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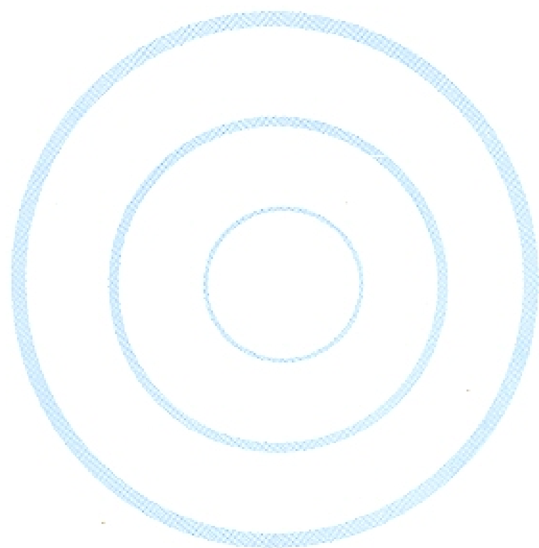

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Centre for Health and Population Research

ICDDR,B, or 'The Centre', was established in 1978 as successor to the Cholera Research Laboratory created in 1960 to study the epidemiology, treatment, and prevention of cholera. The Centre is an independent, international, non-profit organization for research, education, training, clinical services, and information dissemination. Located in Dhaka, the capital city of Bangladesh, the Centre is the only truly international health research institution based in a developing country. The results of research conducted over the years at the Centre provide guidelines for policy makers, implementing agencies, and health professionals in Bangladesh and around the globe. Researchers at the Centre have made major scientific achievements in diarrhoeal disease control, maternal and child health, nutrition, and population sciences. These significant contributions have been recognized worldwide.

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(See inside of the back cover...)

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Announcement

The Editorial Board announces that Professor David A. Sack, MD, has assumed the responsibility of the Editor-in-Chief of the Journal of Diarrhoeal Diseases Research (JDDR). Prof. Sack is also the current Director of the ICDDR,B: Centre for Health and Population Research. Before joining the Centre, he was a Professor of International Health at the Johns Hopkins University, USA. Prof. Sack was born in November 1943 in Alabama, USA, obtained his MD from the University of Oregon Medical School in 1968, and started his professional career in 1969. Previously he also served the Centre from 1977 to 1987 as the Head of Laboratory Sciences Division. The Editorial Board sincerely hopes that under his able and dynamic leadership, the Centre and the JDDR will continue to flourish and excell in its scopes and objectives.

At the same time the Editorial Board also recalls the excellent services provided by Professor Roger Eeckels, the previous Editor-in-Chief of the Journal in improving the quality of the JDDR since 1995. Prof. Eeckels has put in tremendous efforts to enrich the scientific quality and reliability of the Journal.

We all wish wonderful health and joyful life for both of them in the future.

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REVIEW ARTICLE

Food as a Vehicle of Transmission of Cholera

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ABSTRACT

Cholera has been recognized as a killer disease since earliest time. Since 1817, six pandemics have swept over the world, and the seventh one is in progress. The disease is caused by infection of the small intestine by *Vibrio cholerae* O1 and O139 and is characterized by massive acute diarrhoea, vomiting, and dehydration: death occurs in severe, untreated cases. Cholera is a highly contagious disease, and is transmitted primarily by ingestion of faecally-contaminated water by susceptible persons. Besides water, foods have also been recognized as an important vehicle for transmission of cholera. Foods are likely to be faecally contaminated during preparation, particularly by infected food handlers in an unhygienic environment. The physico-chemical characteristics of foods that support survival and growth of *V. cholerae* O1 and O139 include high-moisture content, neutral or an alkaline pH, low temperature, high-organic content, and absence of other competing bacteria. Seafoods, including fish, shellfish, crabs, oysters and clams, have all been incriminated in cholera outbreaks in many countries, including the United States and Australia. Contaminated rice, millet gruel, and vegetables have also been implicated in several outbreaks. Other foods, including fruits (except sour fruits), poultry, meat, and dairy products, have the potential of transmitting cholera. To reduce the risk of food-borne transmission of cholera, it is recommended that foods should be prepared, served, and eaten in an hygienic environment, free from faecal contamination. Proper cooking, storing, and re-heating of foods before eating, and hand-washing with safe water before eating and after defaecation are important safety measures for preventing food-borne transmission of cholera.

Key words: Cholera; *Vibrio cholerae*; Food; Disease transmission; Review literature

INTRODUCTION

Although cholera had been prevalent in many parts of the world for centuries, it is only during the last 50 years that we have learnt important aspects of the disease, including its pathogenic mechanism, treatment, and prevention. Robert Koch, during work in Alexandria and Calcutta, isolated *Vibrio cholerae*, and, in 1883, was the first to conclusively show that it was the cause of cholera. In 1953, SN De, a bacteriologist in Calcutta, discovered the crude cholera toxin, responsible for stimulating fluid secretion from the small intestine (1). Since then, rapid advances in research have illuminated the pathogenic mechanisms of fluid secretion and its regulation, the modalities of treatment, immune mechanisms, and have stimulated vaccine development.

V. cholerae O1, the causative organism of cholera, is a Gram-negative bacterium which infects and colonizes

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the small intestine, stimulating massive outpouring of fluid and electrolytes and leading to severe watery diarrhoea, dehydration, vomiting, electrolyte abnormalities, and metabolic acidosis. Although the majority of infections with *V. cholerae* lead to inapparant infection or mild disease, death occurs in 50 to 70% of the severe cases if they are not adequately rehydrated (2), either by intravenous infusion or, more simply, by oral rehydration solutions (ORS) (3). Antimicrobial agents, including tetracycline, are effective in reducing the volume of fluid loss and the duration of diarrhoeal illness (4). Although cholera occurs in individuals of all ages, children aged 2 to 9 years have the highest incidence of the disease in endemic areas, such as Bangladesh (5). Short-lived, protective immunity develops in adults in the endemic areas due to repeated exposures to infection, but long-term protection is rare.

Cholera is a disease of great epidemic potential. It is endemic along the Ganges valley, particularly in eastern India and Bangladesh. Since 1817, six cholera pandemics have swept over the world, exacting a heavy toll of human lives. The seventh pandemic started in Sulawesi, Indonesia in the early 1960s, and has now spread to most parts of the world. This last pandemic was the first caused by the El Tor biotype of *V. cholerae*.

Epidemiological evidence indicates that cholera is primarily a water-borne disease (6). Its faecal-oral transmission usually occurs by the ingestion of faecally contaminated water by susceptible individuals. Besides drinking water, food has also been recognized to be an important vehicle of transmission of cholera. In developing countries, where both poverty and poor sanitation are common, faecal contamination of domestic and commercial food is likely to occur, and in many outbreaks the infection has been traced to consumption of faecally-contaminated foods. In the developed countries, food-borne outbreaks of cholera have on many occasions occurred due to consumption of contaminated seafoods.

FOOD AS A MEDIUM FOR SURVIVAL OF V. CHOLERAE

The survival and growth of *V. cholerae* in foods depend on the physico-chemical properties of the particular foodstuff that has been contaminated. Food characteristics, which enhance the growth of *V. cholerae,* are low temperature, high-organic content, neutral or alkaline pH, high-moisture content, and absence of other competing micro-organisms in the food (7-10). *V. cholerae* are very sensitive to heat, and are rapidly killed when exposed to temperature of 100 °C. Drying and exposure to sunlight is also an effective means of killing *V. cholerae* (8,11). Domestic freezing is usually ineffective in sterilizing foods, and the organisms can survive for a long period in a frozen state.

Fruits and vegetables: In many countries, the practice of fertilizing gardens with untreated night soil and the habit of consuming uncooked vegetables have often resulted in cholera outbreaks. Vegetables may be contaminated during washing with polluted water. This can also occur when contaminated water is injected into fruits, such as water melons, to preserve their weight and taste (12). The pH of a specific fruit is an important factor that influences contamination by *V. cholerae.* Sour fruits, such as lemons and oranges, with lower pH (below 4.5) do not support the growth of *V. cholerae*, and, thus, do not pose risk of cholera transmission. Fruit pulp and concentrate preserved in cans are also less likely to be contaminated if they have an acidic pH. Spices, including raw onions and garlic, can support the survival of *V. cholerae* for 2 to 3 days at ambient temperature (8,13).

Seafoods: The importance of fish and shellfish as a vehicle of transmission of cholera has been recognized by early observers. Fishes are likely to be contaminated by *V. cholerae* when the surrounding water is contaminated by sewage or other environmental sources of *V. cholerae* O1. It has been shown that *V. cholerae* can survive in seawater in association with zooplankton (copepods). Zooplankton secrete a self-protective coat of chitin that can be dissolved by chitinase, an enzyme produced by *V. cholerae* O1. Seafoods, including molluscs, crustaceans, crabs, and oysters, feed on plankton and can become infected with *V. cholerae* (14). Once infected, particularly clams and oysters can harbour *V. cholerae* for weeks, even if refrigerated (7). In crabs, the organisms can rapidly multiply at ambient temperature, and boiling for less than 10 minutes or steaming for less than 30 minutes does not completely kill *V. cholerae* (7).

Dairy products: *V. cholerae* O1 can survive for more than two weeks in different dairy products, including milk, milk products, soft deserts, and cakes. Addition of sugar and eggs enhances bacterial survival. Although *V. cholerae* is killed by pasteurization of milk, the organisms can persist in raw milk as long as four weeks, even if refrigerated (13).

Poultry and meat: Contamination of meat of animal origin occurs exogenously during processing, cooking, storage, or consumption. It has been shown that *V. cholerae* can live and grow on cooked chicken, an increase in numbers of *V. cholerae* from 10^3 to 10^6 within 16 hours has been demonstrated (15). An early observation by Seligmann indicates that consumption of improperly cooked horsemeat was incriminated in a

small outbreak of cholera in Berlin in 1918 (16). The meat had been prepared by an infected butcher who succumbed to cholera the next day.

There are many other types of food that may be contaminated with *V. cholerae*. *V. cholerae* can survive on cooked rice, potatoes, eggs, and pasta for up to 5 days, and can also survive in spices, including pepper and cinnamon, for up to several days (8).

LABORATORY CHARACTERIZATION OF V. CHOLERAE O1

The mere presence of *V. cholerae* in food does not implicate food as the vehicle of cholera transmission. The organism must be tested in the laboratory to identify its biological characteristics with regard to pathogenicity, toxin production, antigenic type, and genetic structure. Application of modern molecular techniques to detect *V. cholerae* in food samples should be considered. Molecular probes of natural DNA fragments (17,18) and synthetic oligonucleotides have been developed to detect *V. cholerae* toxin genes (19). Polymerase chain reaction (PCR), a very sensitive tool, can detect trace amounts of DNA fragments, even from dead or lysed organisms. This assay may be very useful in investigating food-borne cholera outbreaks. Epidemiologic and medical implications should be considered to establish the cause-and-effect relation between the suspected source of infection and the outbreak of the disease. In this regard, the laboratory plays an important role with respect to characterization of the organism. When a specific food is suspected for contamination, a sufficient amount (25 g) of the food should be sampled, and specific laboratory procedures should be employed for the isolation and characterization of *V. cholerae*. Specific guidelines for laboratory examination of contaminated foodstuff, developed by the U.S. Food and Drug Administration, Department of Public Health, may be useful for proper identification of the suspected pathogens.

EPIDEMIOLOGY OF FOOD-BORNE TRANSMISSION OF CHOLERA

The epidemiological evidence reviewed by Politzer (20) suggests drinking water as the primary vehicle of transmission of cholera. However, early observations as well as those made during the last 30 years indicate that many outbreaks of cholera have been traced to consumption of contaminated foods throughout the world (21,22). The role of food as a vehicle of cholera transmission depends on several factors, including the likelihood of contamination of a specific food item, and the gastric acid-neutralizing capacity of a particular food.

Table 1. Food-borne outbreaks of cholera mostly in countrie other than USA*

Country	Year	Infected food	References
Philippines	1961	Raw shrimps	26
Malaysia	1963	Seafood/shellfish	36
Israel	1970	Raw vegetables	44
Bahrain/Sydney	1972	Hors d'oeuvres	46
Italy	1973	Mussels	37
Portugal	1974	Raw cockles/bottled water	39/59
Guam	1974	Salted fish	60
Gilbert Island	1977	Raw shellfish/salted fish	61/62
Louisiana, USA	1980	Inadequately steamed crabs	63
Sardinia	1980	Shellfish	35
Singapore	1982	Seafood	33
Gulf of Mexico	1983	Water-contaminated rice	42
Micronesia	1984	Food	64

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Seafood and shellfish: Seafoods, particularly fish and shellfish, have been incriminated in many cholera outbreaks since the nineteenth century (Table 1). Fish becomes infected with *V. cholerae* either due to sewage contamination of water or by ingestion of aquatic vegetation and zooplankton infested with *V. cholerae* (23). In a food survey in Taiwan, 1,088 vibrios, including *V. cholerae* and other species, were isolated from seafoods and aquacultured foods (24). In many countries, fish is eaten raw or undercooked (25,26). Outbreaks of cholera due to consumption of raw fish have been reported from Japan as early as 1886 (27) and from the Philippines in 1908 (28). Fish may serve as an important vehicle of transmission of cholera in the endemic areas

of Asia, where it is a major food item and is likely to be contaminated by *V. cholerae* due to both poor environmental sanitation and poverty prevailing in this region. Pandit and Hora (29) observed that, in India, the transmission of endemic cholera is maintained through infection of *hilsa* fish**,** which breeds abundantly in the Hoogly river that runs through Calcutta.

During the course of the seventh cholera pandemic, contaminated seafoods have been identified as the source of infection in several outbreaks. Seafoods and seafood products most frequently incriminated are raw shrimps, crabs, oysters, clams, shellfish, and mussels. These foods have been identified as a source of repeated outbreaks in the Unites States and elsewhere (30-32). In 1978, Singapore experienced another cholera outbreak, which was traced to consumption of prawns and squid, which were likely to be contaminated by infected food handlers (34). Again in Singapore, a food-borne cholera outbreak occurred in 1982 among 37 construction workers after they ate contaminated seafoods at a local cafeteria, where two food handlers were found to be infected with *V. cholerae* O1 (33). In 1979, an outbreak of cholera occurred in Sardinia; the source of infection was traced to eating of bivalves from which *V. choleare* O1 were isolated (35). In 1974 in the Pacific island of Guam, eating of small, salted fish was thought to be the vehicle of transmission of cholera (60).

The importance of shellfish as a vehicle of cholera transmission has been conclusively shown by a statistically significant association of cholera with shellfish consumption in Italy in 1973 (37,38). One year later, a severe cholera epidemic occurred in Portugal with 2,467 reported cases and 48 deaths (39). *V. cholerae* O1 was isolated from 42% of shellfish tested, and consumption of raw or poorly cooked cockles were significantly associated with cholera cases.

Reports of cholera outbreaks from the U.S. Gulf Coast during the last two decades provide epidemiological evidence of a definitive role of fish in the transmission of cholera (Table 2). In the United States, the first case of cholera after 1911 occurred in a shrimp fisherman in Texas in 1973 (40). The isolate was identified as *V. cholerae* O1, biotype El Tor. In spite of extensive investigations, the source of infection remained unidentified. Later, in 1978, 11 persons were attacked with *V. cholerae* El Tor in Louisiana after eating cooked crabs in different sites of the coastal marshes. In 1984 in Maryland, one person who had consumed infected crabs collected along the Texas coast developed cholera (41). In Florida (25) and Georgia (26), two isolated cases of cholera occurred in 1978 due to consumption of contaminated raw oysters. In the 1986 Louisiana cholera outbreak, 18 persons were attacked in 12 different clusters; all were infected by eating crabs and shrimps collected from different sites along the Louisiana coast (32). In the fall of 1991, a single cholera case was identified in an oil rig barge in Texas, which was followed by 13 secondary cases of cholera and one asymptomatic infection (42). The source of infection in the index case was traced to consumption of infected seafoods from local water. The secondary cases were infected by consuming rice prepared with water contaminated by the faeces of the index case through cross-connection between a sewer drain and the drinking water supply. Since 1973, a total of 65 cholera cases have been associated with the Gulf Coast reservoir in the United States. In all cases, the *V. cholerae* O1 isolated were of El Tor biotype and Inaba serotype; all strains produced cholera toxin and haemolysin on blood agar,

Table 2. Cholera cases in the Unites States associated with consumption of seafoods from Gulf Coast waters (1973-1992)*

State	Year	No. of cases	Implicated food	References
Texas	1973	1	Raw oysters	40
Louisiana	1978	11	Cooked crabs	63
Texas	1981	2	Fish/shrimps	65
Texas	1981	16	Cooked rice	53
Maryland	1984	1	Crabs	41
Florida	1986	1	Raw oysters	25
Louisiana	1986	18	Crabs/shrimps	63
Georgia	1986	18	Raw oysters	26
Louisiana	1987	2	Crabs	31
Colorado	1988	1	Raw oysters	66
Total	1973-1992	71		

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and possessed a characteristic bacteriophage, VcA3 (19). Genetically, all strains had the same restriction digestion pattern and ribotype pattern. All these data support the hypothesis that *V. cholerae* O1 strains have been persisting as a free-living organism along the Gulf Coast water for the last 20 years, independent of exogenous introduction during the pandemics.

Other foods. The African continent had been free of cholera for 70 years, but in 1971 cholera reappeared in Africa, and 30 of the 46 countries started reporting cholera. During a cholera outbreak in Mali in 1984, a case-control study showed that eating leftover millet gruel by villagers in an arid region was associated with cholera (43). In this community, millet is a major food item, which is prepared once a day and stored at room temperature for many hours and is often consumed without proper cleanliness by people in groups. In another outbreak in Guinea, consumption of leftover rice with peanut sauce has been incriminated as the vehicle of transmission of cholera. In contrast, leftover rice eaten with tomato sauce, having an acidic pH, unfavourable for *V. cholerae*, was not associated with cholera cases (43). There were also two reports of cholera outbreaks due to contamination of rice during preparation of a funeral feast by women who had cleaned the bodies of patients who died of cholera.

The importance of contaminated vegetables as a vehicle of cholera transmission is indicated by an outbreak in Jerusalem (44). In August 1970, an epidemic of cholera broke out in Jerusalem with 256 cases, the initial cases appearing simultaneously in three different places. However, the later cases appeared sporadically without specific primary cases; and the cases were limited to the Jerusalem region, despite considerable movement of people. Careful investigation of the epidemic could not establish a source of infection for the initial cases. However, the later cases were shown to be infected by secondary spread of *V. cholerae* through consumption of vegetables contaminated by faeces from the initial cases. In Jerusalem, vegetables are supplied from the surrounding villages, where sewage and night soil are used as fertilizer.

In the United States, an outbreak of cholera occurred in Maryland in 1991, in which four persons were infected, three of them having diarrhoea (45). Consumption of Thai-style rice topped with coconut milk was the source of infection. Laboratory examination of the same brand of coconut milk revealed its contamination by *V. choleare*, which differed in molecular characteristics from those strains causing the seventh pandemic in Asian and Latin American countries, and in the Gulf Coast of the U.S.A. These findings suggest that the coconut milk was contaminated during its preparation in the country of origin.

Table 3. U.S. cases of cholera in persons returning from Latin America or eating food from Latin America.*

State	Year	Nos. of cases	Suspected food	Country of food origin	References
Georgia	1991	1	Cooked crab meat	Peru	67
New Jersey	1991	8	Cooked crab/salad	Ecuador	68
Florida	1991	1	Raw oysters	Ecuador	68
New York	1991	4	Cooked crab/salad	Ecuador	69
New Jersey	1991	1	Crabs	Ecuador	CDC, Unpub.
California	1992	2	Seafood at stand	El Salvador	CDC, Unpub.
California	1992	76	Shrimp/fish in salad	Peru	53
California	1992	1	Raw seafood	El Salvador	CDC, Unpub.
Total	1991-1992	94			

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An outbreak of cholera was reported in 47 of the 331 passengers of an airline flight en route from London to Sydney in 1972. The source of the infection was traced to consumption of contaminated hors d'oeuvre served on the aircraft and obtained from a caterer in Bahrain, where an epidemic of cholera was going on (47).

Recent cholera outbreaks in Latin America: For the first time in the 20th century, cholera appeared in Latin America in 1991. Peru was the first country to report cholera during the epidemic (48). The disease spread quickly to other countries. By August 26, 1992, 640,000 cholera cases and 5,600 deaths were reported by 19 countries (48-50); these numbers were more than those reported for the entire world during the former five years (51,52). In the same wave of the epidemic, cholera

entered into the United States with travellers returning from Latin America (Table 3). Food has been incriminated as the vehicle of transmission of cholera from Latin America to the United States in several instances.

An index case of cholera was identified in New York in 1991. This case was followed by three secondary cases of symptomatic infection with *V. cholerae* O1 (69). The index case had travelled to Ecuador and brought with him boiled crabs which were found to be contaminated by the *V. cholerae* strain prevailing in Latin America, as detected by sensitive assays, including PCR and DNA ribotyping (42). The molecular characteristics of this strain of *V. cholerae* O1 were completely different from those isolated from the coastal areas of Texas and Louisiana. In another instance in 1991, 76 airline passengers returning from Argentina to Los Angeles were infected with *V. cholerae* O1 by eating contaminated shrimp and fish salads prepared in Lima, Peru (53). Although no secondary spread occurred, 37 passengers became ill, and of them one died. A total of 41 imported cases of cholera have been documented in the United States during 1961-1990. Most of these cases were associated with travel to Latin American countries or with eating of seafoods brought from there.

These observations indicate that there is a possibility of cholera transmission through eating contaminated food in another country. However, the risk of transmission of cholera through imported commercial foods seems to be small. There have been no cases of cholera in the United States as a result of importing commercial foodstuffs from Latin American countries.

FOOD-BORNE CHOLERA IN ENDEMIC REGIONS

In cholera endemic regions of Asia, including Bangladesh, contamination of food may be an important factor in the transmission of cholera. Case-control studies have shown that, in Bangladesh, the rate of contamination of household water with *V. cholerae* O1 is significantly higher in water used for cooking than in water used for drinking (54). It is likely that water may serve as a source of secondary contamination of food during its preparation. A household survey carried out in Bangladesh indicates that only 0.13% of the food samples cultured were contaminated with *V. cholerae* O1. This indicates that the risk of food-borne transmission of cholera during the non-epidemic season in the endemic areas may be small. Although fish and shellfish have been shown to be an important vehicle of cholera transmission in non-endemic areas (U.S. Gulf Coast, Africa, Latin America), in the endemic areas of India and Bangladesh, fish-borne transmission seems to be rare. In these communities, most people eat fresh water fish rather than salt water fish and other marine species, such as shellfish, clams, and oysters. Nevertheless, *V. cholerae* O1 has been isolated from aquatic flora and fauna in this region (55). It has also been found that blue-green algae can act as a reservoir of *V. cholerare* O1 in the aquatic environment of Bangladesh (55). These algae are eaten by fish. Since fish is usually well-cooked before eating and never consumed raw, fish-borne infections must be rare. However, cross contamination of foods through handling of infected fish remains a possible risk of transmission.

In endemic areas, transmission of cholera through contaminated foods served by street vendors and restaurants should be considered. In Dhaka, the capital city of Bangladesh, there were two outbreaks of cholera in 1974 and 1975 (56). The results of a case-control study indicated that the attack rates of cholera were significantly associated with eating in restaurants. Moreover, the free food distribution centres established in the city to feed the famine-affected people also played a significant role in the transmission of the disease. In the Hong Kong outbreak of cholera in 1990, the source of infection has been traced to consumption of a special rice dish called "moonsalus" (57).

CONCLUSIONS AND RECOMMENDATIONS

Cholera is a disease of great public health importance. The mainstay of treatment of cholera is the replacement of fluid and electrolytes lost in the stools. Rehydration can be easily achieved either by intravenous infusions or more simply by oral rehydration solutions. Treatment with an effective antimicrobial agent reduces fluid loss and the duration of diarrhoea, but this form of treatment is not considered a substitute for rehydration therapy. Since untreated diarrhoeal stools from cholera patients are the primary source of environmental contamination, including water sources and foods, proper treatment of cholera cases and safe disposal of faeces would reduce secondary spread and faecal contamination of the environment. Transmission of cholera occurs by the faecal-oral route, and water has been recognized as the primary vehicle of cholera transmission. Thus, to interrupt the transmission cycle, effective public health measures should be undertaken to prevent faecal contamination of drinking water supplies, as well as to establish sanitary disposal and sewage treatment systems. This is not an easy task in most situations, and, thus, calls for a considerable investment and commitment by the government and the community leaders.

Beside water, foods constitute an important vehicle for transmission of cholera in the environment. Consumption of *V. cholerae*-contaminated foods, particularly seafoods and fish, has been implicated in a large number of cholera outbreaks throughout the world. Seafoods can be infected if the surrounding water is faecally contaminated or during its processing by the infected food handlers. After cooking, food can be infected during storage and consumption in an unclean environment.

To prevent food-borne transmission of cholera, the foods must be safe and free from *V. cholerae* before consumption. The World Health Organization recommends the following food safety measures to prevent the spread of cholera (58):

- avoid raw food (exception: undamaged fruits and vegetables from which the peel can be removed are safe if hygienically handled);
- cook food until it is hot throughout;
- eat food while it is still hot, or reheat food thoroughly before eating;
- wash and thoroughly dry all cooking and serving utensils after use;
- handle and prepare food in a way that reduces the risk of contamination (e.g. cooked food and eating utensils should be kept separate from uncooked foods and potentially contaminated utensils); and
- wash hands thoroughly with soap (or ash) after defaecating, or after contact with faecal matter, and before preparing or eating food, or feeding children.

With regard to the risk of cholera transmission through food trade, the WHO recommends: "… although there is a theoretical risk of cholera transmission associated with international food trade, the weight of evidence suggests that this risk is small and can normally be dealt with by means other than an embargo on importation."

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A Prediction Model for Moderate or Severe Dehydration in Children with Diarrhoea

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ABSTRACT

A hospital-based unmatched case-control study (387 cases and 387 controls) was carried out at the Government Medical College Hospital, Nagpur, India, to devise and validate a risk-scoring system for predicting the development of moderate or severe dehydration in children, aged less than five years, with acute watery diarrhoea. On unconditional multiple logistic regression, 12 risk factors--infancy, minority religion, undernutrition, not washing hands by mother before preparation of food, frequency of stools >8/day, frequency of vomiting >2/day, measles in previous 6 months, withdrawal of breast-feeding/other feedings, withdrawal of fluids during diarrhoea, not giving oral rehydration solutions (ORS), home available fluids and both during diarrhoea--were significant. Based on regression coefficients, these factors were ascribed statistical weights of 5, 5, 4, 4, 22, 9, 11, 13, 5, 5, 5, and 7 respectively. The receiver-operating characteristic curve suggested a total score of 48 to be the best cut-off for predicting the development of moderate or severe dehydration. At this cut-off, the sensitivity, specificity, positive predictive value, Cohen's kappa, and overall predictive accuracy were 0.81, 0.81, 0.81, 0.61, and 0.86 respectively. If substantiated by further validation, this system can be used for predicting the development of dehydration at the earlier stage, thereby reducing the mortality associated with life-threatening dehydration.

Key words: Diarrhoea, Infantile; Diarrhoea, Acute; Dehydration; Case-control studies; Risk factors; Epidemiology

INTRODUCTION

Diarrhoeal diseases are still one of the leading causes of morbidity and mortality in children in developing countries (1), and are responsible for approximately one-third of the 15 million deaths, each year, of children aged less than five years (2). Although the majority of episodes are self-limited, a small proportion quickly lead to dehydration and death (3,4). Most deaths due to diarrhoea are attributed to development of moderate or severe dehydration (5,6). Little is relatively known about what puts a child at risk of developing life-threatening dehydration (7). A number of hypothesized risk factors have been shown to be associated with the development of moderate or severe dehydration (2,7). Their relative contribution to the development of moderate or severe dehydration reportedly varies from study to study and from population to population. Moreover, the combined effect of these risk factors can help in better prediction of development of moderate or severe dehydration in

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children, aged less than five years, with acute watery diarrhoea compared to the individual effect. The present study was, thus, undertaken (a) to identify the important risk factors contributing to the development of moderate or severe dehydration in children, aged less than five years, with acute watery diarrhoea, (b) to develop a simple risk-scoring system for prediction of development of moderate or severe dehydration, and (c) to validate the risk-scoring system so devised.

METHODS AND MATERIALS

A hospital-based unmatched case-control study, comprising children aged less than five years suffering from acute watery diarrhoea and admitted to the Diarrhoea Treatment Unit (DTU), Government Medical College Hospital, Nagpur, India, was carried out during a 15-month period from October 1996 to December 1997.

Sample size

To calculate the sample size required for the study, an information from an earlier study (7) was considered. It was observed in the earlier study that the prevalence of "not giving ORS [oral rehydration solutions] during diarrhoea episode" in controls was 0.47, and the odds ratio was 2.1 for development of moderate or severe dehydration. With an a error of 0.05 and 90% power, the sample size was calculated to be 387 cases and an equal number of controls. The study subjects were selected sequentially.

Cases

Cases comprised 387 children, aged less than five years, admitted to the DTU with diarrhoea, who were assessed as having severe or moderate dehydration. Moderate or severe dehydration was diagnosed when a child had definite signs of reduced skin elasticity and, in addition, had one or more of the following: sunken eyes, rapid and weak pulse, sunken anterior fontanelle (provided open), and no urine output for at least 6 hours (8). Due care was given for evaluating reduced skin elasticity in the malnourished (marasmic) child on admission which was re-checked after the child was fully hydrated. The reduced skin elasticity was corroborated with relevant history from a responsible family member, mostly the mother. At the time of discharge, the mothers were further asked regarding skin elasticity, and if the response was definite and positive, the reduced skin elasticity was attributed to malnutrition.

Controls

Controls comprised 387 children, aged less than five years, attending the DTU with diarrhoea, who were assessed as having no or mild dehydration, when there were no clear signs of dehydration with or without thirst (8).

Risk factors

A trained investigator interviewed the mothers using a close-ended questionnaire containing information on risk factors included in the study. The current study included 17 hypothesized risk factors (7) for development of moderate or severe dehydration in children, aged less than five years, with acute watery diarrhoea, i.e. infancy, female sex, religion, residing in urban slums or rural area, undernutrition, cessation of breast-feeding/other feeding during diarrhoeal episode, fluid intake decreased/stopped during diarrhoeal episode, ORS not received, home available fluids (HAF) not received, both ORS and HAF not received, not washing hands by the mothers before preparation of food, after defaecation and after disposal of faeces, history of measles in previous 6 months, frequency of stools >8 per day, frequency of vomiting more than 2 per day and temperature more than 37.2 °C. The scheme of categorization and measurement of these risk factors is shown in Table 1.

Statistical analysis

Univariate analysis included Pearson's chi-square, crude odds ratios, and Cornfield estimates for 95% confidence intervals for the odds ratios (9). Unconditional multiple logistic regression (MLR) analysis was carried out. The full model of MLR included all risk factors incorporated in the current study. However, the final model included the risk factors which were significant in the full model at a level of 0.1. The level of significance was fixed at a=0. 05 for judging the significance in the final model.

The risk factors in the final model were then given a statistical weight. The weight for a factor was calculated by using the following linear transform on the regression coefficient of the variable in the final unconditional logistic regression model: Statistical weight=Round (bx10). This transform was necessary to make the scoring system easy to use.

All the 774 study subjects were then scored individually using the developed risk-scoring system. The predictive accuracies, sensitivities, specificities, and Cohen's kappas of the risk-scoring system at various cut-offs of the total score were calculated (10). The overall predictive accuracy was calculated as an equivalent of the Wilcoxon statistic as described by Hanley and McNeil (11). The best cut-off for the total score was obtained graphically by plotting receiver-operating characteristic (ROC) curve (10). Statistical analysis was carried out by using CHISQ (1987 PC

Table 1. Univariate analysis of risk factors for development of moderate or severe dehydration in children, aged less than five years, with acute watery diarrhoea

Risk factors	Cases (n=387)	Controls (n=387)	Odds ratio	95% CI	Chi-square	p value
Demographic factors						
Age group (months)						
0-12	145	120	1.33	0.98-1.79	3.58	0.0582
>12	242	267				
Sex						
Female	212	199	1.14	0.86-1.51	0.88	0.0349
Male	175	188				
Religion						
Muslim	76	59	1.35	0.93-1.96	2.59	0.1073
Hindu	311	328				
Area of residence						
Rural	126	95	2.23	1.56-3.18	20.15	<0.0001
Urban slum	149	103	2.44	1.73-3.44	26.44	<0.0001
Urban	112	189	1.0			
Nutritional status (IAP[a] classification)						
<60	120	51	5.76	3.72-8.91	65.97	<0.0001
61-70	122	99	3.01	1.69-5.37	31.30	<0.0001
71-80	80	78	2.50	1.63-3.82	18.38	<0.0001
>80	65	159	1.0			
Hygiene practices						
Washing hands by mothers before preparation of food						
No	236	206	1.37	1.03-1.82	4.74	0.0293
Yes	151	181				
after defaecation						
No	248	200	1.67	1.25-2.23	12.21	0.0004
Yes	139	187				
after disposal of faeces						
No			1.53	1.18-1.98	8.45	0.0036
Yes						
Clinical features on admission						
Frequency of stool/day						
>8	309	106	10.5	7.52-14.67	214.08	<0.0001
[8	78	281				
Frequency of vomiting/day						
>2	260	162	2.84	2.12-3.81	50.04	<0.0001
[2	127	225				
Temperature						
>37.2 °C	36	29	1.23	0.73-2.05	0.82	0.3643
[37.2 °C	351	358				
History of measles in previous 6 months						
Yes	49	26	2.01	1.20-3.37	7.81	0.0052
No	338	361				
Management of diarrhoea						
Breast-feeding/other feedings during diarrhoea						
Stopped	104	27	4.89	3.11-7.68	54.48	<0.0001
Increased/continuing	283	360				
Fluid						
Stopped	79	33	2.83	1.56-5.12	12.17	0.0004
Increased inadequate	270	309	1.03	0.64-1.63	0.02	0.8846
Increased adequate	38	45	1.0			
ORS[b]						
Not received	263	190	2.19	1.64-2.93	28.36	<0.0001
Received	124	197				
HAF[c]						
Not received	259	210	1.71	1.28-2.29	12.99	0.0003
Received	128	177				
ORS[b] and HAF[c]						
Not received	205	110	2.84	2.11-3.82	48.31	<0.0001
Received	182	277				

[a] Indian Academy of Paediatrics; [b] Oral rehydration solution; [c] Home available fluids

Table 2. Multivariate analysis (unconditional multiple logistic regression) of risk factors for development of moderate or severe dehydration in children, aged less than five years, with acute watery diarrhoea (full model)

Risk factors	Odds ratio	95% Cl	p value
Age [12 months	1.53	1.02-2.28	0.038
Female sex	1.18	0.80-1.73	0.389
Muslim religion	1.64	1.01-2.70	0.048
Area of residence in rural/urban slums	0.98	0.77-1.24	0.884
Undernutrition	1.56	1.31-1.86	<0.001
Not washing hands by mothers before preparation of food	1.45	0.97-2.16	0.064
Not washing hands by mothers after defaecation	1.33	0.90-1.97	0.144
Not washing hands by mothers after disposal of faeces	1.44	0.97-2.12	0.063
Frequency of stool >8/day	8.76	5.88-13.04	<0.001
Frequency of vomiting >2/day	2.57	1.74-3.78	<0.001
Temperature >37.2 °C	0.91	0.47-1.76	0.797
History of measles in previous 6 months	2.87	1.47-5.56	0.001
Withdrawal of breast-feeding/other feedings during diarrhoea	3.61	2.11-6.16	<0.001
Withdrawal of fluids during diarrhoea	1.61	1.09-2.37	0.016
Not giving ORS[a] during diarrhoea	1.59	1.08-2.34	0.018
Not giving HAF[b] during diarrhoea	1.62	1.09-2.40	0.015
Not giving both ORS and HAF during diarrhoea	1.98	1.34-2.91	<0.001

[a]Oral rehydration solution; [b]Home available fluids

version), EPIINFO (CDC, Atlanta and WHO, Geneva, version 6.04b, 1997), MULTLR (Ludwig Institute for Cancer Research, Release 5/89 and SCREEN (an indigenously developed) software packages.

RESULTS

Univariate analysis resulted in the identification of the significance of residence area in rural/urban slums, undernutrition, poor hygienic practices, frequency of stool >8/day, more than 2 vomiting per day, previous history of measles, withdrawal of breast-feeding/other feedings, withdrawal of fluids and not giving ORS, HAF and both during diarrhoea, in the outcome of moderate or severe dehydration (Table 1). Of the total 17 risk factors included in the full model, except female sex, residence area in rural/urban slums, not washing hands by the mothers after defaecation and temperature above 37.2 °C, all other risk factors were significant at a level of 0.1 (Table 2). In the final model (Table 3), which included 13 significant risk factors from the full model, except not washing hands by the mothers after disposal of faeces, the remaining 12 risk factors were significantly associated with the development of moderate or severe dehydration at the 0.05 significance level. Table 4 describes the regression coefficients for the significant factors and their respective statistical weights calculated by using afore-mentioned linear transform. Because of the multiple categories in the classification of some variables (undernutrition and withdrawal of fluids during diarrhoea), the possible range of the total score for an individual by this risk-scoring system was 0 to 108. Table 5 shows the classification of cases and controls by the total risk score categories using a total score of 12 as the class interval. It is apparent that most controls were clustered in less than 48 total score category, while the opposite was true of cases. This finding was also highlighted by the ROC curve (figure below). Table 6 shows the validity characteristics of the risk-scoring system at various cut-offs. It can be seen that the Cohen's kappa was maximum (0.6150) at the total score of 48. The overall predictive accuracy of the risk-scoring system as calculated by Wilcoxon statistic was 0.8633 (95% CI=0.8370-0.8895).

DISCUSSION

Looking at the demographic characteristics of the study subjects, it is not surprising to see the significant role of 12 identified risk factors in the ultimate outcome of moderate or severe dehydration. Among the demographic characteristics, infants and muslims were at risk of development of dehydration. Infancy as a risk factor was also recognized by other investigators (7,12). Several workers have

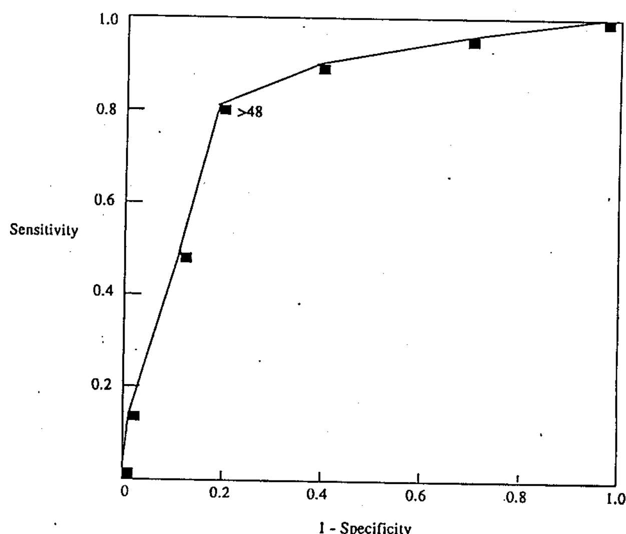

Fig. Receiver-operating characteristic curve for the risk-scoring system

Table 3. Multivariate analysis (unconditional multiple logistic regression) of risk factors for development of moderate or severe dehydration in children, aged less than five years, with acute watery diarrhoea (final model)

Risk factors	Odds ratio	95% Cl	p value
Age [12 months	1.57	1.05-2.35	0.025
Muslim religion	1.64	1.01-2.69	0.048
Undernutrition	1.56	1.31-1.86	<0.001
Not washing hands by mothers before preparation of food	1.54	1.04-2.28	0.027
Not washing hands by mothers after disposal of faeces	1.46	0.99-2.15	0.054
Frequency of stool >8/day	8.73	5.90-12.93	<0.001
Frequency of vomiting >2/day	2.56	1.74-3.76	<0.001
History of measles in previous 6 months	2.95	1.52-5.74	0.001
Withdrawal of breast-feeding/other feedings during diarrhoea	3.58	2.11-6.08	<0.001
Withdrawal of fluids during diarrhoea	1.64	1.12-2.42	0.010
Not giving ORS[a] during diarrhoea	1.57	1.07-2.30	0.020
Not giving HAF[b] during diarrhoea	1.67	1.13-2.47	0.009
Not giving both ORS and HAF during diarrhoea	2.00	1.36-2.94	<0.001

[a]Oral rehydration solution; [b]Home available fluids

Table 4. The scoring system

Risk factors	Regression coefficient	Weight	Maximum score
Age [12 months	0.4560	5	5
Muslim religion	0.4964	5	5
Undernutrition	0.4475	4	12
Not washing hands by mothers before preparation of food	0.4372	4	4
Frequency of stool >8/day	2.1675	22	22
Frequency of vomiting >2/day	0.9409	9	9
Measles in previous 6 months	1.0847	11	11
Withdrawal of breast-feeding/ other feedings during diarrhoea	1.2764	13	13
Withdrawal of fluids during diarrhoea	0.5004	5	10
Not giving ORS[a] during diarrhoea	0.4540	5	5
Not giving HAF[b] during diarrhoea	0.5149	5	5
Not giving both ORS and HAF during diarrhoea	0.6957	7	7

[a]Oral rehydration solution; [b]Home available fluids

shown that malnutrition is strongly associated with fatal diarrhoea (13,14). The present study also endorsed the role of undernutrition in moderate or severe dehydration.

Table 5. Classification of study subjects by the total risk score

Score	Cases (n=387)	Controls (n=387)
0-12	0	14
13-24	10	109
25-36	27	115
37-48	38	75
49-60	122	52
61-72	130	20
73-84	52	2
85-96	8	0
97-108	0	0

Table 6. Validity characteristics of the risk-scoring system

Cut-off for total score	Sensitivity	Specificity	Positive predictivity	Cohen's kappa
>12	1.0000	0.0362	0.5092	0.0362
>24	0.9742	0.3178	0.5881	0.3440
>36	0.9044	0.6149	0.7014	0.5194
>48	0.8062	0.8088	0.8083	0.6150
>60	0.4910	0.9432	0.8962	0.4342
>72	0.1550	0.9948	0.9677	0.1498
>84	0.0207	1.0000	1.0000	0.0208

The risk-scoring system ascribed a statistical weight of 12 to severe undernutrition which is next to the statistical weights attributed to frequency of stool >8/day and withdrawal of breast-feeding during diarrhoea. It is not surprising to find only not washing hands by mothers before preparation of food was a marginally significant factor among the three personal hygienic factors included in the study. This could be due to the fact that the role of these practices is more relevant in the outcome of diarrhoea (15) than development of dehydration.

Except temperature more than 37.2 °C, other two clinical features on admission, i.e. frequency of stool more than 8 per day and frequency of vomiting more than 2 per day, were significantly associated with moderate or severe dehydration. The significance of these two factors was also highlighted by the statistical weights of 22 and 9 ascribed to them respectively. According to Victoria *et al.* (2), among others, frequent passage of stools and vomiting had the highest sensitivities for development of life-threatening dehydration. A child with history of measles in preceding six months and currently suffering from diarrhoea is 3 times at more risk than a child suffering from diarrhoea without history of measles. This factor was attributed a statistical weight of 11. This study also emphasized the importance of continued breast-feeding, an increased fluid intake, use of ORS, HAF and both from the beginning of diarrhoea to prevent the development of

life-threatening dehydration. Withdrawal of breast-feeding/other feedings during diarrhoeal episode was given a statistical weight of 13, which is the second highest of all the weights. Earlier studies also emphasized the importance of continued breast-feeding and use of oral rehydration therapy from the beginning of diarrhoea to prevent the development of life-threatening dehydration and death (7,12).

It appears quite logical, therefore, that these 12 factors represent the main substance of contributory factors leading to or associated with the development of moderate or severe dehydration. This was also confirmed by the overall predictive accuracy of the risk-scoring system, which can predict an individual to be at a higher risk of developing moderate or severe dehydration by taking into account only three (frequency of stool >8/day, withdrawal of breast-feeding/other feedings during diarrhoea and history of measles in previous six months) of the 12 risk factors and any one of the remaining nine factors, with a probability of 86%. The fact that a total score of >48 was the point from which there was a higher risk of developing moderate or severe dehydration emphasized the importance of above-stated three factors in predicting the child at a higher risk.

This risk-scoring system can obviously be used for an early identification of episodes at the highest risk of developing moderate or severe dehydration, which is important basically for two reasons. Firstly, knowledge of early warning signs would be useful to target health-education messages to mothers of at-risk children aged less than five years, encouraging them to seek an early treatment. Secondly, it would enable health workers to recognize the potential severe cases, treat them appropriately, and keep them under a close surveillance. An early identification of at-risk children and timely effective intervention can, therefore, help reduce mortality associated with severe dehydration. Further studies are, however, needed to verify whether the same risk factors apply to other countries, where the aetiology and clinical presentation of diarrhoea may vary considerably.

Although the present study developed a risk-scoring system, the predictive accuracy of which was about 86%, this method of validation as described by Herman *et al.* (16) is known as back validation. This means that the same data from which the risk-scoring system was developed have been used for validation. It may obviously lead to an optimistic overestimate of the predictive accuracy. Still, the high-predictive accuracy encountered in this study may also have been due to the use of a robust unconditional multiple logistic regression model.

To develop a simple risk-scoring system, a statistical weight, based on the estimate of regression coefficient using a linear transform, was used. The logistic regression linearly predicts the log of the odds ratio. Hence, whether logistic regression coefficients should be used in a straight linear way is a questionable issue. But by assessing the predictive accuracy of this system, it seems that it worked well by using even a linear transform in developing the model.

The risk-scoring system that we developed is simple and has high-predictive accuracy; these are its obvious advantages. Moreover, it is based on 12 important hypothesized risk factors. But whether these results can be readily generalized depends, however, on the demographic characteristics of the population under consideration. Further studies are, thus, needed to be undertaken to validate the risk-scoring system by using longitudinal, population-based, heterogenous epidemiological studies. The inherent quality of such a risk-scoring system is that it may help in an early prevention of development of moderate or severe dehydration. Of particular interest is the point that the majority of the factors included in this risk-scoring system are the modifiable risk factors, and the implication of the risk-scoring system is, therefore, underscored.

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Outcome of Children Rehydrated in a Hospital ORT Corner in Bangladesh: A Follow-up Study

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ABSTRACT

This prospective follow-up study was carried out during 1996 to identify the outcome of children rehydrated in the ORT Corner of the Chittagong Medical College Hospital. In total, 269 children, aged less than five years, who attended the ORT Corner, accompanied by their mothers, for treatment of uncomplicated acute watery diarrhoea with 'no signs of dehydration' and 'some dehydration,' were selected for the study. Mothers of the children were interviewed at the ORT Corner, and children were followed-up at home on the 5th post-ORT Corner visit day. The follow-up was completed for 260 cases (96.7%). At follow-up, 227 cases (87.3%) were found to be cured, and 33 cases (12.7%) still had diarrhoea. Of the cured, the mean duration of the episode was 5.84±2.34 days (95% CI.5.55-6.13 days). All the cases received oral rehydration therapy (ORT) after attending the ORT Corner. Eighty-one cases (31.2%), however, reconsulted the private doctors. Of those who reconsulted, only 3 (3.7%) received WHO-recommended treatment of acute watery diarrhoea, and the rest 78 (96.3%) received medication. The medication prolonged the episode of diarrhoea significantly (6.22 days vs 5.48 days, $p<0.001$). None of the cases, who reconsulted the private doctors, revisited the ORT Corner. Treatment by 'ORS only' was mentioned by 64% of the mothers as the reason for non-revisit of the ORT Corner, and 20% of the mothers denied the receipt of any advice about when to revisit the ORT Corner which calls for further strengthening of communication activities of the ORT Corner.

Key word: Diarrhoea, Infantile; Diarrhoea, Acute; Dehydration; Oral rehydration therapy; Rehydration; Prospective studies; Follow-up studies

INTRODUCTION

Diarrhoeal disease has long been recognized as a leading cause of morbidity and mortality of children aged less than five years, specially in developing countries (1,2). A child has a median number of 2.6 episodes of diarrhoea per year which is equivalent globally to an estimated one billion episodes each year resulting in 3.3 million deaths (2). In Bangladesh, a child aged less than five years has 3.5 to 4.0 diarrhoeal episodes per year, and more than a quarter of deaths are diarrhoea-related, i.e. about 260,000 children die due to diarrhoea each year in Bangladesh (3,4), and most of them die due to dehydration (5). Numerous studies show that oral rehydration therapy (ORT) cures dehydration and prevents death (6-9). It is simple, highly effective, inexpensive, and technologically appropriate (10).

A ORT Corner was organized at the Chittagong Medical College Hospital in Bangladesh as a cost-effective strategy for the promotion of case management of diarrhoeal diseases (11). Experiences from the Dhaka Medical College Hospital showed that almost all cases of 'no signs of dehydration' and more than 90% cases of 'some dehydration' were managed at the ORT Corner,

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using diarrhoea case management-related guidelines of WHO, and were sent back to their homes educating their mothers (12). It is not known what happened to these cases after getting back home after visiting the ORT Corner. This study, therefore, aims to identify the outcome of the diarrhoea patients after rehydration at the ORT Corner.

MATERIALS AND METHODS

This prospective follow-up study was carried out in the ORT Corner of the Chittagong Medical College Hospital during 1996. In total, 269 children aged less than five years were selected for the study. These were the cases who attended the ORT Corner of Chittagong metropolitan area, accompanied by their mothers, for the treatment of uncomplicated acute watery diarrhoea with 'no signs of dehydration' and 'some dehydration.' The cases were selected consecutively but not more than five cases a day. The mothers of the children were interviewed by an appointed staff nurse for information about the present diarrhoeal episode using a pretested questionnaire. The patients were examined and managed according to the WHO diarrhoea case management protocol. The instruction about home management was given to the mothers. All the cases were followed-up at home on the 5th post-ORT Corner visit day. A separate questionnaire was used for collecting follow-up information by a different interviewer.

The recommended fluids, such as oral rehydration salts (ORS) solution, labon-gur solution, sugar-salt solution, rice water, and coconut water when used for ORT, were taken as correct fluids. Correct amount of ORT fluid was assessed by the response of 10-20 tsp and 20-40 tsp of fluid given to a child aged less than two years and aged over two years respectively after each loose stool, and the feeding of ORT fluid by a spoon to a child aged less than two years and by cup/glass to an older child was taken as the correct method (13).

The follow-up at home was completed for 260 cases (96.7%), and the rest 9 cases (3.3%) could not be traced, and were, therefore, excluded from follow-up analysis.

RESULTS

Characteristics of patients

The characteristics of the mother-child pairs are presented in Table 1. The majority of the cases belonged to the illiterate mothers (55.4%), poor socioeconomic background (64.2%), age group of 6-23 months (63.5%), and malnutritional status (81.2%). The duration of diarrhoea and the state of dehydration on the day of attendance at the ORT Corner are shown in Table 2. The mean duration of the diarrhoeal episode at presentation was 2.96±1.19 days, 248 cases (92.2%) had 'no signs of dehydration,' and 21 cases (7.8%) had 'some dehydration.' Cough and fever were associated in 18.0% and 14.2% of the cases respectively.

Table I. Characteristics of children with acute watery diarrhoea

Characteristics	No.	%
Mother's age (years)		
<20	05	1.9
20-29	199	78.5
30-39	55	21.2
40 and above	01	0.4
Mother's education		
Illiterate	144	55.4
Primary	64	24.6
Secondary	44	17.0
College	04	1.5
University	04	1.5
Socioeconomic status		
Poor	167	64.2
Lower middle	68	26.2
Upper middle	21	8.1
Rich	04	1.5
Age of children (months)		
0-5	23	8.8
6-11	78	30.0
12-23	87	33.5
24-59	72	27.7
Sex of children		
Male	150	57.7
Female	110	42.3
Nutrition status (Gomez)		
Normal	49	18.8
Mild malnutrition	44	18.9
Moderate malnutrition	94	36.2
Severe malnutrition	71	27.3
Overweight	02	0.8

Outcome at follow-up

The outcome of cases recorded on the day of follow-up at home is shown in Table 3. It is evident from the table that, at follow-up, 227 cases (87.3%) were found to be cured, and 33 cases (12.7%) still had diarrhoea. The mean duration of the diarrhoeal episode of those who were cured was 5.84±2.34 days (95% CI.5.55-6.13 days). None had developed any blood dysentery. Only 3 cases (1.2%) required hospital admission, but were found to be cured. All the cases received ORT, but 251 (97.0%), 187 (72.0%) and 199 (79.0%) cases received correct type and amount of ORT fluid by correct method respectively.

Eighty-one cases (31.2%) reconsulted the private doctors after attending the ORT Corner as they were not getting well (87.0%) and for the associated condition

Table 2. Duration of diarrhoea and state of dehydration at the ORT Corner

Duration of diarrhoea (days)	Total no. of patients	State of dehydration: No sign of dehydration No.	%	Some dehydration No.	%
01	34	31	91.2	3	8.8
02	61	52	85.2	9	14.8
03	86	83	96.5	3	3.5
04	56	52	92.8	4	7.2
05	32	30	93.8	2	6.2
Total	269	248	92.2	21	7.8
Mean±SD		2.96±1.19 days			

(13.0%) developed during the episode. Of the cases reconsulted the private doctors, only 3 cases (3.7%) received WHO-recommended treatment of acute watery diarrhoea, 53 cases (65.4%) received one drug, 18 cases (22.2%) received two drugs, 6 cases (7.4%) received three to four drugs, and one case received five drugs. The commonly-prescribed drugs were metronidazole (51.0%), cotrimoxazole (10.0%), nalidixic acid (14.0%), furazolidine (11.0%), antiemetics (10.0%), and rifampicin (4.0%).

Table 3. Outcome of diarrhoea cases at follow-up

Duration (days)	Cured No.	%	Not cured No.	%	Total No.	%
03	11	4.2	-	-	11	4.2
04	39	15.0	-	-	39	15.0
05	46	17.7	-	-	46	17.7
06	55	21.2	03	1.2	58	22.4
07	40	15.4	02	0.8	42	16.2
08	26	10.0	06	2.3	32	12.3
09	10	3.8	16	6.1	26	9.9
10	-	-	06	2.3	6	2.3
Total	227	87.3	33	12.7	260	100.0

Table 4. Medication and the outcome of diarrhoea cases at follow-up

Medication	Outcome: Cured No.	(%)	Not cured No.	(%)	Total (%)
Yes	110	(83.3)	23	(16.7)	133 (100.0)
No	117	(91.4)	10	(8.6)	127 (100.0)
Total	227	(87.3)	33	(12.7)	260 (100.0)
X2=5.201, df - 1, p< 0.05					

One hundred thirty-three cases (51.2%) received medication during the episode either before or after attending the ORT Corner. Table 4 shows the effects of medication on the outcome of diarrhoea cases, and it is evident from the table that significantly ($p<0.05$) more children were found to be cured at follow-up when medication was not received (91.4%) than those who received medication (83.3%). The records of children, who were found to be cured at follow-up, were reviewed to know the effects of medication on the duration of diarrhoeal episode. It was found that the duration (mean±SD) of the diarrhoeal episode was 6.22+1.55 days when medication was received in contrast to 5.48+1.42 days when medication was not received, and the difference is statistically significant (Z=4.88, $p<0.001$).

The effect of nutritional status of the children on the outcome of the diarrhoeal episode is shown in Table 5. It is evident from the table that 39 (79.6%) children with normal nutritional status and 188 (89.1%) children with malnutrition were found to be cured at follow-up. The difference is not statistically significant. The mean duration of the diarrhoeal episode of those who were found to be cured at follow-up was 5.86+1.77 days with normal nutritional status and 5.85+1.48 days with malnutrition (Z=0.0326, p value not significant). Interestingly, it was also found that 63.2% of the children with normal nutritional status received medication compared to 40.7% of the children with malnutrition (Z=2.551, $p<0.05$).

Table 5. Nutritional status and outcome of diarrhoeal cases at follow-up

Nutritional status	Outcome: Cured (%)	Not cured (%)	Total (%)
Normal	39 (79.6)	10 (20.4)	49 (100.0)
Malnutrition	188 (89.1)	23 (10.9)	211 (100.0)
Total	227 (87.3)	33 (12.7)	260 (100.0)
X2=2.70, df - 1, p-NS. NS=Not significant			

None of the mothers, who reconsulted, revisited the ORT Corner. Treatment by 'ORS only' was mentioned by 64% of the mothers as the reason of non-revisit to the ORS Corner, and 20% of the mothers denied the receipt of any advice about when to revisit the ORT Corner.

DISCUSSION

The children were followed-up at home on the 5th day after attendance at the ORT Corner. In total, 227 (87.3%) cases were found to be cured, and 33 (12.7%) cases still had diarrhoea. The mean duration of the episode among the cured was 5.84±2.34 days which is about the same as seen in children of a community-based study by Bari *et al.* (14). The duration of diarrhoea and the proportion of children found to be cured at follow-up were also in agreement with the findings of Riyad *et al.* of Egypt

(15). In their study, one child had died, and 15% remained at risk of dehydration; in contrast, in our series none had died, and only 1.2% required hospital admission after attendance at the ORT Corner. The good outcome in our series might be due to the fact that all mothers practized ORT at home, and this reflects the effectiveness of educating the mothers on home management of diarrhoea carried out at the ORT Corner.

Eighty-one cases (31.2%) reconsulted the private doctors after attending the ORT Corner, only 3 (3.7%) cases received WHO-recommended treatment of acute watery diarrhoea, and the rest received treatment which was not recommended by WHO. This type of deviation is widespread (16-19) which calls for appropriate intervention to improve prescribing. Such intervention must, however, address the factors responsible for the observation, and a model of learning process and behaviour should be kept in mind (20). The prescription pattern found was similar to two other community-based studies carried out in Bangladesh (16,17), except prescription of rifampicin, and is a matter of concern. It was also found that medication prolonged the duration of acute watery diarrhoeal episode significantly. Contrary to the usual findings of an association between diarrhoea and malnutrition, no significant association was found in our study population. These findings might be explained partially by the fact that, in this study sample, significantly more children with normal nutritional status received medication, and medication prolonged the diarrhoeal episode of the children of normal nutritional status with bad outcome.

None of the cases, who reconsulted the private doctors, revisited the ORT Corner, and 64% of the mothers mentioned treatment by 'ORS only' as the reason for non-revisit of the ORT Corner, and 20% of the mothers denied the receipt of any advice about when to revisit the ORT Corner. This evidence is indicative of communication gap. Further strengthening of communication activities of the ORT Corner is, therefore, recommended.

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Analysis of Human Rotavirus G Serotype in Bangladesh by Enzyme-Linked Immunosorbent Assay and Polymerase Chain Reaction

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ABSTRACT

Distribution of human rotavirus G serotype was investigated by enzyme-linked immunosorbent assay (ELISA) and polymerase chain reaction (PCR) with faecal specimens obtained from children with diarrhoea in Bangladesh. By ELISA, subgroup and G serotype were determined for 59.5% and 28.6% of group A rotavirus-positive specimens respectively. However, of the 120 specimens, the G serotype of which was not determined by ELISA, serotype of the 112 specimens was typed by PCR. In total, G serotype was assigned for 95.2% of all the specimens, showing the highest rate of G4 (41.7%), followed by G1 (23.2%) and G2 (14.9%). Twenty-four specimens showed mixed types, such as G2 with G1, G8 or G9, or G1 with G4. These results indicate that PCR combined with ELISA is highly effective for G serotyping of rotavirus.

Key words: Rotavirus; Diarrhea, Infantile; Enzyme-linked immunosorbent assay; Polymerase chain reaction; Serotyping

INTRODUCTION

Human rotavirus (HRV) is the major aetiologic agent of severe diarrhoea in infants and young children worldwide (1,2). Although detection of rotavirus from clinical specimens has become a routine diagnostic procedure, characterization of these strains for serotype or specific gene products has remained difficult despite the need for such information before the introduction of a human rotavirus vaccine.

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The rotavirus particle consists of outer and inner capsid, containing 11 segments of double-stranded RNA. The outer capsid is composed of a major glycoprotein VP7 and minor trypsin-sensitive protein designated VP4. Both proteins appear to be involved in virus neutralization and define different antigenic specificities. VP7 defines a G serotype of rotavirus, and four G serotypes (GI, G2, G3, and G4) are most frequently detected in wild strains of human rotavirus. The antigenic specificity of VP4, which is independent of G serotype specificity, has been designated as P serotype (1,3-7). VP6, the sole component of the inner capsid, carries group-specific and subgroup-specific antigens. In group A rotaviruses, two subgroup specificities, I and II, are recognized (8).

Due to high morbidity and mortality of children due to rotavirus infection, global effort to develop and

evaluate rotavirus vaccines is in progress; the development of a new tetravalent reassortant vaccine is now close to licensure in the USA (9,10). To design a vaccine strategy and to evaluate success of candidate vaccines, it is essential to know the G serotypes of prevailing rotavirus strains before and after its introduction.

An enzyme-linked immunosorbent assay (ELISA), using serotype-specific monoclonal antibodies directed against VP7, has recently been developed as a rapid and simple method for serotyping rotavirus strains in faecal samples (10,11). In epidemiological studies, rotavirus G serotype in faecal specimens has been determined mostly by ELISA, using G serotype-specific monoclonal antibodies (Mabs) directed to VP7 (12,13). However, as reported previously and observed also in this study, G serotypes of a considerable proportion of specimens could not be determined by ELISA probably due to lack of a sufficient amount of double-shelled particles therein.

Comparative analysis of the amino-acid sequences of VP7 proteins of various rotavirus serotypes has revealed that VP7 protein has six variable regions (A to F) in which amino-acid sequences are highly divergent among strains of different serotypes, but are highly conserved among strains of the same serotypes (16,17).

To compensate the afore-mentioned defect in ELISA, PCR, using serotype-specific primers set appropriately in these variable regions, was applied for G serotyping, and high sensitivity and specificity of the method have been demonstrated (14,15). Although in Bangladesh, distribution and frequency of HRV G serotype have been examined until 1990 (13,19,20), it is necessary to understand the long-term trend of G serotype distribution, since it often changes from year to year (21-23).

In the present study, rotavirus specimens of Bangladesh were analyzed by PCR, in addition to ELISA, to obtain more complete information of G serotype distribution during 1992-1994.

MATERIALS AND METHODS

Stool specimens and screening of group A rotavirus

In total, 1,260 stool specimens were collected from children, aged less than five years, with watery diarrhoea and/or vomition. The patients came mostly from rural areas around Mymensingh town, and the distribution of age is typical for rotavirus. There has been no marked seasonal variation in the occurrence of diarrhoea. These patients were admitted into the Mymensingh Medical College hospital and infectious disease ward of S.K. hospital in Mymensingh during 1992-1994. Group A rotavirus was first screened by latex agglutination commercial test kit (Rotalex, Orion Diagnostics, Finland) and was further confirmed by ELISA with Mab YO-156 which is reactive with antigenic epitope on VP6 of group A rotavirus (24).

ELISA

ELISA for subgrouping and serotyping of HRV was carried out according to the procedure described previously, employing subgroup I- and II-specific and G serotype 1-, 2-, 3- and 4-specific monoclonal antibodies (10,12,24). Briefly, the ELISA employed anti-VP7 serotype-specific monoclonal antibodies, namely KU-4, S2-2G10, YO-1E2 and ST-2G7, as capture antibodies, rabbit antiserum (a pool of hyperimmune antisera to serotype 1-4 HRV strains) as detector antibody, and peroxidase-conjugated goat anti-rabbit 1gG.

Extraction of double-stranded RNA (dsRNA)

Rotavirus dsRNA was extracted by the method of Gentsch *et al.* (17) with some modifications. Three hundred µL of 10% stool suspension were mixed well with an equal volume of fluorocarbon and centrifuged at 8000 g for 5 minutes. The 250 µL of the aqueous phase, 320 µL of 6 M guanidine thiocyanate and 10 µL of RNA matrix in RNAID kit (BIO 101, Inc., LaJolla) were added and mixed on a mixer for 10 minutes. The mixture was centrifuged at 900 g for 1 minute, and the pellets were washed three times with 400 µL of wash buffer supplied in the RNAID kit by sequential centrifugation at 2000 g, 3500 g, and 8000 g. The final pellet was dried under vacuum, suspended in 50 µL of distilled water, and incubated at 65 °C for 10 minutes. The suspension was centrifuged at 8000 g for 1 minute, and the supernatant was stored at -20 °C until use.

PCR typing

Rotavirus dsRNA was extracted from stool suspension or virus-infected tissue culture fluid in the same way commonly used for RNA electropherotyping in polyacrylamide gels (25). Virus in 200 µL of 10% stool suspension or virus-infected culture fluid was disrupted by incubating at 55 °C for 30 minutes with 50 µL of disrupting solution, containing 50 mM Tris HC1 (pH 8.0), 5 mM EDTA, 5 percent Nonidet P-50, and 500 mg/mL proteinase K. Proteinase K was then inactivated by heating at 95 °C for 10 minutes. After phenol and chloroform extraction and ethanol precipitation, rotavirus dsRNA was suspended in 100 µL of distilled water. Stool specimens, negative for rotavirus, were also processed as described above for negative control.

The procedure for PCR typing was similar to the method developed by Gouvea *et al.* (15). To increase the sensitivity and specificity, amplification was carried out in two stages. Amplification of the full-length VP7 gene in the first stage was followed by a second amplification of the DNA fragments using serotype-specific primers and the copy of the full-length VP7 gene as template. Serotype-specific primers were set in variable regions, so that the PCR products of different sizes are amplified depending on different G serotypes (16).

RESULTS

Subgrouping and serotyping by ELISA

Subgrouping and serotyping of group A rotaviruses in stools collected from children hospitalized with watery diarrhoea and/or vomition were carried out with an ELISA with subgroup and serotype-specific monoclonal antibodies. Of the 1,260 specimens, 168 (13.3%) were positive for group A rotavirus. Of the 168 rotavirus-positive specimens, subgroup was determined for 100 specimens (59.5%) with 48 specimens (28.6%) each being subgroup I and subgroup II specificity, and 4 specimens (2.4%) showed dual subgroup specificity. As to the G serotype specificity, 48 (28.6%) rotavirus-positive specimens were determined in ELISA. The results are shown in the table.

Of the 48 specimens, the serotype of which was determined, 6 were assigned to serotype G1, 12 to serotype G2, 2 to serotype G3, and 27 to serotype G4. One specimen reacted with G2+G4. The remaining 120 specimens remained undetermined.

PCR typing

G serotyping by PCR employing G1, G2, G3, G4, G8 or G9-specific primers was carried out for 120 specimens which were untypeable in ELISA.

The PCR products from strains with different serotype specificity exhibited different migration which corresponded to the expected size of DNA fragments.

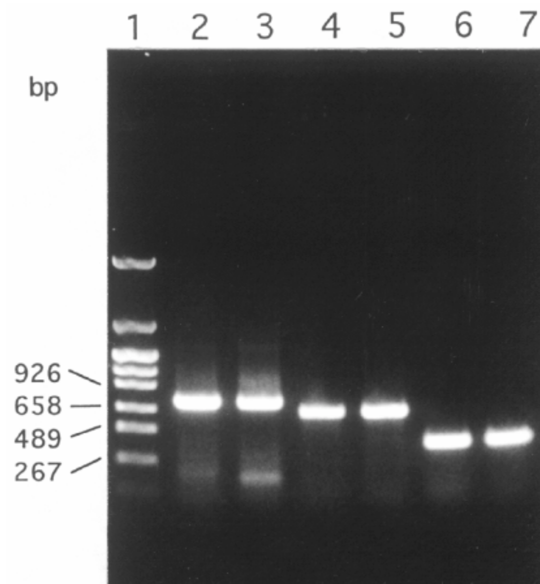

Fig. Agarose gel electrophoresis of PCR product derived from specimens with representative G serotype. Lane 1, molecular weight marker; lane 2 and 3, G serotype 1 specimens (749 bp); lane 4 and 5 G serotype 2 specimens (657 bp); lane 6 and 7, G serotype 4 specimens (394 bp)

The figure shows the PCR products amplified from HRV specimens with representative G serotypes. In total, 112 (93.3%) of the 120 rotavirus-positive specimens, whose G serotype was not detained by ELISA, were typed by PCR (Table). Of these, 33 were serotype G1, 13 serotype G2, 43 serotype G4, 2 serotype G1+G2, 17 serotype G1+G4, 3 serotype G2+G4, and 1 reacted with G2+G9. Eight specimens could not be serotyped by PCR (Table).

DISCUSSION

To understand the epidemiologic features of individual rotavirus serotypes prevailing worldwide, especially in developing countries, and to make use of the results for vaccination programme, worldwide surveys on the

Table. Frequency of HRV G serotype as determined by ELISA and PCR

Method for serotyping	No. of specimens examined	G serotype (%) G1	G2	G3	G4	G1+G2	G1+G4	G2+G4	G2+G9	ND†
ELISA	168	6 (3.6)	12 (7.1)	2 (1.2)	27 (16.1)			1 (0.6)		120 (71.4)
PCR	120*	33 (27.5)	13 (10.8)		43 (35.8)	2 (1.7)	17 (14.2)	3 (2.5)	1 (8.3)	8 (6.7)
ELISA or PCR	168	39 (23.2)	25 (14.9)	2 (1.2)	70 (41.7)	2 (1.2)	17 (10.1)	4 (2.4)	1 (0.6)	8 (4.8)

* *G serotype of these specimens was not determined by ELISA;* † *Not detected*

distribution of rotavirus serotypes are essential. A large number of surveillance of rotavirus serotypes, performed globally by ELISA with serotype-specific monoclonal antibodies against four important rotavirus serotypes, have provided useful information on the rotavirus-associated epidemiology (12,13,16,27). At present in human rotaviruses, four major G serotypes have been identified among the 14 human and animal rotaviruses (1,28). Serotype is, by definition, a classification based on neutralization of viral infectivity. Rotavirus G serotyping has been performed recently by ELISA (4,10,11), using G serotype-specific monoclonal antibodies which are not yet available for all serotypes.

However, in most studies, serotype of about 20-30 percent (in some cases over 50%) of rotavirus strains in stool specimen was left untyped. There may be several possible reasons for the reduced rate of serotyping, using serotype-specific monoclonal antibodies by ELISA. One major reason is that the serotype-specific monoclonal antibodies may not detect strains within a given serotype which have antigenic variation on VP7 protein (11,29,30), because monoclonal antibody is directed against a single epitope. The other reason is that the virion outer capsides, consisting of VP7 and VP4, are not stable in faeces, and the VP7 molecule released from the virion is considered to be a poor antigen, because the serotype-specific epitopes on VP7 were found to be conformational (29).

To compensate this defect in ELISA, PCR was applied for G serotyping, and high sensitivity and specificity of the method have been demonstrated (14,15). Although in Bangladesh, distribution and frequency of HRV G serotypes have been examined until 1993 (13,19,20,31), it is necessary to understand the long-term trend of G serotype distribution since it often changes from year to year (21-23), Gouvea *et al.* (15) first applied the PCR method for rotavirus serotyping by adding DMSO to the reaction buffer to prevent reannealing of viral dsRNA. In our study, we applied the Gouvea's method modified by Taniguchi *et al.* (16) where sensitivity of the reaction was increased. The concentration of DMSO was decreased to 3.5 percent, RNase inhibitor was added to the reaction buffer, second PCR was routinely employed, and serotype G2, G3 and G4-specific new primers were prepared (16). In the present study, rotavirus specimens in Bangladesh were analyzed by PCR, in addition to ELISA, to obtain more complete information on distribution of G serotype.

G serotyping by PCR employing G1-, G2-, G3-, G4-, G8- or G9-specific primers was carried out for 120 specimens which were untypeable in ELISA according to the procedure described previously (15). As a result, G serotype was assigned for 112 of the 120 specimens (Table). In total, 160 of the 168 rotavirus-positive specimens were typed by ELISA and PCR (Table), indicating the highest detection rate of G4 (41.7%), followed by G1 (23.2%), and G2 (14.9%). In the previous studies on HRV serotypes prevailing in Bangladesh, G1 or G2 was generally the predominant serotype, and an occasional increase of G4 was observed (13,19,20,31). G3 was always the rarest serotype as seen in our present study, suggesting that HRV with G3 is not endemic in Bangladesh. However, unlike previous findings, G4 was the commonest strain in the present study. Further investigation is needed to assess whether it was an occasional event. Although ELISA has been widely employed for G serotyping since the development of G serotype-specific Mabs (24), the rate of typeable specimens varies considerably depending on the study, e.g. approximately 70% in a Japanese study (12), in contrast to about 40% in the previous study in Bangladesh (20). These inconsistent results seem to be caused by difference in the amount of double-shelled particles in the stool samples, which may be influenced by condition of sample preservation, since a sufficient amount of virion is required for ELISA.

However, this deficiency of ELISA may be compensated by PCR G serotyping which requires only a little amount of viral genome, not virion itself. In this study, G serotype of 93.3% of the specimens which were untypeable in ELISA was indeed determined by PCR, and a similar increase in the typing rate by PCR, compared to ELISA, was reported (15). Hence, it should be emphasized that PCR is effective for G serotyping of rotavirus in combination with ELISA.

It was noted that mixed serotypes were found in 19.2% of the samples examined in PCR and that a minor serotype, G9, was found in a single specimen as a mixed type (G2+G9). The G9 serotype was also found in the stool specimens of Bangladesh in our previous study (unpublished data). Some genetic variation of G9-VP7 gene has been reported, and G9 virus is suggested to be prevalent much more than our previous estimation. Thus, it is probable that most undetermined G serotype of rotavirus may belong to G9. However, it is preferable to use some different set of primers to detect G9 by PCR because of its genetic variation. We have no data on electropherotype of the G9 rotavirus. In our previous report on HRV infections in Bangladesh, mixed patterns of RNA profiles were frequently observed, especially after flood (20). Mixed infecfion with different rotavirus strains may reflect frequent contamination of water with viruses and may facilitate generation of novel rotavirus strains through reassortment (32). Therefore, the frequency of mixed infection of rotaviruses and its influence against efficacy of rotavirus vaccine should be further investigated.

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Binding of *Escherichia coli* Heat-stable Toxin and Rise of Guanylyl Cyclase Activity in the Brush-border Membranes of Rabbit Intestinal Epithelial Cells

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ABSTRACT

The study examines the age-related differences in the density of *Escherichia coli* heat-stable enterotoxin (STa) receptors in the small intestine of rabbits. The number of STa receptors was found to be 1.7x10^{12} in 14-day old rabbits compared to 2.4x10^{9} in 14-week old rabbits per milligram brush-border membrane protein. The STa-induced guanylyl cyclase activity in the intestinal brush-border membranes was found to be stimulated by 6.2 folds over the basal enzyme activity in 14-day old rabbits, whereas in the 14-week old rabbits, it was 4 folds over the basal activity. Moreover, the enzyme activity remained lower in the adult rabbits compared to the younger ones. Autoradiographic analysis of sodium dodecyl sulphate polyacrylamide gel electrophoresis showed two STa-binding proteins of apparent molecular weights of 140 and 38 kDa in the intestinal brush-border membranes of rabbits.

Key words: *Escherichia coli*; Enterotoxins; Epithelial cells; Membranes; Membrane proteins; Cyclic AMP; Guanylate cyclase

INTRODUCTION

Heat-stable enterotoxin (STa), produced by enterotoxigenic *Escherichia coli*, is a small peptide of 18-19 amino-acid residues [1,2] which causes secretory diarrhoea in animals and human [3,4]. After binding to its receptor on the intestinal mucosa, STa leads to the rise of intracellular levels of cyclic GMP, subsequently resulting in intestinal hypersecretion [5,6]. STa also causes inositol triphosphate-mediated calcium mobilization in the intestinal epithelial cells of rat [7,8] and in the human colonic cell line [9]. Most studies on STa-receptor interactions have been carried out with the rat intestine, which reveal the structural and functional heterogeneity of STa receptors [10].

Several groups of workers have been studying the density and distribution of STa receptors in different animal models. Beside rats [11], the distribution of STa receptors has been shown in North American opossum [12], porcine [13], and chicken [14]. Recently, the distribution of STa receptors was also studied on different avian [15] and reptilian species [16]. Moreover, it has been reported that the density of STa receptors remains high in immature rats [11] and pigs [13] compared to adults.

Although STa causes diarrhoea in laboratory animals [3,17], no studies were done to find out the species-

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specific variation of distribution of STa receptors in laboratory animals.

The effect of STa on guanylyl cyclase activity was shown in the small intestine of rabbits [5,18], but no reports are available on the association and density of STa receptors in the intestine of rabbits.

The present study was undertaken to examine the association and density of STa receptors, and to evaluate the STa-induced guanylyl cyclase activity in the small intestinal epithelial cells of rabbits.

MATERIALS AND METHODS

Purification and radiolabelling of STa

STa was purified from *E. coli* SK1 strain (kindly provided by Dr. T. Takeda, National Children's Medical Center, Tokyo 154, Japan) to homogeneity and was radiolabelled with $Na^{125}I$ (Bhabha Atomic Research Centre, Mumbai, India) to a specific activity of 1000-1200 Ci/mmol as described earlier [19].

Selection of animals

Immature 14-day and 14-week old rabbits (Newzealand white), obtained from the Animal Facility Division of the National Institute of Cholera and Enteric Diseases, were taken for the present study.

Preparation of enterocytes

The enterocytes were isolated from the small intestine of rabbits by the method of Weiser [20]. All operations were done at 4 °C. The jejunums of rabbits were excised and taken into beakers containing 20 mL of 0.9% NaCl. After removing the debris with 20 mL of 0.9% NaCl, the jejunums were rinsed with 20 mL of 0.9% NaCl containing 1 mM DTT. Each jejunum was then filled with 5 mL of isolation buffer (pH 7.3) containing 0.23 mM PMSF and 1 mM DTT and incubated at 37 °C for 15 minutes in the same buffer which did not contain DTT and PMSF. Subsequently, the jejunums were emptied and filled with 5 mL of EDTA buffer (pH 7.6) containing 0.5 mM DTT and 0.23 mM PMSF, making sure that both the ends of the intestines were well tied and devoid of any leak. Each jejunum was then tapped very gently with fingers, and the cells were emptied. Each jejunum further received 5 mL of EDTA buffer plus 0.5 mM DTT and 0.23 mM PMSF, and was tapped similarly to obtain a higher number of enterocytes. The cells were spun down at 300xg (Sorvall RC5C) for 5 minutes, washed twice with 20 mL of balanced salt solution (BSS), and finally suspended in 2 mL of BSS. The cells were examined under a phase contrast microscope (Leitz, Laborlus S, Germany), and the viabilities were checked by the trypan blue exclusion technique.

Preparation of intestinal brush-border membranes

The brush-border membranes were prepared essentially by the procedure of Hauser *et al.* [21] with slight modifications. The enterocytes were washed in BSS containing 10 mM HEPES buffer (pH 7.4), 135 mM NaCl, 4.5 mM KCl, 0.5 mM $MgCl_2$, 1 mM $CaCl_2$, 1% D-glucose and resuspended in a buffer, consisting of 300 mM mannitol, 10 mM HEPES (pH 7.4), 1 mM EDTA and 5 mM EGTA. The cells were then disrupted by four to six bursts of sonication in a sonicator (MSE) at 4 °C and subsequently diluted six-fold by ice-cold deionized water. Solid $MgCl_2$ was gradually added to the mixture to a final concentration of 10 mM and after 15 minutes, the preparations were centrifuged at 2000xg for 15 minutes at 4 °C in a sorvall RC5C SWTi rotor to get rid of the nuclear materials. The supernatants were finally centrifuged at 1,00,000xg for 1 hour at 4 °C. The pellets, comprising purified brush-border membranes, were homogenized with 10-15 strokes in a homogenizer, kept in BSS (pH 7.4) containing 1 mM DTT, 0.23 mM PMSF and soybean trypsin inhibitor (5 µg/mL) to a concentration of 5-10 mg/mL and stored at -70 °C. The relative purity of the brush-border membrane was determined by measuring brush-border marker enzyme sucrase [22]. The levels of sucrase activity in the membranes prepared from 14-day and 14-week old rabbits were comparable (6.54"0.82 µmoles.h^{-1}.mg^{-1} protein vs. 6.80"0.97 µmoles.h^{-1}.mg^{-1} protein.

Receptor-binding assay

Binding of ^{125}I-STa to the brush-border membranes was done with 50-100 µg proteins in a reaction mixture comprising 50 mM HEPES buffer (pH 7.4), 3 mM $MgCl_2$, 1 mM EDTA, 0.1% BSA and a cocktail of protease inhibitors consisting of 1 µg/mL leupeptin, 1 µg/mL aprotinin and 1 mM PMSF [19]. The binding reaction (total binding) was initiated by adding ^{125}I-STa at 37 °C in a total of 200 µL reaction mixture. Reaction was terminated by adding ice-cold HEPES buffer (pH 7.4), followed by filtration through Millipore EAWP membrane filters with the help of Manifold vacuum system (Millipore, Bedford, USA). In parallel incubations, in addition to ^{125}I-STa, 1,000-fold molar excess of unlabelled STa was added to get the non-specific bindings. It was noted that about 95% of ^{125}I-STa were displaced by 1000-fold molar excess of unlabelled STa in the competition of receptor occupancy by unlabelled and labelled (^{125}I)-STa; non-specific binding was less than 5%. Specific bindings were obtained by subtracting non-specific binding data from the total binding data. The reactions were done in triplicate.

Guanylyl cyclase assay

The reaction mixture for guanylyl cyclase determination comprised 25-50 µg membrane proteins in 50 mM Tris-HCl (pH 7.6), 10 mM theophylline, a GTP-regenerating system containing 15 mM creatine phosphate and 2.5 units of creatine phosphokinase. Reactions were initiated by adding various concentrations of STa, 1 mM GTP and 5 mM $MgCl_2$ to a final volume of 150 µL. Reactions were carried out at 37 °C for 10 minutes and subsequently terminated by adding 100 µL of cold sodium acetate (pH 4.5). This was rapidly followed by boiling the samples at 95 °C for 3 minutes. The supernatants were then carefully collected to the volume of 100 µL and immediately used as the source of cyclic GMP produced by the guanylyl cyclase-induced catalysis of 1 mM GTP. The cyclic GMP generated by this procedure was quantified by radioimmunoassay using the [^{3}-H]cGMP radioimmunoassay kit (Amersham, UK).

Receptor cross-linking and autoradiography

Binding of ^{125}I-STa to the intestinal brush-border membranes was carried out in presence and absence of unlabelled STa with 150 µg of membrane proteins in 1 mL binding buffer at 37 °C for 40 minutes. The reaction mixtures were then centrifuged at 1,00,000xg for 60 minutes at 4 °C, and the pellets were then resuspended in HEPES buffer (pH 7.4). Dissucinimidyl suberate (DSS; Pierce, USA), a homobifunctional cross-linker (10 mM in DMSO), was added to a final concentration to the mixtures of 1.0-2.0 mM, and the reactions were carried out at 25 °C for 30 minutes, followed by centrifugation at 1,00,000xg for 60 minutes at 4 °C. The resulting pellets were dissolved in electrophoresis buffer [23], boiled for 10 minutes, and were subjected to 5-20% gradient sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE). After electrophoresis, the gel was dried, exposed to Kodak X-ray films at -70 °C and developed.

Protein determination

The concentrations of proteins were measured according to the protocol of Bradford [24], using bovine serum albumin as standard.

RESULTS

Equilibrium binding of ^{125}-STa to the intestinal brush-border membranes

STa was found to bind to the brush-border membranes in a specific, time and temperature-dependent manner. Maximum binding occurred at 37 °C over a 60-minute period (data not shown). To determine whether there were any differences in association of the binding of ^{125}I-STa to the intestinal brush-border membranes in 14-day and 14-week old rabbits, equilibrium bindings were done with 50-100 µg of membrane proteins with different concentrations of ^{125}I-STa at 37 °C for 60 minutes. Scatchard analysis of the stoichiometric data yielded linear plot, defining a single class of receptors and STa bound with association constants of $0.85 \times 10^{11} M^{-1}$ (Fig. 1) and $0.40 \times 10^{12} M^{-1}$ (Fig. 2) in 14-day and 14-week old animals respectively. The receptor densities per milligram brush-border membrane protein at equilibrium were found to be 1.7×10^{12} and 2.4×10^9 in 14-day and 14-week old animals respectively.

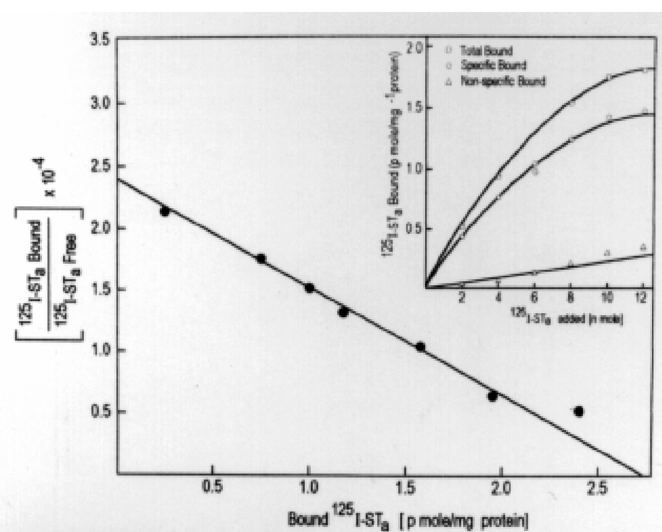

Fig 1. Scatchard analysis of ^{125}I-STa to the brush-border membranes of 14-day old rabbit. Inset shows equilibrium binding.

Correlation of STa receptor-binding and guanylyl cyclase activation

To determine the effect of STa on guanylyl cyclase activity, the intestinal guanylyl cyclase activities in response to graded doses of STa (1-100 ng/mL) were measured. The guanylyl cyclase activity was found to be higher in 14-day old rabbits compared to 14-week old rabbits. It was observed that, compared to the basal guanylyl cyclase activity of the intestinal brush-border membranes of 14-day old rabbits (4.8"1.10 pmol cGMP $mg^{-1}protein.min^{-1}$), the enzyme activity was raised by 3 and 6.2 folds at toxin concentration of 10 and 100 ng STa/mL respectively (14.40"3 vs. 29.76"4 pmol cGMP $mg^{-1}protein.min^{-1}$). In case of 14-week old rabbits, the guanylyl cyclase activity was increased over basal (3.2"0.63 pmol cGMP $mg^{-1}protein.min^{-1}$) by 2.12 and 4 folds at toxin concentration of 10 and 100 ng STa/mL respectively (6.8"0.95 vs. 12.80"1.67 pmol cGMP $mg^{-1}protein.min^{-1}$) (Fig.3).

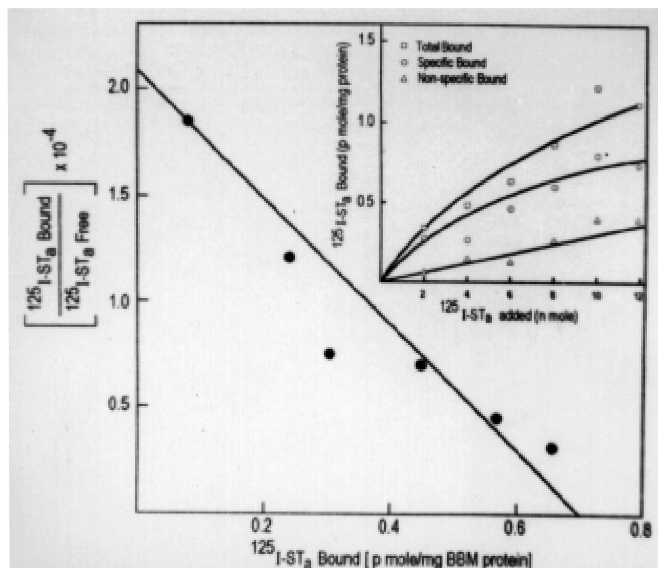

Fig.2: Scatchard analysis of 125I-STa to the brush-border membranes of 14-week old rabbit. Inset shows equilibrium binding.

Identification of STa-binding proteins by SDS-PAGE

The molecular masses of the STa receptors on the intestinal brush-border membranes of the rabbit were compared with those of the rat by SDS-PAGE analyses, followed by autoradiography. As shown in Fig. 4, 125I-STa bound to two different masses of the intestinal brush-border membranes with apparent molecular weights of 140 and 35 kDa in the rabbit which is in contrast to 160 kDa found in the rat.

DISCUSSION

The present study examined the age-related differences in the density of receptors for *E.coli* STa in the small intestine of rabbits. The experimental analysis demonstrated that STa bound to a single class of receptors in both 14-day old rabbits and 14-week old rabbits in a specific, time, temperature and dose-dependent manner. However, the density of STa receptors was found to be higher in the 14-day old rabbits compared to the 14-week old rabbits as found by our experimental analysis. This observation corroborates with the earlier reports on rats [11] and pigs [13]. Since the higher density of STa receptors was found in younger animals, we compared the STa-induced signal transduction in these animals with that of adult ones. In response to graded doses of STa, the guanylyl cyclase activity was found to be optimum at toxin concentration of 100 ng/mL. The guanylyl cyclase activity remained higher in 14-day old rabbits compared to 14-week old rabbits. That the receptor density would be directly correlated with the enzyme activation has been further supported by the observation of Cohen *et al.* [11], where they showed that an increased receptor density in immature rats concomitantly increased the guanylyl cyclase activity. To know the molecular mass(s) of STa-binding protein(s), SDS-PAGE analysis of the 125-STa-cross-linked intestinal brush-border membranes was done. Interestingly, two STa-binding proteins of apparent molecular weights of 140 and 38 kDa were found which were in contrast to one 160 kDa as found in the rat intestinal brush-border membranes. The 160-kDa STa-binding protein in the rat intestinal membranes was also demonstrated by other investigators [10, 25]. These data clearly showed the heterogeneity of STa-binding proteins. The observation of heterogenicity of STa-binding proteins can be explained in support of the following facts. It has been suggested [26] that there might be differences in the binding affinities of various receptor populations which are not apparent by classic Scatchard analysis because of relative abundance or paucity of each type of receptor. A similar picture has been observed with the receptors for atrial natriuretic peptide where the receptors were found to share a similar affinity for the native peptide [27,28], but the ligand has been found to interact with at least three different

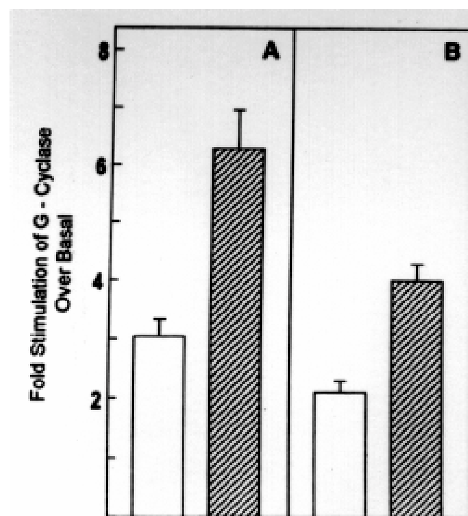

Fig.3: Activation of guanylyl cyclase following application of STa in 14-day old (A) and 14-week old (B) rabbits. Purified STa at concentrations of 10 ng/mL (□) and 100 ng/mL (▨) were applied to intestinal brush-border membranes of rabbit, and the intracellular cGMP concentrations accumulated were expressed in terms of fold activation of G-cyclase over basal. Each point represents mean of three separate experiments.

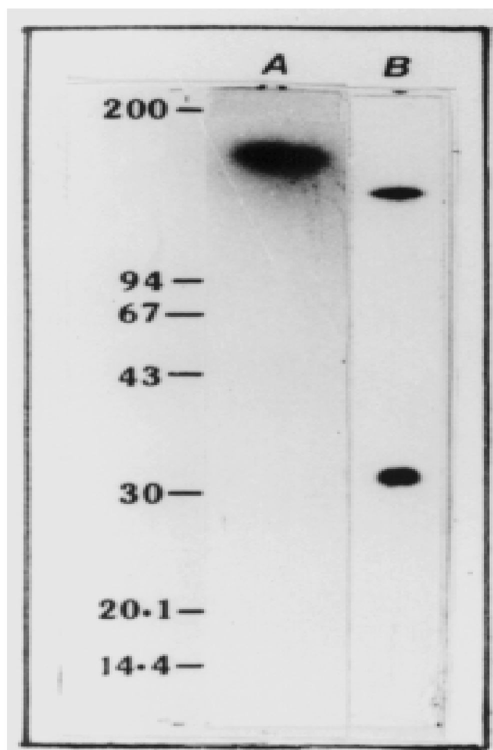

Fig.4: Autoradiographic demonstration of STa-binding proteins after SDS-PAGE. Brush-border membranes of rat (Lane A) and rabbit (Lane B) after cross-linking with ^{125}I-STa were run in 5-20% gradient SDS-PAGE, and the ^{125}I-STa-binding proteins were detected as described in the text. In the control lane (not shown), ^{125}I-STa was reacted with brush-border membranes in presence of 1000-fold molar excess of unlabelled STa.

receptors which were distinguishable on the basis of their molecular masses [29]. Moreover, Ivans *et al.* [26] suggested that the STa receptor might represent different gene products with a convergent function or might be the product of a single gene that undergoes divergent post-transcriptional and post-translational modifications which resulted in the heterogeneity of subunit organization of STa receptors.

In conclusion, this study showed a higher number of STa receptors and guanylyl cyclase activity in younger rabbits compared to adults. In contrast to the rat, two Sta-binding proteins were found to incorporate in the intestinal brush-border membranes of the rabbits.

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SHORT REPORT

Combined Infection of Norwalk-like Virus and Verotoxin-producing Bacteria Associated with a Gastroenteritis Outbreak

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ABSTRACT

Detection of multiple pathogens, particularly a combination of viruses and bacteria, is infrequently documented in outbreaks of gastroenteritis. This paper reports the presence of Norwalk-like virus (NLV) and enterohaemorrhagic verotoxin-producing *Escherichia coli* in one individual, and NLV and verotoxin-producing *Aeromonas sobria* in another individual, both part of a large gastroenteritis outbreak. The causes of gastroenteritis in such outbreaks may be more complex than previously thought.

Key Words: Norwalk virus; Gastroenteritis; Bacterial toxins; Verotoxins; *Escherichia coli*; *Aeromonas*; Disease outbreaks

INTRODUCTION

Outbreaks of gastroenteritis due to the Norwalk-like group of viruses (NLV) are regularly reported around the world, and NLVs are accepted as causative agents of gastroenteritis (1).

In recent years, verotoxin (VT)-producing bacteria, notably *Escherichia coli* and *Aeromonas* spp., have also emerged as important or potentially important causes of gastroenteritis (2,3). In this report, we describe an outbreak of gastroenteritis in which NLV was the chief infectious agent detected, but in which VT-producing organisms were also found in some individuals. The results emphasize the need to test for all possible causes of gastroenteritis in an outbreak.

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MATERIALS AND METHODS

The outbreak

In October 1997, approximately 600 adults attended a dinner dance at a major function centre in Melbourne, Victoria, Australia. About 48 hours later, approximately 60 individuals (10%) became ill with symptoms of vomiting, diarrhoea, aches, and pains. In general, the illnesses lasted for 24 to 48 hours. Specimens were collected from 8 individuals with these general

symptoms. Faeces for this study were typically collected within 4 days of the onset of symptoms.

Two of the eight specimens were positive for both NLV and VT-producing bacteria as discussed in the results section. Patient PR had vomiting and diarrhoea for 48 hours, then cramps for nine days. Patient WA had vomiting and diarrhoea for 24 hours, then cramps for a few days. Neither PR nor WA had bloody diarrhoea.

Virology

Virological studies were carried out on the eight faecal specimens (including those from PR and WA) by negative staining electron microscopy (EM) and polymerase chain reaction (PCR) methodologies (4).

Bacteriology

The same eight faeces were examined for bacterial pathogens by standard microbiological procedures for *Aeromonas* spp., *Bacillus cereus*, *Campylobacter* spp., *E. coli*, *Listeria monocytogenes*, *Plesiomonas* spp., *Salmonella* spp., *Shigella* spp., *Yersinia* spp., *Staphylococcus aureus*, and *Vibrio* spp. Colonies from MacConkey agar, which resembled *E. coli*, were examined further by checking for agglutination with polyvalent antisera against commonly-recognized enteropathogenic *E. coli* serovars, as well as specific *E. coli* O157 antisera. The presence of VT-producing strains (also called Shiga-like toxin-producing strains) was determined by placing a filtrate of an overnight shake-culture onto VT-sensitive HeLa cells. Any possible verotoxigenic strains were further examined, being subjected to full biochemical analysis and serotyping, as well as testing for VT production by both Vero cell assay and enzyme-linked immunosorbent assay. The VT-producing *E. coli* strain was fully serotyped for O and H antigens.

RESULTS

Virology

By EM NLVs were detected in two cases (individuals PR and CB); other viruses, including rotavirus, adenovirus, astrovirus, and classic calicivirus, could not be detected in any individual. For PCR, RNA was extracted from partially-purified faecal material (4) and subjected to a reverse transcription-hemi-nested PCR specific for NLV genogroup 2 (1). All eight faecal samples were positive.

Faecal specimens from the two individuals PR and WA, which were positive for the presence of enterohaemorrhagic (VT-producing) *E.coli* (EHEC) and *Aeromonas sobria* respectively (see below), were subjected to further study. Sequence analysis of the ORF 1 cDNA fragment of NLV PCR product, performed using the method of Cauchi *et al.* (4), indicated that the NLVs detected were Camberwell/Lordsdale-like members of the NLV genogroup 2 (1). Similarly, sequence analysis of PCR products from the faecal specimen of individual CB (positive for NLV by EM; negative for *E. coli* or *A. sobria*) also showed the presence of a Camberwell/ Lordsdale-like NLV.

Bacteriology

No *B. cereus*, *Campylobacter* spp., *L. monocytogenes*, *Plesiomonas* spp., *Salmonella* spp., *Shigella* spp., *Vibrio* spp. *Staphylococcus aureus,* or *Yersinia* spp. were found.

EHEC were isolated from the faeces of a female (PR) aged over 60 years who yielded a strain of *E. coli* O128:H2-producing VT1 and enterohaemolysin (EHly). Another female (WA), aged 50 years, yielded a strain of *A. sobria*-producing VT but not EHly.

DISCUSSION

Detection of multiple pathogens, particularly a combination of both viruses and bacteria in a given individual, is infrequently documented in outbreaks of gastroenteritis. In a study of rotavirus-associated gastroenteritis, Unicomb *et al.* (5) concluded that the severity of mixed infections of rotavirus and *E. coli* was the same as that of infections with rotavirus alone. However, in contrast to this study, none of the *E. coli* described by Unicomb *et al.* (5) were EHEC. With one exception--a report linking *B. cereus* and Norwalk agent in an outbreak of gastroenteritis (6)--there are no detailed reports in the mainstream literature linking EHEC and/ or toxigenic *Aeromonas* with NLVs in outbreaks of gastroenteritis.

In this paper, we report the presence of both EHEC (VT-producing) and NLV in samples from one individual (PR) associated with a large outbreak of gastroenteritis. A second individual (WA) who was excreting NLV also carried a VT-producing bacterium, a strain of *A. sobria.* Testing of NLVs from this outbreak indicated that they were Camberwell/ Lordsdale-like members of genogroup 2.

Both NLVs and these VT-producing bacteria are individually likely causes of gastroenteritis in humans.

NLVs, a group of highly infectious viruses within the family *Caliciviridae*, are commonly associated with gastroenteritis in humans and generally cause mild and self-limiting illness (1,7). NLVs have been responsible for outbreaks of gastroenteritis as well as sporadic cases (1,4,7). Camberwell/Lordsdale-like NLVs appear to be commonly associated with outbreaks of gastroenteritis in Victoria (1).

EHEC belonging to serotype O128:H2 have been isolated from human patients with diarrhoea in many parts of the world (8,9,10,11) although they have also been isolated from healthy humans in Germany (12) and healthy sheep (10). Strains of *Aeromonas* producing VT-like toxins have been reported (3), but their significance as causes of gastroenteritis is unclear. The strain of *A. sobria* isolated from patient WA closely resembled *E. coli* on all primary isolation media and was treated as an *E. coli* until the full biochemical analysis was done.

The typical clinical illness associated with this outbreak, and the detection of NLV in all the specimens examined, support a causative role for NLV. The clinical significance of the bacterial enteropathogens is less clear, but the fact that both *E.coli* O128:H2 and *A. sobria* can produce VT-like toxins implies that they must be considered as potential pathogens (10), and they would have been considered potentially causative had they been detected in isolation.

The unusual results of this study were only obtained because of the close collaborative work of several public health reference laboratories and because of the availability of more sensitive techniques for detection of NLV and of procedures for examination for VT-producing organisms. It is possible that the phenomenon described in this study is more common, and further studies are needed to determine whether the linkage between verotoxin detection and NLV is more widespread. The results of our study emphasize the need for testing for a range of organisms when determining the cause of an outbreak of gastroenteritis.

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SHORT REPORT

Virulence Patterns of *Aeromonas eucrenophila* Isolated from Water and Infected Fish

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ABSTRACT

Six isolates of *Aeromonas eucrenophila*–2 from water and 4 from superficial skin ulcer of cat fish–were examined for haemagglutination, serum sensitivity, chitinase production and enterotoxicity, and correlation, if any, between them; only one strain showed haemagglutination and was inhibited by both D-mannose and L-fucose. All the strains showed resistance to normal human serum, but produced chitinase; one of them elaborated inducible chitinase. All these strains caused fluid accumulation only after 1-4 serial passages through rabbit ileal loops, of which one strain that elaborated inducible chitinase caused significantly more ($p<0.005$) fluid accumulation. These observations indicate that there is no correlation between enterotoxicity and haemagglutination and/or serum resistance, and these properties did not change after animal passage. However, a correlation could be observed between elaboration of inducible chitinase and enterotoxin production.

Key words: *Aeromonas eucrenophila;* Haemagglutination; Enterotoxins; Virulence

INTRODUCTION

All the currently recognized 13 hybridization groups (HGs) of *Aeromonas*, except *Aeromonas eucrenophila* and *A. sobria* (HG 7) (1), have been described as a pathogen of fish (2-5) and human (6-9). These organisms have also been implicated in extra-intestinal infection and diarrhoea in man (10,11)--the strains often originating from water. Several of these genospecies, such as *A. hydrophila* (HG 1), *A. veronii* bv *sobria* (HG 8), but rarely *A. caviae* (HG 4), produced enterotoxin and haemolysin (12-17) and also showed resistance to normal human serum (NHS) (18-20). Moreover, enterotoxigenic diarrhoeal isolates of *A. hydrophila* showed haemagglutination (HA) which was not sensitive to D-mannose and L-fucose. But *Aeromonas* strains showing HA sensitive to D-mannose and L-fucose or no haemagglutination (NHA) were non-toxigenic strains of *A. caviae*, commonly from non-diarrhoeal infection or environment (21). Although the production of haemolysin and enterotoxin by *A. eucrenophila* has been demonstrated in this laboratory (22), the link between fish and water isolates of *A. eucrenophila* and human illness has not yet been established. Therefore, this study examined the possible virulence factors, such as haemagglutination, serum resistance and chitinase production, associated with *A. eucrenophila* and their correlation with enterotoxicity.

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MATERIALS AND METHODS

Bacterial strains

Strains of *Aeromonas* were isolated from water and from superficial skin ulcers of cat fish. Samples were plated on nutrient agar plate and incubated at 37 ^{0}C for overnight. Suspected colonies were tested for oxidase reaction. The oxidase-positive colonies were further tested with biochemical substrates for identification following the criteria of Popoff (23). These strains were identified as *A. eucrenophila* as per Schubert and Hegazi (24) and Abbott *et al.* (25), according to their ability to hydrolyze aesculin, ferment salicin, glycerol and mannose and to produce gas (weak) and H_2S (weak), and to use citrate. Reference strain of *A. eucrenophila* NCMB 74, provided by JM Janda, USA, was used in this study for comparison. A toxigenic strain 569B of *Vibrio cholerae* O1 and a non-toxigenic strain 265 of *Escherichia coli,* respectively, served as positive and negative controls. Strains were maintained in peptone agar stab cultures at room temperature and did not undergo more than three subcultures prior to the experiments.

Haemagglutination and inhibition of haemagglutination by sugars

The method of Atkinson and Trust (26) was used. Human 'O' group erythrocytes were collected by venipuncture and stored in Alsever's solution at 4 ^{0}C. Before use, they were washed three times with phosphate-buffered saline (PBS, 0.004 M, pH 7.4) and then a 3% suspension was prepared in PBS.

Colonies of overnight-grown cultures of *Aeromonas* strains on nutrient agar plates were inoculated in brain heart infusion broth (BHIB, Difco) and incubated at 37 ^{0}C for 18 hours to yield Ca 10^9 bacteria mL^{-1}. These cultures were centrifuged and washed twice in PBS and re-suspended the bacteria in PBS.

HA test was performed at room temperature by mixing 20 µL suspension of erythrocytes with 20 µL of bacterial suspension on a slide alongside a control suspension of erythrocytes and PBS, and gently rocking by hand. Strains were considered HA-negative, if agglutination did not occur within 5 minutes.

Sensitivity of HA to sugar was studied in a similar three-volume test with 20 µL suspension of erythrocytes (3% v/v), 20 µL of sugar (1% w/v), and 20 µL of bacterial suspension. Reaction in the presence of D-mannose (M), L-fucose (F), and D-galactose (G) was compared with positive control (20 µL each of erythrocytes, bacteria, and PBS) and negative control (20 µL each of erythrocytes and 40 µL of PBS). The reaction was recorded as sensitive (S), if previously positive strain became negative in the presence of sugar, and resistant (R) if it remained positive.

Susceptibility to normal human serum

Group 'O' blood was obtained by venipuncture from healthy individuals with no history of infection with aeromonads; pooled sera were separated and used immediately or stored at –70 ^{0}C. Fresh or thawed NHS was used unaltered. Serum-sensitive *E. coli* K12 strain served as control for each experiment.

Aeromonas strains were challenged against 65% NHS in a microcolorimetric assay (27). The strains tested were transferred to microdilution well containing 100 µL of peptone (1% v/v) and glucose (1% w/v) broth (PGB). After overnight incubation at 37 ^{0}C, 20 µL of each PGB culture was transferred to 200 µL fresh PGB and incubated at 37 ^{0}C for 2-3 hours. Log phase bacteria were then inoculated (20 µL, Ca 10^7 bacteria) into 100 µL PGB containing 65% NHS and 0.5% of 1.5% stock solution of bromothymol blue (final concentration 0.0075%). Serum resistance was assayed by measuring a colorimeter change from green (inhibition) to yellow (growth) of the PGB containing NHS. Control, consisting of PGB with 65% heat-inactivated serum (HIS, 56 ^{0}C for 30 minutes) and bacteria. Incubation was done at 37 ^{0}C for 4-5 hours.

In addition to the microcolorimetric assay, the representative strains of *A. eucrenophila* were challenged against 65% NHS in the tube assay according to the procedure described by Carruthers and Kabat (28), with plate counts determined after 30, 60, and 120 minutes. Strains exhibiting a ten-fold (one log) decrease in viable count after 120 minutes (when compared with t=0) were considered serum-sensitive.

Preparation of culture supernatant and estimation of chitinase activity

Culture supernatant (CS) of each strain was prepared according to the method described by Dastidar and Narayanaswami (29), and was tested for chitinase activity, following the method of Jeuniaux (30). In brief, each CS in 0.5 mL amounts in two tubes mixed with 0.1 mL of 0.8 M potassium tetraborate (Sigma) was boiled in a water bath for (exactly) 3 minutes and brought to room temperature. One percent dimethyl amino benzaldehyde (p-DMAB, Sigma, prepared in glacial acetic acid containing 1.25% 10 N HCl) in 3.0 mL amount was added to each tube, mixed well and allowed to stand for 20 minutes at 37 ^{0}C. The samples were brought to room temperature, and absorbance was noted at 540 nm in a colorimeter within 10 minutes. A

calibration curve was established with 10, 20, 40, 80 and 160 mg μL^{-1} concentration of N-acetyl glucosamine (Sigma). Enzyme activity was expressed in terms of mg of N-acetyl glucosamine (N-AG) mL^{-1}.

Enterotoxicity test

The culture filtrates (CFs), prepared with each strain following the method of Annapurna and Sanyal (31), were passaged through millipore membrane (0.22 µm pore size) and stored at 4 ^{0}C until use. These CFs were tested for accumulation of fluid in rabbit ileal loop (RIL), following the method of De and Chatterje (32). CF was prepared only when the live cells of a particular strain gave positive ileal loop reaction, whether at the initial test or after passage through the gut of a susceptible host. (33).

RESULTS

Of the 6 strains of *A. eucrenophila*, only one was HA-positive and showed sensitivity to both D-mannose and L-fucose (MFS-HA) (Table).

In the initial tests in RILs, live cells of all these strains caused no accumulation of fluid. These non-toxigenic strains whether showing NHA or MFS-HA became toxic without change in their HA properties after serial passages through RILs (Table). CFs also caused accumulation of fluid in the range similar to those of live cells

All the strains of *A. eucrenophila* were resistant to bactericidal action of NHS. Accumulation of fluid was not caused by these strains when tested initially, but became positive after 1-4 serial passages through the gut of rabbit (Table).

Strains of *A. eucrenophila* elaborated chitinase in their CS. Enhancement of chitinase activity in CS was also observed on prolongation of the incubation period (Table). One of the 6 strains showed significant increased production of enzyme chitinase in CS containing chitin. This strain caused significantly more ($p<0.005$; student '*t*' test) accumulation of fluid than those elaborated by constitutive chitinase (Table).

DISCUSSION

The finding of the present study that *A. eucrenophila* strains showed NHA or MFS-HA indicating HA pattern may be correlated with the source of isolation. These observations corroborate with the finding of Burke *et al.* (21) who reported that the majority of diarrhoeal isolates of *A. hydrophila* showed HA pattern other than MFS-HA and those of *A. caviae* mostly from non-diarrhoeal or water sources showed either NHA or MFS-HA.

It was reported that strains of *Aeromonas* showing NHA or MFS-HA were non-toxic (21). However, the observation that strains of *A. eucrenophila* were non-toxic in the initial tests but became toxic after serial passages through RILs suggests a repression-de-repression phenomenon influencing the toxin gene. Moreover, none of the non-haemagglutinating strains showed HA after RIL passage although they became enterotoxic; this suggests that repression-de-repression may not apply to the haemagglutinin gene. This observation clearly indicates that there is no correlation between HA pattern and enterotoxicity, and that they are not probably genetically linked.

Table. Correlation between haemagglutination, serum sensitivity, chitinase production and enterotoxicity in *Aeromonas eucrenophila* strains

Strain no.	HA pattern	Serum susceptibility	Chitinase activity (µg of N-AG mL-1) Brain Heart Infusion Broth without chitin		Brain Heart Infusion Broth with chitin		Mean (SD) volume of fluid accumulation after animal passage* ($mLcm^{-1}$ of RIL)
			Day 3	Day 9	Day 3	Day 9	
W-2	NHA	R	12	14	14	18	0.31 (0.05)
+W-30	NHA	R	16	24	36	44	0.72 (0.05)
CF-13	NHA	R	32	36	34	40	0.27 (0.05)
CF-35	NHA	R	20	24	24	28	0.36 (0.06)
CF-67	MFS-HA	R	24	28	28	32	0.42 (0.04)
CF-68	NHA	R	18	20	24	28	0.37 (0.02)
NCMB 74	NHA	ND	8	10	6	8	ND
$V. cholerae 569B						1.35	(0.30)
#*E. coli* 265							0.00 (0.00)

**Each test was done in two rabbits; +Strain produced inducible chitinase; $Positive control; #Negative control; HA: haemagglutination; NHA: no haemagglutination; M: D-mannose; F: L-fucose; R: serum resistant; ND: not determined*

Several workers (10,11,34,35) have reported that *A. hydrophila* and *A. sobria* are more virulent and are serum-resistant than *A. caviae* in their potential to cause intestinal and extra-intestinal infections. However, *A. caviae* strains have been increasingly implicated in intestinal (11,36) and extra-intestinal infections, including septicaemia and bacteraemia (37) in immuno-compromised hosts. The observation that isolates of *A. eucrenophila* from water and infected fish were serum-resistant compared to other species of *Aeromonas* (20) may account for even higher pathogenic potential in extra-intestinal infections and also versatility in the role of this species in various diseases.

It was observed that all strains of *A. eucrenophila* produce constitutive chitinase, like those of other species (38,39). However, one strain that elaborated significantly higher amount of chitinase in the presence of chitin indicates the inducible nature of this enzyme. This phenomenon may probably be explained by an earlier observation in relation to *V. cholerae* and *V. parahaemolyticus* which adsorb to chitin particles and copepod (40-42). It has been suggested that a primary role of vibrios could be colonization and initiation of degradation of chitinous material in the aquatic ecosystem (43). Such a role was postulated by Hood and Mayers (44) who demonstrated the role of *Vibrio* species in chitin turnover and metabolism in marine crustacean. The observation of the present study that the strain of only water origin, but not of fish produced inducible chitinase may suggest that this isolate may use the chitin in aquatic life, such as crustaceans and arthropods, may adhere to them with the help of this enzyme, as vibrios and aeromonads do (45, 46). The data of this study that *A. eucrenophila* strain W30 which elaborated inducible chitinase caused significantly more ($p<0.005$) fluid accumulation suggest a correlation between production of inducible chitinase and enterotoxin. This observation further strengthens our earlier finding with *Aeromonas* spp. that there is a correlation between production of inducible chitinase and enterotoxin (39). Chitinase production is probably an important prerequisite for survival of *Aeromonas* on chitinous fauna in the environment, but may not be so while in gut. Similar phenomenon of association of enterotoxicity with virulence factors, such as haemolysin (16,17), serum resistance, and haemagglutination (20) in *Aeromonas* spp., has been reported earlier from this laboratory.

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CASE REPORT

Pigmented nails and *Strongyloides stercoralis* infestation causing clinical worsening in a patient treated for immunoproliferative small intestinal disease: two unusual observations

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ABSTRACT

Immunoproliferative small intestinal disease (IPSID) is commonly reported from developing countries with poor socioeconomic conditions, hygiene, and high frequency of gastrointestinal infections and infestations. The disease requires anti-malignant chemotherapy in lymphomatous stage. Reported here is a 20-year old man with IPSID lymphoma who responded to anti-malignant chemotherapy initially, but later deteriorated due to *Strongyloides stercoralis* infestation, which was treated successfully with mebendazole. Importance of an early recognition and adequate treatment for gastrointestinal infections and infestations before anti-malignant chemotherapy for this disease is highlighted considering the occurrence of this disease in the developing world. The patient developed alternate brown black and white lines in the finger nails after combination chemotherapy, which has not been reported earlier in this disease; the nail changes disappeared 6 months after the withdrawal of doxorubicin suggesting this drug as the cause for such nail changes during anti-malignant combination chemotherapy.

Key words: Immunoproliferative small intestinal disease; *Strongyloides stercoralis*; Nails

INTRODUCTION

Patients with immunoproliferative small intestinal disease (IPSID) usually present with chronic diarrhoeal malabsorption, abdominal pain, cachexia and clubbing of finger and toes, and require anti-cancer chemotherapy in most cases (1). We report here a patient with the IPSID whose clinical condition worsened due to *Strongyloides stercoralis* infestation. He developed alternate brown-black and white lines in the nails following anti-cancer chemotherapy; both the observations are unusual in this disease.

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CASE REPORT

A 20-year old man presented in April 1995 with history of severe diarrhoea, anorexia, nausea, occasional

vomiting, weakness and wasting of three years. On examination, he was grossly emaciated (body weight 29 kg) and pale, and had clubbed fingers and toes. Investigations revealed: haemoglobin (Hb)-95 g/L, dimorphic erythrocytes on peripheral smear, total leucocyte count (TLC)–7.5x10^9/L , polymorphonuclear leucocytes 72%, eosinophils 1%, lymphocytes 25%, monocytes 2%, platelets adequate; liver function tests: serum bilirubin–20.5 µmol/L (normal 2-18), serum albumin–25 g/L (normal 40-60), aspartate and alanine aminotransferases 44 and 52 U/L (normal 0-35), alkaline phosphatases–730 U/L (normal 70-110), creatinine–97.2 µmol/L (normal 50-110), calcium–1.1 µmol/L (normal 2.2-2.6), and postprandial blood sugar normal. Stool microscopy revealed larvae of *S. stercoralis.* His faecal fat excretion was 6 g in 24 hours (normal 6 g), and urinary D-xylose excretion following oral ingestion of 5 g of D-xylose was 0.8 g (normal $\geq$1 g).

Chest radiograph and abdominal ultrasonogram were normal. Barium follow through series revealed mucosal nodularity and irregular thickening of mucosal folds in the small bowel showing "postage stamp" appearance; the changes were more marked in the proximal small bowel. An endoscopic examination showed nodularity of duodenal mucosa. Biopsy from these lesions revealed diffuse poorly differentiated small cell lymphocytic lymphoma. Immunoelectrophoresis, using polyclonal affinity-purified mono-specific antisera against *kappa*, *lambda*, *gamma, mu* and *alpha* chains, revealed normal patterns with *kappa*, *lambda, gamma* and *mu* antisera. The pattern for *alpha* chain showed an anodic extension which was not seen with normal control serum, suggesting the presence of abnormal *alpha* chains. A diagnosis of IPSID lymphoma was made.

Following a course of mebendazole 200 mg/day for three days to treat strongyloidosis, anti-cancer combination chemotherapy with cyclophosphamide, doxorubicin, vincristine, and prednisolone every third week was stated; symptoms subsided, he gained body weight (55 kg after the third session of chemotherapy), and Hb rose from 95 g/L to 110 g/L; he remained well till the fifth chemotherapy cycle after which the symptoms, including diarrhoea, recurred, and body weight fell to 38 kg. Stools were passing in a large volume without blood and mucus. Investigations revealed: Hb–120 g/L, TLC–20x10^9/L, polymorphonuclear leucocytes 30%, eosinophils 28%, lymphocytes 41%, and monocytes 1%. Stool microscopy revealed larvae of *S. stercoralis* and ova of *Trichuris trichiura.* Endoscopic duodenal and gastric biopsies repeated at this stage revealed dense eosinophilic infiltrate in mucosa. A bone-marrow examination revealed hypercellularity with a marked increase in cells of eosinophilic series. The patient was treated with two courses of mebendazole (200 mg/day) for two weeks each with an interval of one week between the courses. Stool microscopy repeated two months later did not show any ova or parasite; Hb–105 g/L, TLC–5.2x10^9/L, polymorphonuclear leucocytes 46%, eosinophils 4%, and lymphocytes 50%. The original regime of anti-cancer chemotherapy was reinstituted. After instituting three more courses of the drugs, alternate brown-black and white lines were noted in the nails (Fig.). Since nail changes have been reported earlier following combination chemotherapy (2,3), and doxorubicin has been thought to be the likely agent

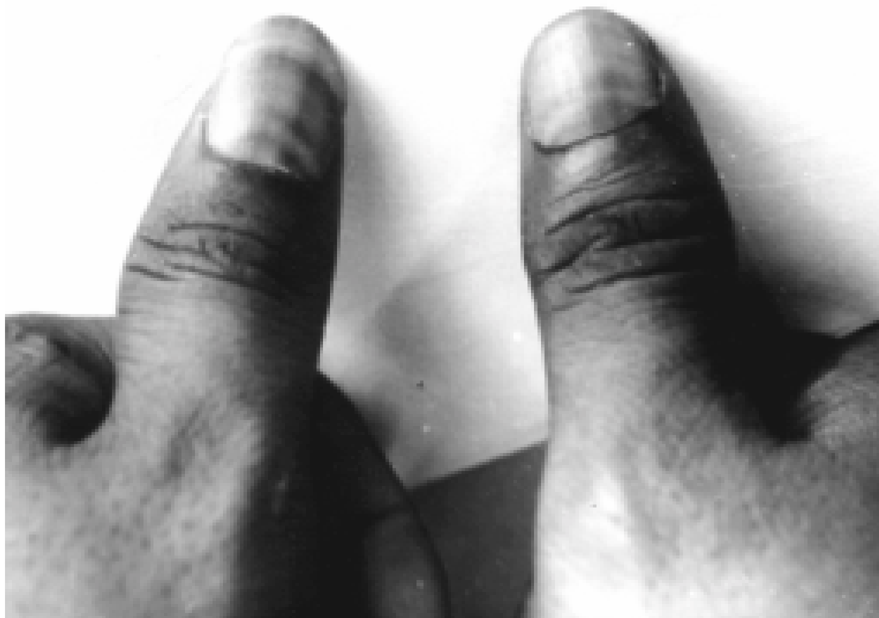

Fig. Photograph of the hands showing alternate brown-black and white lines in the nails which developed following combination chemotherapy.

in children (4), this drug was omitted from the regime and three more cycles given without this drug. The nail changes gradually disappeared beginning from the base of the nail. Six months later the nails were found to be normal.

The patient remained well for two years after presentation, passing formed stool once daily, weight 55 kg, Hb–130 g/L, D-xylose excretion after oral intake of 5 g of D-xylose-1.6 g (normal $\leq$1 g). However, in July 1997, he came with recurrence of diarrhoea and generalized lymphadenopathy; biopsy from these lymph nodes revealed non-Hodgkin's lymphoma. Repeated stool microscopy did not reveal any ova or cyst. The patient died before the second chemotherapy cycle could be completed.

DISCUSSION

Immunoproliferative small intestinal disease is commonly reported from less-developed countries, including India. Our patient, however, had two unusual features which made this case interesting. The first unusual feature is the unusual nail change following eight sessions of anti-cancer chemotherapy. Since such nail changes have been related earlier to combination chemotherapy for nodal lymphoma (2,3) and doxorubicin was thought to be the most likely agent causing it (4), we omitted doxorubicin from our chemotherapy regime. The nail changes disappeared in six months beginning from the base of the nail; normally, nails grow at a rate of 0.1 mm a day, and takes about 5 months to grow from the base to the tip (5). Therefore, our case confirms the role of doxorubicin as a single agent in causing such nail changes.

Although the reoccurrence of the nail changes following rechallenge with the drug would have made our point stronger, we could not do it, because the patient remained in remission initially, and as such, further administration of anti-malignant chemotherapy was not justified, and during later recurrence, he died before receiving adequate chemotherapy. However, the nail toxicity of anti-malignant chemotherapy might not be of much clinical significance as this did not lead to any morbidity; further studies need to be done to see whether nail changes predict the development of major toxicities of doxorubicin therapy.

The second unusual feature is the deterioration of clinical course due to infestation by *S. stercoralis* while the patient was improving following anti-cancer chemotherapy. The majority of the patients with the IPSID have been reported from various countries (1.6) with low socioeconomic conditions, poor hygiene, and high frequency of intestinal infection and infestation.

Importance of an early recognition of infections and infestations, particularly before and during anti-malignant chemotherapy in a patient with the IPSID, is enormous, since these can cause sudden worsening of the clinical condition of the patient (as in our case) following anti-cancer chemotherapy associated depression of immune system and serious morbidity and mortality (6).

Temporal correlation with administration of anti-malignant chemotherapy, demonstration of the larvae and cysts of the worms in the stool, development of marked eosinophilia, and response to anthelmintic therapy suggest the possibility of the parasites being responsible for the worsening of the condition of our patient. *S. stercoralis* was the likely agent causing it, since *T. trichiura* infestation is often asymptomatic or associated with dysentery syndrome due to involvement of colon which was lacking in our patient. Although mebendazole is not very effective in strongyloidiasis, we used this drug to treat our patient as he had mixed infection, and an extended course of mebendazole has been found effective in patients with strongyloidiasis (7).

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"Enteropathogenic *Escherichia coli* (EPEC) belongs to a family of related bacterial pathogens, including enterohemorrhagic *Escherichia coli* (EHEC) O157:H7 and other human and animal diarrheagenic pathogens that form attaching and effacing (A/E) lesions on host epithelial surfaces. Bacterial secreted Esp proteins and a type III secretion system are conserved among these pathogens and trigger host cell signal transduction pathways and cytoskeletal rearrangements, and mediate intimate bacterial adherence to epithelial cell surfaces in vitro. However, their role in pathogenesis is still unclear. To investigate the role of Esp proteins in disease, mutations in *espA* and *espB* were constructed in rabbit EPEC serotype O103 and infection characteristics were compared to that of the wild-type strain using histology, scanning and transmission electron microscopy, and confocal laser scanning microscopy in a weaned rabbit infection model. The virulence of EspA and EspB mutant strains was severely attenuated. Additionally, neither mutant strain formed A/E lesions, nor did either one cause cytoskeletal actin rearrangements beneath the attached bacteria in the rabbit intestine. Collectively, this study shows for the first time that the type III secreted proteins EspA and EspB are needed to form A/E lesions in vivo and are indeed virulence factors. It also confirms the role of A/E lesions in disease processes."

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"Enterotoxigenic *Escherichia coli* (ETEC) are diverse pathogens that express heat-labile (LT) and/or heat-stable (ST) enterotoxins, yet little is known about whether epidemiologic patterns of pediatric ETEC diarrhea vary by the expressed ETEC toxin phenotype. In total, 242 Egyptian children aged <3 years were prospectively followed in 1993-1995. ETEC episodes were detected during twice-weekly home visits, and asymptomatic ETEC excretion was identified from monthly cross-sectional surveys. ETEC episodes were 0.6 per child-year. ST-only ETEC was 2.6 times ($P<.001$) more common in warmer than cooler months, while LT-only ETEC showed no seasonal variation. Ownership of a household sanitary latrine, but not breast-feeding, was associated with a lower risk of both enterotoxin phenotypes. Coexpression of a colonization factor by LT- or ST-only ETEC strengthened the association with diarrhea. These findings indicate that the epidemiologic patterns of LT-only and ST-only ETEC are not identical and that disease interventions should include improved household sanitation."

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"Enteropathogenic *Escherichia coli* (EPEC) strains are a common cause of infantile diarrhea in developing countries. EPEC strains induce a characteristic attaching and effacing (A/E) lesion on epithelial cells. A/E lesion formation requires intimin, an outer membrane adhesin protein. The cell-binding activity of intimin is localized at the C-terminal 280 amino acids of the polypeptide (Int280). So far, four distinct Int280 types (a, b, g, and d) have been identified. The aim of this study was to identify immunodominant regions within the Int280a and Int280b domains. Recombinant DNA was used to construct and express overlapping polypeptides spanning these domains. Rabbit anti-Int280 antisera and human colostral immunoglobulin A were reacted with these polypeptides in Western blots and enzyme-linked immunosorbent assays. The results obtained with the rabbit antisera showed the presence of two separate immunodominant regions which are common to both Int280a and Int280b. The first localized within the N-terminal region of Int280, and the second localized between amino acids 80 and 130. The results with the human colostra revealed one reactivity pattern against the Int280a fragments but two different reactivity patterns against the Int280b domain"

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"In children, the treatment of acute diarrhoea with the World Health Organization (WHO) standard oral rehydration solution (ORS) provides effective rehydration but does not reduce the severity of diarrhoea. In community practice, carob bean has been used to treat diarrhoeal diseases in Anatolia since ancient times. In order to test clinical antidiarrhoeal effects of carob bean juice (CBJ), 80 children, aged 4-48

months, who were admitted to SSK Tepecik Teaching Hospital with acute diarrhoea and mild or moderate dehydration, were randomly assigned to receive treatment with either standard WHO ORS alone or a combination of standard WHO ORS and CBJ. Three patients were excluded from the study because of excessive vomiting. In the children receiving ORS + CBJ the duration of diarrhoea was shortened by 45%, stool output was reduced by 44% and ORS requirement was decreased by 38% compared with children receiving ORS alone. Weight gain was similar in the two groups at 24 h after the initiation of the study. Hypernatraemia was detected in three patients in the ORS group but in none of those in the ORS + CBJ group. The use of CBJ in combination with ORS did not lead to any clinical metabolic problem. We therefore conclude that CBJ may have a role in the treatment of children's diarrhoea after it has been technologically processed, and that further studies would be justified."

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"One-hundred and six male children aged 6-23 months with a history of acute watery diarrhoea of less than 72 h duration were randomized to receive either folic acid in a dose of 5 mg at 8-h intervals or placebo for 5 d. There were 54 children in the folic acid group and 52 in the placebo group. The admission characteristics were comparable between the two groups. No significant differences were observed in the intake of oral rehydration solution or stool output between the groups. The mean ± SD of total stool output (g kg^{-1}) was 532 ± 476 vs 479 ± 354 and the duration (h) of diarrhoea was 108 ± 68 vs 103 ± 53 in the folic acid vs placebo group, respectively. The findings, therefore, should have a positive influence on preventing the inappropriate use of folic acid in acute diarrhoea."

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"*Vibrio cholerae* 638 (El Tor, Ogawa), a new CTXF-negative hemagglutinin/protease-defective strain that is a cholera vaccine candidate, was examined for safety and immunogenicity in healthy adult volunteers. In a double-blind placebo-controlled study, no significant adverse reactions were observed in volunteers ingesting strain 638. Four volunteers of 42 who ingested strain 638 and 1 of 14 who received placebo experienced loose stools. The strain strongly colonized the human small bowel, as evidenced by its isolation from the stools of 37 of 42 volunteers. *V. cholerae* 638, at doses ranging from 4 x 10^7 to 2 x 10^9 vibrios, elicited significant serum vibriocidal antibody and anti-Ogawa immunoglobulin A antibody secreting cell responses."

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"The need for a rotavirus vaccine in any particular country depends primarily on the number of hospitalized cases. Since only limited data are available for Germany, we undertook a retrospective hospital-based analysis in order to gather further information. From 1987 through 1996, a total of 3618 inpatients were hospitalized with a diagnosis of gastroenteritis (ICD 9). In 892 (25%) of them the causative organism was a rotavirus. During the same period, 1886 (out of 8383; 22%) stool specimens tested in the hospital laboratory were obtained from rotavirus-positive inpatients. In 49.2% the infection was community-acquired, and in the remainder of nosocomial origin. Infants under 4 months of age (n=709; 38%) predominated among both the nosocomial and community-acquired infections. Premature neonates made up 26% of the nosocomial, but only 2% of the community-acquired cases of diarrhoea. The winter peak (January) was most pronounced in the age group 4-12 months, but in those more than 1 y old the peak came a month later. The median hospitalization time for community-acquired cases was 4 d (mean 5.9 d). The mortality was 0.1%. Rotavirus infection must therefore be regarded as a considerable burden, particularly with regard to infants and young children. Furthermore, the morbidity due to nosocomial infection with the rotavirus, analysed here in a long-term observational study, is unexpectedly high."

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018 Boerlin P, McEwen SA, Boerlin-Petzold F, Wilson JB, Johnson RP, Gyles CL. Associations between virulence factors of *Shiga* toxin-producing *Escherichia coli* and disease in humans. J Clin Microbiol 1999 Mar;37(3):497-503. 43 ref, Eng. Institut fur Veterinar-Bakteriologie der Universitat Bern, Langgassstrasse 122, CH-3012 Bern, Switzerland

"Associations between known or putative virulence factors of Shiga toxin-producing *Escherichia coli* and disease in humans were investigated. Univariate analysis and multivariate logistic regression analysis of a set of 237 isolates from 118 serotypes showed significant associations between the presence of genes for intimin *(eae)* and Shiga toxin 2 (str2) and isolates from serotypes reported in humans. Similar associations were found with isolates from serotypes reported in hemorrhagic colitis and hemolytic-uremic syndrome. The enterohemorrhagic *E. coli* (EHEC) hemolysin gene was significantly associated with isolates from serotypes found in severe diseases in univariate analysis but not in multivariate logistic regression models. A strong association between the intimin and EHEC-hemolysin genes may explain the lack of statistical significance of EHEC hemolysin in these multivariate models, but a true lack of biological significance of the hemolysin In humans or in disease cannot be excluded. This result warrants further investigations of this topic. Multivariate analysis revealed an interaction between the *eae and stx2* genes, thus supporting the hypothesis of the synergism between the adhesin intimin and Shiga toxin 2. A strong statistical association was observed between the str, gene and severity of disease for a set of 112 human isolates from eight major serotypes. A comparison of 77 isolates of bovine origin and 91 human isolates belonging to six major serotypes showed significant associations of the genes for Shiga toxin I and EspP protease with bovine isolates and an increased adherence on HEp-2 cell cultures for human isolates, particularly from diarrheic patients and healthy persons."

019 Boone JH, Wilkins TD, Nash TE, Brandon JE, Macias EA, Jerris RC, Lyerly DM. TechLab and Alexon *Giardia* enzyme-linked immunosorbent assay kits detect cyst wall protein 1. J Clin Microbiol 1999 Mar;37(3):611-4. 19 ref, Eng. TechLab, Inc., 1861 Pratt Dr., Corporate Research Center, Blacksburg, VA 24060-6364, USA

"A *Giardia lamblia* antigen detected by the TechLab *Giardia Test* (TechLab, Inc., Blacksburg, Va.) and the Alexon ProSpecT *Giardia* microplate assay (Alexon, Inc., Sunnyvale, Calif.) was purified by immunoaffinity chromatography from supernatant fluids of encystment cultures. Two major proteins (M_r 22,000 and 26,000) were observed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and Coomassie staining that did not resemble the GSA65 antigen reportedly detected by the Alexon test. These proteins reacted intensely with the monoclonal antibodies used in both commercial enzyme-linked immunosorbent assays (ELISAs). Both proteins had identical N-terminal amino acid sequences and were identified as cyst wall protein I (CWPI). The 26-kDa form appeared early during encystment followed by the appearance of the 22-kDa form. Recombinant CWPI (M_r 26,000) was strongly positive in both commercial tests. CWPL was stable in human stool specimens, resistant to degradation by proteases and N- and 0-glycanases, and unaffected by oxidation with sodium periodate. Two minor proteins with M_rs of 32,000 and 39,000 were detected in CWPL preparations by using a sensitive fluorescent protein stain. Both were identified as CWP2, and neither reacted with the monoclonal antibodies from the commercial tests. We analyzed 535 stool specimens for CWPI by using both commercial ELISAs and resolved discrepant results by using routine ova and parasite examination (O&P) and on immunofluorescence antibody assay. The presence of CWPI correlated well between both ELISAs (98.7% correlation). Our results demonstrate that both commercial ELISAs detect CWPI, which is a useful diagnostic marker because it is highly

stable, is secreted in large amounts by encysting trophozoites, and correlates well with O&P.

020 Bourke B, Sherman PM. Gastrointestinal infections in children. Curr Opin Gastroenterol 1999 Jan;15(1):79-84. 60 ref, Eng. Department of Paediatrics, University College Dublin, Children's Research Centre, Our Lady's Hospital for Sick Children, Dublin 12, Ireland

021 Byun R, Elbourne LDH, Lan R, Reeves PR. Evolutionary relationships of pathogenic clones of *Vibrio cholerae* by sequence analysis of four housekeeping genes. Infect Immun 1999 Mar;67(3):1116-24. 52 ref, Eng. Department of Microbiology (GO8), The University of Sydney, Sydney, New South Wales 2006, Australia

"Studies of the *Vibrio cholerae* population, using molecular typing techniques, have shown the existence of several pathogenic clones, mainly sixth-pandemic, seventh-pandemic, and U.S. Gulf Coast clones. However, the relationship of the pathogenic clones to environmental *V. cholerae* isolates remains unclear. A previous study to determine the phylogeny of *V. cholerae* by sequencing the *asd* (aspartate semialdehyde dehydrogenase) gene of *V. cholerae* showed that the sixth-pandemic, seventh-pandemic, and U.S. Gulf Coast clones had very different *asd* sequences which fell into separate lineages in the *V. cholerae* population. As gene trees drawn from a single gene may not reflect the true topology of the population, we sequenced the *mdh* (malate dehydrogenase) and *hlyA* (hemolysin A) genes from representative of environmental and clinical isolates of *V. cholerae* and found that the *mdh* and *hlyA* sequences from the three pathogenic clones were identical, except for the previously reported 11-bp deletion in *hlyA* in the sixth-pandemic clone. Identical sequences were obtained, despite average nucleotide differences in the *mdh* and *hlyA* genes of 1.52 and 3.25%, respectively, among all the isolates, suggesting that the three pathogenic clones are closely related. To extend these observations, segments of the *recA* and *dnaE* genes were sequenced from a selection of the pathogenic isolates, where the sequences were either identical or substantially different between the clones. The results show that the three pathogenic clones are very closely related and that there has been a high level of recombination in their evolution."

022 Callender JE, Walker SP*, Grantham-McGregor SM, Cooper ES. Growth and development four years after treatment for the *Trichuris* dysentery syndrome. Acta Paediatr 1998 Dec; 87(12):1247-9. 14 ref, Eng. *Tropical Metabolism Research Unit, University of the West Indies, Mona, Kingston 7, Jamaica

"A follow-up study is reported of 18 children 4 y after treatment for the *Trichuris* dysentery syndrome (TDS) and matched control children. The TDS children were initially severely stunted and had extremely low developmental levels. They showed catch-up in height of 1.9 *z*-scores even though they remained in very poor environments. Their intelligence quotients, school achievement and cognitive function remained significantly lower than those of the controls. Controlling for their earlier developmental levels, the TDS children showed a small improvement in mental development relative to the controls."

023 Cegielski JP, Ortega YR, McKee S, Madden JF, Gaido L, Schwartz DA, Manji K, Jorgensen AF, Miller SE, Pulipaka UP, Msengi AE, Mwakyusa DH, Sterling CR, Reller LB. *Cryptosporidium*, *Enterocytozoon*, and *Cyclospora* infections in pediatric and adult patients with diarrhea in Tanzania. Clin Infect Dis 1999 Feb;28(2):314-21. 69 ref, Eng. Centers for Disease Control and Prevention, Mailstop E-10, 1600 Clifton Road, Atlanta, Georgia 30333, USA

"Cryptosporidiosis, microsporidiosis, and cyclondsporiasis were studied in four groups of Tanzanian inpatients: adults with AIDS-associated diarrhoea, children with chronic diarrhea (of whom 23 of 59 were positive [$^+$] for human immunodeficiency virus [HIV]), children with acute diarrhea (of whom 15 of 55 were HIV$^+$), and HIV$^-$ control children without diarrhea. *Cryptosporidium was* identified in specimens from 6/86 adults, 5/59 children with chronic diarrhea (3/5, HIV$^+$), 7/55 children with acute diarrhea (0/7, HIV$^+$), and 0/20 control children. Among children with acute diarrhea, 7/7 with cryptosporidiosis were malnourished, compared with 10/48 without cryptosporidiosis (P<*.01). Enterocytozoon* was identified in specimens from 3/86 adults, 2/59 children with chronic diarrhea (1 HIV$^+$), 0/55 children with acute diarrhea, and 4/20 control children. All four controls were underweight (P<*.01). Cyclospora* was identified in specimens from one adult and one child with acute diarrhea (HIV$^-$). Thus, *Cryptosporidium* was the most frequent and *Cyclospora* the least frequent pathogen identified. *Cryptosporidium and Enterocytozoon* were associated with malnutrition. Asymptomatic fecal shedding of *Enterocytozoon* in otherwise healthy, HIV$^-$ children has not been described previously."

024 Chakrabarti S, Sengupta N, Chowdhury R. Role of DnaK in in vitro and in vivo expression of virulence factors of *Vibrio cholerae*. Infect Immun 1999 Mar;67(3): 1025-33. 46 ref, Eng. Biophysics Division, Indian Institute of Chemical Biology, 4 Raja S.C. Mullick Road, Calcutta 700032, India

"The *dnaK* gene of *Vibrio cholerae* was cloned, sequenced, and used to construct a *dnaK* insertion mutant which was then used to examine the role of DnaK in expression of the major virulence factors of this important human pathogen. The central regulator of several virulence genes of *V. cholerae* is ToxR, a transmembrane DNA binding protein. The *V. cholerae dnaK* mutant grown in standard laboratory medium exhibited phenotypes characteristic of cells deficient in ToxR activity. Using Northern blot analysis and *toxR* transcriptional fusions, we demonstrated a reduction in expression of the *toxR* gene in the *dnaK* mutant strain together with a concomitant increase in expression of a *htpG*-like heat shock gene that is located immediately upstream and is divergently transcribed from *toxR*. This may be due to increased heat shock induction in the *dnaK* mutant. In vivo, however, although expression from heat shock promoters in the *dnaK* mutant was similar to that observed in vitro, expression of

both *toxR* and *htpG* was comparable to that by the parental strain. In both strains, in vivo expression of *toxR* was significantly higher than that observed in vitro, but no reciprocal decrease in *htpG* expression was observed. These results suggest that the modulation of *toxR* expression in vivo may be different from that observed in vitro."

025 Chiang SL, Mekalanos JJ. *rfb* mutations in *Vibrio cholerae* do not affect surface production of toxin-coregulated pili but still inhibit intestinal colonization. Infect Immun 1999 Feb;67(2):976-80. 40 ref, Eng. Department of Microbiology and Molecular Genetics and Shipley Institute of Medicine, Harvard Medical School, 200 Longwood Ave., Boston, MA 02115, USA

"The toxin-coregulated pilus (TCP) of *Vibrio cholerae* is essential for colonization. It was recently reported that *rfb* mutations in *V. cholerae* 569B cause the translocation arrest of the structural subunit of TCP, raising the possibility that the colonization defects of lipopolysaccharide mutants are due to effects on TCP biogenesis. However, an *rfbB* gene disruption in either *V. choterae* O395 or 569B has no apparent effect on surface TCP production as assessed by immunoclectron microscopy and CTX phage transduction, and an *rfbD*::Tn*5ac* mutant of O395 also shows no defect in TCP expression. We conclude that the colonization defect associated with *rfb* mutations Is unrelated to defects in TCP assembly."

026 Clausen MR, Jørgensen J, Mortensen PB. Comparison of diarrhea induced by ingestion of fructooligosaccharide idolax and disaccharide lactulose: role of osmolarity versus fermentation of malabsorbed carbohydrate. Dig Dis Sci 1998 Dec;43(12):2696-707. 37 ref, Eng. Department of Medicine CA 2121, Division of Gastroenterology, Rigshospitalet Blegdamsvej 9, DK-2100 København Ø, Denmark

"Whether carbohydrate malabsorption causes diarrhea probably depends on the balance between the osmotic force of the carbohydrate and the compensatory capacity of the colon to dispose of the carbohydrate by bacterial fermentation. The present study evaluated the specific role of the osmolarity by comparing the severity of diarrhea after ingestion of two nonabsorable carbohydrates, the fructooligosaccharide idolax and the disaccharide lactulose. Both carbohydrates are readily fermented by the colonic flora but differ in osmolarity, the osmotic force being twice as high for lactulose as for idolax. Twelve subjects were given increasing doses (0, 20, 40, 80, 160 g/d) of idolax and lactulose in a crossover design. Every dose level was administered for three days with intervals of one week. Stools were collected on the third day to determine 24-hr volume, concentrations of short-chain fatty acids, L- and D-lactate, residues of idolax or lactulose, sodium, potassium, pH, osmolarity, and *in vitro* productions of organic acids. Measured by short-chain fatty acid and lactate formation in a fecal incubation system, the fermentation of idolax and lactulose was identical and very rapid compared with a range of reference carbohydrates. A laxative effect of both idolax and lactulose was demonstrated. The increment in fecal volume as a function of the dose administered was twice as high for lactulose (slope of the regression line = 7.3, r = 0.64, $P<10^{-5}$) as for idolax (slope = 3.7, r = 0.51, $P<10^{-3}$), ie, isosmolar doses of lactulose and idolax had the same effect on fecal volume. The variation in fecal volume was substantial (lactulose 80 g/day: 110-1360 g/day; idolax 160 g/day; 130-1440 g/day). High responders had earlier and larger fecal excretions of the saccharide compared with low-responders. Fecal volume in carbohydrate-induced diarrhea is proportional to the osmotic force of the malabsorbed saccharide, even though all or the majority of the saccharide is degraded by colonic bacteria. The capacity to modify the diarrhea varies considerably from person to person and is associated with colonic saccharide disposal, whereas the variation in response to isosmolar amounts of different saccharides is small within the same individual."

027 Cobelens FGJ, Leentvaar-Kuijpers A, Kleijnen J, Coutinho RA. Incidence and risk factors of diarrhoea in Dutch travellers: consequences for priorities in pre-travel health advice. Trop Med Int Health 1998 Nov;3(11):896-903. 25 ref, Eng. KVB A0-137, Academic Medical Center, PO Box 22700, 1000 DF Amsterdam, The Netherlands

"A cohort of 742 Dutch short-term travellers (1-6 weeks) to various (sub) tropical areas was studied to assess incidences of travellers' diarrhoea (TD) and risk factors to guide prevention policies. The occurence of TD was ascertained retrospectively by questionnaire; independent risk factors were identified by logistic regression analysis. The overall attack rate (AR, 95% CI) of TD was 52% (49-56); 11% (9-14) reported two or more episodes. The overall incidence rate (IR) per 100 person weeks of travel (pwt) (95% CI) was 22 (20-24). IRs were highest for travellers to the Middle East (48, 33-71), lowest for South-east Asia (17, 15-20) and East Africa (18, 14-24) and intermediate for South America and West Africa (both 26, 19-36), Central America (29, 23-37) and the Indian subcontinent (32, 26-39). Compared to first episodes of TD, subsequent episodes were of longer duration and more frequently accompanied by faecal blood loss, abdominal cramps or systemic symptoms. After adjustment for travel duration and destination, independent risk factors (OR, 95% CI) for TD were recent treatment for gastrointestinal (GI) disorders (4.6, 1.2-17.2), history of GI surgery (3.9, 1.4-11.1) and, possibly, current use of medication reducing gastric acidity (6.9, 0.7-67.4). The risk was reduced for extensive travel experience (0.4, 0.3-0.7) and organized travel (0.7, 0.5-0.9). Regarding prevention and/or antibiotic self-treatment of TD, priority should be give to travellers who may suffer major health or other consequences from TD and to those with pre-existing GI disorders, particularly when visiting a high or intermediate-risk area on individual journeys with limited travel experience."

028 Collington GK, Booth IW, Donnenberg MS, Kaper JB, Knutton S. Enteropathogenic *Escherichia coli* virulence genes encoding secreted signalling proteins are essential for modulation of Caco-2 cell electrolyte transport (note). Infect Immun 1998 Dec;66(12):6049-53. 34 ref, Eng. Institute of Child Health, University of Birmingham, Clinical Research Block, Whittall Street, Birmingham B4 6NH. UK

029 Cox MA, Lewis KO, Cooper BT*. Measurement of small intestinal permeability markers, lactulose, and mannitol in serum: results in celiac disease. Dig Dis Sci 1999 Feb;44(2):402-6. 21 ref, Eng. *Gastroenterology Unit, City Hospital, Dudley Road, Birmingham B18 7QH, UK

030 Cunliffe NA, Kilgore PE, Bresee JS, Steele AD, Luo N, Hart CA, Glass RI. Epidemiology of rotavirus diarrhoea in Africa: a review to assess the need for rotavirus immunization. Bull WHO 1998;76(5):525-37. 117 ref, Eng. Malaria Project and Wellcome Trust Centre, College of Medicine, Blantyre, Malawi

"Rapid progress towards the development of rotavirus vaccines has prompted a reassessment of the disease burden of rotavirus diarrhoea in developing countries and the possible impact of these vaccines in reducing diarrhoeal morbidity and mortality among infants and young children. We examined the epidemiology and disease burden of rotavirus diarrhoea among hospitalized and clinic patients in African countries through a review of 43 published studies of the etiology of diarrhoea. The studies were carried out from 1975 through 1992, and only those in which a sample of more than 100 patients with diarrhoea were specifically screened for rotavirus by using an established diagnostic test were included. Rotavirus was detected in a median of 24% of children hospitalized for diarrhoea and in 23% who were treated as outpatients; 38% of the hospitalized patients with rotavirus were <6 months and 81% were <1 year of age. Rotavirus was detected year-round in nearly every country and generally exhibited distinct seasonal peaks during the dry months. In 5 countries where rotavirus strains had been G-typed, 74% of strains were of one of the four common serotypes (G1 to G4), G1 was the predominant serotype, and 26% were non-typeable. This cumulative experience from 15 African countries suggests that rotavirus is the most important cause of severe diarrhoea in African children and that most strains in circulation today belong to common G types that are included in reassortant vaccines. Wherever large numbers of cases of rotavirus diarrhoea occur early in infancy, immunization at birth may protect the children before their first symptomatic infection."

031 Dalsgaard A, Forslund A, Tam NV, Vinh DX, Cam PD. Cholera in Vietnam: changes in genotypes and emergence of class I integrons containing aminoglycoside resistance gene cassettes in *Vibrio cholerae* O1 strains isolated from 1979 to 1996. J Clin Microbiol 1999 Mar;37(3):734-41. 45 ref, Eng. Department of Verterinary Microbiology, The Royal Veterinary and Agricultural University, Bulowsvej 13, 1870 Frederiksberg C, Denmark

"The number of cholera cases and the mortality rates reported from different regions of Vietnam varied considerably in the period from 1979 to 1996, with between 2,500 and 6,000 cases reported annually from 1992 to 1995. Annual mortality rates ranged from 2.0 to 9.6% from 1979 to 1983 to less than 1.8% after 1983. Major cholera outbreaks were reported from the High Plateau region for the first time in 1994 and 1995; this is an area with limited access to health services and safe drinking-water supplies. All cases were associated with *Vibrio cholerae* O1. Using ribotyping, cholera toxin (CT) genotyping, and characterization of antibiotic susceptibility patterns and antibiotic resistance genes by PCR, we show that strains isolated after 1990 were clearly different from strains isolated before 1991. In contrast to strains isolated before 1991, 94% of 104 strains isolated after 1990 showed an identical ribotype R1, were resistant to sulfamethoxazole and streptomycin, and showed a different CT genotype. Furthermore, PCR analysis revealed that sulfamethoxazole-resistant strains harbored class I integrons containing a gene cassette *ant(3")-1a* encoding resistance to streptomycin and spectinomycin. This is, to our knowledge, the first report of class I integrons in *V. cholerae*. The development of cholera and the changes in the phenotypic and genotypic properties of *V. cholerae* O1 shown in the present study highlight the importance of monitoring *V. cholerae* O1 in Vietnam as in other parts of the world. In particular, the emergence of the new ribotype R1 strain containing class I integrons should be further studied."

032 Das A, Variyam EP. Intestinal parasitic infections. Curr Opin Gastroenterol 1999 Jan;15(1):59-65. 68 ref, Eng. Gastroenterology Section, 111E(W), V.A. Medical Center, 10701 East Boulevard, Cleveland, OH 44106, USA

033 Desenclos JC, Rebiere I, Letrillard L, Flahault A, Hubert B. Diarrhoea-related morbidity and rotavirus infection in France. Acta Paediatr 1999 Jan;(Suppl 426):42-7. 23 ref, Eng. Réseau National de Santé Publique, 14 rue du Val d'Osne, 94415 Saint-Maurice, France

"To assess the importance of diarrhoea in France and, specifically, rotavirus-related diarrhoea among children, we reviewed data obtained from three complementary sources: (1) general practitioner (GP) sentinel surveillance; (2) hospital discharge data from paediatric hospitals; and (3) laboratory based surveillance. The GP sentinel network is based on 500 physicians who electronically notify new cases of eight illnesses, including diarrhoea, each week. It was estimated that about 3.3 million patients seek medical attention for diarrhoea from their GP each year, with a winter outbreak associated with an increased rate of isolation of rotavirus. A national system of hospital discharge diagnosis was used to estimate the burden of diarrhoeal morbidity in two paediatric wards in Tours, France. Between 1994 and 1996, 1164 patients under 15 y of age (9.7% of all admissions) were admitted for diarrhoea, of whom 83% were reported as having viral gastroenteritis; 14.3% were dehydrated and 52% were under 1 y old. Hospital admissions had a seasonal pattern similar to notifications from sentinel GP for children under 5 y old. A centralized laboratory surveillance network representing 17 of the 22 French regions describes a rotavirus outbreak each winter that is concomitant of outbreaks detected by the GP sentinel network and seen in hospitals. Most of the isolates (985) identified through this surveillance system are among children under 5 y of age. Al the data reviewed in this

study indicate that the epidemiology of rotavirus diarrhoea in France fits well with what has been reported in other developed countries."

034 Desselberger U. Viral gastroenteritis. Curr Opin Infect Dis 1998 Oct;11(5): 565-75. 115 ref, Eng. Clinical Microbiology and Public Health Laboratory, Addenbrooke's Hospital, Cambridge CB2 2QW, UK

035 Djuretic T, Ramsay M, Gay N, Wall P, Ryan M, Fleming D. An estimate of the proportion of diarrhoeal disease episodes seen by general practitioners attributable to rotavirus in children under 5 y of age in England and Wales. Acta Paediatr 1999 Jan;(Suppl 426):38-41. 20 ref, Eng. Public Health Laboratory Services, Communicable Disease Surveillance Centre, 61 Colindale Avenue, London NW9 5EQ, UK

"Mean weekly incidence rates for a 4-week period of new episodes of infectious intestinal disease (IID) and laboratory reports of faecal isolations in children under 5 y of age presenting in general practice were used to estimate the incidence of IID due to rotavirus infection in England and Wales. Between January 1992 and December 1996, a total of 92452 new episodes of IID were seen at sentinel general practices and reported to the Royal College of General Practitioners (RCGP) Research Unit in Birmingham, UK. Of these 32% (29592) were in children under 5 y of age. During the same period the Communicable Disease Surveillance Centre (CDSC) in London, UK received 159532 reports of faecal identifications in children under 5 y of age; 69219 (43%) of these were due to rotavirus. By modelling RCGP data and laboratory reports, the proportion of episodes attributable to rotavirus infection was estimated to be 29% (95% CI: 24% to 34%). By extrapolation of RCGP data it was estimated that rotavirus accounted for 762000 of new episodes of IID nationally in children under 5 y of age between January 1992 and December 1996. Implementation of a rotavirus vaccination programme could substantially reduce the incidence of childhood diarrhoea."

036 Dodson JM, Lenkowski PW, Jr., Eubanks AC, Jackson TFGH, Napodano J, Lyerly DM, Lockhart LA, Mann BJ, Petri WA, Jr. Infection and immunity mediated by the carbohydrate recognition domain of the *Entamoeba histolytica* Gal/GalNAc lectin. J Infect Dis 1999 Feb;179(2):460-6. 38 ref, Eng. Division of Infectious Diseases, Room 2115 MR4 Bldg., University of Virginia Health Sciences Center, Charlottesville, VA 22908, USA

037 Erku WA, Ashenafi M. Prevalence of food-borne pathogens and growth potential of *Salmonella* in weaning foods from Addis Ababa, Ethiopia. East Afr Med J 1998 Apr;75(4): 215-8. 24 ref, Eng. Department of Biology, Faculty of Science, Addis Ababa University, PO Box 1176, Addis Ababa, Ethiopia

038 Falbo V, Carattoli A, Tosini F, Pezzella C, Dionisi AM, Luzzi I. Antibiotic resistance conferred by a conjugative plasmid and a class I integron in *Vibrio cholerae* O1 El tor strains isolated in Albania and Italy (note). Antimicrob Agents Chemother 1999 Mar;43(3):693-6. 37 ref, Eng. Laboratory of Ultrastructures, Istituto Superiore di Sanita, Viale Regina Elena, 299, 00161 Rome, Italy

039 Fankhauser RL, Noel JS, Monroe SS, Ando T, Glass RI. Molecular epidemiology of "Norwalk-like viruses" in outbreaks of gastroenteritis in the United States. J Infect Dis 1998 Dec;178(6):1571-8. 43 ref, Eng. Viral Gastroenteritis Section, Mailstop G04, Centers for Disease Control and Prevention, 1600 Clifton Road, N.E., Atlanta, GA 30333, USA

"Fecal specimens from 90 outbreaks of nonbacterial gastroenteritis reported to 33 state health departments from January 1996 to June 1997 were examined to determine the importance of and to characterize "Norwalk-like viruses" (NLVs) in these outbreaks. NLVs were detected by reverse transcription-polymerase chain reaction in specimens from 86 (96%) of 90 outbreaks. Outbreaks were most frequent in nursing homes and hospitals (43%), followed by restaurants or events with catered meals (26%); consumption of contaminated food was the most commonly identified mode of transmission (37%). Nucleotide sequence analysis showed great diversity between strains but also provided evidence indicating the emergence of a common, predominant strain. The application of improved molecular techniques to detect NLVs demonstrates that most outbreaks of nonbacterial gastroenteritis in the United States appear to be associated with these viruses and that sequence analysis is a robust tool to help link or differentiate these outbreaks."

040 Faruque SM, Asadulghani, Saha MN, Alim ARMA, Albert MJ, Islam KMN, Mekalanos JJ. Analysis of clinical and environmental strains of nontoxigenic *Vibrio cholerae* for susceptibility to CTXF: molecular basis for origination of new strains with epidemic potential. Infect Immun 1998 Dec;66(12):5819-25. 37 ref, Eng. ICDDR,B: Centre for Health and Population Research, GPO Box 128, Dhaka 1000, Bangladesh

"Toxigenic *Vibrio cholerae* strains are lysogens of CTXF, a filamentous phage which encodes cholera toxin. The receptor for CTXF for invading *V. cholerae* cells is the toxin-coregulated pilus (TCP), the genes for which reside in a larger genetic element, the TCP pathogenicity island. We analyzed 146 CTX-negative strains of *V. cholerae* O1 or non-O1 isolated from patients or surface waters in five different countries for the presence of the TCP pathogenicity island, the regulatory gene *toxR*, and the CTXF attachment sequence *attRS*, as well as for susceptibility of the strains to CTXF, to investigate the molecular basis for the emergence of new clones of toxigenic *V. cholerae*. DNA probe or PCR assays for *tcpA, tcpI, acfB, toxR,* and *attRS* revealed that 6.85% of the strains, all of which belonged to the O1 serogroup, carried the TCP pathogenicity island, *toxR*, and multiple copies of *attRS*, whereas the remaining 93.15% of the strains were negative for TCP but positive for either one or both or neither of *toxR* and *attRS*. An analysis of the strains for susceptibility

to CTXF, using a genetically marked derivative of the phage CTX-Km F, showed that all TCP-positive CTX-negative strains and 1 of 136 TCP-negative strains were infected by the phage either in vitro or in the intestines of infant mice. The phage genome integrated into the chromosome of infected *V. cholerae* O1 cells forming stable lysogens. Comparative analysis of rRNA gene restriction patterns revealed that the lysogens derived from nontoxigenic progenitors were either closely related to or distinctly different from previously described clones of toxigenic *V. cholerae*. To our knowledge, this is the first demonstration of lysogenic conversion of naturally occurring nontoxigenic *V. cholerae* strains by CTXF. The results of this study further indicated that strains belonging to the O1 serogroup of *V. cholerae* are more likely to possess the TCP pathogenicity island and hence to be infected by CTXF, leading to the origination of potential new epidemic clones."

041 Faruque SM, Albert MJ, Mekalanos JJ. Epidemiology, genetics, and ecology of toxigenic *Vibrio cholerae*. Microbiol Mol Biol Rev 1998 Dec;62(4):1301-14. 150 ref, Eng. ICDDR,B: Centre for Health and Population Research, GPO Box 128, Dhaka 1000, Bangladesh

042 Foss DL, Murtaugh MP. Mucosal immunogenicity and adjuvanticity of cholera toxin in swine. Vaccine 1999 Feb 26;17(7-8):788-801. 28 ref, Eng. University of Minnesota, Department of Veterinary Pathobiology, 1971 Commonwealth Ave., St. Paul, MN 55108, USA

043 Fine KD, Ogunji F, Florio R, Porter J, Ana CS. Investigation and diagnosis of diarrhea caused by sodium phosphate. Dig Dis Sci 1998 Dec;43(12):2708-14. 25 ref, Eng. Baylor University Medical Center, GI Research, 2nd floor Hoblitzelle, 3500 Gaston Ave., Dallas, Texas 75246, USA

"Because there are no published reference values for fecal phosphate concentration or output, diagnosing surreptitious use of phosphate laxatives has been difficult. The purposes of this study were to determine normal fecal phosphate levels and to quantitate and chemically analyze diarrhea produced by sodium phosphate. Timed stool collections were obtained from 20 normal subjects during 25 study periods (normal controls), from 27 normal subjects with diarrhea induced by a variety of laxatives not containing phosphate during 234 study periods (diarrhea controls), and from 10 normal subjects during 14 periods after ingestion of 45 or 22.5 ml of a commercially available 66% sodium phosphate solution (Fleet Phospho-Soda). All stools were analyzed for soluble phosphate concentration, and daily output was calculated. The upper limits of normal for soluble fecal phosphate concentration and output, derived from the normal controls and diarrhea controls, respectively, were 33 mmol/liter and 15 mmol/day. Diarrhea produced by 45 ml of sodium phosphate was watery and voluminous, with fecal weights averaging 1078 g/day (range 601-1713 g/day). Measured fecal phosphate concentrations and outputs averaged 85 mmol/liter and 92 mmol/day, respectively, and all values were significantly elevated. Soft, less voluminous stools were produced with 22.5 ml of sodium phosphate but all had an abnormally high soluble phosphate concentration and 24-hr output. In conclusion, the upper limits of normal for soluble fecal phosphate concentration and output established in this study should be useful in the chemical diagnosis of phosphate-induced diarrhea."

044 Finlay BB, Abe A. Enteropathogenic *E. coli* interactions with host cells. Jpn J Med Sci Biol 1998;51(Suppl 1):S91-100. 17 ref, Eng. Department of Bacteriology, The Kitasato Institute, Minato-ku, Tokyo 108-8642, Japan

045 Fujii Y, Nomura T, Kanzawa H, Kameyama M, Yamanaka H, Akita M, Setsu K, Okamoto K. Purification and characterization of enterotoixin produced by *Aeromonas sobria*. Microbiol Immunol 1998;42(10):703-14. 33 ref, Eng. Department of Biochemistry, Faculty of Pharmaceutical Sciences, Tokushima Bunri University, Yamashiro, Tokushima, Tokushima 770-8514, Japan

046 Fullner KJ, Mekalanos JJ. Genetic characterization of a new type IV-A pilus gene cluster found in both classical and El Tor biotypes of *Vibrio cholerae*. Infect Immun 1999 Mar;67(3):1393-404. 59 ref, Eng. Department of Microbiology and Molecular genetics, Harvard Medical School, Building D1-427, 200 Longwood Ave., Boston, MA 02115, USA

"The *Vibrio cholerae* genome contains a 5.4-kb *pil* gene cluster that resembles the *Aeromonas hydrophila tap* gene cluster and other type IV-A pilus assembly operons. The region consists of five complete open reading frames designated *pilABCD* and *yacE*, based on the nomenclature of related genes from *Pseudomonas aeruginosa* and *Escherichia coli* K-12. This cluster is present in both classical and El Tor biotypes, and the *pilA* and *pilD* genes are 100% conserved. The *pilA* gene encodes a putative type IV pilus subunit. However, deletion of *pilA* had no effect on either colonization of infant mice or adherence to HEp-2 cells, demonstrating that *pilA* does not encode the primary subunit of a pilus essential for these processes. The *pilD* gene product is similar to other type IV prepilin peptidases, proteins that process type IV signal sequences. Mutational analysis of the *pilD* gene showed that *pilD* is essential for secretion of cholera toxin and hemagglutinin-protease, mannose-sensitive hemagglutination (MSHA), production of toxin-coregulated pili, and colonization of infant mice. Defects in these functions are likely due to the lack of processing of N termini of four Eps secretion proteins, four proteins of the MSHA cluster, and TcpB, all of which contain type IV-A leader sequences. Some *pilD* mutants also showed reduced adherence to HEp-2 cells, but this defect could not be complemented in *trans*, indicating that the defect may not be directly due to a loss of *pilD*. Taken together, these data demonstrate the effectiveness of the *V. cholerae* genome project for rapid identification and characterization of potential virulence factors."

047 Gafanovich I, Shir Y, Tsvang E, Ben-Chetrit E*. Chronic diarrhea—induced by celiac plexus block? J

Clin Gastroenterol 1998 Jun;26(4):300-2. 12 ref, Eng. *Department of Medicine, Hadassah University Hospital, Jerusalem, PO Box 12000, Israel

048 Germani Y, Minssart P, Vohito M, Yassibanda S, Glaziou P, Hocquet D, Berthelemy P, Morvan J. Etiologies of acute, persistent, and dysenteric diarrheas in adults in Bangui, Central African Republic, in relation to human immunodeficiency virus serostatus. Am J Trop Med Hyg 1998 Dec;59(6):1008-14. 41 ref, Eng. Institut Pasteur de Bangui, Laboratoire des Maladies Infectieuses Opportunistes, Boite Postale 923, Bangui, Central African Republic

"A study of the etiologies of diarrhea in adults in relation to their human immunodeficiency virus (HIV) serostatus and number of CD4+ cells was carried out in the Central African Republic. In cases and controls, multiparasitism was observed. *Salmonella* spp. were identified mainly during acute diarrhea, with 50% of the *S. enteritidis* isolated during the study being responsible for septicemia and/or urinary tract infection in immunodeficient patients. Enteroaggregative *Escherichia coli* (EAggEC) were the most frequently identified agent in HIV+ patients with persistent diarrhea; 42.8% of the patients with EAggEC as sole pathogens had bloody diarrhea, and these strains were negative for the presence of a virulence plasmid. Coccidia were found in those with acute and persistent diarrhea. Blood was observed in 53.3% of infections involving coccidia as the sole pathogen. *Microsporidium* spp. and *Blastocystis hominis* were found only in HIV+ patients with persistent diarrhea. *Shigella* spp., *Campylobacter* spp., and *Entamoeba histolytica* were found in HIV+ and HIV- dysenteric patients; bacteria resembling spirochetes that could not be cultivated were identified only in HIV+ cases with dysentery. Shiga-like toxin-producing *E. coli* O157:H-was isolated from two cases with hemolytic-uremic syndrome. Fungi were identified as the sole pathogen in 6.4% of the HIV+ patients with persistent diarrhea. Most of enteropathogenic bacteria identified were resistant to ampicillin and trimethoprim-sulfamethoxazole, remained susceptible to ampicillin plus clavulanic acid, and were susceptible to amikacin, gentamicin, and ciprofloxacin."

049 Gibreel A, Skold O. High-level resistance to trimethoprim in clinical isolates of *Campylobacter jejuni* by acquisition of foreign genes (*dfr1* and *dfr9*) expressing drug-insensitive dihydrofolate reductases. Antimicrob Agents Chemother 1998 Dec;42(12)): 3059-64. 45 ref, Eng. Division of Microbiology, Department of Pharmaceutical Biosciences, P.O. Box 581, Biomedical Center, Uppsala University, S-751 23 Uppsala, Sweden

050 Gibreel A, Sjogren E, Kaijser B, Wretlind B, Skold O. Rapid emergence of high-level resistance to quinolones in *Campylobacter jejuni* assoicated with mutational changes in *gyrA* and *parC* (note). Antimicrob Agents Chemother 1998 Dec;42(12):3276-8. 24 ref, Eng. Division of Microbiology, Department of Pharmaceutical Biosciences, P.O. Box 581, Biomedical Center, Uppsala University, S-751 23 Uppsala, Sweden

051 Glass RI, Bresee JS, Parashar UD, Holman RC, Gentsch JR. First rotavirus vaccine licensed: is there really a need? Acta Paediatr 1999 Jan;(Suppl 426):2-8. 47 ref, Eng. Viral Gastroenteritis Section, Mailstop G04, Centers for Disease Control and Prevention, 1600 Clifton Road, Atlanta GA 30333, USA

"The first rotavirus vaccine was licensed in the United States on 31 August 1998 for the prevention of severe rotavirus diarrhea in children. Despite this landmark in new vaccines, many pediatricians and public health professionals in Europe are uncertain of the need for this vaccine for the routine immunization of infants. In Europe, ample evidence suggests that rotavirus is the most common cause of hospitalizations for severe diarrhea among children, but proper studies documenting the disease burden of rotavirus or the cost-effectiveness of a rotavirus immunization program have only been conducted in the United Kingdom following epidemiologic models used in the United States. All children are infected with rotavirus during their first few years of life, 30-50% of diarrheal hospitalizations among children <5 years are due to this agent, and, by the age of 5 years, between 1 in 40 and 1 in 77 children in Europe and the United States may be hospitalized for rotavirus. The first vaccine is a live, oral preparation combining four different serotypes of rotavirus and administered in three doses with other childhood immunizations. The good efficacy against severe rotavirus diarrhea, the low risk of adverse side effects and the positive cost-effectiveness equation have led the two major immunization advisory groups in the U.S. to recommend this vaccine for routine use in American infants. European physicians and policy-makers should re-examine the epidemiology and disease burden of rotavirus diarrhea now that an effective method of prevention is at hand."

052 Hadidjaja P, Bonang E, Suyardi MA, Abidin SAN, Ismid IS, Margono SS. The effect of intervention methods on nutritional status and cognitive function of primary school children infected with *Ascaris lumbricoides*. Am J Trop Med Hyg 1998 Nov;59(5):791-5. 11 ref, Eng. Department of Parasitology, University of Indonesia, Jakarta, Indonesia

"The prevalence rate of ascariasis in primary school children in northern Jakarta, Indonesia varies from 60% to 90%. An association between helminthic infection and educational achievement has long been recognized. This study was carried out in the northern part of Jakarta among primary school children 6-8 years of age. Treatment of ascariasis and health education were used as the interventions. Before the interventions, basic data on socioeconomic status, epidemiology, infection with *Ascaris lumbricoides*, nutritional status, and cognitive function were collected. After the interventions, only data on infection with *A. lumbricoides*, nutritional status, and cognitive function were collected. The children were divided into five groups. Group I was given an anthelminthic (mebendazole), group II was provided with health education, group III was given an antihelminthic and provided with health education, group IV was given a placebo (controls), and group V consisted of egg-negative children, who also served as controls. Data from 336 students were

analyzed by analysis of covariance. Parasitologic examinations showed a mean prevalence rate of 58.4% for *A. lumbricoides* infection in the pre-intervention children and a mean prevalence rate of 40.6% in the post-intervention children. Concerning nutritional status, approximately 80% of the children showed good scores in the pre- and post-treatment data, and only a small percentage (0.9-16.2%) showed mild or moderate malnutrition. No significant difference was found between the pre- and post-treatment nutritional status. The results of the cognitive test showed that the group treated with mebendazole showed significant improvement in the Colored Progressive Matrices and Coding test. Children also showed an improvement in their learning ability, concentration, and eye-hand coordination after five months of receiving this intervention."

053 Horii T, Barua S, Kimura T, Kasugai S, Sato K, Shibayama K, Ichiyama S, Ohta M. Heterogeneity of phenotypic and genotypic traits including organic-acid resistance in *Escherichia coli* O157 isolates. Microbiol Immunol 1998;42(12):871-4. 29 ref, Eng. Department of Bacteriology, Nagoya University School of Medicine, 65 Tsurumai-cho, Showa-ku, Nagoya, Aichi 466-8550, Japan

054 Ingole KV, Jalgaonkar SV, Fule RP. Study of proteases and other enzymes of *Vibrio cholerae* O1 E2 Tor and O139 serotypes isolated in Yavatmal (Maharashtra). Indian J Pathol Microbiol 1998 Oct;41(4):419-22. 11 ref, Eng. Department of Microbiology, V.N. Govt. Medical College, Yavatmal 445001, Maharashtra, India

"*V. cholerae* O1 El Tor isolated during cholera epidemic of 1994 and *V. cholerae* O139 serotype isolated during 1993 epidemic were subjected to the study of proteases and other enzymes. Out of 26 strains of *V. cholerae* O1 studied, gelatinase and caseinase activity was seen in 100 and 69.23 percent strains respectively. All strains showed catalase and oxidase activity. Of the other enzymes studied 19.23, 65.38 and 57.69 percent strains were positive for DNAse, lipase and phosphatase respectively. None of the strains showed lecithinase activity. Similar findings were observed in 22 strains of *V. cholerae* O139 except all strains were positive for phosphatase activity. Role of enzymes in virulence is suggested."

055 Isenbarger DW, Bodhidatta L, Hoge CW, Nirdnoy W, Pitarangsi C, Umpawasiri U, Echeverria P. Prospective study of the incidence of diarrheal disease and *Helicobacter pylori* infection among children in an orphanage in Thailand. Am J Trop Med Hyg 1998 Nov; 59(5):796-800. 14 ref, Eng. Department of Bacteriology, Armed Forces Research Institute of Medical Sciences, 315/6 Rajavithi Road, Bangkok 10400, Thailand

"To evaluate the hypothesis that gastric infection with *Helicobacter pylori* increases risk for diarrheal disease in children, we conducted a yearlong prospective study among 160 orphanage children <5 years of age in Nonthaburi, Thailand. Serum samples collected at six-month intervals were examined by ELISA for antibodies to *H. pylori*, and children were followed daily for the development of diarrhea. Seven percent of children were seropositive on enrollment, 59% were seronegative, and 34% were indeterminate. Among the seronegative children, seroconversion occurred at a rate of 7% per six months. Forty-six percent of children developed 214 total episodes of diarrhea. By age group, children <18 months, 18-24 months and >24 months of age experienced 2.6, 1.1, and 0.2 mean diarrhea episodes per six months. The incidence of diarrhea was not significantly different between children by *H. pylori* serostatus. We conclude that *H. pylori* infection was not associated with an increased risk of diarrheal disease."

056 Izumikawa K, Hirakata Y, Yamaguchi T, Yoshida R, Nakano M, Matsuda J, Mochida C, Maesaki S, Tomono K, Yamada Y, Tashiro T, Kohno S, Kamihira S. Analysis of genetic relationships and antimicrobial susceptibility of verotoxin-producing *Escherichia coli* strains isolated in Nagasaki Prefecture, Japan in 1996. Microbiol Immunol 1998;42(10): 677-81. 12 ref, Eng. Department of Laboratory Medicine, Nagasaki University School of Medicine, 1-7-1 Sakamoto, Nagasaki, Nagasaki 852-8501, Japan

057 Jeppesen PB, Staun M, Tjellesen L, Mortensen PB. Effect of intravenous ranitidine and omeprazole on intestinal absorption of water, sodium, and macronutrients in patients with intestinal resection. Gut 1998 Dec;43(6):763-9. 22 ref, Eng. Department of Medicine CA, Section of Gastroenterology, 2121, Rigshospitalet, Blegdamsvej 9, DK-2100 Copenhagen, Denmark

058 Jones MA, Wood MW, Mullan PB, Watson PR, Wallis TS, Galyov EE. Secreted effector proteins of *Salmonella dublin* act in concert to induce enteritis. Infect Immun 1998 Dec;66(12):5799-5804. 29 ref, Eng. Institute for Animal Health, Compton, Berkshire RG 20 7NN, UK

059 Jordan SL, Glover J, Malcolm L, Thomson-Carter FM, Booth IR, Park SF. Augmentation of killing of *Escherichia coli* O157 by combinations of lactate, ethanol, and low-pH conditions. Appl Environ Microbiol 1999 May;65(3):1308-11. 27 ref, Eng. Institute of Food Research, Reading Laboratory, Earley Gate, Reading RG6 6BZ, UK

060 Kader HA, Piccoli DA, Jawad AF, Mcgowan KL, Maller ES. Single toxin detection is inadequate to diagnose *Clostridium difficile* diarrhea in pediatric patients. Gastroenterology 1998 Dec;115(6):1329-34. 42 ref, Eng. Divisions of Gastroenterology and Nutrition, Children's Hospital of Philadelphia, 34th Street and Civic Center Boulevard, Philadelphia, Pennsylvania 19104, USA

"***Background and Aims***: *Clostridium difficile* is an important cause of symptomatic diarrhea in pediatric patients. The bacterium produces two toxins, although many laboratories

assay for only one. We questioned this diagnostic approach when patients had positive results for *C. difficile* at our institution, but initially had tested negative at outside laboratories. ***Methods:*** We retrospectively analyzed relative frequencies of *C. difficile* toxin A alone, toxin B alone, and toxins A and B from pediatric patients with diarrhea. Results were stratified according to toxin detection and patient age. ***Results:*** Of 1061 specimens, 276 (26.8%) were positive for *C. difficile* toxin(s). Fifty-one (18.5%) were positive for toxin A alone, 133 (48.2%) for toxin B alone, and 92 (33.3%) for both toxins. Assaying for toxin B identified *C. difficile* infection more frequently than did assaying for toxin A ($P < 0.0001$). The frequency of toxin B detection was significantly higher for older children but not for infants. ***Conclusions:*** Testing for *C. difficile* toxin A or toxin B alone will result in more frequent misdiagnosis than testing for both toxins. This practice may lead to inappropriate further invasive investigations in children, although this finding may not be applicable to adults."

061 Kang G, Shanthakumari S, Ramakrishna BS. *Cryptosporidium* carriage in asymptomatic rural south Indians. Indian J Med Microbiol 1998 Jul;16(3):112-4. 6 ref, Eng. Department of Gastrointestinal Sciences, Christian Medical College and Hospital, Vellore 632004, India

062 Karmali MA, Petric M, Bielaszewska M. Evaluation of a microplate latex agglutination method (verotox F assay) for detecting and characterizing verotoxins (Shiga toxins) in *Escherichia coli*. J Clin Microbiol 1999 Feb;37(2):396-9. 31 ref, Eng. Institute of Medical Microbiology, The 2nd Medical Faculty, Charles University, Vuvalu 84, 150 06 Prague 5-Motol, Czech Republic

063 Karmali MA. The nature of immunity to the *Escherichia coli* Shiga toxins verocytotoxins and options for toxoid immunization. Jpn J Med Sci Biol 1998;51(Suppl 1):S26-35. 50 ref, Eng. Department of Laboratory Medicine and Pathobiology, University of Toronto, Toronto, Ontario M2N3S9, Canada

064 Kato N, Liu C, Kato H, Watanabe K, Nakamura H, Iwai N, Ueno K. Prevalence of enterotoxigenic *Bacteroides fragilis* in children with diarrhea in Japan (note). J Clin Microbiol 1999 Mar;37(3):801-3. 14 ref, Eng. Institute of Anaerobic Bacteriology, Gifu University School of Medicine, 40 Tsukasa-machi, Gifu 500-8705, Japan

065 Kellogg JA, Elder CJ. Justification for use of a single trichrome stain as the sole means for routine detection of intestinal parasites in concentrated stool specimens (note). J Clin Microbiol 1999 Mar;37(3):835-7. 15 ref, Eng. Clinical Microbiology Laboratory, York Hospital, 1001 S. George St., York, PA 17405, USA

066 Kelly P, Farthing MJG. Bacterial infections of the gut (excluding enteric fever). Curr Opin Infect Dis 1998 Oct;11(5):577-82. 55 ref, Eng. Digestive Diseases Research Centre, St Bartholomew's and the Royal London School of Medicine and Dentistry, Turner Street, London E1 2AD, UK

067 Kelly P, Davies SE, Mandanda B, Veitch A, McPhail G, Zulu I, Drobniewski F, Fuchs D, Summerbell C, Luo NP, Pobee JOM, Farthing MJG. Enteropathy in Zambians with HIV related diarrhoea: regression modelling of potential determinants of mucosal damage. Gut 1997 Dec;41(6):811-6. 38 ref, Eng. Digestive Diseases Research Centre, St Bartholomew's and the Royal London School of Medicine and Dentistry, 2 Newark Street, London E1 2AT, UK

"***Background:*** AIDS is characterised by small intestinal mucosal damage, but its aetiopathogenesis is poorly understood. Enteric infections in Africa differ from those in northern countries, where protozoan infections have been associated with severe enteropathy in AIDS patients. ***Aims:*** To characterise enteropathy in Zambian AIDS patients compared with local controls, and to assess relative contributions of enteric infection, nutritional impairment, and immune dysfunction. ***Methods:*** Computer aided mucosal morphometry of small intestinal biopsy specimens from 56 HIV infected Zambians with persistent diarrhoea and 26 diarrhoea free controls, followed by regression modelling. ***Results:*** Patients with HIV related diarrhoea had reduced villous height and increased crypt depth compared with controls. There was no difference between HIV positive and negative controls. In regression models applied to AIDS mucosal measurements, villous height and crypt depth were related to nutritional parameters and to serum soluble tumour necrosis factor receptor p55 concentration. Crypt depth was also related to lamina propria plasma cell count. Intestinal infection was found in 79%, which consisted predominantly of microsporidia in 34%, *Isospora belli* in 24%, and *Cryptosporidium parvum*n in 21%, but detection of these enteropathogens was not related to severity of enteropathy. *Conclusions*¾Nutritional and immune disturbances were associated with enteropathy, accounting for over one third of the variation in mucosal morphometric parameters."

068 Keusch GT. The rediscovery of Shiga toxin and its role in clinical disease. Jpn J Med Sci Biol 1998;51(Suppl 1):S5-22. 66 ref, Eng. Division of Geographic Medicine and Infectious Diseases, Tupper Research Institute, New England Medical Center, Boston, Massachusetts 02111, USA

069 Khetawat G, Bhadra RK, Nandi S, Das J. Resurgent *Vibrio cholerae* O139: rearrangement of cholera toxin genetic elements and amplification of *rrn* operon. Infect Immun 1999 Jan;67(1):148-54. 42 ref, Eng. Biophysics Division, Indian Institute of Chemical Biology, 4 Raja S.C. Mullick Rd., Calcutta 700032, India

"The unprecedented genesis of a novel non-O1 *Vibrio cholerae* strain belonging to serogroup O139, which caused an epidemic in late 1992 in the Indian subcontinent, and its subsequent by El Tor O1 vibrios after 18 months initiated a

renewed investigation of the aspects of the organism that are related to pathogenesis. The reappearence of *V. cholerae* O139 with altered antibiotic sensitivity compared to O139 Bengal (O139B) in late 1996 has complicated the epidemiological scenario of *V. cholerae* and has necessitated an examination of possible rearrangements in the genome underlying such rapid changes in the phenotypic traits. With a view to investigating whether the phenotypic changes that have occurred are associated with alteration in the genome, the genome of the resurgent *V. cholerae* O139 (O139R) strains were examined. Pulsed-field gel electrophoresis analysis of *Not*I- and *Sfi*I-digested genomic DNA of O139R isolates showed restriction fragment length polymorphism including in the cholera toxin (CTX) genetic element locus and with O139B isolates. Analyses of the organization of the CTX genetic elements in O139R strains showed that in contrast to two copies of the elements connected by two direct-repeat sequences (RS) in most of the genomes of O139B isolates, the genomes of all O139R strains examined, except strain AS192, have three such elements connected by a single RS. While the RS present in the upstream of the CTX genetic elements in the genome of O139R is of O139B origin, the RS connecting the cores of the elements has several new restriction sites and has lost the *BglH* site which is supposed to be conserved in all O1 strains and O139B. The endonuclease I-*Ceu*I, which has sites only in the *rrn* operons in the genomes of all organisms examined so far, has 10 sites in the genomes of O139R strains, compared to 9 in the genomes of O139B strains. The recent isolates of *V. cholerae* O139 have thus gained one *rrn* operon. This variation in the number of *rrn* operons within a serogroup has not been reported for any other organism. The results presented in this report suggest that like the pathogenic El Tor O1 strains, the genomes of O139 strains are undergoing rapid alterations."

070 King AJ, Sundaram S, Cendoroglo M, Acheson DWK, Keusch GT. Shiga toxin induces superoxide production in polymorphonuclear cells with subsequent impairment of phagocytosis and responsiveness to phorbol esters. J Infect Dis 1999 Feb;179(2):503-7. 18 ref, Eng. Box 390, New England Medical Center, 750 Washington St., Boston, MA 02111, USA

071 Kobari K. Clinical aspects of shigellosis in reference of sigmoidoscopic findings and histopathological changes of the colon. Jpn J Med Sci Biol 1998;51(Suppl 1):S23-5. Eng. Ryukyu University, Uehara 207, Nishihara-machi, Nakagami-gun, Okinawa, 903-0125, Japan

072 Konkel ME, Gray SA, Kim BJ, Garvis SG, Yoon J. Identification of the enteropathogens *Campylobacter jejuni* and *Campylobacter coli* based on the *cadF* virulence gene and its product. J Clin Microbiol 1999 Mar;37(3):510-7. 26 ref, Eng. Department of Microbiology, Washington State University, Pullman, WA 99164-4233, USA

"*Campylobacter jejuni* and *Campylobacter coli* are common cases of gastroenteritis in humans. Infection with *C. jejuni* or *C. coli* is commonly acquired by eating undercooked chicken. The goal of this study was to develop specific detection assays for *C. jejuni* and *C. coli* isolates based on the *cadF* virulence gene and its product. The *cadF* gene from *C. jejuni* and *C. coli* encodes a 37-kDa outer membrane protein that promotes the binding of these pathogens to intestinal epithelial cells. A fragment of approximately 400 bp was amplified from 38 of 40 (95%) *C. jejuni* isolates and 5 of 6 (83.3%) *C. coli* isolates with primers designed to amplify an internal fragment of the *cadF* gene. PCR was then used to amplify *Campylobacter* DNA from store-bought chickens. A 400-bp band was amplified from 26 of the 27 chicken carcasses tested by the PCR-based assay. The CadF protein was detected in every *C. jejuni* and *C. coli* isolate tested, as judged by immunoblot analysis with a rabbit anti-*C. jejuni* 37-kDa serum. In addition, methanol-fixed samples of whole-cell *C. jejuni* and *C. coli* were detected with the rabbit anti-37-kDa serum by using an indirect-immunofluorescence microscopy assay. These findings indicate that the *cadF* gene and its product are conserved among *C. jejuni* and *C. coli* isolates and that a PCR assay based on the *cadF* gene may be useful for the detection of *Campylobacter* organisms in food products."

073 Lahiri KK, Ayyagari A. *Vibrio cholerae* O139 in Lucknow (letter). Indian J Med Microbiol 1998 Jul;16(3):133. 3 ref, Eng. Department of Microbiology, Sanjay Gandhi Postgraduate Institute of Medical Sciences, Raebareli Road, Lucknow 226014, India

074 Lindo JF, Levy VA, Baum MK, Palmer CJ. Epidemiology of giardiasis and cryptosporidiosis in Jamaica. Am J Trop Med Hyg 1998 Nov;59(5):717-21. 37 ref, Eng. Department of Microbiology, The University of the West Indies, Kingston 7, Jamaica

"We report the findings of a cross-sectional epidemiologic study of *Giardia lamblia* and *Cryptosporidum* infections in Jamaica. Three hundred twenty eight stool samples from patients less than one to 81 years of age were examined using formalin-ether concentration for *G. lamblia*. Zeihl-Neelsen staining for *Cryptosporidium*, and the Prospect® rapid enzyme immunoassay (EIA; Alexon, Sunnyvale, CA) for parasite diagnosis. The Prospect® *Giardia* rapid assay detected 17 cases of *G. lamblia* infection compared with six by formalin-ether concentration. However, the Prospect® *Cryptosporidum* EIA did not increase the rate of detection of *Cryptosporidum* when compared with Zeihl-Neelsen staining. *Cryptosporidum* infections were most frequently diagnosed in children less than five years old and prevalence decreased with age. In contrast, the prevalence of giardiasis increased as children became older. There were no associations between the infections and stool consistency, clinical manifestations, or sex of the individuals. The contribution of the parasites to childhood morbidity will depend on accurate laboratory diagnosis."

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Department of Microbiology, 109 Lewis Hall, Montana State University, Bozeman, MT 59717, USA

076 Ljungh Å. Bacterial infections of the small intestine and colon. Curr Opin Gastroenterol 1999 Jan;15(1):43-52. 72 ref, Eng. Department of Infectious Diseases and Medical Microbiology, Lund University, SE-223 62, Lund, Sweden

077 Ludwig SL, Brundage JF, Kelley PW, Nang R, Towle C, Schnurr DP, Crawford-Miksza L, Gaydos JC. Prevalence of antibodies to adenovirus serotypes 4 and 7 among unimmunized US army trainees: results of a retrospective nationwide seroprevalence survey. J Infect Dis 1998 Dec;178(6):1776-8. 15 ref, Eng. AMSA-US Army Center for Health Promotion and Preventive Medicine, Building T-20, Room 213, Walter Reed Army Medical Center, Washington, DC 20307-5100, USA

078 Lumadue JA, Manabe YC, Moore RD, Belitsos PC, Sears CL, Clark DP*. A clinicopathologic analysis of AIDS-related cryptosporidiosis. AIDS 1998;12(18):2459-66. 42 ref, Eng. *Department of Pathology, The Johns Hopkins School of Medicine, Room 406, Pathology Building, 600 N. Wolfe Street, Baltimore, MD 21287, USA

079 Magambo JK, Zeyhle E, Wachira TM. Prevalence of intestinal parasites among children in southern Sudan. East Afr Med J 1998 May;75(5):288-90. 8 ref, Eng. Department of Zoology, Jomo Kenyatta University of Agriculture and Technology, PO Box 62000, Nairobi

080 Maguire AJ, Green J, Brown DWG, Desselberger U, Gray JJ. Molecular epidemiology of outbreaks of gastroenteritis associated with small round-structured viruses in East Anglia, United Kingdom, during the 1996-1997 season. J Clin Microbiol 1999 Jan; 37(1):81-9. 44 ref, Eng. Clinical Microbiology and Public Health Laboratory, Level 6, Addenbrooke's Hospital, Hills Road, Cambridge, CB2 2QW. UK

"During the winter season from November 1996 to May 1997, 550 fecal specimens were submitted from 94 outbreaks of gastroenteritis occurring in East Anglia, United Kingdom. These specimens were tested for the presence of small round-structured viruses (SRSVs) by electron microscopy, reverse transcriptase PCR, or both methods. SRSVs were shown to be associated with 64 of 94 (68%) of these outbreaks, of which 16 (25%) outbreaks occurred at a single location (Southend) within the region. Twenty-four specimens from 13 of the 16 SRSV-positive outbreaks occurring in southend were available for genomic analysis, in which divergence within the RNA polymerase region of the SRSV genome was investigated. A further 27 specimens from 17 other SRSV-associated outbreaks, occurring at different locations within East Anglia but at the same time as those at southend, were also studied. Fifty of the total of 51 (98%) specimens studied were shown to belong to genogroup II, and within this genogroup, 49 of 50 (98%) specimens were shown to be Grimsby-like viruses, with only one Mexico-like strain. Furthermore, phylogenetic analysis of the Grimsby-like viruses indicated clusterings according to the geographical location of the outbreak. One specimen contained a virus belonging to genogroup I, and this had the greatest sequence identity (83%) with Southampton virus."

081 Mahon BE, Slutsker L*, Hutwagner L, Drenzek C, Maloney K, Toomey K, Griffin PM. Consequences in Georgia of a nationwide outbreak of *Salmonella* infections: what you don't know might hurt you. Am J Public Health 1999 Jan;89(1):31-5. 18 ref, Eng. Foodborne and Diarrheal Diseases Branch, Mailstop A-38, Centers for Disease Control and Prevention, 1600 Clifton Road, Atlanta, GA 30333, USA

082 Marsh JW, Taylor RK. Genetic and transcriptional analyses of the *Vibrio cholerae* mannose-sensitive hemagglutinin type 4 pilus gene locus. J Bacteriol 1999 Feb;181(4):1110-7. 59 ref, Eng. Department of Microbiology, Dartmouth Medical School, Hanover, NH 03755, USA

083 Martinez MB, Taddei CR, Ruiz-Tagle A, Trabulsi LR, Girón JA. Antibody response of children with enteropathogenic *Escherichia coli* infection to the bundle-forming pilus and locus of enterocyte effacement-encoded virulence determinants. J Infect Dis 1999 Jan; 179(1):269-74. 15 ref, Eng. Faculdade de Ciencias Farmaceuticas, Depto. de Analises Clinicas e Toxicologia, Universidade de São Paulo, Av. Lineu Prestes, 580, bl. 17, Cidade Universitaria, Butanta, São Paulo, Brazil

"Enteropathogenic *Escherichia coli* (EPEC) express a plasmid-encoded type IV pilus termed bundle-forming pilus, which is associated with the formation of bacterial microcolonles on cultured epithelial cells. Bacterial attachment and effacement of the enterocyte brush border membrane is attributed to a surface outer membrane protein adhesin termed intimin and EPEC-secreted proteins EspA, EspB, and EspD. Except for intimin, production in vivo or antibody response against these virulence determinants during natural EPEC infections in young children has not been demonstrated. Antibody responses against BfpA, intimin, EspA, an d EspB were investigated in Brazilian children naturally infected with EPEC. Generally, IgG antibodies against BfpA and EspB were the most commonly found, followed by antiEspA and intimin antibodies. Thus, bundle-forming pilus and locus of enterocyte attachment-encoded products are produced in vivo during natural EPEC infections and elicit an immune response against heterologous EPEC virulence determinants. These findings have important Implications in the immunoprophylaxis against EPEC infections."

084 Meng G, Smith PD. Gastrointestinal infections in the immunocompromised host. Curr Opin Gastroenterol 1999 Jan;15(1):85-9. 46 ref, Eng. Division of Gastroenterology and Hepatology, Department

of Medicine, University of Alabama at Birmingham School of Medicine and the VA Medical Center, University of Alabama Station, 633 Zeigler Research Building, 703 South 19th Street, Birmingham, AL 35294, USA

085 Merchant RH, Shroff RC. HIV seroprevalence in disseminated tuberculosis and chronic diarrhea. Indian Pediatr 1998 Sep;35(9):883-7. 14 ref, Eng. Division of Neonatology, Bai Jerbai Wadia, Hospital for Children, Acharya Donde Marg, Parel, Mumbai 400012, India

086 Mizunoe Y, Wai SN, Takade A, Yoshida S-I. Isolation and characterization of rugose form of *Vibrio cholerae* O139 strain MO10 (note). Infect Immun 1999 Feb; 67(2):958-63. 43 ref, Eng. Department of Bacteriology, Faculty of Medicine, Kyushu University, Fukuoka 812-8582, Japan

087 Moulton LH, Staat MA, Santosham M, Ward RL. The protective effectiveness of natural rotavirus infection in an American Indian population. J Infect Dis 1998 Dec;178(6): 1562-6. 34 ref, Eng. Department of International Health, 615 N. Wolfe St., Baltimore, MD 21205, USA

"The degree of protection conferred by natural rotavirus infection was estimated through analyses of data gathered as part of a 2-year rotavirus vaccine study of 1185 Native American infants. In 292 placebo recipients with complete serum sample sets, rotavirus IgA antibody indicative of infection before 2 months of age was associated with a 58% decrease in symptomatic infections throughout the trial. In all 391 placebo recipients, the preventive effectiveness of an initial symptomatic infection was 72% overall and 94% within 6 months following the infection. In contrast to studies conducted at other sites in the United States, serotype G3 was the predominant serotype associated with gastrointestinal episodes (80%). The effectiveness of an initial serotype G3 episode with respect to preventing subsequent serotype G3 episodes was 91%."

088 Mrukowicz JZ, Krobicka B, Duplaga M, Kowalska-Duplaga K, Domanski J, Szajewska H, Kantecki M, Iwanczak F, Pytrus T. Epidemiology and impact of rotavirus diarrhoea in Poland. Acta Paediatr 1999 Jan;(Suppl 426):53-60. 30 ref, Eng. II Department of Paediatrics, Polish-American Children's Hospital, Jagiellonian University School of Medicine, ul. Wielicka 265, 30-663 Krakow, Poland

"Hospital and laboratory data were analysed in three hospitals to estimate rotavirus disease burden in 1994-96. Community acquired gastroenteritis was diagnosed in 757 children of whom 41% tested positive for rotavirus. A total of 196 children had rotavirus nosocomial infections (39% of all rotavirus community-acquired and nosocomial cases). Infants less than 24 months old and children less than 3 months old comprised 74% and 11.9% of admissions for rotavirus, respectively. Almost 94% of children with rotavirus infection had severe gastroenteritis (score ^{3}11). The annual rate of rotavirus associated hospitalization in Poland in 1996 was 3.1/1000 children under the age of 60 months and 5.2/1000 infants under 24 months of age. The mean hospital stay was 9.5 d (±9.8 d). We estimated that 8918 children under 60 months of age were hospitalized for rotavirus gastroenteritis in 1996; they accounted for 84899 inpatient days. We conclude that rotavirus is a leading aetiological agent of severe gastroenteritis in young children in Poland and that the burden of this infection is significant. Rotavirus vaccine could significantly decrease the hospitalization rate and the financial impact of rotavirus gastroenteritis in Poland."

089 Muennig P, Pallin D, Sell RL, Chan M-S. The cost effectiveness of strategies for the treatment of intestinal parasites in immigrants. N Engl J Med 1999 Mar 11;340(10):773-9. 52 ref, Eng. Refugee Health Program, New York City Department of Health, 125 Worth St., Box 21-A, New York, NY 10013, USA

"***Background:*** Currently, more than 600,000 immigrants enter the United States each year from countries where intestinal parasites are endemic. At entry persons with parasitic infections may by asymptomatic, and stool examinations are not a sensitive method of screening for parasitosis. Albendazole is a new, broad-spectrum antiparasitic drug, which was approved recently by the Food and Drug Administration. International trials have shown albendazole to be safe and effective in eradicating many Parasites. In the United States there is now disagreement about whether to screen all immigrants for parasites, treat all immigrants presumptively, or do nothing unless they have symptoms. ***Methods:*** We compared the costs and benefits of no preventive intervention (watchful waiting) with those of universal screening or presumptive treatment with 400 mg of albendazole per day for five days. Those at risk were defined as immigrants to the United States from Asia, the Middle East, sub-Saharan Africa, Eastern Europe, and Latin America and the Caribbean. Cost effectiveness was expressed both in terms of the cost of treatment per disability-adjusted life-year (DALY) averted (one DALY is defined as the loss of one year of healthy life to disease) and in terms of the cost per hospitalization averted. ***Result:*** As compared, with watchful waiting, presumptive treatment of all immigrants at risk for parsitosis would avert at least 870 DALYs, prevent at least 33 deaths and 374 hospitalization and save at least $4.2 million per year. As compared with watchful waiting, screening would cost $159,236 per DALY averted. ***Conclusions:*** Presumptive administration of albendazole to all immigrants at risk for parasitosis would save lives and money. Universal screening, with treatment of persons with positive stool examinations, would save lives but is less cost effective than presumtive treatment."

090 Murphy GS, Echeverria P. Treatment of gastrointestinal infections. Curr Opin Gastroenterol 1999 Jan;15(1):90-4. 17 ref, Eng. Viral and Rickettsial Disease Program, Naval Medical Research Institute, Code 41, Building 17,8901 Wisconsin Avenue, Bethesda, MD 20889, USA

091 Ndubani P, Kelly P*, Farthing MJG, Wallman S. Local understandings of adult diarrhoeal disease

and its treatment in an area of high HIV-seroprevalence in Zambia. Trop Med Int Health 1998 Oct;3(10):783-7. 14 ref, Eng. *Digestive Diseases Research Centre, St Bartholomew's and Royal London School of Medicine and Dentistry, Turner Street, London E1 2AD, UK

"We set out to investigate the extent to which cultural constructs might determine treatment-seeking for diarrhoea in the poorer populations of Lusaka, Zambia. This paper describes these concepts and perception and outlines a classification of such illnesses, together with an analysis of its implications for understanding treatment choice. Data were derived from focus group discussions, a household survey, a survey of practitioners of traditional medicine and interviews with local residents attending an urban health centre with persistent diarrhoea. The classification is based on symptoms and perceptions of aetiology. While resulting categories convey imperatives for treatment choice, it is clear that individuals with diarrhoeal illnesses seek treatment from multiple sources. This may be because any single illness may fit more than one category, or because unrelenting ill-health engenders desperate behaviour. The cultural constructs do not fully explain treatment choice and attitudes to prevention, but could be used to improve communication regarding public health and treatment strategies."

092 Noriega FR, Liao FM, Maneval DR, Ren S, Formal SB, Levine MM. Strategy for cross-protection among *Shigella flexneri* serotypes. Infect Immun 1999 Feb;67(2): 782-8. 42 ref, Eng. Clinical Development, Pasteur Merieux Connaught, Discovery Drive, Swiftwater, PA 18370-0187, USA

"Based upon the lipopolysaccharide (LPS) structure and antigenicity of *Shigella* group B, a strategy for broad cross-protection against 14 *Shigella flexneri* serotypes was designed. This strategy involves the use of two *S. flexneri* serotypes (2a and 3a), which together bear the all of the major antigenic group factors of this group. The novel attenuated strains used in these studies were *S. flexneri* 2a strain CVD 1207 (D*guaB-A* D*virG* D*setl* D*sen*) and *S. flexneri* 3a strain CVD 1211 (D*guaB-A* D*virG* D*sen*). Guinea pigs were immunized with an equal mixture of these strains and later challenged (sereny test) with a wild-type *S. flexneri* serotype 1a, 1b, 2b, 4b, 5b, Y, or 6 strain of demonstrated virulence in the same model. Guinea pigs that were immunized with these two vaccine strains produced serum and mucosal antibodies that cross-reacted with all the *S. flexneri* serotypes tested (except of *S. flexneri* serotype 6) as assessed by enzyme-linked immunosorbent assay, immunoblotting, and slide agglutination. Furthermore, the combination vaccine conferred significant protection against challenge with *S. flexneri* serotype 1b, 2b, 5b, and Y but not with serotypes 1a, 4b, or (as predicted) 6."

093 Oberhelman RA, Gilman RH, Sheen P, Taylor DN, Black RE, Cabrera L, Lescano AG, Meza R, Madico G. A placebo-controlled trial of *Lactobacillus* GG to prevent diarrhea in undernourished Peruvian children. J Pediatr 1999 Jan;134(1):15-20. 13 ref, Eng. Department of Tropical Medicine, Tulane School of Public Health, 1501 Canal St, New Orleans, LA 70112, USA

"***Objective:*** *Lactobacillus* GG (L-GG), and acid- and bile-resistant strain that colonizes the intestinal mucosa, has been used to manage diarrhea in children. Our objective was to evaluate the prohylactic use of L-GG to prevent diarrhea in children at high risk from a developing country in a randomized, placebo-controlled trial. ***Study design:*** Two hundred four undernourished children 6 to 24 months old from an indigent peri-urban Peruvian town received either L-GG or placebo in flavored gelatin once daily, 6 days a week, for 15 months. Episodes of diarrhea were documented by daily home visits, and diagnostic studies were done in a subset of cases. Recovery of L-GG in stool from subjects and from family contacts was examined. ***Results:*** Subjects in the L-GG group had significantly fewer episodes of diarrhea (5.21 episodes diarrhea/child/year ["ecy"] L-GG group, 6.02 ecy placebo group; P=.028). The decreased incidence of diarrhea in the L-GG group was greatest in the 18- to 29-month age group (P=.004) and was largely limited to nonbreastfed children (Breastfed: 6.59 ecy L-GG, 6.32 ecy placebo, P=.7; nonbreastfed: 4.69 ecy L-GG, 5.86 ecy placebo, P=.005). The duration of diarrhea episodes and the causes of diarrhea were similar in both groups, except adenovirus was more common in the placebo group. ***Conclusion:*** L-GG supplementation may be useful as a prophylactic measure to control diarrhea in undernourished children at increased risk, especially nonbreastfed children in the toddler age group."

094 Øktedalen O, Skar V, Dahl E, Serck-Hanssen A. Changes in small intestinal structure and function in HIV-infected patients with chronic diarrhoea. Scand J Infect Dis 1998;30(5):459-63. 21 ref, Eng. Department of Medicine, Ullevaal University Hospital, Kirkeveien 166, N-0407 Oslo, Norway

"Human immunodeficiency virus (HIV) is often combined with unexplainable diarrhoea and weight loss. This study was designed to see if changes in the intestinal mucosal structure could explain the malabsorption found in HIV-infected patients with diarrhoea. Twenty acquired immunodeficiency system (AIDS) patients, 19 men and 1 women, CD4 <0.01, with severe weight loss and with non-infectious chronic diarrhoea, were evaluated using a new intestinal function test (D-xylose breath test). Fifteen of the subjects were examined with an upper intestinal endoscopy with biopsy specimens taken from the duodenal mucosa. The function test showed that the D-xylose uptake was markedly decreased to the same extent as for patients with coeliac disease (breath index AIDS patients 9.4 (4.3-14.4), coeliac patients 15.6 (7.6-23.6), reference level 2.5 (2.4-2.9), urine excretion AIDS patients 20% (13-26), coeliac patients 22% (14-24), reference level 37% (32-42). The severe malabsorption could not be explained by the slight mucosal changes occasionally seen by light microscopy with small mucosal inflammation and almost normal villi. However, electron microscopy showed enterocytes with signs of hypofunction and degeneration correlating better to the intestinal malabsorption found in patients with advanced HIV infection and chronic diarrhoea."

095 Orden JA, Ruiz-Santa-Quiteria JA, Garcia S, Cid D, De La Fuente R. In vitro activities of cephalosporins and quinolones against *Escherichia coli* strains isolated from diarrheic dairy calves. Antimicrob Agents Chemother 1999 Mar;43(3):510-3. 36 ref, Eng. Departamento de Patologia Animal I, Facultad de Veterinaria, Universidad Complutense, 28040 Madrid, Spain

096 Peterson JW, Finkelstein RA, Cantu J, Gessell DL, Chopra AK. Cholera toxin B subunit activates arachidonic acid metabolism. Infect Immun 1999 Feb;67(2):794-9. 47 ref, Eng. Department of Microbiololgy and Immunology, University of Texas Medical Branch, Galveston, TX 77555-1070, USA

097 Pradhan L, Sahu KL. *Ascaris lumbricoides*: unusual exit. Indian Pediatr 1998 Apr;35(4):371. Eng. Government Hospital, Udala, Mayurbhanj, Orissa 757041, India

098 Rabbani GH, Albert MJ, Rahman ASMH, Islam MM, Islam KMN, Alam K. Short-chain fatty acids improve clinical, pathologic, and microbiologic features of experimental shigellosis. J Infect Dis 1999 Feb;179(2):390-7. 52 ref, Eng. ICDDR,B: Centre for Health and Population Research, GPO Box 128, Dhaka 1000, Bangladesh

"Because of the metabolic and antibacterial actions of short-chain fatty acids (SCFA), their roles in modifying the clinicopathologic features of shigellosis were evaluated in a rabbit model of shigellosis. Acute colitis was induced in adult rabbits by intracolonic administration of *Shigella flexneri* 2a. After 24 h, rabbits were given 6-h colonic infusions of SCFA (acetate, propionate, n-butyrate; 60:30:40 m*M*) or SCFA-free solution (control); groups of rabbits were killed in batches of 2 or 3 animals at 24, 48, 72, and 96 h after treatment, for histologic and bacteriologic assessment. SCFA significantly reduced fecal blood and mucus and improved clinical symptoms. Histologically, SCFA significantly ($P<.01$) reduced mucosal congestion, cellular infiltration, and necrotic changes. SCFA also significantly ($P<.05$) reduced the number of shigellae in the colon. No such improvements occurred in the control group. SCFA may be useful agents in improving clinicopathologic features of shigellosis and should be clinically evaluated."

099 Riggs MW, McNeil MR, Perryman LE, Stone AL, Scherman MS, O'Connor RM. *Cryptosporidium parvum* sporozoite pellicle antigen recognized by a neutralizing monoclonal antibody is a b-mannosylated glycolipid. Infect Immun 1999 Mar;67(3): 1317-22. 36 ref, Eng. Department of Veterinary Science and Microbiology, Veterinary Science and Microbiology Building, University of Arizona, Tucson, AZ 85721, USA

100 Rios M, Prado V, Trucksis M, Arellano C, Borie C, Alexandre M, Fica A, Levine MM. Clonal diversity of Chilean isolates of enterohemorrhagic *Escherichia coli* from patients with hemolytic-uremic syndrome, asymptomatic subjects, animal reservoirs, and food products (note). J Clin Microbiol 1999 Mar;37(3):778-81. 20 ref, Eng. Programa Microbiologia y Micologia, Instituto de Ciencias Biomedicas, Facultad Medicina-Oriente, Universidad de Chile, Avenida Condell 303, Santiago, Chile

101 Rivera WL, Tachibana H, Kanbara H. Field study on the distribution of *Entamoeba histolytica* and *Entamoeba dispar* in the northern Philippines as detected by the polymerase chain reaction. Am J Trop Med Hyg 1998 Dec;59(6):916-21. 37 ref, Eng.

102 Rousset E, Harel J, Dubreuil JD. Sulfatide from the pig jejunum brush border epithelial cell surface is involved in binding of *Escherichia coli* enterotoxin b. Infect Immun 1998 Dec;66(12):5650-8. 46 ref, Eng. Groupe de Recherche sur les Maladies Infectieuses du Porc, Département de Pathologic et Microbiologie, Faculté de Médecine Vétérinaire, Université de Montréal, C.P. 5000, Saint-Hyacinthe, Québec, Canada J2S 7C6

103 Roy SK, Tomkins AM, Mahalanabis D, Akramuzzaman SM, Haider R, Behrens RH, Fuchs G. Impact of zinc supplementation on persistent diarrhoea in malnourished Bangladeshi children. Acta Paediatr 1998 Dec;87(12):1235-9. 32 ref, Eng. ICDDR,B: Centre for Health and Population Research, GPO Box 128, Dhaka 1000, Bangladesh

"To evaluate the impact of zinc supplementation on the clinical recovery and body weight of children with persistent diarrhoea, a randomized, double-blind, controlled trial was conducted in 190 children with persistent diarrhoea aged between 3 and 24 months. Children were randomly allocated to receive either zinc (20 mg d^{-1}) syrup with multivitamin (2 x RDA) or multivitamin alone in three divided daily doses for 2 weeks. The trial was conducted in a diarrhoeal disease hospital in Dhaka, Bangladesh. Duration until clinical recovery (d), impact on body weight and serum zinc level after 2 weeks of zinc supplementation were recorded. The duration of illness was significantly reduced (33%) with zinc supplementation among children who were underweight (£70% wt/age, p=0.03). Supplemented male children also had a significant reduction (27%) in duration for recovery compared with unsupplemented children (p=0.05). From baseline to convalescence, zinc-supplemented children maintained their serum zinc concentration (13.4 vs 13.6 mmol l^{-1}, $p<0.03$). The mean body weight of the children in the supplemented group was maintained (5.72 vs 5.70 kg, p=0.62) during hospitalization, unlike that of the control group, in which there was a reduction in body weight (5.75 vs 5.67 kg, p=0.05). Five children in the unsupplemented group and one child in the zinc-supplemented group died during the 2 weeks of supplementation (p=0.06). Zinc supplementation in persistent diarrhoea significantly reduced the length of the recovery period in malnourished children and prevented a fall in body weight and serum zinc concentration, indicating that zinc is a beneficial therapeutic strategy in this high-risk childhood illness."

104 Ruggeri FM, Declich S. Rotavirus infection among children with diarrhoea in Italy. Acta Paediatr 1999 Jan;(Suppl 426):66-71. 75 ref, Eng. Laboratorio di Ultrastrutture, Istituto Superiore di Sanita, Viale Regina Elena 299, 00161 Rome, Italy

"Despite the absence of a nationwide surveillance system for rotavirus infection, relevant information concerning the epidemiology of this pathogen in Italy can be obtained from hospital-based studies carried out since the early 1980s on patients with acute diarrhoea. A review of more than 50 papers and congress proceedings published in both international and national literature indicates that rotavirus is the most important cause of diarrhoea in Italy among young children requiring hospitalization, with a prevalence ranging from approximately 20% to 40% in different studies. Infection is predominant among children aged 6-24 months, although cases are also common in younger children and in children 2-3 y of age. Despite differences among studies in geographical area, years and age group under investigation, an increase in rotavirus cases is consistently reported in the winter months, with a peak in February through April. Although a few studies have been conducted in non-hospitalized patients, rotavirus infection is significantly less frequent among outpatients with enteritis than among inpatients. Most circulating rotavirus strains typed from 1981 to 1992 belong to serotype 1 and, to a lesser extent, 4. However, untypable rotavirus strains have been found in these years, with prevalences up to 27%, suggesting a possible spread of non-serotype 1 through 4 strains."

105 Runyen-Janecky LJ, Hong M, Payne SM. The virulence plasmid-encoded *impCAB* operon enhances survival and induced mutagenesis in *Shigella flexneri* after exposure to UV radiation. Infect Immun 1999 Mar;67(3):1415-23. 59 ref, Eng. Department of Microbiology and Institute of Cellular and Molecular Biology, University of Texas at Austin, Austin, TX 78712-1095, USA

"Upon exposure to UV radiation, *Shigella flexneri* SA100 displayed survival and mutation frequencies comparable to those of *Escherichia coli* AB1157, which contains a functional UmuDC error-prone DNA repair system. Survival of SA100 after UV irradiation was associated with the presence of the 220-kb virulence plasmid, pVP. This plasmid encodes homologues of ImpA and ImpB, which comprise an error-prone DNA repair system encoded on plasmid TP110 that was initially identified in *Salmonella typhimurium*, and ImpC, encoded upstream of ImpA and ImpB. Although the *impB* gene was present in representatives of all four species of *Shigella*, not all isolates tested contained the gene. *Shigella* isolates that lacked *impB* were more sensitive to UV radiation than isolates that contained *impB*. The nucleotide sequence of a 2.4-kb DNA fragment containing the *imp* operon from *S. flexneri* SA100 pVP was 96% identical to the imp operon from the plasmid TP110. An SA100 derivative with a mutation in the *impB* gene had reduced survival following UV irradiation and less UV-induced mutagenesis relative to the parental strain. We also found that *S. flexneri* contained a chromosomally encoded *umuDC* operon; however, the *umuDC* promoter was not induced by exposure to UV radiation. This suggests that the *imp* operon but not the *umuDC* operon contributes to survival and induced mutagenesis in *S. flexneri* following exposure to UV radiation."

106 Salam MA. Antimicrobial therapy for shigellosis: issues on antimicrobial resistance. Jpn J Med Sci Biol 1998;51(Suppl 1):S43-62. 98 ref, Eng. ICDDR,B: Centre for Health and Population Research, GPO Box 128, Dhaka 1000, Bangladesh

107 Sansonetti PJ. Pathogenesis of shigellosis: from molecular and cellular biology of epithelial cell invasion to tissue inflammation and vaccine development. Jpn J Med Sci Biol 1998;51(Suppl 1):S69-80. 54 ref, Eng. Unite de Pathogenie Microbienne Moleculaire, INSERM U389, Institut Pasteur, Parix, Cedex 15, France

108 Savarino SJ, Hall ER, Bassily S, Brown FM, Youssef F, Wierzba TF, Peruski L, El-Masry NA, Safwat M, Rao M, Mohamady HE, Abu-Elyazeed R, Naficy A, Svennerholm A-M, Jertborn M, Lee YJ, Clemens JD. Oral, inactivated, whole cell enterotoxigenic *Escherichia coli* plus cholera toxin B subunit vaccine: results of the initial evaluation in children. J Infect Dis 1999 Jan;179(1):107-14. 38 ref, Eng. c/o Research Publications Office, US Naval Medical Research Unit no. 3, PSC 452, Box 5000, FPO, AE, 09835-0007, USA

"Two randomized, double-blinded trials assessed the safety and immunogenicity of an oral, killed enterotoxigenic *Escherichia coli* (ETEC) plus cholera toxin B subunit vaccine in Egyptian children. Two doses of vaccine or *E. coli* K-12 were given 2 weeks apart to 105 6- to 12-year-olds and 97 2- to 5-year-olds. Safety was monitored for 3 days after each dose. Blood was collected before immunization and 7 days after each dose to measure immune responses. Few children reported postdosing symptoms, with no differences in the frequency of symptoms between treatment groups. Most vaccinees had an IgA antibody-secreting cell response against colonization factor antigen I (100%, 6-12 years; 95%, 2-5 years), coli surface antigen 2 (92%, 6-12 years; 83%, 2-5 years), and coli surface antigen 4 (93%, 6-12 years). Vaccination evoked a ³4-fold rise in antitoxic IgA and IgG titers in 93% and 81% of children, respectively. In conclusion, the oral ETEC vaccine was safe and immunogenic in 2- to 12-year-old children, justifying further evaluation in infants."

109 Schmidt H, Geitz C, Tarr PI, Frosch M, Karch H. Non-O157:H7 pathogenic Shiga toxin-producing *Escherichia coli*: phenotypic and genetic profiling of virulence traits and evidence for clonality. J Infect Dis 1999 Jan;179(1):115-23. 54 ref, Eng. Institut für Hygiene und Mikrobiologie der Universitat Würzburg, Bau 17, Josef-Schneider-Str. 2, D-97080 Würzburg, Germany

110 Semenza JC, Roberts L, Henderson A, Bogan J, Rubin CH. Water distribution system and diarrheal disease transmission: a case study in Uzbekistan. Am

J Trop Med Hyg 1998 Dec;59(6):941-6. 16 ref, Eng. Department of Medicine, Epidemiology Division, University of California, 224 Irvine Hall, Irvine, CA 92697-7550, USA

"Deteriorating water treatment facilities and distribution systems pose a significant public health threat, particularly in republics of the former Soviet Union. Interventions to decrease the disease burden associated with these water systems range from upgrading distribution networks to installing reverse osmosis technology. To provide insight into this decision process, we conducted a randomized intervention study to provide epidemiologic data for water policy decisions in Nukus, Uzbekistan, where drinking water quality is suboptimal. We inverviewed residents of 240 households, 120 with and 120 without access to municipal piped water. Residents of 62 households without piped water were trained to chlorinate their drinking water at home in a narrow-necked water container with a spout. All study subjects (1583 individuals) were monitored biweekly for self-reported diarrheal illness over a period of 9.5 weeks. The home chlorination intervention group had the lowest diarrheal rate (28.8/1,000 subject/month) despite lack of access to piped water in their homes. Compared with the two groups that did not receive the intervention this rate was one-sixth that of the group with no piped water (179.2/1,000 subjects/month) and one-third that of the households with piped water (75.5/1,000 subjects/month). More than 30% of the households with piped water lacked detectable levels of chlorine residues in their drinking water, despite two-stage chlorination of the source water, and were at increased risk of diarrhea. Forty-two percent of these municipal users reported that water pressure had been intermittent within the previous two days. The dramatic reduction in diarrheal rates in the home-chlorination intervention group indicates that a large proportion of diarrheal diseases in Nukus are water-borne. The home-chlorination group had less diarrhea than the group with piped water, implicating the distribution system as a source of disease transmission. Taken together, these epidemiologic data would support the hypothesis that diarrhea in the piped water group could be attributed to cross-contamination between the municipal water supply and sewer, due to leaky pipes and lack of water pressure. Relatively inexpensive steps, including chlorination, maintain water pressure, and properly maintaining the distribution system, rather than reverse osmosis technology, should reduce diarrheal rates."

111 Sharma A, Pradhan RK. Comparative study of rice-based oral rehydration salt solution *versus* glucose-based oral rehydration salt solution (WHO) in children with acute dehydrating diarrhoea. J Indian Med Assoc 1998 Dec;96(12):367-8. 13 ref, Eng. Department of Paediatric Medicine – Unit 11, Pt BD Sharma Postgraduate Institute of Medical Sciences, Rohtak 124001, India

"One hundred children with acute dehydrating diarrhoea were studied. They were divided into two groups: Group A (n=50) were given rice-based oral rehydration salt (ORS) solution and group B (n=50) were given glucose-based ORS solution (WHO). There was no significant decrease in mean stool output and percentage weight gain with rice-based ORS. Both the groups were comparable for volume of ORS solution consumed, time taken for initial rehydration, mean stool output and for correcting biochemical abnormalities."

112 Sharma AK, Holder FE. *Clostridium difficile* diarrhea after use of tacrolimus following renal transplantation (brief report). Clin Infect Dis 1998 Dec;27(6):1540-1. 3 ref, Eng. Magnolia Medical Center, 101 South 11th Street, Suite 4, Leesburg, Florida 34748, USA

113 Shaw RD. Viral infections of the gastrointestinal tract. Curr Opin Gastroenterol 1999 Jan;15(1):53-8. 47 ref, Eng. Research Service (151), Northport VA Medical Center, Northport, NY 11768, USA

114 Shi L, Miyoshi S-I, Hiura M, Tomochika K-I, Shimada T, Shinoda S. Detection of genes encoding cholera toxin (CT), zonula occludens toxin (ZOT), accessory cholera enterotoxin (ACE) and heat-stable enterotoxin (ST) in *Vibrio mimicus* clinical strains. Microbiol Immunol 1998;42(12):823-8. 29 ref, Eng. Faculty of Pharmaceutical Sciences, Okayama University, Tsushima-Naka, Okayama, Okayama 700-8530, Japan

115 Shyu R-Y, Jiang S-Y, Lai C-H, Hsu C-T, Young T-H, Yeh M-Y. High frequency of cytotoxin-associated gene A in *Helicobacter pylori* isolated from asymptomatic subjects and peptic ulcer patients in Taiwan. J Clin Gastroenterol 1998 Jul;27(1):54-9. 30 ref, Eng. Section of Gastroenterology, Tri-Service General Hospital, PO Box 90055, Taipei, Taiwan 100, Republic of China

116 Sinha P, Naik S, Ayyagari A, Naik SR. Isoenzyme and molecular characterization of *Entamoeba histolytica* and *Entamoeba dispar* isolates from symptomatic and asymptomatic subjects. Indian J Gastroenterol 1999 Jan-Mar; 18(1):18-21. 25 ref, Eng. Department of Gastroenterology, Sanjay Gandhi Postgraduate Institute of Medical Sciences, Raebareli Road, Lucknow 226014, India

117 Sunabe T, Honma Y*. Relationship between O-serogroup and presence of pathogenic factor genes in *Escherichia coli*. Microbiol Immunol 1998;42(12):845-9. 16 ref, Eng. *Department of Bacteriology, Faculty of Medicine, University of the Ryukyus, 207 Uehara, Nishihara, Okinawa 903-0215, Japan

"A total of 383 isolates of serogroup-based enteropathogenic and enteroinvasive *Escherichia coli* (310 strains of EPEC and 73 strains of EIEC) were examined for the presence of corresponding pathogenic genes. The serogroup-based EPEC consisted of 232 strains isolated from diarrhea patients and of 78 strains from healthy carriers. The gene encoding intimin, *eaeA*, was detected in 42 of the 232 EPEC strains from patients (18.1%) and 9 of the 78 strains from carriers (11.5%). The difference was not significant. The *bfp* gene of the EAF plasmid was detected in 7 of the 42 *eaeA*-positive EPEC strains from patients but was not detected in the 9 strains from carriers. In serogroup-based EIEC, a chromosomal *ipaH*

gene encoding one of the invasive plasmid antigens was detected in 4 of the 60 strains from patients (6%) but not in the 13 strains from carriers. The 4 *ipaH*-positive strains possessed the invasive plasmid. These results suggested that the serogroup-based diagnosis of EPEC and EIEC is not sufficient for identifying strains carrying the *eaeA* or *ipaH* gene."

118 Suzuki T, Sasakawa C. N-WASP is an important protein for the actin-based motility of *Shigella flexneri* in the infected epithelial cells. Jpn J Med Sci Biol 1998;51(Suppl 1):S63-8. 22 ref, Eng. Institute of Medical Science, University of Tokyo, Minato-ku, Tokyo 108-8639, Japan

119 Syngal S, Kaptain S. A 50-year-old woman with severe diarrhea during radiation treatment for resected metastatic melanoma. N Engl J Med 1999 Mar 11; 340(10):789-96. 53 ref, Eng. (Address not available)

120 Threlfall EJ, Graham A, Cheasty T, Ward LR, Rowe B. Resistance to ciprofloxacin in pathogenic *Enterobacteriaceae* in England and Wales in 1996. J Clin Pathol 1997 Dec; 50(12):1027-28. 5 ref, Eng. Laboratory of Enteric Pathogens, Central Public Health Laboratory, 61 Colindale Avenue, London NW9 5HT, UK

121 Trajman A, MacDonald TT, Elia CCS. Intestinal immune cells in *Strongyloides stercoralis* infection. J Clin Pathol 1997 Dec;50(12):991-5. 27 ref, Eng. Department of Internal Medicine, University Hospital, Universidade Federal do Rio de Janeiro, 22461-150 Brazil

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132 Yuhas Y, Shulman L, Weizman A, Kaminsky E, Vanichkin A, Ashkenazi S. Involvement of tumor necrosis factor alpha and interleukin-1b **in enhancement of pentylenetetrazole-induced seizures caused by *Shigella dysenteriae*. Infect Immun 1999 Mar;67(3):1455-60. 36 ref, Eng.** The Basil and Gerald Felsenstein Medical Research Center, Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel

"Neurologic manifestations, mainly convulsions, are the most frequent extraintestinal complications of shigellosis. We used an animal model to study the roles of tumor necrosis factor alpha (TNF-a) and interleukin-1b (IL-1b) in *Shigella*-related seizures. Administration of *Shigella dysenteriae* 60R sonicate enhanced the sensitivity of mice to the proconvulsant pentylenetetrazole (PTZ) within 7 h. This was indicated by a significantly higher mean convulsion score and an increased number of mice responding with clonic-tonic seizures in the *Shigella*-pretreated group. Preinjection of mice with anti-murine TNF-a (anti-mTNF-a) or anti-murine IL-1b (anti-mIL-1b) 30 min prior to administration of *Shigella* sonicate abolished their enhanced response to PTZ at 7 h. Mean convulsion scores were reduced by anti-mTNF-a from 1.2 to 0.8 (P=0.017) and by anti-mIL-1b from 1.3 to 0.7 (P=0.008). Preinjection of anti-mTNF-a also reduced the percentage of mice responding with clonic-tonic seizures, from 48 to 29% (P=.0.002), and preinjection of anti-mIL-1b reduced it from 53 to 21% (P=0.012). Neutralization of TNF-a or IL-1b did not protect the mice from death due to *S. dysenteriae* 60R. These findings indicate that TNF-a and IL-1b play a role in the very early sensitization of the central nervous system to convulsive activity after *S. dysenteriae* administration. Similar mechanisms may trigger neurologic disturbances in other infectious diseases."

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"Following the successful rehydration of two moderately dehydrated patients with transient glucose intolerance (TGI) using rice-oral rehydration solution (R-ORS), R-ORS has been used in Hacettepe University Ihsan Dogramaci Children's Hospital Diarrhea Training and Treatment Unit (DTTU) to rehydrate moderately dehydrated children with TGI. The files of children with moderate dehydration and glucose intolerance admitted to the unit were reviewed retrospectively within two periods according to the availability of R-ORS. The clinical and laboratory findings were analysed where available. Before R-ORS became available (September 1993) 6 patients were admitted, all of whom deteriorated with glucose (G)-ORS treatment in 7.0 ± 3.8 h and were hospitalized for i.v. fluid treatment. During the second period 22 moderately dehydrated children with TGI were admitted. The clinical and laboratory characteristics on admission of the children in the two periods were not statistically different (p>0.05). Among the 22 patients admitted during the second period 10 were administered G-ORS in the unit and 12 had already received G-ORS at home. Clinical and laboratory deterioration was observed in these 10 patients while receiving G-ORS in the unit within 6.3 ± 3.7 h and rehydration was continued with R-ORS. Clinical and laboratory improvement were demonstrated in 8 patients within 18.2 ± 6.5 h. Overall, 17 patients were rehydrated successfully with R-ORS, with a mean time of 18.0 ± 7.2 h. Five patients were hospitalized. The overall success rate of R-ORS was 77.3%. R-ORS may be considered as an alternative mode of therapy to i.v. treatment in the rehydration of moderately dehydrated children with TGI."

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