for secretory activity similar to cholera toxin.

Shigella species produce similar toxins, albeit in lesser amounts (O'Brien et al., 1977).

A toxin distinct from these others has been isolated from S. dysenteriae (Takeda et al., 1977). In Chinese hamster ovary cells the toxin will stimulate morphologic changes similar to those stimulated by cholera toxin. The relative importance of these virulence determinants is unclear. It is known that if Shigella do not have the ability to invade epithelial cells, disease will not result. In contrast, S. dysenteriae variants that do not produce toxin but do invade epithelial cells, can cause disease (Levine et al., 1973). Bacillary dysentery is often preceded by a phase of watery diarrhoea: a toxin inducing fluid secretion may be responsible (in a similar fashion to the watery diarrhoea induced by cholera toxin).

We are still some way from a full understanding of bacillary dysentery.

Escherichia coli

Certain strains of Escherichia coli are known to cause diarrhoea. On the basis of the method of pathogenesis it is possible to divide these strains into three groups. It is intriguing that these strains should adopt very different virulence mechanisms.

Enterotoxigenic E. coli

In 1968 we were unaware of the existence of enterotoxigenic E. coli (ETEC), since ^{then} we have made advances in the understanding of this organism. The ETEC cause diarrhoea mostly in infants, in adults in developing countries and in travellers to these countries. Their importance is demonstrated by the fact that in Bangladesh ETEC are the most common cause of adult and the second most common cause of infantile diarrhoea (after rotavirus). Disease is less frequent in the developed countries, however some nursery outbreaks have been documented, as have occasional food and water borne outbreaks (Bull. WHO, 1980) Plasmids are of critical importance in the pathogenesis of ETEC diarrhoea. Two toxins have been isolated from ETEC strains: both are coded for by plasmids. Heat labile (LT) toxin resembles cholera toxin in both structure and action. Heat stable (ST) toxin is much smaller (2000 daltons as opposed to 83,000 daltons) and appears to act by stimulation of guanylate cyclase, causing accumulation of cyclic GMP in mucosal cells. (Carpenter, 1982). However the exact mode of action of ST is still to be defined.

In both animal and some human strains plasmids have been found that code for factors that mediate adhesion - probably pili. These "colonisation factors" are necessary if an ETEC strain is to cause disease. In cattle, immunisation against these factors ("K antigens") will protect the animals against diarrhoea caused by these strains. This approach could also be attempted in humans, however the colonisation factors discovered

at present are only found in a limited number of ETEC stains. The other strains probably also will be found to have similar mechanisms that promote adhesion (although not necessarily antigenically similar).

We are still left with questions about the ETEC. Why are these plasmids found in a limited number of (O;K;H;) serogroups? In theory any E. coli strain could harbour such plasmids and so become pathogenic. Can exchange occur between animal strains and human strains of ETEC? If this were to occur this might expose us to a wide variety of pathogenic mechanisms adopted by E. coli throughout nature.

Enteroinvasive E. coli

These utilise a radically different pathogenic mechanism. Whilst the ETEC resemble Vibrio cholerae, the enteroinvasive E. coli (EIEC) resemble Shigella in invading the intestinal cells of the gut. The finding that one of the commoner EIEC serogroups (strain 0124) shares a common somatic antigen with Shigella dysenteriae (strain 3) has led to the suggestion that they may share a common pathogenic route. However the details of this mechanism are unclear. The EIEC are relatively rare causing sporadic outbreaks, sometimes related to contamination of food. (Bull. WHO, 1980).

Enteropathogenic E. coli

The pathogenic mechanism of enteropathogenic E. coli (EPEC) is poorly understood. It appears to be distinct from both ETEC and EIEC. Colonisation of the duodenum, jejunum and ileum occurs. The organisms can be shown to be cytotoxic in cell culture and a fluid flux demonstrated in a model: the ligated loop. (Bull. WHO, 1980).

The epidemiology of EPEC is particularly interesting. Between 1940 and 1961 neonatal epidemics of diarrhoea were shown to be due to certain "O" serogroups of E. coli. However since 1971 epidemic disease has been absent from both the U.K. and the U.S.A., with sporadic cases only. 50% of children possess antibodies to EPEC by

one year of age-asymptomatic infections would seem common (Bull. WHO, 1980).

Why has the pattern of EPEC disease changed? Could the organisms be evolving into a less virulent form? Perhaps the virulence was previously associated with a plasmid that the prevalent strains have since lost. Or does the decreased incidence represent improving control of infection in neonatal nurseries?

Salmonella

Salmonella infections are well recognised causes of diarrhoea. The genus may be divided into three groups:

1. Those species primarily adapted to man: S. typhi and S. paratyphi.
2. Those species primarily adapted to animals as a host: eg. S. dublin (horses).
3. Those species unadapted to any particular host.

All three groups may infect man. However the second two are particularly associated with diarrhoea. The first group cause typhoid fever and the related paratyphoid fever: diarrhoea is one symptom of these conditions.

Once again we have a good understanding of the epidemiology of Salmonella infections, whilst a limited knowledge of the pathogenesis.

There are over 1700 serotypes of salmonella; however only about 20 are responsible for the majority of human infections. For infection to occur a large number of bacteria must be ingested, for S. typhi this means a minimum dose of 10^3 - 10^4 organisms (Mims 1982). This is thought necessary to overcome the barrier of gastric acid; patients with achlorhydia suffer from more severe disease than normal (Lambert 1984). For these numbers of bacteria extensive multiplication must have occurred in the contaminated food or water.

Once the salmonella have penetrated the gastric acid barrier they are free to colonise the gut. Both invasion and toxin production appear to play a role. Electron microscopy has shown how salmonella adhere to mucosal cells, followed by the local degeneration of microvilli, the formation of a cavity and subsequent internalisation of the salmonella in a membrane bound vacuole. Somehow the salmonella advance through the cell and penetrate to reach the lamina propria. Surprisingly the cell does not appear to be damaged by this process (Turnbull and Richmond 1978).

Two exotoxins have been found (Peterson and Sandefur

1979). The precise role of such factors in the pathogenesis of disease has not been elucidated. DuPont (1978) postulates that once the salmonella have reached the lamina propria then the type of immune response mounted determines the nature of the resulting disease. In typhoid fever predominantly mononuclear cells are stimulated, these transport the bacteria to local lymph nodes without destroying them, where further multiplication occurs. In salmonella food poisoning the chief cells stimulated are polymorphonuclear phagocytes which are capable of destroying the bacteria in situ. Compelling though this theory may be, there is no strong evidence to support it.

We are left with many uncertainties concerning Salmonella. Why does S. typhi localise in the gall bladder in patients with chronic infection? How do salmonella penetrate through intestinal cells?

However one clear feature to emerge is that many of the estimated 200,000 salmonella infections in England and Wales each year (Bull. WHO, 1980) could be prevented. We can never hope to eliminate the organism: its prevalence in a large number of animal hosts ensures this. The 1700+ serotypes makes a vaccine impracticable. However adequate cooking with basic regard to hygiene, could ensure that the minimum infective dose of organisms is never ingested.

Campylobacter

Today Campylobacter is well established as a cause of significant numbers of cases of diarrhoea. However it has only come to our attention within the last ten years. In spite of being described in 1931 (Jones et al., 1931), it was not until 1962 that the first cases of human enteritis caused by campylobacter were reported (King 1962). The bacteria were not confirmed as major pathogens until 1973, when techniques for their selective culture were developed (Dekeyser et al., 1973).

Campylobacter jejuni is the species responsible for most instances of campylobacter enteritis. Infections have been reported from all over the world in both developed and under-developed countries (Skirrow 1977; Svedhem and Kaijser 1980; Olusanyo et al., 1980).

The prevalence is variable but may be as high as 12% in some places. Sources of infection include animals (eg. chickens, sheep and domestic animals), unpasteurised milk and water. The bacteria are comparable to Salmonella: infections peaking in the summer, causing zoonoses and occasional epidemics (Lancet 1983). Unlike Salmonella person to person spread rarely occurs, and sporadic cases are much more common (Butzler et al., 1979).

Like many other intestinal pathogens C. jejuni causes a wide spectrum of clinical illness, from asymptomatic infection to systemic infection. Generally the illness is self-limiting and resolves spontaneously without treatment.

The jejunum and ileum are thought to be the major sites of infection, although the colon may be involved.

Disagreement exists about the mode of pathogenesis.

Butzler et al., (1979) claim that invasion accounts for the majority of symptoms, although some strains have been shown to produce a toxic factor. Guerrant et al., (1978) claim that no toxins are produced and the bacteria do not demonstrate typical invasiveness. An antiphagocytic capsule has also been described (Winter et al., 1978).

So where does Campylobacter stand in 1984? The diagnosis

should certainly be considered in any case of diarrhoea. Laboratory isolation can confirm the diagnosis. When indicated erythromycin presents a suitable treatment. The epidemiology of the organisms continues to be elucidated: recent reports of campylobacter-like organisms on the gastric antrum of patients with atrophic gastritis may indicate a wider distribution than previously thought (Lancet 1983). The pathogenesis remains mysterious. No vaccine exists and some authorities believe there is no urgent need for one. However the outer membrane has been shown to be immunogenic should the need for a vaccine arise (Lancet 1983).

Yersinia enterocolitica

Yersinia enterocolitica has been reported from cases of diarrhoea from all over the world. Its overall incidence is unknown, there has however been an increase in its identification (Vantrappen et al., 1982). This may represent a real increase in incidence or it could be the result of greater awareness and diagnostic ability. Numerous sources have been reported including water supplies, milk, oysters; most authorities believe animals to be the major reservoirs of infection. Both sporadic and widespread outbreaks have been described. The clinical picture is varied: diarrhoea is common; symptoms mimicking acute appendicitis may also occur- one series in Sweden reported that 5% of all cases of appendicitis had evidence of Y. enterocolitica infection (Nilehn and Sjostrom 1967).

The diarrhoea may result from the actions of a heat stable toxin (similar to Escherichia coli ST) (Pai and Mors 1978). Invasiveness has also been documented- there appears to be some relationship with the possession of certain "O" antigens (Vantrappen et al., 1982).

In summary, Y. enterocolitica is now recognised as a pathogen. Disease tends to be mild although severe manifestations can occur. Tetracycline may be effective in such cases. Pathogenesis is poorly documented. There have been suggestions of associations with idiopathic inflammatory bowel disease, but little evidence supports this.

Clostridium perfringens

Clostridium perfringens is the most common cause of bacterial food poisoning after Salmonella. Classically, spores are thought to contaminate a cooked meat dish and multiply as it cools. In the gut sporulation occurs and the bacilli release an exotoxin. The mode of action is not so well understood. Like cholera it seems to mediate a form of secretory diarrhoea: inducing secretion of sodium and chloride ions. Unlike cholera however, the toxin interferes with metabolic processes and does not seem to induce new pathways. Changes of morphology can be observed in intestinal villi, with damage to the mucosa (M^CDonel 1979).

Prevention of disease should be simple: adequate cooking of food and refrigeration if it is not to be served immediately.

Clostridium difficile

Clostridium difficile is associated mainly with antibiotic associated pseudomembranous colitis. Patients typically present with diarrhoea approximately nine days after treatment has begun with antibiotics. Proctosigmoidoscopy reveals the pseudomembranous plaques of the colitis. However the colitis and diarrhoea do not always present together, and correlate poorly with each other. In addition, sporadic cases of diarrhoea, unassociated with antibiotic therapy have been reported due to C. difficile. (Tedesco 1982).

C. difficile has only recently been established as the cause of pseudomembranous colitis. Animal models initially demonstrated that a toxin could transmit the disease to animals previously unaffected by the disease. (Rifkin et. al., 1978). Later a cytotoxin and an enterotoxin elaborated by C. difficile were found, however their exact role in the pathogenesis of the disease is unclear. (Taylor 1979).

Another area of uncertainty concerns the transmission

of the infection. C.difficile is widespread in the environment: on the hands of hospital personnel, toilet seats (Tedesco 1982). Infection could be acquired exogenously, or endogenously from the patient's own intestinal flora. Why does the use of antibiotics predispose to this organism? After treatment, with vancomycin, relapses can occur. Does this represent reinfection, or germination of spores (released by the original infecting organisms) in the gut?

Bacillus cereus

This organism has already been mentioned as a cause of food poisoning, typically associated with fried rice. Two syndromes appear to operate: one resembling Staphylococcus aureus in causing vomiting after a short incubation period; the other resembling Clostridium perfringens. This is marked by a longer incubation period and causes diarrhoea.

Bacillus licheniformis has also been described in some cases of food-related diarrhoea (Lambert 1984).

Aeromonas hydrophila

This organism is an inhabitant of water and has been implicated as a cause of diarrhoea in West Australia and Indonesia. Both invasion and toxin production may be involved (Lambert 1984).

Viruses and Diarrhoea

The last decade has witnessed an awakening of interest in viral causes of diarrhoea. The electron microscope can frequently reveal the presence of virus particles in the faeces of patients with diarrhoea. Numerous virus types have now been described. However the presence of many of these viruses in asymptomatic controls sheds doubt on their causative role. Ideally, to assign a pathogenic role to any microorganism, it must satisfy Koch's postulates.¹ Those viruses implicated as a cause of infectious diarrhoea satisfy these postulates to different extents. Here the evidence implicating these viruses is examined and where appropriate the epidemiology and pathogenesis considered.

Rotavirus

In 1973 duodenal biopsies from patients with acute nonbacterial gastroenteritis revealed the presence of virus particles (Bishop et al., 1973). Simultaneously similar viruses were found in large numbers in the stools of patients with diarrhoea (Flewett et al., 1973). In view of its wheel-like appearance the virus was named "rotavirus".²

Of the viruses implicated as causes of diarrhoea, rotavirus comes the closest to satisfying Koch's postulates.

1 Koch's postulates (introduced by Henle in 1840) supply the criteria to prove that a microorganism is the cause of a given disease. They are:

- a. The microbe should be found in the body in all cases of the disease.
- b. It can be isolated and grown in vitro.
- c. Inoculation of this culture should reproduce this disease in susceptible animals.

2 This name was only arrived at after much confusion. The earlier literature abounds with names such as "duovirus", "reo-like virus", "orbivirus" and "infantile gastroenteritis virus". Frequently it is unclear whether the authors were referring to the same or different viruses.

It is repeatedly found in high concentrations in the stools of children with diarrhoea (Steinhoff 1980). It is found significantly less often in children with no diarrhoea. The virus can be propagated in cell culture (albeit with some difficulty), (Flewett, 1976). When adults were given oral doses, evidence of infection occurred (Middleton et al., 1974).

Infants appear to be the group most at risk.¹ There are many accounts of epidemics of diarrhoea in nurseries and paediatric wards (Bishop et al., 1976). Infections are uncommon during the first six months of life; when they occur they are often asymptomatic (Chrystie et al., 1978). Older children and adults may be infected: rotavirus has been described in both traveller's diarrhoea (DuPont 1981) and outbreaks of diarrhoea in schoolchildren (Hare et al., 1978). Adult contacts of paediatric patients often acquire an asymptomatic infection (Kim et al., 1977).

Infection occurs world-wide. In temperate countries infection rates are highest in the winter. Tropical countries show this seasonal variance less clearly. It has been assumed that the faecal-oral route provides the mode of transmission, however this seasonal variance is reminiscent of respiratory tract infections, and has led to the suggestion that rotavirus too may be transmitted by the respiratory route.

Diarrhoea may be the result of an osmotic shift of fluid into the intestinal lumen. Duodenal biopsy shows a shortening and blunting of villi, resulting in a patchy irregularity of the mucosa (Bishop et al., 1973). This diminishes the disaccharidase activity of the brush border, resulting in accumulation of disaccharide in the lumen, causing a shift of fluid to equalise the osmotic pressure (Steinhoff 1980).

1 Rotavirus may account for half of all paediatric hospital admissions for acute diarrhoea and one million deaths throughout the world, due to dehydration (Steinhoff 1980).

There is no specific treatment. WHO recommend the use of oral re^hydration therapy (Bull WHO 1982). There is a need for a vaccine- one has been used in cattle for many years. Several approaches are being tried: attenuated animal or human strains or hybrid reassortment types. Local (IgA) antibody correlates better with resistance to infection than systemic antibody (Kapikian et al., 1980). At least three serotypes have been described. The vaccine should then stimulate local antibody against all serotypes.

Norwalk-like viruses

Whilst rotavirus typically causes sporadic (and occasionally epidemic) diarrhoea in infants, another group of viruses typically cause epidemics in both adults and children. Many of these outbreaks appear to be caused by a group of 27 nm viruses: the Norwalk-like viruses. These have never been cultured, hence Koch's postulates have not been fulfilled. Nevertheless the epidemiological evidence strongly implicates these viruses as a cause of diarrhoea.

Norwalk virus was discovered following an outbreak of diarrhoea at a public school in Norwalk, Ohio (Kapikian et al., 1972). Other agents morphologically identical yet antigenically distinct have also been described, including Hawaii Agent, Montgomery County Virus and Marin County Virus. These may be different serotypes of a common agent. Study of their physical properties led to the suggestion that they might be parvoviruses. Recently it has been suggested that they may in fact be a variant of calicivirus (Barnett 1983).

Infection occurs world-wide: 50-80% of adults have antibody to Norwalk virus (Greenberg et al., 1979). 42% of all epidemics of gastroenteritis may be caused by Norwalk virus (Kaplan et al., 1982). Many infections are associated with contaminated water supplies (Taylor et al., 1981). Foodborne outbreaks have also been

described (Murphy et al., 1979). Person to person spread probably also occurs. The viruses may play a role in ^{the} aetiology of traveller's diarrhoea. Illness tends to be mild and self-limiting, the patients rarely needing hospitalisation.

Adenovirus

Adenovirus is frequently seen in the stools of patients with diarrhoea, sometimes as many as 10^{11} viruses/g (Bryden 1975). Yolken et al. (1982) claim that adenovirus is found in 52% of children with diarrhoea, as compared to 1.4% of asymptomatic controls. However other studies show no such difference between patient and control (Davidson et al., 1975; Spratt et al., 1978). Their role is still unclear. The entero-associated ^{adeno} adenoviruses like the Norwalk and rotaviruses, are fairly resistant to in vitro cultivation. This contrasts to the adenoviruses associated with respiratory tract infection.

Astrovirus

Astrovirus was first described by Madeley and Cosgrove (1975) in an outbreak of diarrhoea in infants. The virus has not been cultured and its epidemiology is largely unknown. It is transmissible; in adults it appears to be of low pathogenicity (Kurtz et al., 1979).

Calicivirus

There have been several accounts of infantile diarrhoea due to calicivirus (Cubitt et al., 1979; Spratt et al., 1978; Madeley and Cosgrove 1977). Nausea and vomiting are also common complaints. The presence of virus in the stool does not always correlate with symptoms.

Coronavirus

These club-shaped viruses are known to cause diarrhoea in pigs and cows. They have been detected in large numbers in the stools of patients with diarrhoea (Clarke et al., 1979). However they are also seen in normal adults (Flewett 1976). Like adenovirus they are also associated with infection of the respiratory tract.

Miscellaneous viruses

Enteroviruses are probably unimportant as causes of diarrhoea- in contrast to their importance in systemic infections.

Minirota, Otofuke agent, Minireovirus and a variety of agents termed "small round viruses" have also been described (Madeley et al., 1977). Their roles remain speculative. Some authors have argued that the small round viruses may be artefactual (Flewett 1976). Alternatively, they may be bacteriophages, infecting the commensal flora of the gut. Until culture techniques are available, the role of these and the other less well established viruses shall probably remain unclear.

The prevalence of viral diarrhoea is unknown. However in up to 75% of all cases of infantile diarrhoea no bacterial pathogen can be isolated. (Lancet 1975).

The relative importance of viruses in these cases remains to be established.

Parasite Associated Diarrhoea

World-wide, parasites (ie protozoa, helminths) are important causes of diarrhoea. This is a complex field of tropical medicine: here the more significant organisms are briefly surveyed.

Entamoeba histolytica

Amoebiasis, caused by Entamoeba histolytica, has a wide clinical spectrum. 80% of intestinal infections are asymptomatic; a minority present with a fatal dysentery. In addition there may be extra-intestinal manifestations. Chronic diarrhoea is a fairly common presentation. What is the situation in 1984? Amoebiasis is globally distributed: the prevalence in the tropics from 2-60%; in the U.K. from 2-5%¹ (Bull. WHO 1980). Epidemics have been associated with food and water contamination. In developed countries it is generally believed that high levels of sanitation control spread of the infection. However 1984 may see a new risk group: the male homosexual population. A carriage rate of 32% in male homosexuals for E. histolytica, as compared to 3% in the population as a whole, was reported in New York (Kean et al., 1979). This is of epidemic proportions- and in a city in a developed country, presumably with adequate sanitation control.

The virulence of E. histolytica may be an unstable phenomenon this might explain the variable clinical picture in different parts of the world. In Mexico, amoebiasis is often severe and fulminating. In temperate countries it tends to be mild. It may also explain how organisms carried harmlessly in the gut may seemingly transform into a virulent state.

¹ In the U.K. E. histolytica caused 31 recorded deaths between 1962-71. It is responsible for about 300 hospitalised cases a year (Bull. WHO 1980).

Associated bacteria or viruses may convert a harmless commensal into an invasive pathogen (Patterson and Schoppe 1982). Alternatively, E. histolytica might exist in two morphologically identical forms: an invasive and noninvasive strain. Evidence of two types of E. histolytica comes from isoenzyme studies on organisms from symptomatic and non-symptomatic patients. (Lancet 1979).¹

A combination of invasion and toxin secretion may be involved in the pathogenesis of invasive amoebiasis. The protozoa are cytotoxic in cell culture- possibly due to a toxin on the microfilaments. A phospholipase may be involved. A toxin having secretory activity has also been described (Patterson and Schoppe 1982).

Giardia lamblia

Giardia lamblia is a common cause of diarrhoea. In the U.S.A. it is the most frequently diagnosed intestinal protozoa (Wolfe 1982). It is globally distributed: the prevalence ranging from under 1% to 20%. Infants are commonly infected and may pass on the infection to other family members.

Effective hygiene should keep infection at relatively low levels. Food and water epidemics have been described (Bull.WHO 1980). Zoonoses (eg between dog and man) are thought to have caused some water-borne epidemics. Cysts can survive for up to two months in water and are resistant to chlorination.

In most people infection is mild and self-limiting. In some people chronic infection can occur. The reasons for this are obscure; sometimes a selective IgA deficiency can give rise to troublesome infections (Mims 1982).

¹ These studies demonstrated a phosphoglucomutase found only in E.histolytica from patients with active infection. However this does not exclude either of the two hypotheses: the phosphoglucomutase could be a marker for a different strain, or it may represent derepression of genetic material on transformation of a noninvasive form to an invasive state.

To further complicate the pathogenesis of the diarrhoea, infection frequently occurs in conjunction with decreased gastric and pancreatic secretion, bacterial colonisation of the small bowel and malnutrition- all of which may give rise to diarrhoea. So the contribution of G. lamblia to the symptoms is not always known.¹

Trichuris trichuria

Trichuris trichuria is the commonest gastrointestinal nematode, responsible for an estimated $\frac{1}{2}$ billion infections world-wide. Its prevalence may be 90% or more in some tropical countries (Bull. WHO 1980). The majority of infections are asymptomatic.

The most important factor determining whether a helminthic infection is symptomatic is population density of the helminths. In common with most helminths, Trichuris does not multiply in the gut; its numbers reflect the degree and frequency of exposure to soil containing T. trichuria eggs. The major age group at risk are the 1-5 year old children. If they harbour 1000 or more worms they are likely to be symptomatic- presenting with chronic diarrhoea and dysentery. The diarrhoea may be due to impaired water reabsorption by the colon, or due to necrosis of mucosal cells seen in heavy infections. (Bull. WHO 1980).

Strongyloides stercoralis

This nematode may also cause diarrhoea with heavy intestinal infection. The larvae penetrate the skin and migrate to reach the mucosa of the duodenum and jejunum. In well-nourished, immunocompetent individuals a mild episode of diarrhoea may result. However in immunosuppressed, malnourished, or individuals with a concomitant infection the nematodes may cause overwhelming infection. Chronic diarrhoea may result from autoinfection (even in healthy

¹ Again male homosexuals are at risk. In the New York study there was a reported carriage rate of 18%, compared to a rate of 3% in the rest of the population.

individuals). The infection rate world-wide is not known (Bull. WHO 1980).

Capillaria philippinensis

This severe and rapidly fatal infection has been described in the Philippines and Thailand. Capillaria philippinensis invades the jejunal mucosa, leading to a protein-losing enteropathy due to a persistent diarrhoea. The infection may result from eating uncooked fish (Bull. WHO 1980).

The other helminthic infections may on occasion cause diarrhoea, although this is not a typical presentation. These include: hookworm, ascariasis, taeniasis, Hymenolepsis.

Schistosoma

Schistosomiasis affects an estimated 250 million people (Bull. WHO 1980). Diarrhoea is a common symptom of intestinal infection. Chronic infections may result since the trematodes are adept at avoiding the host immune response (Mims 1982).

Rarer protozoal causes of diarrhoea include:

Balantidium coli: infection may be a zoonosis, pigs being the natural hosts. Infections are mostly mild, except in malnourished children.

Isopora belli: the cause of human coccidiosis. It has a global distribution, the infection rate being 1: 1000 (Bull. WHO 1980). Diarrhoea results when intestinal enterocytes are invaded and destroyed. It is rarely fatal.

Sarcocystis suhominis may be ingested on eating raw meat. It invades enterocytes, however is frequently asymptomatic. Unlike the other parasitical infections there is no treatment.

Various fluke infections, if intensive enough, may cause diarrhoea. These include: Fasciolopsis bushi, Heterophyes heterophyes, Metagonimus yoleogani, Echinostoma ilocanum, Trichinella spiralis.

Plasmodium falciparum may present with diarrhoea, especially in young children.

Diarrhoea is an uncommon presentation of Leishmania donovani and also African trypanosomiasis.

Diarrhoea in the Compromised Host

Certain parasites cause severe infections in the compromised host. Bacterial overgrowth is not a major problem in this group. Bacterial colonisation of the small intestine can certainly give rise to diarrhoea (Simon and Gorbach 1982). However in the compromised host population, bacterial overgrowth correlates poorly with diarrhoea (Boyd and Bachman 1982). Of greater importance is fungal colonisation, particularly by Candida albicans (Boyd and Bachman 1982). All parts of the gastrointestinal tract are susceptible to infection by this organism.

Conclusion

This essay has shown that many organisms may cause infectious diarrhoea. Some have long been known to be pathogenic: as a result we frequently have some knowledge as to the method of pathogenesis. Other organisms are only now emerging as potential pathogens: we are largely ignorant as to their mechanisms. However what is the purpose of the diarrhoea? It may serve as a host defence mechanism to rid the host of an infectious agent. It may be an unwanted side-effect of any invasion of the mucosa by a pathogenic microorganism. However some organisms appear to possess specific mechanisms to produce the diarrhoea, cholera toxin for example. Presumably these organisms derive some advantage from possessing such mechanisms. The diarrhoea may aid the organism in exiting the host, facilitating transmission to the next susceptible host. Alternatively, the diarrhoea may disturb the host defence mechanisms (or the commensal flora) and allow a pathogen to establish itself. Whatever the reason, diarrhoeal infection is still an important cause of morbidity and mortality throughout the world.

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