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Report of a Sub-group of the
Scientific Working Group on Epidemiology and Etiology

(Copenhagen, 15-16 January 1979)

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1. INTRODUCTION

Diarrhoea is an important cause of mortality and morbidity in infants and young children in the developing countries. Until recently, the etiology of diarrhoea could be determined in only about 20% of cases. In the last ten years intensive research into the causes of acute diarrhoea has led to the widespread recognition, *inter alia*, of three groups of *Escherichia coli* as important diarrhoeal pathogens: (1) *enterotoxigenic* *E. coli* which produce enterotoxins and are important causes of diarrhoea in infants, young children and adults in developing countries, and also in travellers to these countries; (2) *enteropathogenic* *E. coli* which have been responsible for frequent outbreaks of infantile diarrhoea in many parts of the world, especially in the period from the late 1940's to the early 1960's, and are known to belong to specific serotypes; and (3) *enteroinvasive* *E. coli* which are invasive and have a pathogenesis similar to that of shigellosis.

A clearer understanding of the pathogenesis and virulence factors of these types of *E. coli* is important for the development of specific measures for treatment and prophylaxis of the diarrhoeal illnesses they cause. There is also a need to develop simple tools for the detection of these organisms which would facilitate the research required to obtain a more comprehensive understanding of the epidemiology of the three groups of *E. coli*. Such epidemiological data will help to identify the population groups at greatest risk of disease and the means of transmission of these agents, so that specific control measures, including immunization, can be developed and applied as appropriate.

The purpose of the meeting, therefore, was to review the available information about the epidemiology, clinical features, and laboratory characteristics of these three groups of *E. coli* and determine the research priorities in these fields. Because of their potential relevance to the development of a vaccine against enterotoxigenic *E. coli* in man, the meeting also reviewed the important features of enterotoxigenic *E. coli* diarrhoea in various species of young animals and the recent advances made in the development of vaccines for use in animals.

2. REVIEW OF RECENT KNOWLEDGE

2.1 Enterotoxigenic *Escherichia coli* (ETEC)

2.1.1 Epidemiology of disease in man

Our understanding of the importance of ETEC as a cause of diarrhoeal disease has expanded remarkably in the last 10 years. Much of this rapid advancement in knowledge was facilitated by studies of cholera and cholera toxin. In 1968 we were unaware of ETEC as a cause of diarrhoea; we now know that these organisms are a major cause of diarrhoeal illness in children in the developing world, are by far the most common cause of travellers' diarrhoea, and are responsible for severe cholera-like disease in children and adults in a few cholera-endemic areas from which information is available.

Laboratory aspects

ETEC produce one or both of two recognized enterotoxins: a heat-labile enterotoxin that is immunologically related to cholera toxin (LT), and a heat-stable, low molecular weight, non-antigenic enterotoxin (ST); thus there are LT strains, ST strains, and LT/ST strains. The genetic material that controls the production of these enterotoxins is located in a bacterial plasmid, and is thus relatively easily transferable to recipient strains, at least in the laboratory.

Unfortunately there are no biochemical markers by which ETEC can be easily distinguished from non-ETEC strains, and thus no selective media can be used to facilitate their identification. Therefore, the ability to recognize these strains has rested largely on demonstration of the enterotoxins they produce.

A variety of assays have been developed for identification of these enterotoxins (Table 1). Some of the assays were originally designed and used for the identification of cholera enterotoxin and later adapted for the ETEC enterotoxins. Some tests show the presence of both LT and ST, others are only applicable to LT and one demonstrates only ST. The ones that have been used most extensively for the detection of LT are the Y1 mouse adrenal cell and CHO cell assays; for the detection of ST the infant mouse assay has been most commonly utilized. Because all these assays require some specialized procedure, the number of laboratories able to identify ETEC has been limited.

Recent evidence suggests that it may be easier to identify many of the ETEC by serotyping, as a high proportion of ETEC strains isolated from cases of cholera-like disease, travellers' diarrhoea and infantile diarrhoea in many different geographical areas belong to a limited number of well defined O:K:H serotypes. Some of the common ETEC serotypes are listed in Table 2. This association was first and most convincingly demonstrated in LT/ST strains, but is also apparent in ST strains. In studies in one area (Bangladesh), the percentage of LT/ST strains within the most common LT/ST serotypes was considerably higher than the frequency of ST strains within the most common ST serotypes, but some serotypes (e.g., O78:H11, O78:H12) were common to both. Several of these serotypes have characteristic fermentative and other physiological characters; this observation strongly suggests that certain serofermentative types - special clones - are adapted as carriers of the plasmids determining enterotoxin production. To date, only a limited number of human non-ETEC strains have been K typed and thus the occurrence of these special ETEC serotypes among non-ETEC strains is not known; the currently available data indicate that these serotypes are not common among non-ETEC strains, although few studies have been done in those geographical areas where ETEC strains are commonly found. It also appears that some O groups (e.g., O8, O25, O78, O115 and O128) occur more frequently among the ETEC in combination with different H and K antigens. It is of interest that some of these O antigens are also found in strains associated with other types of _E. coli_ disease (e.g., O128 in human enteropathogenic _E. coli_ strains, O78 in neonatal calf septicaemia).

ETEC possess other surface properties called colonization factors that might also prove useful in their identification. Some of these are referred to as K antigens, e.g., K88, K99 in animal strains (see 2.1.3) but they are _protein_ K antigens and are not related to the _polysaccharide_ K antigens.

Clinical characteristics

In spite of the difficulties in identifying ETEC, considerable progress has been made in defining the features of the illness caused by these organisms. The clinical illness caused by ETEC is largely the same whether the strains produce one or both of the enterotoxins, and ranges from mild diarrhoea to a severe cholera-like disease. There is some evidence to suggest that ST-mediated disease is shorter in duration than that caused by LT/ST strains. Both enterotoxins produce a secretory diarrhoea in the small intestine although in the case of LT, like cholera enterotoxin, the secretion is cyclic AMP mediated while the secretion induced by ST is cyclic GMP mediated.

In a one-year study conducted in a cholera-endemic area in Bangladesh, about 30% of children treated for ETEC diarrhoea at a rural hospital had moderate to severe dehydration; among the adults treated for the same disease, the proportion with severe dehydration was even higher, and these cases were often indistinguishable from cholera. In travellers, ETEC disease is usually mild and only occasionally is dehydration severe enough to require intravenous fluid replacement therapy; this is true regardless of the location of the traveller (e.g., Bangladesh or Mexico). However, the mean duration of disease caused by ETEC in travellers (3-4 days) is generally longer than the severe cholera-like disease (1-2 days) observed in India and Bangladesh; the reason for this is unclear but may be related to the fact that adults living in areas endemic for cholera and ETEC disease have acquired immunity. In studies done in the parts of the developing world where cholera is not endemic, ETEC diarrhoea in adults was usually found to be relatively mild and rarely required intravenous rehydration. Careful studies have, however, been rather few.

Antibiotics have been evaluated in ETEC diarrhoea in only one study conducted in Bangladesh. Tetracycline was shown to decrease the duration of illness caused by LT/ST strains. This effect, however, was of a small magnitude and was not felt to be clinically important. No studies have been done in patients with ETEC diarrhoea of longer duration, such as that seen in travellers.

Geographical distribution

In general, ETEC are a common cause of diarrhoea in the developing countries (e.g., Mexico, Morocco, Kenya, Brazil, Peru, Bangladesh, India) and are a relatively infrequent cause in the developed world, except in areas where sanitation is poor.

From limited studies there seem to be geographical differences in the type of enterotoxin produced by strains: for example, in Mexico and Morocco LT/ST strains predominate, in Kenya LT strains are most common, and in Bangladesh ST strains are slightly more common than LT/ST strains and LT strains are less common. However, all three enterotoxin types have been found in all geographical areas.

Incidence

From the few studies that have been done to date, it appears that in developing countries the incidence of ETEC diarrhoea is highest in children under two years of age. The incidence rapidly declines by the age of four years and remains at a lower level throughout life, suggesting that immunity has been acquired.

In the Bangladesh study cited, ETEC were the most frequent pathogen in adults with diarrhoea, and second only to rotaviruses in children. The rate of hospitalized ETEC diarrhoea cases was 8.1/1000 - the highest of all the enteropathogens. The Bangladesh study is the only one so far that has characterized the seasonality of the disease, the period of highest incidence being March through September which includes both hot dry and rainy periods.

Mode of transmission

Volunteer studies have indicated that a relatively large inoculum is required to cause illness, and that a decrease in gastric acidity may increase susceptibility. ETEC have been isolated from water and food sources, some of which have been associated with illness. There is little evidence, however, except in nurseries, of person-to-person spread. In a family study in Bangladesh, 13% of household contacts of cases visiting a hospital were found to be infected with ETEC, and most of these were under five years of age.

A number of large outbreaks of diarrhoeal illness due to ETEC have been related to contamination of water (e.g., Crater Lake National Park in the USA) or food (aboard cruise ships). There have also been well documented nursery outbreaks of ETEC diarrhoea in the USA and England.

Reservoir

Asymptomatic carriers of ETEC have been identified, especially in studies conducted in the USA, and it is presumed that humans are the major reservoir of the organisms; few definitive studies have been done, however, to test that hypothesis. Since many young animals (calves, pigs) also have severe diarrhoeal disease due to ETEC, though of different serotypes (see 2.1.2), it is interesting to speculate that there might be a relationship between human and animal E. coli strains or the plasmids they carry.

Travellers' diarrhoea

Studies carried out in Mexico, Kenya, Morocco and Honduras indicate that ETEC account for about 60-70% of diarrhoeal episodes in travellers from the industrialized countries who visit the developing world. These ETEC are the same organisms that cause the disease in small children, and it is probable that the incidence of travellers' diarrhoea reflects the extent of ETEC diarrhoea in children in any particular geographical area.

Doxycycline has been 85-90% effective in the short-term (3 weeks) prophylaxis of travellers' diarrhoea in Kenya and Morocco. In these areas, the ETEC were generally sensitive to the drug. No studies have yet been done in areas where ETEC are known to be frequently resistant to antibiotics.

2.1.2 ETEC infection in animals

Enterotoxic colibacillosis (ETEC infection) is one of four types of E. coli colibacillosis seen in animals caused by E. coli, the others being septicaemic, local-invasive and enterotoxaemic illnesses. ETEC infections tend to be confined to animals a few days or weeks old and faecal-oral transmission is predominant. In so far as they have been investigated, the associated E. coli tend to be clustered globally into serogroups characteristic of host species (Table 3). Most outbreaks appear to be initiated by the introduction of asymptomatic adult carriers into an adverse environment (crowded conditions; cold or rapidly changing temperatures; damp; filth accumulated in prolonged occupancy; or changes in pen, pen mates, and diets associated with recent handling and shipping). The major source of continuing transmission is ill animals of the same age and species. Animals infected with ETEC are frequently also infected with enteropathogenic viruses and/or protozoa; it is thought that synergism in these mixed infections increases morbidity and mortality. Enterotoxic colibacillosis in animals is of major economic significance. Similarly as in ETEC diarrhoea in humans, this disease is characterized by proliferation of E. coli in the small intestine, where they produce LT and ST enterotoxins which cause the small intestine to secrete fluid. To date, ETEC strains isolated from calves produce only ST while those isolated from pigs produce ST or LT and ST. The LT enterotoxin produced by animal ETEC strains is antigenically related to the LT toxin produced by human ETEC (and thus to cholera toxin). However, there appear to be several minor variations in the animo-acid structure of ST infecting different animal species and man. Thus, dependence on a single assay system, such as the infant mouse, may have led to underestimates of the prevalence of ST-producing strains in animals.

2.1.3 Pili (colonization factors) and their potential role as protective antigens

Pili (fimbriae) are non-flagellar, filamentous appendages which are protein in nature, are produced by a wide variety of bacteria, and can be classified according to their numerous distinctive physical, chemical, antigenic and functional characteristics. Several functional characteristics of pili and piliated bacteria are believed to contribute to virulence. Their ability to adhere to each other and to things with the attendant potential advantage for colonizing epithelial surfaces is perhaps the most widely recognized. Apparently, both specific receptor mediated and non-specific (hydrophobic and charge interactions) types of adhesion occur. Peristalsis has long been considered as a major defence mechanism of the small intestine and the advantages of an "adhesive" pathogen in this environment seem obvious.

There are several lines of evidence indicating that colonization of pig small intestine by ETEC is facilitated by a family of pili designated K88, K99 and 987P (987 pili) and that the presence of such pili is necessary for the production of disease. These pili are physically, chemically, functionally, and antigenically distinct from each other and are also antigenically distinct from the two pili similarly implicated in colonization of the human intestine (CFA I and CFA II). Since these five identified pili are necessary for ETEC colonization of the small intestine, they have been functionally termed colonization factors. The most widespread of the recognized E. coli pili are the type 1 pili ("common pili" or "common fimbriae"); these pili are also antigenically distinct, and although some animal and human ETEC produce type 1 pili, their role in intestinal colonization is unknown. Acquisition of type 1 pili is chromosomally determined while that of the colonization factor pili is plasmid-mediated.

Pili can be differentiated by their pattern of adhesion to epithelial cells or haemagglutination with erythrocytes from a variety of animal species. Intestinal epithelial cells from a single individual apparently contain specific receptors for several different types of E. coli pili. Type 1 pili cause mannose sensitive haemagglutination of guinea-pig erythrocytes. K88, K99, 987P, CFA I and CFA II either do not haemagglutinate guinea-pig erythrocytes or do so in mannose resistant fashion.

Pilus antigens behave serologically like K antigens and thus K88 and K99 were designated as K antigens even though they were recognized as protein. (E. coli K antigens are polysaccharide). A broad definition of pili has been operationally useful as it has led to the search for certain types of surface antigens facilitating intestinal colonization (protein filaments which are host and tissue specific adhesins). This approach led to the recognition of 987P functionally and morphologically. Since the morphology of 987 pili was similar to that of type 1 pili, the pili on strain 987 were designated 987P.

A considerable amount of information is available about the three recognized colonization factor pili in animals. K88 has been found frequently on swine ETEC worldwide. Many ETEC associated with enterotoxic colibacillosis of neonatal pigs in North America lack K88, but all of these strains investigated to date have had either K99 or 987P, suggesting that the family of pili that facilitate colonization of the neonatal pig intestine by ETEC is small. K99 or 987P have not so far been found on wild-type non-ETEC or on strains associated with disease in weanling swine; this suggests that 987P and K99 in swine are confined to strains that infect the neonate. In contrast, K88 occurs commonly, but not universally, among strains of E. coli associated with diarrhoea in weanling swine.

K99 is also common among ETEC that are enteropathogenic for calves and lambs and is probably the major pilus facilitating intestinal colonization in neonates of these species. $K88^+$ and $987P^+$ ETEC have been isolated also from calves, although the best evidence available indicates that $K88^+$ and $987P^+$ ETEC are not enteropathogenic in calves or lambs.

Many E. coli strains shift to the nonpiliated phase during culture on agar, and towards the piliated phase during prolonged culture in still broth. Strains that produce 987P are typical in this regard; furthermore, they shift dramatically to the piliated phase during growth in pig small intestine. Thus, with most isolates, specific techniques are required to demonstrate 987P in culture. Serological detection of K99 in culture can also be difficult. $K88^+$ ETEC tend to colonize intensively (and thus form adherent layers readily demonstrable by light microscopy) throughout the small intestine. In contrast, $987P^+$ and $K99^+$ ETEC tend to do so mainly in the lower half of the small intestine. Immunofluorescent staining (using specific 987P, K99 and K88 antisera) of bacteria in sections or scrapings directly from the ileum of the colonized host is useful as a sensitive and simple method for the detection of these pili. Examination of ETEC directly from colonized intestine, or with minimal passage on artificial media, may also be a useful method to search for additional pili involved in colonization.

The colonization factors in human ETEC strains, CFA I and CFA II, have only recently been discovered. They are immunogenic and appear to be more heat-stable than the three pili described in animals. Diagnostic antisera for CFA I and CFA II can be produced in rabbits and used to identify these factors in simple bacterial agglutination tests. Although only a limited number of ETEC strains have been examined for CFA I and CFA II, there appears to be some relationship to serotype (Table 2). In addition, for the present, it appears that a majority of strains with these factors produce LT and ST. In veterinary diagnostic bacteriology much reliance is laid on the capacity for haemolysin production, another plasmid determined character usually found in ETEC strains from baby pigs. No such character useful for the screening of ETEC has so far been found among human strains.

Volunteers fed a mutant of a LT/ST, CFA I-positive ETEC strain (H-10407) which lacked CFA I did not become ill and did not develop a rise in antibody to CFA I antigen as was observed in volunteers receiving the parent strain. Although the mutant strain also lacked the capacity for ST production, this study gave strong support to the importance of CFA I in pathogenesis.

Vaccination with pili: A considerable amount of experimental work has been done in animals to develop a vaccine for protection against ETEC using pili as the protective antigen.

Piglets suckling dams parenterally vaccinated with partially purified K88 were protected against fatal diarrhoeal disease caused by oral challenge with a K88$^+$ ETEC. The protective antigen was probably K88 because the vaccine was prepared from a nonenterotoxigenic, nonmotile strain of E. coli with different O and polysaccharide K antigens from those of the challenge strain, and vaccination stimulated the production of K88 antibodies in colostrum and milk. Piglets suckling dams parenterally vaccinated with purified 987P were protected against fatal diarrhoeal disease caused by orogastric challenge with the vaccine strain or another 987P$^+$ ETEC which was nonmotile and had O and polysaccharide K antigens different from those of the vaccine strain. In both instances, protection correlated with 987P antibodies in colostrum and with impaired colonization of the ileum by the ETEC. Furthermore, most of the ETEC recovered from the ileum of the protected pigs were in the nonpiliated phase, while most of those from the controls were in the piliated phase. In contrast, vaccination with 987P did not protect against challenge with an ETEC bearing heterologous K99 pili.

Piglets suckling dams vaccinated parenterally with purified K99 pili were protected against fatal diarrhoeal disease caused by orogastric challenge with a K99$^+$ ETEC. The only demonstrable surface antigen common to the challenge strain and the strain used to purify the pili was K99. Protection correlated with K99 antibodies in colostrum and with slightly impaired intestinal colonization by the ETEC. In contrast, vaccination with purified K99 did not protect against challenge with a 987P$^+$ ETEC.

In these experiments, vaccination with 987P and K99 apparently boosted pre-existing levels of antipilus antibodies. In most dams serum K99 and 987P agglutinin titres were low prior to vaccination and rose sharply after the initial parenteral vaccination, but changed little following the second parenteral vaccination two weeks later.

Immunization by vaccination with purified K99 was recently confirmed and extended by the finding that calves (suckling cows vaccinated with purified K99 material cited above) were protected against fatal diarrhoeal disease caused by K99$^+$ ETEC. There is also evidence that K99 can be a protective antigen in whole-cell bacterial vaccines for cattle.

A system of immunizing suckling newborn pigs against ETEC by feeding live ETEC to pregnant dams has been successfully applied. Although the protective antigens in this system are unknown, it is clear that immune pigs are protected from colonization by ETEC. Very recent work demonstrated protection when the vaccine strains and the challenge strains were serotypically heterologous except for the 987 or K99 pilus antigen (both vaccine and challenge strains produced only ST). However, nonpiliated mutants of one challenge strain also protected when they were used as the vaccine and the pigs were challenged with the piliated parent strain. These preliminary data are consistent with the hypothesis that pili can be protective antigens when given orally as live cultures and also indicate that there are protective antigens in addition to pili.

These veterinary studies support the hypothesis that pilus vaccines protect through antibody blockage of specific pilus-mediated adhesion of ETEC to the intestinal epithelium. Alternative or additional mechanisms of protection by pilus antibodies are possible. For example, antibody on the bacterial surface might protect even if the antigen were not a virulence attribute of the ETEC. Thus, it may be that pilus vaccines protect just because pili are produced in vivo and provide an abundant surface lattice of good protein antigen for the deposition of specific antibody. If this is true, than any pilus produced by ETEC in the small intestine is a potential protective antigen, regardless of whether or not that specific pilus is a virulence attribute. The material reviewed above, however, is, in aggregate, strong evidence that certain specific pili (K88, K99 and 987P), which are virulence attributes, can be protective antigens in vaccines for the control of ETEC infections in young animals; it further suggests that similar studies should be undertaken in man.

2.2 Enteropathogenic E. coli (EPEC)

Epidemiology

The attempt to implicate E. coli in the etiology of infantile enteritis commenced when Escherich first isolated the organism in diarrhoea of infants in 1885. During the 1920's and 1930's several workers tried to identify specific types of E. coli as etiological agents but no significant progress was made until a definitive serotyping scheme was produced by Kaufmann in the 1940's. Epidemiological investigation of serious outbreaks of infantile enteritis in London and Aberdeen in the late 1940's clearly showed that certain serological types were responsible.

Additional serotypes were identified in subsequent years by epidemiological investigation of outbreaks; by 1961, some 17 'O' serogroups had been recognized as causes of epidemic infantile enteritis in many countries. These were the O serogroups: 18, 20, 25, 26, 28, 44, 55, 86, 111, 112, 114, 119, 125, 126, 127, 128 and 142. Six of these O groups (26, 55, 111, 119, 127, 128) were found to be particularly common. Within most of these O groups there are a number of H antigens which appear more frequently.

Mortality in these early reported EPEC outbreaks was high (e.g., about 50% in Aberdeen). Since 1950 the mortality in EPEC outbreaks in Western Europe and North America has declined; although this was probably due to improved clinical management another possible factor may be a decrease in the pathogenicity of EPEC. Since 1960 there has also been a noticeable decrease in the incidence of epidemic disease due to EPEC in the developed countries, although a few outbreaks occurred in the late 1960's in Great Britain. Since 1971 serious epidemic EPEC disease has been absent from Britain and North America. The reason for this marked decline in frequency of EPEC outbreaks is unknown.

Prior to 1930, in Great Britain and many other countries with a temperate climate, epidemics of infantile diarrhoea affected primarily infants belonging to the poorer socio-economic groups in the industrial towns, and had their peak incidence in the warmer months. The EPEC outbreaks in the 1940's and 1950's mainly affected neonates or young babies in institutions such as maternity units or day nurseries, and usually occurred in the colder months of the year. The outbreak was usually initiated by the admission of a baby with diarrhoea. During these months the number of babies hospitalized for other infections, notably of the respiratory tract, was high and thus overcrowding may have been an important factor in facilitating spread by cross-infection once EPEC disease had been introduced into a unit. The present situation in Britain, where epidemic EPEC disease is absent, shows a seasonal peak of EPEC isolations from sporadic cases of infantile enteritis in the summer months.

The epidemiology of EPEC disease in the developing countries is not so well defined, but it appears to be different from that in the developed countries. Because breast feeding is more common, complete weaning often delayed until the age of 18 months or even later, and hospital deliveries fewer, EPEC are more frequently isolated from diarrhoea cases in the second six months of life. Also, institutional outbreaks have been less frequent and community outbreaks more common.

In both developed and developing countries the etiological significance of the isolation of EPEC from sporadic cases of infantile enteritis is unclear and controversial, and has raised questions about the value of routine serotyping of E. coli from sporadic diarrhoea cases. It is felt that, while research in this area continues, each country must decide on the value of such serotyping taking into consideration the availability, range, quality, and the cost of antisera and the reliability of ongoing surveillance systems.

EPEC diarrhoea in adults

About 50% of children possess haemagglutinating antibodies to EPEC by the age of one year, suggesting that they are exposed to infection with EPEC early in life. It has been suggested that this indicates acquisition of immunity and explains the rare occurrence of EPEC disease and the high frequency of EPEC carriers among adults. Another variable is that bacteriologists do not usually search for EPEC serogroups when investigating outbreaks of diarrhoea in adults. A few outbreaks have occurred recently in adults, including a waterborne outbreak due to E. coli 0111B4 in 1968 in the United States and two foodborne outbreaks in Great Britain, one in 1967 due to E. coli 0126B16 in cold pork and the other in 1973 due to E. coli 0127B8 in pie.

Antibiotic resistance

In the 1950's and 1960's neomycin was often used to treat EPEC diarrhoea. Neomycin-resistant strains were soon reported and were the cause of some epidemics. The serious hospital epidemics that occurred in Britain in the 1960's due to E. coli 0119, 0114 and 0142 were caused by strains that possessed multiple drug resistance. Often the spread of these resistant strains was facilitated by the use of antibiotics for the treatment of other diseases (e.g., respiratory disease). Examination of EPEC from worldwide sources reveals that about 50% have multiple drug resistance. Such strains have frequently been isolated from serious epidemic disease and there is even some suggestion that multiple resistance may be associated with enhanced virulence. Such an association has been observed with Salmonella strains - e.g., S. wien in Europe and S. typhimurium phage type 193 in South America.

Pathogenesis

In early studies with strains from outbreaks of infantile enteritis a number of workers demonstrated that diarrhoea could be produced by oral feeding to adult volunteers. Post-mortem studies following outbreaks demonstrated that an essential feature of EPEC disease was the colonization of the duodenum, jejunum and upper ileum by the pathogenic strain. More recently this same feature has been demonstrated using duodenal intubation techniques.

It has been shown that epidemic EPEC strains do not produce the enterotoxins of ETEC as identified by the CHO and Y1 tissue-culture assays for LT and the infant mouse test for ST. Such strains were recently fed again to adult volunteers and caused severe diarrhoea. Further laboratory studies with the strains using the perfused rat jejunum showed that these isolates caused a net efflux of fluid into the intestinal lumen. These studies demonstrated unquestionably the pathogenicity of the epidemic EPEC and suggested that a different type of toxin may be responsible for the disease, but no virulence factors have yet been identified.

2.3 Enteroinvasive E. coli (EIEC)

Epidemiology

In 1967 a report from Japan suggested that certain E. coli serogroups other than the common infantile EPEC were being isolated from the stools of older children and adults with dysentery-like disease; these serogroups were 0124, 0136 and 0144.

The '0' serogroups to which "dysentery-producing" strains often belong were gradually extended and now include 0 groups: 28ac, 112ac, 124, 136, 143, 144, 152 and 164. There are H antigens that are frequently found with some of these antigens, although these strains are often non-motile.

The best known of these serotypes is 0124. This serotype was first isolated from United States troops in the Mediterranean area between 1943 and 1945 and caused an outbreak of acute diarrhoea in a school in Britain in 1947. Between 1970 and 1974 this serotype was responsible for sporadic cases and four hospital outbreaks in adults in Britain; in some outbreaks cross-infection occurred. It has also been responsible for a number of waterborne community outbreaks in Hungary and a large multi-state outbreak in the USA attributed to imported French cheese. Outbreaks due to E. coli 0164 (previously designated 145/46) have been reported in mothers and babies in a hospital in Australia, in sporadic cases and institutional outbreaks in Britain, and in sporadic cases in Israel.

Pathogenesis

Laboratory studies using tissue cultures and experimental animals (including the rabbit gut loop) showed that these dysentery-producing strains did not produce enterotoxins (ST or LT) but caused epithelial invasion. The dysentery-producing E. coli are now referred to as enteroinvasive, and their ability to produce kerato-conjunctivitis in the guinea-pig eye (the Serény test) is often used to demonstrate this epithelial invasive property. An alternative test that has received limited study uses HeLa cells in a tissue culture technique.

Laboratory identification

EIEC strains are often atypical biochemically. Lactose may be fermented late or not at all and anaerogenic strains occur. Furthermore, the EIEC serogroups often exhibit marked sharing of antigenic components with Shigella. For example, E. coli 0124 and 0164 share major antigenic components with Sh. dysenteriae 3. The somatic antigen of E. coli 0124 is identical to that of Sh. dysenteriae 3, while that of E. coli 0164 is similar but may be distinguished serologically. Similar examples exist for other serotypes of EIEC.

Because of their biochemical and antigenic resemblance to Shigella, it is likely that many outbreaks due to EIEC have been reported as shigellosis. This is most likely to occur in laboratories with limited facilities, but it is noteworthy that the extensive outbreak due to E. coli 0124 in the United States cited above was first identified as being due to Sh. dysenteriae 3. From the clinical point of view it is perhaps of little importance if these enteroinvasive serogroups are misidentified because they produce a disease that may be indistinguishable clinically from bacillary dysentery. However, for epidemiological purposes accurate etiological diagnosis is essential.

3. RESEARCH NEEDS

Taking into account the currently available knowledge on E. coli diarrhoea, the Group identified the following areas as requiring further research, without attempting to place these in any order of priority.

3.1 Enterotoxigenic E. coli (ETEC)

3.1.1 In order to facilitate studies on the epidemiology of ETEC, simple but sensitive tests need to be developed and evaluated to detect ETEC and to identify its properties. These tests should be suitable for use in clinical microbiological laboratories. The following areas in particular require further research:

- Recognition of LT and ST: A particular problem is the detection of ST for which at present only the infant mouse model is available. Furthermore, there is increasing evidence, especially in animals, that some types of ST are not detected in the infant mouse test. Serological tests, such as the ELISA assay, appear to be promising for LT detection but need further evaluation and simplification. If it becomes possible to raise antisera against ST (through the use of ST coupled proteins), high priority should be given to development of a serological test for ST recognition also.

- Colonization factors: Detection of colonization factor antigens, such as CFA I and II, may be useful as a screening procedure. Other surface antigens undoubtedly exist and these need to be identified. The role of type 1 pili should be investigated to determine whether they are produced in the small intestine, whether they facilitate colonization, and whether they are protective antigens. This work on colonization factors should be pursued in human and animal strains as it holds the best promise for the development of a successful vaccine (see 3.1.7).

- Serotypes and biotypes: Additional information is needed on the serotypes of ETEC strains from different geographical areas; this will facilitate the development and evaluation of serotyping as a diagnostic procedure for ETEC diarrhoea. Also needed are additional data on the frequency of ETEC serotypes in the normal population in different geographical areas. A range of antisera that can detect the most common serotypes among ETEC are at present being evaluated; these need further testing in different geographical areas.

- Plasmids: The characters of plasmids from ETEC strains isolated from heterogeneous sources need to be studied. The genes for enterotoxin control (Ent^+) probably occupy only a small portion of the genome and it may be valuable to identify genetic determinants that may be associated with Ent^+ , such as those that determine antibiotic resistance.

- Antibiotic resistance patterns: Strains from different geographical areas should be studied to determine their antibiotic resistance patterns and whether there is any relationship between toxin type and antibiotic resistance. A detailed genetic study to elucidate this possible relationship would be useful to determine the risks and benefits of the use of antibiotics for the treatment and prophylaxis of ETEC disease.

3.1.2 The general epidemiology of ETEC disease needs to be investigated in different geographical areas. Such studies should include the frequency, age-sex incidence and seasonality of ETEC disease, its relationship to nutritional status and breast feeding, and its means of transmission. They should be carried out in a variety of cultural and topographical situations. These epidemiological studies of ETEC should, where possible, include also the search for EPEC, EIEC and viral agents, such as rotavirus.

3.1.3 Longitudinal studies are needed, especially in young children, to define the acquisition of immunity as it relates to ETEC infection and illness. In particular, it is important to learn the relative importance of antibacterial and antitoxic immunity, and the cross protection, if any, that exists between cholera and the disease caused by LT-producing ETEC.

3.1.4 The possible relationships between animal and human strains of ETEC need to be explored further, including detailed studies of Ent^+ plasmids from human and animal strains, a search for human ETEC in animals and a search for animal ETEC in humans.

3.1.5 The clinical picture of diarrhoea caused by ETEC needs to be related to the toxin type(s) produced by ETEC in different geographical areas. Of particular importance is a detailed study of the electrolyte composition of jejunal fluid and stool in children.

3.1.6 It is necessary to evaluate the effect of antibiotics in the treatment of infantile diarrhoea due to ETEC and in the treatment and prevention of travellers' diarrhoea. Studies of prophylaxis should be done in a double-blind manner using appropriate antibiotics. Studies of treatment should include an evaluation of other antidiarrhoeal agents (e.g., antispasmodic drugs).

3.1.7 Development of a suitable vaccine against human ETEC disease deserves high priority and activities in this respect should take into account the following considerations:

- Immunity at the mucosal surface of the intestine is probably the most important mechanism of protection, and therefore oral vaccines deserve thorough study.

- Since ETEC disease incidence is highest in children aged 6 months to 2 years and also in travellers to endemic countries, the induction of active immunity seems most appropriate. However, studies should also evaluate the immunization of mothers near term with the objective of inducing high titres of antibodies in breast milk which might protect their babies.

- The most appropriate antigens for use as vaccines are not yet known. A self-replicating live vaccine strain of E. coli containing the most important protective antigens would be the most desirable. Non-replicating antigens such as colonization factors, cell-wall antigens and toxoids need also to be studied as possible oral vaccines. In this regard the Group supports the priorities outlined in the Report of the Scientific Working Group on Immunity and Vaccine Development.*

- As part of the strategy for the development of a suitable vaccine and an appropriate method for its administration, support should be given to veterinary studies of the type summarized in section 2.1.3. The similarity of ETEC diarrhoea in certain animal species and man offers an unique opportunity for the development of a human vaccine through intensive veterinary research.

3.2 Enteropathogenic E. coli (EPEC)

3.2.1 There is a need to evaluate the role of EPEC in infantile diarrhoea in a range of epidemiological settings, both in the community and in institutions. Studies in developed countries in the temperate zone have provided a clear insight into the spread of EPEC in hospital units housing young babies, but there is a lack of reliable information on EPEC diarrhoea in the community in developed and developing countries. This deficiency in knowledge should be corrected by means of comprehensively planned case-control studies in a variety of geographical areas. Such studies should include a search for other enteropathogens. It is not sufficient to use polyvalent pooled antisera for serotyping EPEC; the determination of O groups is essential and full serotyping of O and H antigens is ideal.

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* Unpublished document WHO/DDC/78.2 (1978)

3.2.2 Evidence from a few outbreaks suggests that EPEC can cause acute diarrhoea in adults, and this aspect requires further assessment through case-control studies using appropriate antisera (see 3.2.1).

3.2.3 The apparent age-related immunity to EPEC diarrhoea has not been explained. Immunological studies should be used to investigate this observation, including the development of assays to measure antibody to these organisms or its products or virulence properties (see 3.2.4), and the application of such assays in sero-surveys. The basis of the observation that breast feeding protects infants against EPEC diarrhoea also needs to be established.

3.2.4 Little is known about the pathogenesis of EPEC disease, although preliminary results using an animal model suggest that a "toxin" is involved. Further work is needed in this field, and if the "toxin" is confirmed, a simple laboratory assay should be developed that could be used in conjunction with serotyping in epidemiological studies.

3.2.5 Likewise, a search should be made for the presence of colonization factors in EPEC. Preliminary work suggests that the currently recognized colonization factor antigens of the ETEC do not occur in EPEC, but undoubtedly some factors are responsible for epithelial adhesion.

3.2.6 As soon as the factors involved in the pathogenesis of EPEC diarrhoea are discovered, it will be necessary to determine the genetic determinants of these virulence factors.

3.2.7 The clinical parameters of EPEC diarrhoea have not been well described. The application in suitable epidemic situations of modern pathophysiological techniques, similar to those already used in cholera and rotavirus disease, should furnish valuable information on faecal electrolyte and protein losses, which in turn may provide a clue to the mechanisms by which EPEC cause diarrhoea.

3.2.8 The role of antibiotics in the treatment of EPEC diarrhoea needs to be assessed. Antibiotics have been used arbitrarily for many years but their effectiveness is controversial.

3.3 Enteroinvasive *E. coli* (EIEC)

3.3.1 With the exception of a few surveys and occasional reports on outbreaks, little is known about the pathogenesis or epidemiology of EIEC diarrhoea. Such information could very easily be obtained from investigations in those countries (e.g., in Central and Eastern Europe) where EIEC seem to be prevalent. Some basic information that is needed includes infectious doses, clinical symptomatology, age-specific incidence and carrier status. This work would be facilitated by the provision of pooled and monovalent diagnostic antisera against the EIEC serogroups.

3.3.2 The guinea-pig eye kerato-conjunctivitis test (Serény test) has been used as a model to test the epithelial invasiveness of *E. coli*. This is a difficult test and alternative test systems should be developed. One possible alternative is to use suitable tissue cultures (e.g., HeLa cells).

TABLE 1. BIOLOGICAL ASSAYS FOR ENTEROTOXIN OF ETEC STRAINS

Assay	Heat-stable (ST)	Heat-labile (LT)	Comments
In vivo			
Rabbit ligated ileal loop			Previously widely used. Difficult and laborious, but best method for general detection of enterotoxin activity.
Read at 6 hours	+	±	
Read at 18 hours	–	+	
Infant rabbit bowel	+	+	Few reports, particularly for ST.
Marker perfusion in rat jejunum, dog loops	+	+	Complicated, only for specialized laboratories.
Adult rabbit skin (vascular permeability factor)	–	+	Very sensitive. Easy to perform. Not widely used. Easiest way to do in vivo neutralization with antitoxin.
Infant mouse intragastric	+	–	Requires large numbers of mice. Easy to perform. May not detect all types of ST.
In vitro			
Y1 mouse adrenal cells	–	+	Very sensitive. Requires training in tissue culture technique.
Chinese hamster ovarian cells (CHO)	–	+	Very sensitive. CHO cells are probably easier to maintain than Y1 cells.
African green monkey cells (Vero)	–	+	Vero cells are easier to handle than Y1 and CHO cells but probably not as sensitive to LT.
Passive immune haemolysis (sheep erythrocytes)	–	+	Sensitive but not widely used. Requires antibody against purified LT and fresh supply of sheep erythrocytes.
Radial passive immune haemolysis	–	+	Measures LT produced by colonies on agar surface. Less sensitive than the Y1 cell assay.
Solid-phase-radioimmunoassay (RIA)	–	+	Very sensitive. Easy to standardize and automate. Equipment expensive.
Enzyme-linked immunosorbent assay (ELISA)	–	+	Very sensitive. At the moment the most promising test for LT for use in developing countries.

TABLE 2. O:K:H OR O:H SEROTYPES OF MAN COMMONLY ASSOCIATED WITH ENTEROTOXIN PRODUCTION (ETEC)

06:K15:H16[b]
07:H18
08:K40:H9[b]
08:K47:H-
08:K25:H9
09:K84:H2[c]
015:H11
020:K+:H-[a]
025:K7:H42[a]
025:"K98":H-
027:H7[c]
027:H20[c]
063:H12[a]
073:H45
078:H12[a]
078:H11[a]
0114:H21
0115:H51
0128:H7[c]
0128:H12[a][c]
0128:H21
0148:H28
0153:H12[a]
0153:H10[c]
0159:H4
0159:H34
0X2:H41

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[a] Serotypes found with CFA I

[b] Serotypes found with CFA II

[c] These strains have hitherto been found as 'ST only'.

TABLE 3. SEROGROUPS COMMONLY ASSOCIATED WITH ENTEROTOXIN PRODUCTION[a)] IN ANIMALS

Serotype		Pigs							
	Pilus antigen	Neonate		Weaned		Calves		Lambs	
		LT	ST	LT	ST	LT	ST	LT	ST
08:K87:H19	K88	+	+	+	+				
08:K85:H-	K99						+		+
08:K85:H-	K99		+				+		+
09:K103:H-	987P		+						
09:K35:H-	K99		+						
020:K101:H-	987P		+						
020:K^+:H-	K99						+		+
0101:K28:H-	K99		+				+		+
0115	K99						+		+
0138:(H14)					+				
0139:K82:H1					+				
0141:H4					+				
0141:H4	K88	+	+	+	+				
0149:H10				+	+				
0149:H10	K88	+	+	+	+				
0157:H19	K88	+	+						

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a) 078 is commonly associated with septicemia in poultry and calves, and with mastitis in cows. It is *not* usually enterotoxigenic in animals.