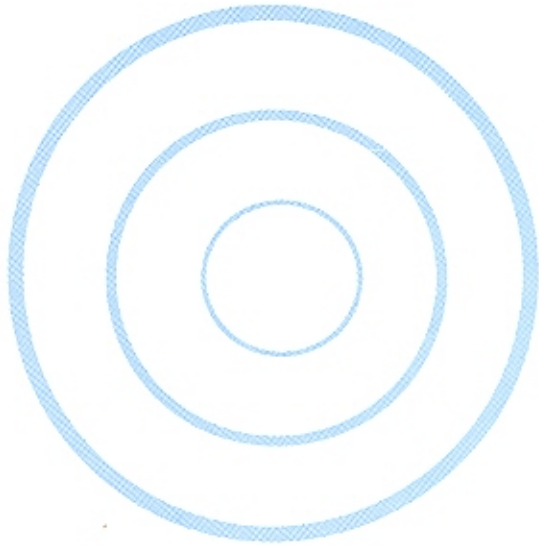
CENTRE
FOR HEALTH AND
POPULATION RESEARCH

Volume 15
Number 4
December 1997

ISSN 0253-8768

# JOURNAL OF Diarrhoeal Diseases Research



INTERNATIONAL CENTRE FOR
DIARRHOEAL DISEASE RESEARCH, BANGLADESH

CENTRE FOR HEALTH AND POPULATION RESEARCH

JOURNAL OF
# Diarrhoeal Diseases Research

Visit us at: http://www.icddrb.org
http://www.icddrb.org.sg

**Subscription information**
Subscriptions to JDDR run for a full calendar year and include air mail postage. The 1998 rates are as follows:

| | | | | | | |
|---|---|---|---|---|---|---|
| SAARC and other developing countries | : | Institution | US$ | 60.00 | Individual | US$ 30.00 |
| Developed countries | : | " | US$ | 80.00 | " | US$ 40.00 |
| Bangladesh | : | " | Taka | 500.00 | " | Taka 500.00 |

Subscription orders may be placed through an agent or directly; 10% commission is allowed for subscription agents. All payments must be made in favour of the **International Centre for Diarrhoeal Disease Research, Bangladesh**. All correspondence regarding subscription should be addressed to: **Managing Editor, Journal of Diarrhoeal Diseases Research, International Centre for Diarrhoeal Disease Research, Bangladesh, Mohakhali, Dhaka 1212 (GPO Box 128, Dhaka 1000), Bangladesh.**


Published by: International Centre for Diarrhoeal Disease Research, Bangladesh
Mohakhali, Dhaka 1212, Bangladesh. E-mail: jddr@icddr.org or msik@icddrb.org
Fax: 880-2-883116 / 880-2-886050 Telex: 675612 ICDD BJ
Phone: (880-2) -882467, 871751-871760 (PABX)
Cable: CHOLERA DHAKA

JOURNAL OF DIARRHOEAL DISEASES RESEARCH

Volume 15 Number 4 December 1997

CONTENTS

REVIEW ARTICLE

# Gastrointestinal Allergy to Food: A Review

**Tahmeed Ahmed and George J Fuchs**

Clinical Sciences Division, International Centre for Diarrhoeal Disease Research, Bangladesh, GPO Box 128, Dhaka 1000, Bangladesh

## SUMMARY

Gastrointestinal food allergy still poses a challenge to the clinician because of its variable symptomatology and lack of reliable diagnostic tests. Its prevalence is estimated at 2~5%, higher in children than in older age-groups. Allergy to food usually diminishes with advancing age. Although a wide variety of foods can cause allergic reactions, cow's milk is the most common cause of food allergy in infants and young children. Depending upon the speed of onset of symptoms, immediate and delayed types of food allergy have been described. Gastrointestinal symptoms in food allergy have been explained by alterations in transport across the intestinal wall (increased secretory and/or decreased absorptive functions), increased permeability, and motility of the intestine. The exact pathogenesis of food allergy is still not clear. However, immediate type of food allergy is believed to be mediated by type I hypersensitivity reaction, involving mast cells and food-specific IgE antibodies. The diagnosis of food allergy is based upon a favorable response to an elimination diet and a response to a challenge with the suspected food. The condition is treated by eliminating the allergenic food from diet for as long as 9-12 months in case of cow's milk allergy. While exclusive breast-feeding for the initial four months or more reduces the chances of development of food allergy, the role of diet restrictions in the mother in reducing the incidence of food allergy in the infant is controversial. Data on food allergy from developing countries are limited. This may be due to lack of diagnosis or less attention given to the condition relative to other diseases including infectious diarrheas and acute respiratory infections. The role of cow's milk allergy in the pathogenesis of persistent diarrhoea, a major problem in the developing world, remains speculative. Frequent intestinal infections and reduced secretory IgA, which are associated with malnutrition, alter intestinal permeability and result in an increased uptake of food antigens. The increased antigenic load combined with factors such as an atopic predisposition may initiate an abnormal mucosal immune response resulting in chronic enteropathy.

***Key words:*** Food hypersensitivity; Food; Gastrointestinal diseases; Diarrhoea

## INTRODUCTION

Due to controversies in definition, protean manifestations of the disease and lack of reliable diagnostic tests, gastrointestinal food allergy remains a challenging problem in paediatric practice. This review summarizes the literature on gastrointestinal food allergy. Food allergy is defined as an adverse clinical reaction due to any type of abnormal immune response resulting from the ingestion of a food or food additive (1). While over 140 different foods have been shown to produce allergic reactions in humans, foods most often documented as a cause of allergy are cow's milk, hen's eggs, legumes (especially peanuts), shrimp, oysters, tomato, chocolate, nuts, and seeds (2). The allergenicity of a particular food is influenced by the manner in which the food is prepared, processed, or stored. For example, heating of cow's milk denatures certain component proteins and alters its allergenicity (3). Denaturation may either reduce allergenicity or increase it by breaking down proteins and exposing epitopes not usually accessible in the intact protein.

Correspondence and reprint requests should be addresed to: Dr. Tahmeed Ahmed. E-mail: tahmeed@icddrb.org

### Common Food Allergens

*Cow's milk:* Cow's milk contains more than 25 proteins capable of inducing a specific antibody response in humans (4). The most allergenic component of cow's milk is b-lactoglobulin, followed by casein, a-lactalbumin and bovine serum albumin (BSA) (Table) (5, 6). Immunoelectrophoretic analysis has shown that casein retains its antigenicity after being heated at 120 °C for 15 minutes, b-lactoglobulin and a-lactalbumin retain theirs up to 100 °C, while BSA and gammaglobulin lose most of their antigenicity between 70 °C and 80 °C (7).

**Table: Characteristics of main protein components of cow's milk**

| Protein component | Molecular weight (kD) | Percent of total protein | Allergenicity | Stability at 100 °C |
|---|---|---|---|---|
| B-lactoglobulin | 18.3 | 10 | +++ | ++ |
| Casein | 20-30 | 82 | ++ | +++ |
| a-lactalbumin | 14.2 | 4 | ++ | + |
| Serum albumin | 67 | 1 | + | + |
| Immunoglobulins | 160 | 2 | + | + |

Hen's egg: Ovalbumin accounts for approximately 65% of the total protein content of hen's egg, and is the most allergenic of all egg proteins as demonstrated by radioallergosorbent test (RAST) studies and histamine release experiments (8). Ovalbumin is relatively heat-labile and, therefore, in patients reacting to cooked eggs, the heat-stable component ovomucoid may be the offending allergen (9).

Legumes: Peanut, known for its potential to elicit severe, life-threatening reactions, is highly heat-stable, maintaining its allergenicity even after being roasted at 145 °C for one hour (10). Peanut oil, containing only peanut lipid and hydrolyzed peanut protein, shows little or no allergenic activity (11). Soybean is a potent allergen which can cause anaphylaxis in sensitized subjects. Soybean oil, like peanut oil, is non-allergenic to soybean sensitive individuals (12).

Fish and shellfish: Fish allergy usually causes urticaria, angioedema, asthma or a combination of these in sensitized subjects within minutes of ingestion. Allergy to fish is easier to diagnose than most other food allergies because of the immediacy of the reaction. The most completely characterized fish allergen is codfish allergen M. It is highly allergenic and cross-reacts with allergens found in other species of fish (13). Shrimp contains potent allergens, and a number of anaphylactic reactions to these allergens have been reported.

Cereals: Wheat flour can result in wheat allergy upon ingestion and baker's asthma upon inhalation, while the gluten fraction is the cause of celiac disease or gluten intolerance. Sutton et al. determined that wheat allergy, baker's asthma, and gluten intolerance are each caused by different wheat protein components (14).

**Cross-Reactivity of Food Allergens**

Foods of the same biological family sometimes share identical or similar epitopes, and may induce cross-reactions in sensitive subjects. Immunologic relationships exist between a-casein, b-lactoglobulin and a-lactalbumin in cow's and goat's milk, which explain the cross-reactivity noted between the two types of milk (15). Cross-reactivity has also been observed between eggs of different birds (16), between different seafoods (17), and between pollens, vegetables and fruits (18).

**Epidemiology of Food Allergy**

The true prevalence of food allergy is unknown because we lack objective, reproducible methods for diagnosing this condition (19). A study from Finland has given a conservative estimate of a 2~5% prevalence in the population, with a higher prevalence (up to 27%) in children (20). Among adults in the general population, a prevalence of 0.2% has been estimated (21).

Cow's milk is the most common food causing allergy in infants and young children (9). The estimated prevalence of cow's milk allergy (CMA) is between 0.5~7.5% (22, 23). CMA is primarily a disease of infancy and early childhood, although well-documented cases of CMA have also been described in teenagers and adults (24). CMA can also rarely occur after years of asymptomatic ingestion of cow's milk. As with other allergic disorders, a familial tendency has been observed (25). The high prevalence of CMA in infancy and childhood is probably due to a number of factors: (a) immaturity of the immune system as well as of the mucosal barrier of the gastrointestinal tract, (b) increased consumption of cow's milk relative to body weight by infants and children, and (c) strong allergenicity of cow's milk proteins (9). Because of its high prevalence, CMA has been the food allergy that has received the most attention from clinicians, researchers, and the general public.

**Clinical Types and Manifestations of CMA**

Depending upon the speed of onset of symptoms or signs following ingestion of cow's milk, two types of CMA have been defined: immediate and delayed (26, 27). In the immediate type, symptoms appear within one hour of ingestion of milk. If symptoms appear one to 48 hours later, then the CMA is of the delayed type. The former is easier to diagnose on the basis of clinical history and laboratory investigations, e.g. a raised titre of allergen-specific serum IgE antibody. By contrast, a diagnosis of delayed type CMA is difficult to establish because currently available investigations lack sensitivity. Further, the delay between ingestion and manifestations makes a cause and effect relationship difficult to

establish (27). The immediate type of CMA is more common, as 40 to 65% of children with CMA show symptoms within one hour of ingestion of cow's milk (5,28).

The most common symptoms of CMA are gastrointestinal, cutaneous or respiratory. The age of the infant at which symptoms start is largely influenced by the total duration of breast-feeding (22). When breast-feeding is of short duration, symptoms usually appear during the first month of life (29), and in 90% by the age of three months (30). In a study of patients with chronic diarrohea due to CMA, the median age at first visit was three months (31). A brief account of the major manifestations of CMA is described below.

Gastrointestinal manifestations: Diarrohea has been reported to occur in 25 to 75% of patients with CMA, while vomiting occurs in 25 to 50%, usually within one hour after ingestion of milk (4). Acute diarrohea is more common than persistent diarrhoea (diarrhoea for more than two weeks) (28), the former being more common in patients with the delayed-type CMA (32). CMA has been suggested to be a cause of infantile colic (33). Protein-losing enteropathy has also been ascribed to CMA (34). A recent study has suggested that CMA may be a cause of chronic constipation in children (35). Cow's milk restriction resulted in resolution of chronic constipation, which reappeared within 40 to 72 hours of milk ingestion. Specific gastrointestinal syndromes of CMA are cow's milk- sensitive enteropathy and cow's milk-sensitive colitis (26).

Cow's milk-sensitive enteropathy: Onset of symptoms occurs within the first six months of life and is uncommon after three years of age. Enteropathy is more frequent in infants born into atopic families and in those exposed to cow's milk during the neonatal period (36). Affected infants have diarrhoea and vomiting which usually persist for many weeks. The infants may have other allergic manifestations, including atopic dermatitis, urticaria, or wheezing. Failure to thrive is a marked feature in certain children. The small intestine is mainly affected and an intestinal biopsy is usually abnormal with patchy lesions (37).

Cow's milk-sensitive colitis: This syndrome is characterized by inflammatory changes in the colon and rectum as a result of immune-mediated reactions to ingested cow's milk proteins (38), and occurs almost exclusively in early infancy. Rectal bleeding is the most common sign. Symptoms of diarrhoea with blood and mucus usually appear within a few days or weeks after starting cow's milk or milk formula feeds. Unlike cow's milk-sensitive enteropathy, the large intestine is mainly involved (39). Cow's milk-free diet leads to dramatic improvement and is a good diagnostic and therapeutic tool. This condition has also been reported to occur in exclusively breast-fed infants (40), and is attributed to small amounts of bovine b-lactoglobulin present in the breast milk of certain mothers who drink cow's milk or eat cheese.

*Dermatologic manifestations:* In a study involving 100 infants with CMA, the most common dermatologic symptom was urticaria, which appeared on ingestion and in some instances upon skin contact with cow's milk (41). Angioedema, subcutaneous swelling caused by an IgE-mediated reaction, involving the larynx and pharynx, may lead to life-threatening upper airway obstruction (36). Atopic dermatitis (AD) has also been associated with CMA. In a double-blind randomized study of 20 children with AD, Atherton *et al.* found that 50% of the children benefited from cow's milk and egg avoidance (42).

*Respiratory Manifestations:* In one survey, one-third of children with CMA were found to have respiratory symptoms (28). All infants with immediate cutaneous manifestations had acute wheezing episodes after milk ingestion. However, another study using double-blind challenges with cow's milk was able to establish CMA in only 19% of children suspected to have wheezing due to CMA (43). Of those with predominantly delayed gastrointestinal symptoms, lower respiratory tract symptoms were found in more than half of the infants. In older children, bronchial asthma may occasionally be due to CMA (22). A particular variant of CMA, Heiner syndrome, is characterized by chronic lung disease with pulmonary hemosiderosis, anemia, growth failure and precipitins to cow's milk (44).

*Anaphylactic shock:* The most extreme manifestation of the immediate type of CMA, anaphylactic shock results from a generalized IgE-mediated reaction to cow's milk. It can be caused by less than one milliliter of cow's milk, and begins with sneezing and itching, followed by generalized urticaria, bronchospasm, and vascular collapse, the whole episode occurring within minutes of milk ingestion. The frequency of anaphylactic reactions varies from none to one-third of cases in studies involving patients with gastrointestinal symptoms (40,45). Anaphylaxis is seen more frequently during clinical challenge after a cow's milk free period (5,40).

*Other Manifestations:* A variety of other symptoms have also been rarely ascribed to CMA, including irritability, hyperactivity, nocturnal enuresis, and insomnia. In a study of eight infants with insomnia, elimination of cow's milk cured all the patients, while a later challenge with cow's milk produced sleeping difficulties in four of them (46). All the infants had high plasma IgE levels, and four of the five tested also had IgE antibodies to b-lactoglobulin.

**Natural History**

Most food allergies diminish with advancing age. Allergy to cow's milk and eggs is more likely to be of shorter duration than allergy to fish, nuts, and peanuts (47). In a study by Esteban *et al.,* 50% of children with CMA were tolerant by three years of follow-up, while 50% with egg allergy became tolerant after five years. In contrast, 80% of children initially allergic to fish remained allergic to fish after eight years of follow-up (48). In children with food allergy followed for seven years, tolerance developed in 44% of those who were younger than three years at the start of their signs and symptoms, but in only 19% of children older than three years at the beginning of the disease (47). Therefore, it appears that children who develop food allergy before the age of three years achieve tolerance earlier than children who develop food allergy at an older age.

In a recent study involving 100 children with CMA diagnosed at a mean age of 16 months, cow's milk tolerance was demonstrated by two years of age in 28% of children, by four years in 56%, and by six years of age in 78% (49). The incidence of tolerance was greater among children with the delayed than with the immediate type of CMA. Only 25% were found to still be allergic to cow's milk alone at the end of follow-up. As many as 58% of the children had reactions to eggs, 47% to soy milk, and 34% to peanuts. These results should be interpreted with caution since the adverse reactions to foods other than cow's milk were not evaluated with challenge tests. The results, however, suggest that if children with CMA do not respond to dietary exclusion of cow's milk, there remains a possibility that they are reacting adversely to other food proteins. At final follow-up, 40% of patients had asthma, 21% atopic dermatitis, and 43% allergic rhinitis. Therefore, exclusion of cow's milk from the diets of cow's milk allergic children may not prevent further development of atopic disorders. From these observations it is not clear whether independent mechanisms control the development of cow's milk allergy and other atopic disorders.

**Pathology**

There are no pathological findings unique to CMA. Since the gastrointestinal system is commonly involved, pathological features of the two variants having mainly gastrointestinal manifestations are discussed.

*Cow's milk-sensitive enteropathy:* Small intestinal mucosal damage is an obvious finding in cow's milk-sensitive enteropathy (50). Although there are reports of normal mucosal findings in patients with cow's milk- sensitive enteropathy (51), this may be due to the patchy distribution of lesions. The enteropathy has been shown to be cow's milk sensitive by serial biopsies after withdrawal of, and subsequent challenge with, cow's milk (52). Histopathological findings in the jejunal biopsy specimens are similar to those found in celiac disease, although less severe. Usually there is patchy villous atrophy with crypt hyperplasia and inflammation both intraepithelially and in the lamina propria (53). The villous atrophy varies in degree from partial to subtotal. The number of intraepithelial lymphocytes is higher than in healthy subjects but not as high as in celiac disease (54). Ultrastructurally, the epithelial cells have short

microvilli, abnormal nuclei and an abundance of lysozymes (52). The mucosal disaccharidase and alkaline phosphatase levels have been found to be depleted (37).

*Cow's milk-sensitive colitis:* On colonoscopy, the colonic mucosa is erythematous and friable with aphthoid ulcers. There is increased nodularity suggestive of lymphoid hyperplasia (38). Microscopically, the overall architecture of the mucosa is well maintained, without features of chronicity typical of inflammatory bowel disease, such as distorted or atrophic crypts, Paneth cell metaplasia, lymphoid aggregates, or diffuse plasmacytosis (55). The most striking histologic feature is the presence of focal infiltrates of eosinophils in the mucosa, particularly in the lamina propria, which resolves after elimination of cow's milk from the diet. Because of subtle similarities in histopathology, this disorder should be distinguished from inflammatory bowel disease on the basis of biopsy findings, clinical course including response to cow's milk elimination, and absence of systemic inflammatory changes (26).

**Pathophysiology**

The consequence of exposure to food allergens is a complex process that is still not well understood. Experiments in animal models have provided an excellent opportunity to study the effects of food allergen challenges, the results of which help in elucidating the mechanisms underlying CMA. Most of these studies have described the effects of exposure to food allergens in terms of transport across the intestinal wall, permeability, and motility of the intestine (56).

*Intestinal transport:* Alteration of intestinal transport (increased secretion and/or decreased absorption) contributes to the symptoms of CMA. Intragastric administration of b-lactoglubulin in mice passively sensitized with monoclonal IgE against b-lactoglobulin, resulted in fluid secretion in the lumen (57). Hooded Lister rats sensitized to egg albumin showed a significantly decreased absorption of $Na^+$, $Cl^-$, $K^+$ and water after the antigen was added to a perfused segment of jejunum (58). These changes were accompanied by mucosal histamine depletion, increased histamine recovery from the perfusates, and villous edema (59). Prevention of these changes by doxantrazole, a mucosal mast cell stabilizer, suggested the involvement of mucosal mast cells (60).

*Permeability:* Increase in permeability to macromolecules is believed to be important in the pathophysiology of CMA. It may occur either as an increase in vascular permeability or in intestinal permeability. Challenge with egg albumin in sensitized Sprague-Dawley rats resulted in increased counts of $^{125}$I-labeled rat serum albumin in the gut wall and contents (61). Changes in vascular permeability were accompanied by increased intestinal secretions, goblet cell mucus, and histamine levels (62). In other studies, evidence of mucosal mast cell degranulation was also seen accompanying permeability changes (63), as well as gross damage to the enterocytes after challenge with food antigen in sensitized animals (64). Increased intestinal permeability to normally unabsorbable molecules has been documented in children with CMA (65).

*Motility:* Substances released from mast cells such as histamine, serotonin, prostaglandins, and leukotrienes cause smooth muscle contraction in sensitized animals

upon challenge with food allergen (66). Altered intestinal motility patterns have been observed after jejunal challenge with food allergen in sensitized rats (67). As the changes were prevented by doxantrazole, the motility responses were attributed to mast cell involvement.

**Pathogenesis**

Two phases can be recognized in food allergic reactions (68). The first is the triggering phase, in which the food or its components interact with host cells or non-cellular systems. The second phase is the activation of the mediators of inflammation, which determines the nature and extent of the disease. Although the exact pathogenetic mechanisms in CMA are still not clear (69), it is believed that they may involve one or more of the four classic types of hypersensitivity reactions described by Gell and Coombs (70).

*Type I, Immediate hypersensitivity:* The majority of allergic reactions to foods are thought to be mediated by immediate hypersensitivity, involving mast cells and food-specific IgE antibodies. The mucosal mast cell, by virtue of its location, mediator content, and IgE receptors, plays a major role in the pathogenesis of responses to allergens presented by the oral route (71). Compared to the other types of immune reactions, type I reaction is relatively well understood. It occurs in atopic individuals who produce excessive levels of total and specific IgE antibodies (72). The incidence of this type of reaction is highest in infancy and declines with age. It is characterized by the onset of clinical symptoms within minutes of ingestion of the food to which the individual is allergic.

IgE binds to Fc receptors on the cell membranes of mast cells. Mast cells have a large number of these receptors, 2~5 x $10^5$ per mast cell, which have extremely high affinity for IgE binding (73). Upon exposure to allergen, the IgE molecules and their receptors are cross-linked, which acts as a signal for a cascade of cellular events leading to mast cell degranulation and mediator release. In the gut, mediator release leads to increased muscle tone, stimulation of pain fibers, increased mucus production, and increased permeability to macromolecules (Figure) (71). Local recruitment of inflammatory cells may also take place, with possible late phase reactions and inflammation. Tests that are usually positive in this type of reaction are skin tests, detection of specific IgE by RAST, and basophil histamine release (74).

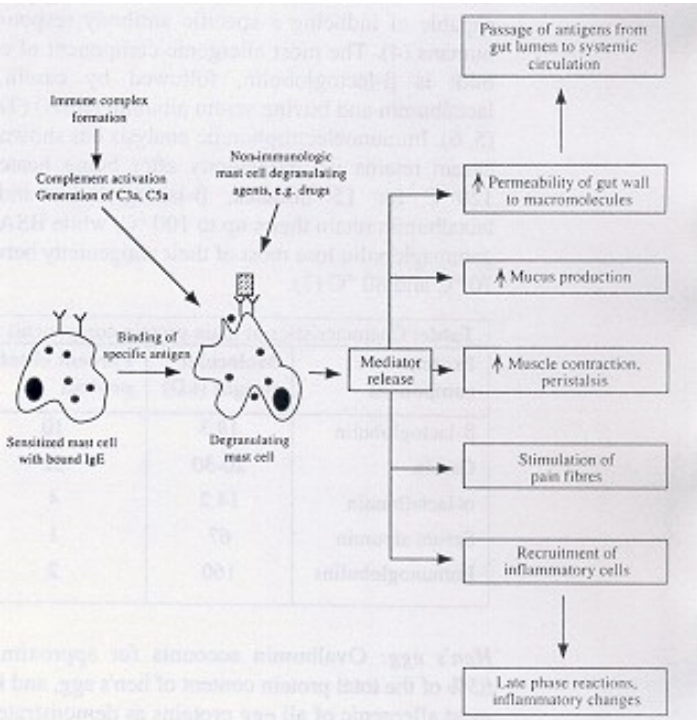


Fig. Activation of mucosal mast cells and its probable local and systemic consequences

Since $IgG_4$ antibodies can release mediators from mast cells, this subclass has also been implicated in the pathogenesis of immediate hypersensitivity (75). But individuals who have $IgG_4$ antibodies to food antigens do not have symptoms or positive skin tests unless they also have specific IgE antibodies. On the contrary, a protective "blocking" role for $IgG_4$ antibodies has also been implicated (76). $IgG_4$ antibodies are non-complement fixing, non-precipitating, and actually inhibit precipitation by $IgG_1$ antibodies. An initial $IgG_1$ response (complement fixing and precipitating) to a food antigen may lead to immune complex disease. Therefore, a progressive change from $IgG_1$ to $IgG_4$ antibodies may reduce the harmful effects of continued exposure to the antigen.

Type II, Antibody-dependent cytotoxic hypersensitivity: In this type of reaction, food allergens stick to the surface of cells and are later bound by specific IgG or IgM antibodies. The cells are injured or destroyed by the activation of complement or the release of cytotoxic substances from activated killer cells. Milk-induced thrombocytopenia is probably mediated by this type of reaction (77).

Type III, Immune complex-mediated hypersensitivity: Immune complex reactions usually involve IgG or IgM antibodies that combine with the antigen and form circulating immune complexes to which complement is fixed. When these complexes reach small blood vessels, especially the post-capillary venules, an Arthus-like reaction occurs resulting in vasculitis and tissue damage (4). Based on histologic and immunofluorescent findings in intestinal biopsies, there is evidence that the Arthus-type reaction is involved in the pathogenesis of cow's milk sensitive colitis (78). The finding of immune complex deposits in lung biopsy specimens taken from patients with Heiner syndrome implicates this type of reaction in the pathogenesis of the syndrome (79).

Type IV, Cell-mediated delayed hypersensitivity: Delayed hypersensitivity lesions mediated by CD4+ T-cells are the cause of tissue damage in various diseases, classically in tuberculosis. The reaction is based on the interaction of antigen with endogenous receptors on the surface of primed T-cells. Cytokines are released as a result, which stimulate other types of cells and produce inflammatory changes. A sub-population of T-cells may also be stimulated to become directly cytotoxic (80). The effects of these events are usually delayed and may not be manifested before 24 to 72 hours after antigen exposure. Circumstantial evidence suggests that T-cell-mediated hypersensitivity may play a role in response to food antigens, especially in the delayed type of reactions (81). Recent studies provided inconclusive results as to whether lymphocyte proliferation occurs in response to cow's milk protein antigens in CMA (82).

Recent in vitro studies suggest that cytokines, released by cow's milk protein-stimulated lymphocytes of patients with CMA, may be responsible for the intestinal dysfunctions. Hill et al. reported that peripheral T-lymphocytes from patients with the delayed-type CMA produced more interferon gamma (IFN-g) than those from patients with the immediate type (83). This finding indicates a tendency towards a Th1 type of response in the delayed type. Heyman *et al.* found that peripheral lymphocytes from infants with CMA, when cultured in the presence of cow's milk proteins, released more tumor necrosis factor (TNF-a) than those from control infants (84). Supernatants of lymphocyte cultures from patients altered intestinal barrier capacity, as evident from the decrease in electrical resistance and the increase in horseradish peroxidase, ($^{14}C$) mannitol, and $^{22}Na^{+}$ fluxes. These results indicate that during CMA the high levels of TNF-a released by lymphocytes increase intestinal permeability.

**Diagnosis**

In the past, diagnosis of CMA was based upon clinical criteria described by Goldman *et al.* (5): (a) development of symptoms which subside after elimination of milk from diet; (b) recurrence of symptoms within 48 hours of milk challenge; (c) reactions to three such challenges should be positive and have similar onset, duration, and clinical features. The criterion of having three positive challenges is now considered impractical for routine diagnosis, particularly in small infants who are more at risk of having acute anaphylaxis (85). The immediate type of CMA is relatively easier to diagnose with a challenge test, because the amount of food antigen required to produce symptoms is small, and the reaction is rapid and clear. In the delayed type, reactions often appear slowly after a challenge and may require a large

amount of antigen (27). Based on these considerations, the two important diagnostic points are a response to an elimination diet and a response to a food challenge (27, 85).

1. *Response to an elimination diet*

This is an absolute criterion for the diagnosis of food allergy, and includes both clinical and laboratory observations. There should be relief of all symptoms and gain in weight if there had been loss of weight due to the disease.

2. *Response to a food challenge*

At present, a single challenge test with food remains the standard for the diagnosis of food allergy. Only natural food (for example, cow's milk but not the isolated proteins) should be used in challenge, because processing may alter allergenicity. The end point of the positive challenge test should be an objective observation, such as vomiting, diarrhoea, colic, erythema, etc. The test should not be done in young infants with severe symptoms because of the danger of anaphylaxis.

**Immunological tests**

The skin prick test with standardized food extracts, and measuring circulating specific IgE antibodies are sensitive methods which help in the diagnosis of immediate type of allergy (86). The usefulness of these tests is poor in the delayed type of allergy. Moreover, allergen-specific IgE antibodies may persist even when the child has achieved tolerance. Studies evaluating the usefulness of IgG and IgA antibodies to food antigens have yielded contradictory results (28,87,88).

**Diagnosis of delayed-type CMA**

Because of the delay in appearance of symptoms, the diagnosis of this type of food allergy, i.e. cow's milk- sensitive enteropathy or colitis, can be done on the basis of typical clinical findings, morphologic changes in the intestinal mucosal biopsy specimens, and favoorable response to elimination of cow's milk from diet. Since the danger of anaphylactic reactions is particularly high in this type, a challenge test should be performed late, preferably 9 to 12 months after the beginning of elimination diet (89).

**Treatment**

The treatment of CMA is based on the elimination of cow's milk and of products containing cow's milk proteins. Elimination should continue for 9 to 12 months. Cow's milk allergic patients with chronic diarrhoea and malnutrition may require prolonged parenteral nutrition because severe intestinal damage may not allow oral feeding for several weeks. For oral feeding, breast milk is best for young infants. Special formulae based on hydrolyzed casein can be given, although they may also cause allergy (90). Children aged more than one year generally do not need special formulae.

**Prevention**

Interventions, including exclusive breast-feeding, maternal avoidance of allergic foods, and use of "hypoallergenic" formulae, have been attempted to reduce the development of atopic disease in children (91). In a five-year follow-up study, Chandra has shown a significantly reduced incidence of atopic diseases, like eczema, asthma, and food allergy, in infants breast-fed for ³4 months, compared to infants fed cow's milk formula until 6 months of age (92). During 18 months of follow-up, Magnusson *et al.* found no difference in the incidence of food allergy and other atopic diseases between infants whose mothers avoided cow's milk and eggs during the last trimester of pregnancy, and those whose mothers did not (93). In another study, maternal avoidance of cow's milk, eggs and fish during the first three months of lactation resulted in a lower incidence of IgE antibodies to cow's milk and eggs, and also a lower

incidence of atopic dermatitis in infants during the first six months of life (94). Because of conflicting results from studies evaluating the role of diet restrictions in the mother in reducing the incidence of atopic disease in the infant, maternal avoidance of food allergens should still be considered investigational (91).

## ROLE OF FOOD ALLERGY IN THE PATHOGENESIS OF OTHER DISEASES

### Inflammatory bowel diseases

Although infectious agents, genetic and psychogenic factors, and dietary influences have all been implicated, the pathogenesis of inflammatory bowel diseases (IBD) is still unclear. Several observations suggest that allergic reactions may contribute to the inflammatory process. Taylor and Truelove initially raised the possibility of CMA as a pathogenetic factor by observing milk precipitins in the sera of patients with ulcerative colitis (95). Increased numbers of mast cells have been observed in the intestinal biopsy samples from patients with IBD (96). Further, a history of CMA in infancy has been found with increased frequency in patients with ulcerative colitis (97). Exclusion of food allergens by giving elemental or semi-elemental diets can induce clinical remission in patients with Crohn disease (98). However, some of these observations have been refuted by subsequent studies, and the role of food allergy in the pathogenesis of IBD remains poorly defined (82).

### Insulin-Dependent Diabetes Mellitus (IDDM)

Epidemiological studies suggest an association between cow's milk consumption and the incidence of IDDM in childhood (99, 100). Animal studies have also shown that the addition of cow's milk proteins to rat feeds increases the incidence of diabetes in diabetes-prone BB rat (101). Over the last six years, several studies have reported increased levels of circulating IgG or IgA antibodies to cow's milk, b-lactoglobulin, and BSA in children with IDDM (102, 103, 104, 105). BSA has been implicated as a possible trigger of the autoimmune response that destroys pancreatic beta cells in genetically predisposed individuals, causing IDDM (106). BSA shares a common epitope with p69, a pancreatic beta cell surface protein, 69 kD in size. The anti-BSA antibodies found in the sera of patients with IDDM bind to p69, giving rise to a cross-reactive immune response.

### Gastrointestinal Allergy to Food in

### Developing Countries

Most of the literature on gastrointestinal allergy to food comes from developed countries, and data from developing countries are scarce. Whatever data there are from the developing world, come mostly from South-east Asia (37,53,107). Till date, there is no published data about the prevalence of food allergy in Bangladesh. The lack of data may be due to under-diagnosis and less attention given to food allergy relative to other killer diseases, such as acute respiratory infections and diarrhoea.

Persistent diarrhoea and malnutrition are mostly encountered in children of developing countries. The pathogenesis of persistent diarrhoea, which accounts for about ten percent of all diarrhoea episodes but is associated with 30 to 50% of all diarrhoeal deaths, is still not clear (108, 109, 110). CMA has been attributed to be one of the probable causes of persistent diarrhoea. As a result of frequent intestinal infections and reduction in secretory IgA, which are associated with malnutrition (111), the intestinal permeability is altered and there is increased uptake of food antigens (112). The increased antigenic load combined with factors such as an atopic background and age at weaning may initiate an abnormal mucosal immune response resulting in chronic enteropathy and persistent diarrhoea. Much more remains to be known about this important issue.

## REFERENCES


1. Metcalfe D. Food hypersensitivity. *J Allergy Clin Immunol* 1984;73:749-60.
2. Galant S. Common food allergens. *In:* Bierman C, Pearlman D, editors. Allergic diseases of infancy and childhood. Philadelphia: Saunders, 1980:211-8.
3. Wahn U, Wahl R, Rugo E. Comparison of the residual allergenic activity of six different hydrolyzed protein formulas. *J Pediatr* 1992;121:S80-4.
4. Bahna S, Gandhi M. Milk hypersensitivity. I. Pathogenesis and symptomatology. *Ann Allergy* 1983;50:218-23.
5. Goldman A, Anderson D Jr, Sellers W *et al.* Milk allergy. I. Oral challenge with milk and isolated milk proteins in allergic children. *Pediatrics* 1963;32:425.
6. Kletter B, Gery I, Freier S, Davies A. Immune responses of normal infants to cow's milk. I. Antibody type and kinetics of production. *Int Arch Allergy Appl Immunol* 1971;40:656.
7. Hanson L, Mansson I. Immune electrophoretic studies of bovine milk and milk products. *Acta Paediatr Scand* 1961;50:484.
8. Anet H, Back J, Baker R, *et al.* Allergens in the white and yolk of hen's egg. A study of IgE binding by egg proteins. *Int Arch Allergy Appl Immunol* 1985;77:364-71.
9. Chiaramonte L, Yalamanchili A. Common food allergens. *In:* Chiaramonte L, editors. Food allergy: a practical approach to diagnosis and management. New York: Dekker, 1988:89-106.
10. Fries J. Peanuts: Allergic and other untoward reactions. *Ann Allergy* 1982;48:220-26.
11. Nordlee J, Taylor S, Jones R, Yunginger J. Allergenicity of various peanut products as determined by RAST inhibition. *J Allergy Clin Immunol* 1982;68:376-82.
12. Bush R, Taylor S, Nordlee J, Busse W. Soybean oil is not allergenic to soybean sensitive individuals. *Allergy Clin Immunol* 1985;76:242-5.
13. Aas K. Studies of hypersensitivity to fish. Immunodiffusion and haemaglutination experiments. *Int Arch Allergy* 1966;30:190-208.
14. Sutton R, Hill D, Baldo B, Wrigley C. Immunoglobulin E antibodies to ingested cereal components: studies with sera from subjects with asthma and eczema. *Clin Allergy* 1982;12:63.
15. Crawford L. Allergenicity of cow's milk proteins. IV. Relationship of goat's milk proteins as studied by serum-agar precipitation. *J Pediatr* 1961;59:347.
16. Langeland T, Aas K. Allergy to hen's egg white: clinical and immunological aspects. *In:* Brostoff J, Challacombe S, editors. Food allergy and intolerance. London: Tindall, 1987:367-74.
17. Lehrer S. The complex nature of food antigens: studies of cross-reacting crustacea allergens. *Ann Allergy* 1986;57:267-72.
18. Dreborg S, Foucard T. Allergy to apple, carrot and potato in children with birch allergy. *Allergy* 1983;38:167-72.
19. Bock S. Food sensitivity. *Am J Dis Child* 1980;134: 973.
20. Kajosaari M. Food allergy in Finnish children aged 1 to 6 years. *Acta Paediatr Scand* 1982;71:815-19.
21. Chandra R. Food allergy: 1992 and beyond. *Nutr Res* 1992;12:93-99.
22. Savilahti E, Kuitunen P, Visakorpi J. Cow's milk allergy. *In:* Lebenthal E, editors. Textbook of pediatric gastroenterology and nutrition in infancy. New York: Raven, 1989:689-708.
23. Young E, Stoneham M, Petruchevitch A, *et al.* A population study of food intolerance. *Lancet* 1994;343:1127-30.

24. Loveless M. Milk allergy: a survey of its incidence; experiments with a masked ingestion test. *J Allergy* 1950;21:489.

25. Meritt RJ, Carter M, Haight M, et al. Whey protein hydrolysate formula for infants with gastrointestinal intolerance to cow milk and soy protein in infant formulas. *J Pediatr Gastroenterol Nutr* 1990;11:78-82.

26. Walker-Smith JA, Ford RP, Phillips AD. The spectrum of gastrointestinal allergies to food. *Ann Allergy* 1984;53:629-36.

27. Savilahti E, Heyman M, Macdonald T, et al. Diagnostic criteria for food allergy with predominantly intestinal symptoms. *J Pediatr Gastroenterol Nutr* 1992;14:108-12.

28. Hill DJ, Firer MA, Shelton MJ, Hosking CS. Manifestations of milk allergy in infancy: clinical and immunologic findings. *J Pediatr* 1986;109:270-6.

29. Powell G. Milk and soy induced enterocolitis of infancy; clinical features and standardization of challenge. *J Pediatr* 1978;93:553-60.

30. Gerrard J, MacKenzie J, Goluboff N, et al. Cow's milk allergy: prevalence and manifestations in an unselected series of newborns. *Acta Paediatr Scand* 1973;234:1-21.

31. Businco L, Benincori N, Cantani A, *et al.* Chronic diarrhea due to cow's milk allergy. a 4- to 10-year follow-up study. *Ann Allergy* 1985;55:844-7.

32. Jakobsson I, Lindberg T. A prospective study of cow's milk protein intolerance in Swedish infants. *Acta Paediatr Scand* 1979;68:853-9.

33. Lothe L, Lindberg T, Jakobsson I. Cow's milk formula as a cause of infantile colic: a double-blind study. *Pediatrics* 1982;7:7-10.

34. Waldmann T, Wochner R, Laster L, Gordon R. Allergic gastroenteropathy: a cause of excessive gastrointestinal protein loss. *N Eng J Med* 1967;276:761-9.

35. Iacono G, Carroccio A, Cavataio F, *et al*. Chronic constipation as a symptom of cow milk allergy. *J Pediatr* 1995;126:34-9.

36. Cant A. The symptomatology of food allergy. *In:* Reinhardt D, Schmidt E, editors. Food allergy. New York: Raven, 1988:127-35.

37. Iyngkaran N, Yadav M, Boey C, Lam K. Severity and extent of upper small bowel mucosal damage in cow's milk protein-sensitive enteropathy. *J Pediatr Gastroenterol Nutr* 1988;7:667-74.

38. Odze R, Wershil B, Leichtner A, Antonioli D. Allergic colitis in infants. *J Pediatr* 1995;126:163-70.

39. Chong S, Sanderson I, Wright V, Walker-Smith JA. Food allergy and infantile colitis. *Arch Dis Child* 1984;59:690-1.

40. Lake A, Whitington P, Hamilton S. Dietary protein-induced colitis in breast-fed infants. *J Pediatr* 1982;101:906-10.

41. Hill DJ, Ford RP, Shelton MJ, Hosking CS. A study of 100 infants and young children with cow's milk allergy. *Clin Rev Allergy* 1984;2:125-42.

42. Atherton D, Sewell M, Soothill J, Wells R, Chilvers C. A double-blind controlled cross-over trial of an antigen avoidance diet in atopic eczema. *Lancet* 1978;1:401-3.

43. Bock S, Lee W. Studies of hypersensitivity reactions to foods in infants and children. *J Allergy Clin Immunol* 1978;62:327-34.

44. Heiner D. Respiratory diseases and food allergy. *Ann Allergy* 1984;53:657-64.

45. Gryboski J. Gastrointestinal milk allergy in infants. *Pediatrics* 1967;40:354-62.

46. Kahn A, Mozin MJ, Casimir G, Montauk L, Blum D. Insomnia and cow's milk allergy in infants. *Pediatrics* 1985;76:880-4.

47. Bock S. The natural history of food sensitivity. *J Allergy Clin Immunol* 1982;69:173-7.

48. Esteban M, Pascual C, Madero R, *et al.* Natural history of immediate food allergy in children. *In:* Businco L, Ruggieri F, editors. Proceedings of first Latin food allergy workshop. Rome, 1985:27-30.

49. Bishop J, Hill D, Hosking C. Natural history of cow milk allergy: clinical outcome. *J Pediatr* 1990;116:862-7.

50. Visakorpi J. Food allergy and the gut. *In:* Reinhardt D, Schmidt E, editors. Food allergy. New York: Raven, 1988:137-49.

51. Verkasalo M, Kuitunen P, Savilahti E. Changing pattern of cow's milk intolerance. *Acta Paediatr Scand* 1981;70:289-95.

52. Kuitunen P, Rapola J, Savilahti E, *et al.* Response of the jejunal mucosa to cow's milk in the malabsorption syndrome with cow's milk intolerance. *Acta Paediatr Scand* 1973;62:585-95.

53. Iyngkaran N, Robinson M, Prathap K, *et al.* Cow's milk protein sensitive enteropathy; combined clinical and histological criteria for diagnosis. *Arch Dis Child* 1987;53:20-6.

54. Maluenda C, Philips A, Briddon A, et al. Quantitative analysis of small intestinal mucosa in cow's milk sensitive enteropathy. *J Pediatr Gastroenterol Nutr* 1984;3:349-56.

55. Odze R, Bines J, Leichtner A, Goldman H, Antonioli D. Allergic proctocolitis in infants: a prospective clinico-pathologic biopsy study. *Hum Pathol* 1993;24:668-74.

56. Crowe S, Perdue M. Gastrointestinal food hypersensitivity: basic mechanisms of pathophysiology. *Gastroenterology* 1992;103:1075-95.

57. Granato D, Piguet P. A mouse monoclonal IgE antibody anti-bovine milk b-lactoglobulin allows studies of allergy in the gastrointestinal tract. *Clin Exp Immunol* 1986;63:703-10.

58. Perdue M, Chung M, Gall D. The effect of intestinal anaphylaxis on gut function in the rat. *Gastroenterology* 1984;86:391-97.

59. Perdue M, Forstner J, Roomi N, *et al*. Epithelial response to intestinal anaphylaxis in rats: goblet cell secretion and enterocyte damage. *Am J Physiol* 1984;247:G632-37.

60. Perdue M, Gall D. Transport abnormalities during intestinal anaphylaxis in the rat; effect of anti-allergic agents. *J Allergy Clin Immunol* 1985;76:498-503.

61. Bloch K, Walker W. Effect of locally induced anaphylaxis on the uptake of a bystander antigen. *J Allergy Clin Immunol* 1981;67:312-6.

62. Lake A, Kagey-Sobotka A, Jakubowicz T, *et al*. Histamine release in acute anaphylactic enteropathy of the rat. *Immunology* 1984;133:1529-34.

63. Turner M, Boulton P, Shields J, *et al.* Intestinal hypersensitivity reactions in the rat. I. Uptake of intact protein, permeability to sugars and their correlation with mucosal mast-cell activation. *Immunology* 1986;63:119-24.

64. D'Inca R, Ramage J, Hunt R, Perdue M. Antigen-induced mucosal damage and restitution in the small intestine of the immunized rat. *Int Arch Allergy Appl Immunol* 1990;91:270-7.

65. Heyman M, Grasset E, Ducroc R, *et al*. Antigen absorption by the jejunal epithelium of children with cow's milk allergy. *Pediatr Res* 1988;24:197-202.

66. Scott R, Gall D, Maric M. Mediation of food protein-induced jejunal smooth muscle contraction in sensitized rats. *Am J Physiol* 1990;259:G6-14.

67. Scott R, Diamant S, Gall D. Motility effects of intestinal anaphylaxis in the rat. *Am J Physiol* 1988;255:G505-11.

68. Kniker W. Immunologically mediated reactions to food: state of the art. *Ann Allergy* 1987;59:60-70.

69. Walker-Smith J, MacDonald T, Barnes R, *et al.* Conclusions and recommendations. Report of a British Society for Allergy and Clinical Immunology special interest group for food allergy and intolerance. *Clin Exp Allergy* 1995;25:43-4.

70. Coombs R, Gell P. Classification of allergic reactions responsible for clinical hypersensitivity and disease. *In:* Gell P, Coombs R, editors. Clinical aspects of immunology. Oxford: Blackwell, 1975:761-81.

71. Barrett R, Metcalfe D. The mucosal mast cell and its role in gastrointestinal allergic diseases. *Clin Rev Allergy* 1984;2:39-53.

72. Stricker W. Food testing in patients with idiopathic anaphylaxis. *J Allergy Clin Immunol* 1986;77:516-9.

73. Boltansky H, Kaliner M. Cells demonstrating Fc receptors for IgE. *Surv Immunol Res* 1984;3:99-102.

74. Johansson S. Immunological mechanisms of food sensitivity. *Nutr Rev* 1984;42:79-84.

75. Halpern G, Scott J. Non-IgE mediated mechanisms in food allergy. *Ann Allergy* 1987;58:14-27.

76. Platts-Mills TAE. The immunobiology of IgE: immediate or type I hypersensitivity. *In:* Lachman P, editors. Clinical aspects of immunology. Oxford: Blackwell, 1993:983.

77. Caffrey E, Sladen G, Isaaca P. Thrombocytopenia caused by cow's milk. *Lancet* 1981;2:316.

78. Saavedra-Delgado A, Metcalfe D. Interactions between food antigens and the immune system in the pathogenesis of gastrointestinal diseases. *Ann Allergy* 1985;55:694-702.

79. Lee S, Kniker W, Cook C. Cow's milk induced pulmonary disease in children. *Adv Pediatr* 1978;25:39-57.

80. Barrett K, Metcalfe D. Immunologic mechanisms in food allergy. *In:* Chiaramonte L, Schneider A, Lifshitz F, editors. Food allergy: a practical approach to diagnosis and management. New York: Dekker, 1988: 23-43.

81. Kay R, Ferguson A. Intestinal T-cells, mucosal cell-mediated immunity and their relevance to food allergic disease. *Clin Rev Allergy* 1984;2:55-68.

82. MacDonald TT. Evidence for cell-mediated hypersensitivity as an important pathogenetic mechanism in food intolerance. *Clin Exp Allergy* 1995;25:10-3.

83. Hill D, Ball G, Hoskings C, Wood P. Gamma-interferon production in cow's milk allergy. *Allergy* 1993;48:75-80.

84. Heyman M, Darmon N, Dupont C, *et al.* Mononuclear cells from infants allergic to cow's milk secrete TNF-a, altering intestinal function. Gastroenterology 1994;106:1514-23.

85. Walker-Smith JA. Diagnostic criteria for gastrointestinal food allergy in childhood. *Clin Exp Allergy* 1995;25:20-2.

86. Ahmed T, Sumazaki R, Nagai Y, *et al.* Immune response to food antigens: kinetics of food-specific antibodies in the normal population. *Acta Paediatr Japon* 1997;39:322-8.

87. Fallstrom S, Ahlstedt S, Carlsson B, *et al*. Serum antibodies against native, processed and digested cow's milk proteins in children with cow's milk intolerance. *Clin Allergy* 1986;16:417-23.

88. Burks A, Williams L, Casteel H, *et al*. Antibody response to milk proteins in patients with milk-protein intolerance documented by challenge. *J Allergy Clin Immunol* 1990;85:921-7.

89. Carter C. Dietary treatment of food allergy and intolerance. *Clin Exp Allergy* 1995;25:34-42.

90. Schwartz R, Amonette M. Cow's milk protein hydrolysate infant formulas not always hypoallergenic. *J Pediatr* 1991;119:839.

91. Kerner JA. Use of infant formulas in preventing or postponing atopic manifestations. *J Pediatr Gastroenterol Nutr* 1997;24:442-6.

92. Chandra RC. Five-year follow-up of high-risk infants with family history of allergy who were exclusively breast-fed or fed partial whey hydrolysate, soy, and conventional cow's milk formulas. *J Pediatr Gastroenterol Nutr* 1997;24:380-8.

93. Magnusson K, Kjellman N. Development of atopic disease in babies whose mothers were receiving exclusion diet during pregnancy - a randomized study. *J Allergy Clin Immunol* 1987;80:869-75.

94. Hattevig G, Kjellman B, Sigurs N, *et al*. The effect of maternal avoidance of eggs, cow's milk, and fish during lactation on the development of IgE, IgG and IgA antibodies in infants. *J Allergy Clin Immunol* 1990;85:108-15.

95. Taylor K, Truelove S. Circulating antibodies to milk proteins in ulcerative colitis. *Br Med J* 1961;2:924.

96. Kirsner J, Shorter R. Recent developments in nonspecific inflammatory bowel disease. Pt. I. *New Eng J Med* 1982;306:775-85.

97. Glassman M, Newman L, Berezin S, *et al*. Cow's milk protein sensitivity during infancy in patients with inflammatory bowel disease. *Am J Gastroenterol* 1990;85:838-40.

98. Greenberg G. Nutritional support in inflammatory bowel disease: current status and future directions. *Scand J Gastroenterol* 1992;27:117-22.

99. Scott F. Cow milk and insulin-dependent diabetes mellitus: is there a relationship? *Am J Clin Nutr* 1990;51:489-91.

100. Borch-Johnsen K, Mandrup-Poulsen T, *et al*. Relation between breast feeding and incidence rates of insulin-dependent diabetes mellitus. *Lancet* 1984;2:1083-6.

101. Daneman D, Fishman L, Carlson C, *et al.* Dietary triggers of insulin-dependent diabetes in the BB rat. *Diabetes Res* 1987;5:93-7.

102. Dahlquist G, Savilahti E, Landin-Olsson M. An increased level of antibodies to b-lactoglobulin is a risk determinant for early-onset IDDM independent of islet cell antibodies and early introduction of cow's milk. *Diabetologia* 1992;35:980-84.

103. Savilahti E, Saukkonen T, Virtala E, *et al.* Increased levels of cow's milk and b-lactoglobulin antibodies in young children with newly diagnosed IDDM*. Diabetes Care* 1993;16:984-89.

104. Krokowski M, Caillat-Zucman S, Larger E, *et al.* Anti-BSA antibodies: genetic heterogeneity and clinical relevance in adult-onset IDDM. *Diabetes Care* 1995;18:170-73.

105. Ahmed T, Kamota T, Sumazaki R, *et al.* Circulating antibodies to common food antigens in Japanese children with IDDM. *Diabetes Care* 1997;20:74-6.

106. Karjalainen J, Martin J, Knip M, *et al*. A bovine albumin peptide as a possible trigger of insulin-dependent diabetes mellitus. *N Engl J Med* 1992;327:302-7.

107. Manuel PD, Walker Smith JA, Soeparto P. Cow's milk sensitive enteropathy in Indonesian infants. *Lancet* 1980;2:1965-6.

108. Fauveau V, Henry RJ, Briend A, *et al.* Persistent diarrhoea as a cause of mortality in rural Bangladesh. *Acta Paediatr* 1992;81(Suppl 381):12-14.

109. Victora CG, Huttly SR, Fuchs SC, *et al.* Deaths due to dysentery, acute and persistent diarrhea among Brazilian infants. *Acta Paediatr* 1992;81(Suppl 381):7-11.

110. Black RE. Persistent diarrhea in children of developing countries. *Pediatr Infect Dis J* 1993;12:751-61.

111. Suskind D, Murthy KK, Suskind RM. The malnourished child: an overview. *In:* Suskind RM, Suskind LL, editors. The malnourished child. New York: Raven, 1990:1-22.

112. Sanderson IR, Walker WA. Uptake and transport of macromolecules by the intestine: possible role in clinical disorders (an update). *Gastroenterol* 1993;104: 622-39.

SEMINAR

# The Acute Infectious Diarrhoeas as Diseases of the Intestinal Mucosa

**Jehan-François Desjeux and Martine Heyman**

**INSERM**, Unit 290 and Chair of Biology, Conservatoire National des Arts et Métiers, Paris, France

**ABSTRACT**

One of the intriguing aspects of the acute infectious diarrhoeas is that, while resulting from an infection of the intestinal tract, they only last from a few hours to a few days. The study of the interactions between infectious agents and intestinal epithelium has allowed a better understanding of the cellular and molecular mechanisms that cause the sudden loss of water and electrolytes, the hallmark of acute diarrhoea. These interactions have also led to formulating oral rehydration solutions used worldwide now. They do not, however, allow understanding the short course of most intestinal infections. For that, one has to consider the intestinal epithelium as part of an anatomical and functional system that, including the many types of cells present in the lamina propria, constitutes the intestinal mucosa. Infectious agents interact with the whole of the mucosa, including the cells of the lamina propria. This leads, among other things, to a change in the functions of the epithelial cells and accelerates their turnover. The pathophysiology of the intestinal mucosa leads to better understand the short and benign course of most intestinal infections. It also leads to better understand the physiology of the intestinal mucosa, and the interactions between the body and its nutritional environment.

**Key words:** Diarrhoea; Infection; Intestinal mucosa; Lamina propria

## INTRODUCTION

One of the intriguing aspects of the acute infective diarrhoeas is that, while resulting of an infection of the intestinal tract, they only last from a few hours to a few days. At first sight, it is easier to understand the far more rare persisting infectious diarrhoeas with their complications than the much more frequent acute diarrhoeas from which most patients recover spontaneously and quickly , without sequels. Hence, understanding the pathophysiology of diarrhoeas requires an understanding how the body attempts, mostly with success, to fight intestinal infections. This understanding is needed to develop appropriate therapeutic strategies, even if these have to take into account other considerations, including symptomatic, sociological and economical ones (1-8).

The infectious agents, viruses, bacteria, or parasites, entering the intestinal tract act directly on the luminal side of the enterocytes. The study of the ensuing interactions has allowed to understand mechanisms of the induction of diarrhoea, and the loss of water and electrolytes resulting in dehydration. This has led to the formulation of oral rehydration solutions (ORS), now used world-wide (9,10). It does not, however, allow to explain the brief course of most intestinal infections. To achieve this, one must consider the intestinal epithelium as part of one anatomical structure, i.e., the intestinal mucosa which includes the intestinal epithelium and the many types of cells of the *lamina propria* adjacent to the enterocytes lining the gut. The intestinal mucosa as a whole must be considered as a functional unit, able to react very quickly to many stimuli.

The study of the pathophysiology of diseases as common as the acute infectious diarrhoeas has led to the rediscovery of the intestinal mucosa as an integrated organ. This, in turn, is at the origin of new insights in the role of the intestine as an exchange system between the body and its nutritional environment.

Correspondence and reprint requests should be addresed to:
Professor J-F Desjeux, Chaire de Biologie, 2, rue Conte, 75003 Paris
E-mail: desjeux@cnam.fr

### 1. The circulation of water in the intestine

The disturbances of the entero-systemic cycle of water explain the signs and symptoms of diarrhoea (Fig. 1) (11-13). Schematically, the enterocytes covering the intestinal wall can present two types of disturbances. Either do infectious agents stimulate secretion to such an extent that it exceeds the re-absorptive capacity for water and electrolytes, or the re-absorption capacity is diminished and becomes unable to cope with the amounts secreted. The first mechanism is that of the secretory diarrhoeas, caused e.g. by the toxins of *Vibrio cholerae* or *Escherichia coli* (14). The second one implies destruction of the enterocytes and their increased regeneration, as seen in rotavirus infections (15,16). Overall, these perturbations of the entero-systemic cycle of water allow to understand the dehydration caused by diarrhoea. They also explain at least in part the fast resolution of infectious diarrhoeas: the infectious agent is diluted and rapidly eliminated by the water flux.

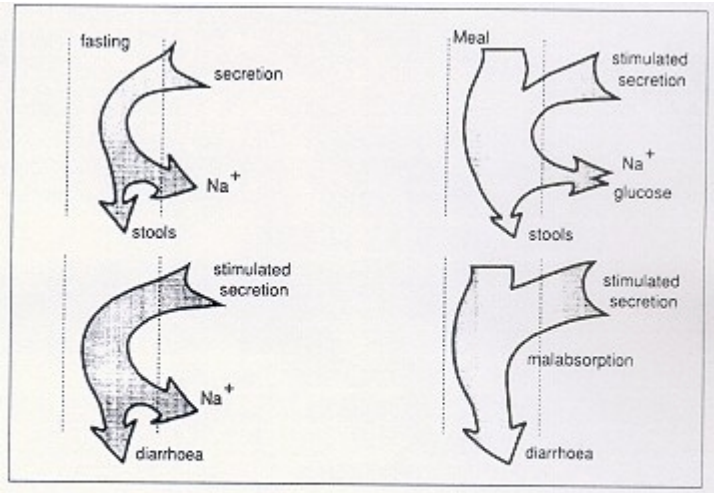



**Fig. 1. Entero-systemic cycle of water**

In the fasting state (upper left), the intestine reabsorbs the water of the digestive fluids trough a mechanism involving active reabsorption of sodium. During a meal (upper right), the nutrients in the intestinal lumen stimulate sodium reabsorption. Diarrhoea results when secretion is stimulated and overwhelms the absorptive capacity (bottom left) or when absorption is decreased (bottom right).

## 2. The role of the enterocytes

Diarrhoea can be considered as a disturbance of the enterocyte transport systems. More particularly, there is an increased $Cl^-$ secretion (Fig. 2, panel 2) and a decreased absorption of NaCl (Fig. 2, panel 3). These two mechanisms have an additive effect, causing an accumulation of water and electrolytes in the intestinal lumen. Important to note is that the glucose-driven sodium absorption is usually conserved (17) (Fig. 1, panel 1). It decreases, however, in rotavirus or cryptosporidium infections (18-20) and may increase in cholera (1). This system is at the basis of the development and the effectiveness of ORS (9,11,21). At the level of the colon it should be possible to stimulate sodium absorption by the short chain fatty acids produced by the fermentation of carbohydrates (22-26).

## 3. The secretory immunoglobulins barrier

An infection of the intestine quickly gives rise to the recognition of microbial antigens by the mucosa-associated lymphoid tissue (MALT) present in the Peyer's patches of the small intestine. Activated specific lymphocytes and memory cells spread all over the intestinal mucosa. The resulting mature plasmocytes produce immunoglobulin A (IgA) that binds to a specific receptor, the secretory piece, derived by proteolytic cleavage from the basal membrane of the enterocytes. The tetravalent dimeric secretory IgA enters the gut and binds specifically to the infecting micro-organisms in the intestinal lumen and the mucus covering the enterocytes. This leads to a very efficient "immunological exclusion" preventing the contact between microbial antigens and enterocytes. Daily, more than 3 gram of IgA reach the mucosal secretions (27), blocking the massive entry of pathogens in the body. There is experimental proof *in vivo* that IgA alone can protect the gut from bacterial colonisation and the ensuing diarrhoea. For example, the induced secretion of a monoclonal IgA directed against a glycosylated epitope of the surface lipopolysaccharide of *V. cholerae* protects infected mice from diarrhoea and death (28). These studies underscore the powerful protective effect on the intestinal mucosa of specific IgA, if present in sufficient amounts. This has been convincingly demonstrated in rotavirus infection (29-31).

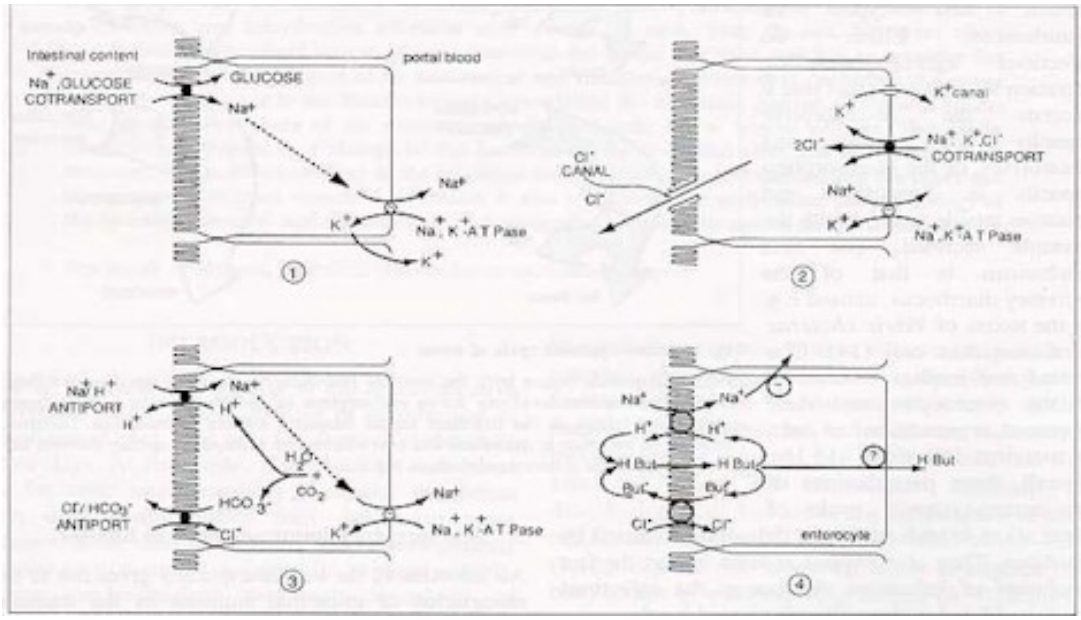



**Fig. 2. Main transport systems in acute infectious diarrhoeas**

All these systems use as source of energy the Na+ electrochemical gradient produced by Na+, K+ ATPase. They are transmembrane proteins, located either at the luminal or the basolateral side of the enterocytes. Their specific distribution explains the absorptive or secretory fluxes through the epithelium. The genes coding for the transmembrane proteins have been identified. They are SGLT1 for the glucose-Na+ cotransporter, CFTR for the Cl– channel, BSC for the 2Cl–/Na+/K+ cotransporter and NHE3 for the Na+/H+ exchanger. The different mechanisms used for the entry of electrolytes through the luminal side and their excretion through the basolateral side explain absorption (panels 1, 3 and 4). Secretion involves a basolateral entry mechanism and a luminal excretion mechanism (panel 2).

### 4. Infectious agents act directly on the epithelium

Despite the protective systems of the mucosa, infectious agents can directly lock on to the enterocytes. This results in changes of the concentrations of intracellular messengers that, in turn, acting on phosphorylation mechanisms, modify the concentration or the activity of the transport systems of the cellular membrane (32,33).

The study of cholera toxin has been the model for all other interactions between pathogens and enterocytes. Indeed, the B-subunit of cholera toxin binds to the $GM^1$ gangliosides of the luminal membranes of the enterocytes. It allows the A-subunit to enter the cell and stimulate adenylate cyclase, an enzyme of the basolateral cell membrane. This causes a rise of cyclic AMP and an increased phosphorylation by protein kinase of the transport proteins located in the luminal membrane.

The transport proteins include CFTR protein and $NHE_3$ protein. The former activates the selective $Cl^-$ secretion channel, and the latter inhibits active coupled NaCl absorption from the mucosal to the serosal side of the gut (Fig. 2).

Beside *V. cholerae*, all other infectious agents that act directly on the enterocytes use similar mechanisms, though the membrane receptors are not necessarily $GM^1$ gangliosides and the intracellular messengers include cyclic GMP, cytosolic $Ca^{++}$ or $IP^3$, all of which modulate the expression of the cellular transport systems (14,34-42).

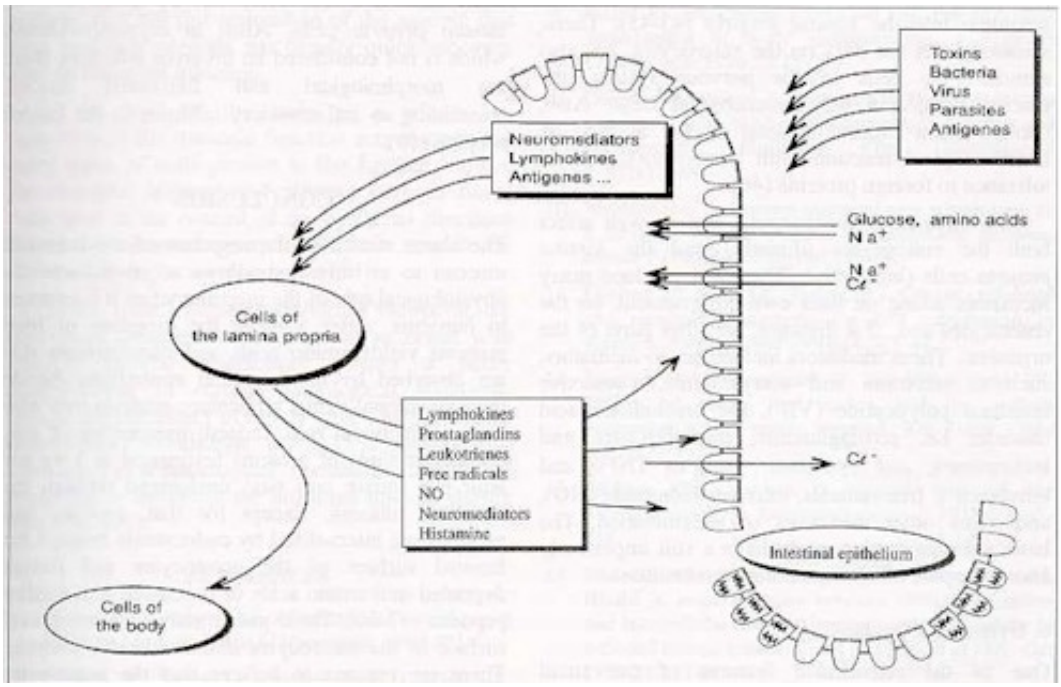



**Fig. 3. Functions of the intestinal mucosa**

The epithelium separates the intestinal content from the rest of the body. Pathological substances act on the functions of this selective barrier by directly interacting with the epithelial cells and indirectly with the cells of the lamina propria. There, numerous messengers are set free that also act on the rest of the body. It is probable that in the absence of intestinal infections, the molecules in the lumen and the lamina propria might play a physiological role: the intestinal mucosa allows man to adapt to his nutritional environment

### 5.Infectious agents also act on the enterocytes by way of the cells of the *lamina propria*

One of the most striking aspects of the pathophysiology of acute infectious diarrhoeas is that the infectious agents act not only directly on the enterocytes and their secretion and absorption of water and electrolytes. They also act on the complex structures of the *lamina propria* surrounding the intestinal epithelium. The *lamina propria* consists of a great variety of cells, including nervous and neuro-endocrine cells, lymphocytes, polymorphonuclear and eosinophilic leukocytes, macrophages, mastocytes, and myofibroblasts. Once stimulated, all these cells interact and influence the function of the enterocytes. Moreover, they also act on other parts of the body, so that infectious diarrhoeas must be considered a generalised disease rather than a localised one. In this sense, it is more correct to consider e.g., a rotavirus infection as rotavirus disease rather than rotavirus diarrhoea.

Taking into account the multiple interactions between the infectious agents and the whole intestinal mucosa (enterocytes and cells of the *lamina propria*), one can divide the pathophysiological mechanisms of diarrhoeas in 4 broad categories (33). They are the action of enterotoxins, the action of cytotoxins, the adherence of infectious agents on the enterocytes, and the invasion of, and effect on, the *lamina propria* through the epithelium. Each of these mechanisms exerts their actions directly on the enterocytes and indirectly on the *lamina propria*.

For example, *V. cholerae* and enterotoxic *E. coli* toxins (LT and ST) act directly on the luminal side of the enterocytes, but also cause the rare endocrine cells located between the epithelial cells to secrete serotonin into the *lamina propria (*43-45). There, serotonin acts not only on the enterocytes, but also activates the cells of the nervous system, the macrophages, and the mesenchymal cells. Also, cholera toxin causes, at least in the animal, an immunological reaction with a transitory loss of tolerance to foreign proteins (46).

The three other mechanisms also as well affect both the enterocytes (directly) and the *lamina propria* cells (indirectly). The latter produce many mediators acting on their own environment, on the enterocytes and, at a distance, on other parts of the organism. These mediators include neuro-mediators, such as serotonin and acetylcholine, vasoactive intestinal polypeptide (VIP), the arachidonic acid cascade, i.e. prostaglandins, thromboxanes and leukotrienes, and cytokines, such as TNFa and interferon g , free radicals, nitrogen monoxide (NO), and many other mediators of inflammation. The latter substances also might have a still imperfectly known trophic effect on the intestinal mucosa.

6. **Dynamic aspects**

One of the remarkable features of the small intestinal epithelium is that the enterocytes covering this considerable surface – when the microvilli are included it has been estimated at not less than 340,000 square cm for a one year old child -- are renewed on average every 72 hours. This is a result of the cellular cycle of the stem cells, located at the bottom of the crypts, and, at least in part, driven by substances secreted by cells of the *lamina propria*. They include cytokines, such as TNFa and IL-6, growth factors, such as epidermal growth factor, (EGF) and hormones, such as norepinephrine and tri-iodothyronine. Immunological diseases, e.g. coeliac disease and host-versus-graft reaction, influence the structure and speed of renewal of the enterocytes. Similar changes occur in acute diarrhoeas. This has been observed in transmissible gastro-enteritis (TGE) of piglets that has many similarities with rotavirus infections in children (18,20). There is a partial destruction of the villi with an accelerated renewal of the enterocytes. The result is a decrease of the absorptive surface, and a reduction of the absorption of sodium-coupled glucose and amino acids, and NaCl. These anomalies are not caused by a direct action of the virus on the enterocytes, but by stimulation of *lamina propria* cells. Also, in cryptosporidiosis, which is not considered an invasive infection, there are morphological and functional lesions, resembling an inflammatory infiltrate in the *lamina propria* (19).

## CONCLUSION

The above sketch of the response of the intestinal mucosa to an infection allows to re-evaluate the physiological role of the intestine when it is exposed to nutrients. After a meal, the digestion of food proteins yields amino acids and oligopeptides that are absorbed by the intestinal epithelium. Beside their nutritional value, alimentary proteins may also have a functional role. Indeed, irrespective of age, minute amounts of proteins (estimated at 1 ng per hour per square cm) pass unchanged through the intestinal mucosa. Except for that, proteins and peptides are internalised by endocytosis through the luminal surface of the enterocytes and further degraded into amino acids or processed into smaller peptides (47-50). These pass through the basolateral surface of the enterocytes into the *lamina propria*. There are reasons to believe that the enterocytes directly present antigenic peptides to T lymphocytes (51). Other protein fragments, or even very small amounts of intact proteins can directly activate the different cells of the *lamina propria*. For example, a peptide sequence of cow's milk b -casein, produced by hydrolysis in the intestinal lumen, can pass through the epithelium to exert an action similar to that of metenkephalin in the *lamina propria* (52,53).

Thus, the role of the intestinal mucosa is to permanently control the exchange of nutrients, water, and electrolytes between the content of the digestive tract and the body proper. This control depends on two major regulation systems -- one at the level of the mucosa and the other at the level of the body as a whole.

At the level of the intestinal mucosa, nutrients taken during a meal, or microbial agents causing an intestinal infection, act directly on the epithelium, which is the exchange organ. They also act directly or indirectly on the cells of the *lamina propria* which control the expression of the transport systems and the speed of renewal of the enterocytes. The result is a constant adaptation of the exchange systems. It is the fast restoration of the mucosa that most probably explains the usually quick recovery after an intestinal infection.

At the level of the body as a whole, the regulation of the mucosal function acts through the many types of cells present in the *lamina propria*. For example, afferent and efferent nervous fibres participate in the control of the epithelial functions via neuro-hormonal synapses that are transitorily disturbed during acute diarrhoeas (54).

Finally, from a pathophysiological viewpoint, the concept of the intestinal mucosa as an organ with multiple cellular interactions allows a better understanding of certain pathological situations, such as the chronicity of cow's milk allergy or other inflammatory conditions (55,56). It should also stimulate pharmacological research aimed at protecting or repairing the intestinal mucosa during infections (57).

## REFERENCES


1. Desjeux JF, Touhami M. Alimentation, Génétique et Santé de l'Enfant. Paris: L'Harmattan, 1994:271.
2. Desjeux D, Favre I, Simongiovanni J, Varge L, Caillol MH, Taponnier S, Desjeux JF. Why is oral therapy associated with drugs in the treatment of diarrhea? *J Pediatr Gastroenterol Nutr* 1996;22:112-4.
3. Desjeux D, Favre I, Simongiovani J. Anthropologie d'une maladie ordinaire. Etude de la diarrhée de l'enfant en Algérie, Thaïlande, Chine et Egypte, Paris: L'Harmattan, 1993
4. Edet EE. Fluid intake and feeding practices during diarrhoea in Odukpani, Nigeria. *East Afr Med J* 1996;73:289-91.
5. Okeke TA, Okafor HU, Amah AC, Onwuasigwe CN, Ndu AC. Knowledge, attitude, practice, and prescribing pattern of oral rehydration therapy among private practitioners in Nigeria. *J Diarrhoeal Dis Res* 1996;14:33-6.
6. Akpede GO, Omotara BA, Shettima AG. Awareness claims versus actual knowledge of oral rehydration therapy and the salt sugar solution in northeastern Nigeria. *J Trop Pediatr* 1996;42:180-2.
7. Fullana Montoro AM, Artero Mora A, Sales Domene C, Mir Plana B, Morales Varela M. Infantile diarrhea: knowledge and attitudes of mothers at a primary care center. *Aten Primaria* 1996;17:284-7.
8. Elliott EJ, Backhouse JA, Leach JW. Pre-admission management of acute gastroenteritis. *J Paediatr Child Health* 1996;32:18-21.
9. Desjeux JF, Briend A, Butzner JD. Oral rehydration solution in the 2000: pathophysiology, efficacy and effectiveness. *Baillère's Clin Gastroenterol* 1997;11:509-27.
10. Mahalanabis D. Current status of oral rehydration as a strategy for the control of diarrhoeal diseases. *Indian J Med Res* 1996;104:115-24.
11. Desjeux JF, Nath SK, Taminiau I. Organic substrate and electrolyte solutions for oral rehydration in diarrhea. *Ann Rev Nutr* 1994;14:321-42.
12. Desjeux JF, Tannenbaum C, Tai YH, Curran PF. Effects of sugars and amino acids on sodium movement across small intestine. *Am J Dis Child* 1977;131:331-40.
13. Phillips SF. Diarrhea: a current view of the pathophysiology. *Gastroenterology* 1972;63:495-518.

14. Nath SK, Huang X, L'helgoualc'h A, Rautureau M, Bisalli A, *et al.* Relation between chloride secretion and intracellular cyclic adenosine monophosphate in a cloned human intestinal cell line HT-29 cl 19A. *Gut* 1994;35:631-6.

15. Davidson GP, Gall DG, Petric M, Butler DG, Hamilton JR. Human rotavirus enteritis induced in conventional piglets. Intestinal structure and transport. *J Clin Invest* 1977;60:1402-9.

16. Davidson GP, Barnes GL. Structural and functional abnormalities of the small intestine in infants and young children with rotavirus enteritis. *Acta Paediatr Scand* 1979;68:181-6.

17. Grasset E, Heyman M, Dumontier AM, Lestradet H, Desjeux JF. Possible sodium and D-glucose cotransport in isolated jejunal epithelium of children. *Pediatr Res* 1979;13:1240-6.

18. McClung HJ, Butler DG, Kerzner B, Gall DH, Hamilton JR. Transmissible gastroenteritis. Mucosal ion transport in acute viral enteritis. *Gastroenterology* 1976;70:1091-5.

19. Argenzio RA, Liacos JA, Levy ML, Meuten DJ, Lecce JG, Powell DW. Villous atrophy, crypt hyperplasia, cellular infiltration, and impaired glucose-Na absorption in enteric cryptosporidiosis of pigs. *Gastroenterology* 1990;98:1129-40.

20. Butler DG, Gall DG, Kelly MH, Hamilton JR. Transmissible gastroenteritis. Mechanisms responsible for diarrhea in an acute viral enteritis in piglets. *J Clin Invest* 1974;53:1335-42.

21. Heyman M, Darmon N, Dupont C, Dugas B, Hirribaren A, Blaton MA, Desjeux JF. Mononuclear cells from infants allergic to cow's milk secrete tumor necrosis factor alpha, altering intestinal function. *Gastroenterology* 1994;106:1514-23.

22. Desjeux JF, Ramakrishna BS. Resistant starch as adjuvant in oral rehydration solutions for the treatment of diarrhea. Nestlé Foundation. Annual report, 1987:75-100.

23. Binder HJ, Mehta P. Short-chain fatty acids stimulate active sodium and chloride absorption in vitro in the rat distal colon. *Gastroenterology* 1989;96:989-96.

24. Phillips SF, Giller J. The contribution of the colon to electrolyte and water conservation in man. *J Lab Clin Med* 1973;81:733-46.

25. Argenzio RA, Moon HW, Kemeny LJ, Whipp SC. Colonic compensation in transmissible gastroenteritis of swine. *Gastroenterology* 1984;86:1501-9.

26. Rajendran VM, Binder HJ. Apical membrane Cl-butyrate exchange: mechanism of short chain fatty acid stimulation of active chloride absorption in rat distal colon. *J Membr Biol* 1994;141:51-8.

27. Kraehenbuhl JP, Neutra MR. Molecular and cellular basis of immune protection of mucosal surfaces. *Physiol Rev* 1992;72:853-79.

28. Winner L, 3d, Mack J, Weltzin R, Mekalanos JJ, Kraehenbuhl JP, Neutra MR. New model for analysis of mucosal immunity: intestinal secretion of specific monoclonal immunoglobulin A from hybridoma tumors protects against *Vibrio cholerae* infection. *Infect Immun* 1991;59:977-82.

29. Guarino A, Casola A, Bruzzese E, Saini M, Nitsch L, Rubino A. Human serum immunoglobulin counteracts rotaviral infection in Caco-2 cells. *Pediatr Res* 1996;40:881-7.

30. Guarino A. Active treatment of viral diarrhea with passive immunotherapy. *J Pediatr Gastroenterol Nutr* 1996;23:337.

31. Mitra AK, Mahalanabis D, Ashraf H, Unicomb L, Eeckels R, *et al.* Hyperimmune cow colostrum reduces diarrhoea due to rotavirus: a double-blind, controlled clinical trial. *Acta Paediatr* 1995;84:996-1001.

32. Powell DW. New paradigms for the pathophysiology of infectious diarrhea (editorial comment). *Gastroenterology* 1994;106:1705-7.

33. Crowe SE, Powell DW. Fluid and electrolyte transport during enteric infections. *In:* Blaser MJ, Smith PD, Ravdin JI, *et al.* editors. Infections of the gastrointestinal tract. New York: Raven; 1995:107-41.

34. Huang XP, Heyman M, Nath SK, Castagna M, Desjeux JF. Distinct signalling mediates chloride secretion induced by tumor promoter bile salts and phorbol esters in human colonic cells. *Int J Oncol* 1995;6:1159-63.

35. Battu S, Clément G, Heyman M, Wal JM, Cook-Moreau J, *et al.* Production of arachidonic acid metabolites by the colon adenocarcinoma cell line HT29 cl.19A and their effect on chloride secretion. *Cancer Lett* 1997;116:213-23.

36. Hyun CS, Binder HJ. Mechanism of leukotriene D4 stimulation of $Cl^-$ secretion in rat distal colon in vitro. *Am J Physiol* 1993;265:G467-73.

37. Peterson JW, Whipp SC. Comparison of the mechanisms of action of cholera toxin and the heat-stable enterotoxins of *Escherichia coli*. *Infect Immun* 1995;63:1452-61.

38. Beubler E, Schirgi-Degen A, Pabst MA, Pothoulakis C, LaMont JT. Effects of purified *Clostridium difficile* toxin A in the small intestine of the rat in vivo. *Nat Toxins* 1993;1:369-75.

39. Peterson JW, Cantu J, Duncan S, Chopra AK. Molecular mediators formed in the small intestine in response to cholera toxin. *J Diarrhoeal Dis Res* 1993;11:227-34.

40. Eklund S, Brunsson I, Jodal M, Lundgren O. Changes in cyclic 3'5'-adenosine monophosphate tissue concentration and net fluid transport in the cat's small intestine elicited by cholera toxin, arachidonic acid, vasoactive intestinal polypeptide and 5-hydroxytryptamine. *Acta Physiol Scand* 1987;129:115-25.

41. Liang YF, Peterson JW, Reitmeyer JC. Inhibitory effect of aspirin on cholera toxin-induced phospholipase and cyclo-oxygenase activity. *FEMS Microbiol Lett* 1990;60:137-41.

42. Farris RK, Tapper EJ, Powell DW, Morris SM. Effect of aspirin on normal and cholera toxin-stimulated intestinal electrolyte transport. *J Clin Invest* 1976;57:916-24.

43. Eherer AJ, Hinterleitner TA, Petritsch W, Holzer-Petsche U, Beubler E, Krejs GJ. Effect of 5-hydroxytryptamine antagonists on cholera toxin-induced secretion in the human jejunum. *Eur J Clin Invest* 1994;24:664-8.

44. Beubler E, Schirgi-Degen A, Gamse R. Inhibition of 5-hydroxytryptamine- and enterotoxin-induced fluid secretion by 5-HT receptor antagonists in the rat jejunum. *Eur J Pharmacol* 1993;248:157-62.

45. Rask-Madsen J, Bukhave K, Beubler E. Influence on intestinal secretion of eicosanoids. *J Intern Med* 1990;732(Suppl):137-44.

46. Gaboriau-Routhiau V, Moreau MC. Gut flora allows recovery of oral tolerance to ovalbumin in mice after transient breakdown mediated by cholera toxin or *Escherichia coli* heat-labile enterotoxin. *Pediatr Res* 1996;39:625-9.

47. Isolauri E, Gotteland M, Heyman M, Pochart P, Desjeux JF. Antigen absorption in rabbit bacterial diarrhea (RDEC-1). In vitro modifications in ileum and Peyer's patches. *Dig Dis Sci* 1990;35:360-6.

48. Heyman M, Ducroc R, Desjeux JF, Morgat JL. Horseradish peroxidase transport across adult rabbit jejunum in vitro. *Am J Physiol* 1982;242:G558-64.

49. Heyman M, Desjeux JF. Antigen handling by intestinal epithelial cells. *In:* Kaiserlian D, editor. Antigen presentation by intestinal epithelial cells. London: Landes, 1996:1-19.

50. Terpend K, Boisgerault F, Blaton MA, Desjeux JF, Heyman M. Protein transport and processing by human HT29-19A intestinal cells: effect of INF gamma. *Gut* 1998 (in press).

51. Kaiserlian D, Vidal K, Revillard JP. Murine enterocytes can present soluble antigen to specific class II-restricted CD4+ T cells. *Eur J Immunol* 1989;19:1513-6.

52. Tome D, Dumontier AM, Hautefeuille M, Desjeux JF. Opiate activity and transepithelial passage of intact beta-casomorphins in rabbit ileum. *Am J Physiol* 1987;253:G737-44.

53. Hautefeuille M, Brantl V, Dumontier AM, Desjeux JF. In vitro effects of beta-casomorphins on ion transport in rabbit ileum. *Am J Physiol* 1986;250:G92-7.

54. Powell DW. Neuroimmunophysiology of the gastrointestinal mucosa: implications for inflammatory diseases. *Trans Am Clin Climatol Assoc* 1994;106:124-38.

55. Saidi D, Heyman M, Kheroua O, Boudraa G, Bylsma P, *et al.* Jejunal response to beta-lactoglobulin in infants with cow's milk allergy. *C R Acad Sci* [III] 1995;318:683-9.

56. Heyman M, Grasset E, Ducroc R, Desjeux JF. Antigen absorption by the jejunal epithelium of children with cow's milk allergy. *Pediatr Res* 1988;24:197-202.

57. Mahraoui L, Heyman M, Plique O, Droy-Lefaix MT, Desjeux JF. Apical effect of diosmectite on damage to the intestinal barrier induced by basal tumour necrosis factor-a. *Gut* 1997;40:339-43.

# Caretakers' Knowledge and Preparation Abilities of Salt-Sugar Solution in North-Eastern Nigeria

**George O Akpede[1], Babatunji A Omotara[2], Glenn D Webb[3], and John O Igene[4]**

*[1]Department of Paediatrics, Edo State University, Ekpoma, Nigeria; [2]Departments of Community Medicine, [3]Sociology and Anthropology, [4]Food Science and Technology, University of Maiduguri, Maiduguri, Nigeria*

## ABSTRACT

Awareness and knowledge of oral rehydration therapy (ORT) and preparation abilities of salt-sugar solution (SSS) were investigated by means of focus group discussions and complemented by a structured questionnaire survey of mothers in rural and urban areas of north-eastern Nigeria. ORT awareness was high with some intra-regional variations. Perception of ORT function was, however, grossly unrealistic, with a third to four-fifth of mothers expecting ORT to stop diarrhoea. At least one quarter of mothers lacked adequate SSS preparation abilities and the materials and ingredients required for its preparation. Re-evaluation of the content and method of imparting health education messages in ORT promotion is recommended. Such messages should emphasise the function of ORT. It is also recommended that standardised cups for water, salt and sugar measurements be provided to households as a ready means of ensuring the correct preparation of SSS in the home-based management of diarrhoea.

***Key Words:*** Oral rehydration solutions; Knowledge, attitudes, practice; Diarrhoea

## INTRODUCTION

The use of home-made fluids is pivotal to the WHO/CDD programme for the appropriate home management of diarrhoea and the prevention of dehydration and related deaths (1). To achieve this goal, the salt-sugar solution (SSS) is recommended for home use in Nigeria (2,3) as in other developing countries (4). The choice of SSS is based on the presumed availability of materials for its preparation, the ease with which the preparation skills can be imparted to mothers and the preference to avoid packaged materials, which might come to be regarded as drugs by mothers (5).

The safety of user-prepared SSS is of prime importance (6,7) and there is an expected decline in preparation skills in the absence of continuous reinforcement. Also, perceptions of the functions of oral rehydration therapy (ORT) are relevant to its use (1). These factors underscore the need for frequent re-appraisals of users' knowledge of SSS preparation abilities and their expectations of treatment with ORT. The aims are to ensure the safety of SSS and to determine new areas in need of emphasis in health education programmes.

In an earlier study in north-eastern Nigeria (8), about one year after the launching of the nation-wide ORT programme in 1986, 20% of mothers had forgotten how to prepare SSS correctly and another 14% lacked the materials required for its preparation. Since 1986, public education campaigns have tried to promote ORT awareness, preparation and use. From March 1993 to April 1994, we evaluated ORT knowledge and SSS preparation abilities and "readiness" in communities in north-eastern Nigeria, using focus group discussions (FGDs) and a survey of mothers, as an assessment of the success of ORT promotion campaigns in the region.

---

*Correspondence and reprint requests should be addresed to*:
George O Akpede,FWACP,Department of Paediatrics, College of Medicine, Edo State University, PMB 14, Ekpoma, Edo State, Nigeria

## SUBJECTS AND METHODS

### Study population

The Kanuris and Buras, two of the major ethnic groups in north-eastern Nigeria, formed the study population. The Kanuris are predominantly Muslims and have a low formal literacy level (9). In contrast, the Buras, a mixed Christian/Muslim group, have a higher literacy level and have also had a longer period of contact with Western education and medicine (9). Members of both ethnic groups frequently practise polygamy and are mostly farmers, although commercial activities are also undertaken in their towns and cities.

### Study site

The study was conducted in Bama (a rural Kanuri settlement), in Hawul (a rural settlement of the Buras) Local Government Areas (LGAs) and in Maiduguri, the Borno State capital and the main urban settlement of both ethnic groups. FGDs among rural Kanuris were held in Banki and Kumshe villages in Bama LGA and among rural Buras in Chuung and Shaffa villages of Hawul LGA. In the Maiduguri urban area, FGDs among the Kanuris were held in Gwange and Hausari wards, and in Wulari ward for urban Buras. The wards are relatively ethnically homogenous.

Maiduguri has a teaching hospital, a specialist hospital (with two annexes) as well as private clinics and hospitals, maternity homes, health centres, pharmacies, and patent medicine shops (chemists). Bama LGA has one general hospital and a comprehensive health centre, dispensaries, chemist shops and a few pharmacies. Hawul LGA has one general hospital, Christian missionary clinics, dispensaries, one pharmacy, and chemist shops.

**Data collection methods**

Both FGDs and a structured questionnaire-based survey were used in data collection. Each FGD took approximately 45 minutes to one hour and was conducted by Kanuri and Bura facilitators and note-takers (postgraduate and graduate students in sociology or anthropology, fluent in the respective languages). Information was sought in FGDs on participants' knowledge of the preparation of SSS (in terms of ratio of salt to sugar, volume of water used, and duration of use of a freshly prepared solution), administration of SSS and knowledge of the function of ORT. Sixteen FGDs were held, four for each ethnic group, in both urban and rural settings. Each focus group consisted of 6 to 8 participants of the same age and sex (younger mothers <35 years, older women/grandmothers >35 years, younger fathers <35 years and older men/grandfathers >35 years). The FGDs were held in the village hall, chief's residences, or school compounds, as dictated by circumstances of availability and convenience.

Qualitative data from the FGDs were analysed by transcribing FGD audio tapes that were then translated into English, coded and subjected to content analysis of common/recurrent themes across FGDs to identify majority as well as minority points of view. The results are presented in narratives.

Two hundred and sixty respondents from each ethnic group, i.e., 130 from urban and 130 from rural areas, were interviewed in the survey. The sample size of 130 per ethnic group per locale was calculated on the basis of a diarrhoea incidence rate of 6 episodes per child per year in northern Nigeria (10) to obtain an adequate number of children in each group who were likely to have had diarrhoea in a recall period of 2 weeks. There were 3 non-responders in the urban areas but none in the rural areas.

A multistage sampling technique was used in the survey. Each LGA was successively stratified into districts, villages and wards, and proportional random samples of "households" were then drawn from the wards; proportional random samples were drawn from the wards in the urban settlements. A household was defined as a family unit consisting of the father, mother(s), children, co-residing unmarried relatives and helpers (if any), and grandmothers (if any). The wife or head wife (in a polygamous family) was the respondent in a sampled household; usually, the first or head wife in a polygamous family in the 2 ethnic groups is responsible for the supervision of domestic activities, including the care of children, hence she was chosen as the respondent in these households.

The survey was conducted during the non-farming period. It used both open-ended and closed questions in a pre-tested and validated structured questionnaire. The questionnaire was administered in both urban and rural areas during a face-to-face interview of respondents by trained female interviewers. Interviewers were selected from among female secondary school graduates, and female teachers, resident in the study areas. Ten interviewers, five resident in the urban areas and five resident in the rural areas, were selected and trained per ethnic group. The questions were administered to the respondents in their languages. The training reference manual for interviewers contained a checklist of materials recommended for the preparation of SSS in Nigeria (a 3-mL teaspoon, coke bottle [300 mL] or beer bottle [600 mL], clean bowl, sugar [granulated or in cube form], and table salt) (3), as well as the steps involved in SSS preparation (hand-washing, preferably with soap and water), measurements of water, sugar and salt (including levelling of the teaspoon measures with the little finger, and mixing and tasting of the solution). The respondents' answers were compared to the correct ones. The proper way of preparing SSS as advocated by the Nigerian CDD programme is to wash the hands with soap and water and dry them with a clean napkin or towel; measure out the water (2 full Coca-Cola bottles [2 x 300 mL] or 1 full beer bottle [1 x 600 mL]) into the clean bowl; measure out the salt and sugar into the water (1 level [levelled to the edge] 3 mL teaspoon of salt and 10 level 3 mL teaspoons of sugar or 5 cubes of sugar); mix thoroughly the solution of salt and sugar with a long spoon or ladle until all particles are dissolved and

taste the solution after completing the preparation (if it tastes more salty than tears, pour away and start again); and make a fresh solution every day.

The following definitions were used in data analysis:

(1) *SSS readiness*: Availability of materials and ingredients required for the preparation of SSS in the home and ability to present all the materials required in the preparation of SSS for on-the-spot inspection;

(2) *Correct preparation of SSS*: Ability to describe the sequence of preparation of SSS correctly;

(3) *Duration of use of freshly prepared SSS*: Respondent's answer is judged as appropriate if she indicates 24 hours (one day) or less;

(4) *Knowledge of the purpose of ORT*: Answers, such as ORT "*replaces water, salt*", or "*gives energy*" were considered appropriate, and answers, such as "*don't know*" or "*stops diarrhoea*" inappropriate.

These definitions were based on an assessment of the answers to questions, including "*what is the purpose of giving ORT/SSS to children with frequent stools?*", "*how do you prepare SSS?*", "*how long do you keep SSS after its preparation?*", and "*can you please show me the materials that you have for preparing SSS?*". These were open-ended questions and the interviewer either wrote the response verbatim or compared it with the description in the training manual in judging the response appropriate or not, depending on what the instruction to the interviewer was in relation to each question in the training manual. When the respondent's answer was judged inappropriate by the interviewer, she noted the respondent's incorrect responses, indicated which steps were missed in the preparation of SSS, or in the case of materials for the preparation of SSS, the missing items.

Survey data were analysed, using the Epi Info statistical programme (11); p values less than 0.05 for chi-squared test with Yates' correction were considered statistically significant.

## RESULTS

### Focus group discussions

The opinions described in the results are majority or consensus opinions. The Kanuris and Buras are considered together, but we comment on marked differences between them when present. In general, knowledge of ORT/SSS and patterns of use were better in the urban areas than in the rural areas, and better among the Buras than the Kanuris in both the FGDs and survey.

### Awareness, acceptability and perceptions of ORT/SSS

In all but one of the FGDs, all the participants had heard of ORT/SSS; the only exception was in the only group containing both men and women (of all ages) in Hawul LGA in which all 4 men present were unaware, whereas, all 5 women were aware. There were otherwise no differences in awareness related to age or sex or domicile (rural versus urban). Participants in rural Kanuri settlements said that they were informed mainly through public demonstrations. All other groups learned about ORT/SSS at formal health facilities; the Church was also described as an important source of information among Buras in the rural areas. Neither ethnic group had a terminology for ORT separate from that for SSS, hence ORT/SSS is used frequently without distinction in the presentation. The Kanuris call SSS *nji manda,* while the Buras call it *yimir una or yimir suga*, which, literally translated, means "sugar water."

ORT/SSS was generally well accepted among both ethnic groups. Rural Kanuris noted that "*we have accepted SSS as a medication prescribed for us by the doctor.*" Urban Kanuris noted that SSS is "*cheap and simple to mix*" and is "*readily available unlike other medicines which are costly.*" Bura participants noted that "*SSS is the first thing you prepare before taking the child to the hospital*" and that "*this treatment is helping many people these days and it may be so effective that there is no need to visit the hospital.*" Two of the 6 young urban Bura men in FGD also noted that SSS is capable of "*stopping diarrhoea without the need to give other medicines.*"

Rural Kanuris were unlikely to use SSS without the advice of a health worker. In contrast, several participants in FGDs among urban Kanuris and both rural and urban Buras reported using SSS on their own before visiting a health facility. Two young rural Bura women noted that, when a child with diarrhoea was taken to the hospital, health workers at the government and mission health facilities usually asked if SSS had been administered at home. However, about half of the participants in FGDs among urban Kanuris noted that whereas SSS was suitable for natural diarrhoea (*yerta allaye*), it was not suitable for teething diarrhoea (*yerta kellinye*) and diarrhoea associated with rectal prolapse (*dankanama*). The types of diarrhoea described by the Kanuris include natural or general diarrhoea (*yerta allaye*), teething diarrhoea (*yerta kellinye*), diarrhoea associated with anal protrusion (*dankanama*), diarrhoea due to hunger (*suro de*), diarrhoea due to whooping cough (*kukkuwu*), diarrhoea due to exposure to the sun (*yerta soro zan kausoye*) and breast-milk diarrhoea (*yerta chambe*). Types of diarrhoea described by the Buras include diarrhoea associated with measles (*zhola*), tonsillitis or "*long uvula disease*" (*belu belu*), teething, onset of the rains (*yabwong*), stomach pain (*kitir kuta*), worms (*jum jum*) and (*hyinna)*: the child is "*sick-looking, has stools with a bad smell and has a rash between the thighs.*" The inter-ethnic variations in the perceptions and treatment of diarrhoea in the study area have been described more fully elsewhere (12).

Generally, the Kanuris had minimal knowledge of the purpose of ORT/SSS. Only in the FGD involving older women in the urban area did 3 of 6 participants adequately describe the function of SSS, stating that it "*stops the body water from finishing*" and "*prevents loss of strength*" when a child has diarrhoea; one participant even noted that "*SSS functions in the same way as the drips given in hospital when given to a child with diarrhoea.*" Participants in the rural areas simply noted that they had not been told by health care workers what the purpose of ORT/SSS and that "*we do not know what this is for, that is for the doctor to bother with.*"

In contrast, the Buras, urban or rural, generally knew what the purpose of ORT/SSS was and used descriptions, such as "*it replaces the salt and water lost from the body.*" However, an urban participant also noted that SSS "*reduces diarrhoea*" and another that "*another medicine is used at times to stop diarrhoea,*" while a younger woman in the rural area stated that herbal remedies were needed to "*help stop diarrhoea.*" A younger female participant in the rural area who disagreed with this latter opinion noted that some people describe SSS as ineffective, because "*the preparation is not made correctly*" and "*some fail to understand that SSS only replaces the lost water of the body.*"

**Survey**

In the survey, 199 (77%) of the 258 Kanuri mothers (there were two non-responders) and 218 (84%) of the 260 Bura mothers were aware of ORT/SSS; the difference in awareness approached statistical significance (uncorrected $c^2$=3.72; p=0.054). There was no significant difference between the two ethnic groups in the proportion of respondents who received their ORT/SSS awareness from formal health facilities, but a higher proportion of Buras gained their awareness from other sources, including religious meetings (Table I).

Perception of the function of ORT/SSS by those respondents who knew about ORT is shown in the Table II. A significantly lower proportion of the Bura respondents expected ORT to stop diarrhoea and a higher proportion expected it to give energy.

One hundred seventy-nine (90%) of the 199 Kanuri and 184 (84%) of the 218 Bura mothers (uncorrected $c^2$=2.84; p=0.092) who were ORT/SSS-aware reported experience with it. Of these, 199 Kanuri mothers, 183 (92%), and 208 (95%) of the 218 Bura mothers (uncorrected $c^2$=2.12; p=0.145) said that they would use SSS in future when their child had diarrhoea. One hundred fifty-one (84%) of the179 Kanuri mothers and 144 (78%) of the 184 Bura mothers (uncorrected $c^2$=7.34; p=0.007) who reported experience with SSS had found it effective. The reasons given for thinking that SSS was effective are listed in Table III, and included cessation of diarrhoea in 96 (64%) of the 151 Kanuri and 41 (29%) of the 144 Bura mothers; these proportions are strikingly different (uncorrected $c^2$=36.52; p<0.00001).

Table I. Sources of ORT/SSS information among ORT/SSS-aware respondents in the survey*

| Information source | Kanuris (n=199) | Buras (n=218) | p** |
|---|---|---|---|
| Formal health facilities | 156 (78%) | 163 (75%) | 0.45 |
| Friends and relations | 33 (17%) | 43 (20%) | 0.48 |
| Electronic media | 9 (5%) | 22 (10%) | 0.048 |
| Public or other demonstrations | 3 (2%) | 17 (8%) | 0.006 |
| Religious meetings | 0 | 10 (5%) | 0.002 |
| Chemists/pharmacies | 3 (2%) | 3 (1%) | 1.00 |

*Total responses larger than sample sizes due to multiple responses
**Probability: chi-squared test per row (Yates corrected; degrees of freedom = 1)

Table II. Function of ORT/SSS described by ORT/SSS-aware survey respondents

| Description of function of ORT/SSS | Kanuris (n=199) | Buras (n=218) | p* |
|---|---|---|---|
| SSS stops diarrhoea | 126 (63%) | 105 (48%) | 0.003 |
| SSS gives energy | 40 (20%) | 75 (34%) | 0.002 |
| SSS gives energy and stops diarrhoea | 18 (9%) | 23 (11%) | 0.73 |
| No idea | 15 (8%) | 15 (7%) | 0.94 |

*Probability: chi-squared test per row (Yates corrected; degrees of freedom =1)

Among the 363 mothers (both groups joined) who had reported experience with ORT/SSS, the majority (80%) liked SSS. The others had complaints about its taste or smell, the need for frequent preparation of the solution, and dislike by children (Table IV). Only the Bura mothers complained of the need to prepare SSS frequently.

**Preparation and administration of SSS**

Knowledge of the preparation of SSS as expressed in FGDs among the Kanuris was generally limited and it was only in the FGD involving older women in the urban area that the participants correctly described SSS preparation. The several recipes given for SSS preparation were descriptions of over-dilute or over-concentrated solutions. Three of the 8 older men/grandfathers and 1 of the 6 older women/grandmothers in the rural areas even described different recipes for adults and children. Ten of the 28 participants in the rural areas did not state the sizes of the recipients and spoons to be used in measuring salt, sugar and water. While in the urban areas, the recipes were nearer to recommended (3) quantities of sugar and salt, they were still largely inadequate (inaccurate quantity of water, no description of levelling of salt and sugar in the spoons). Knowledge of administration of SSS was also limited in the rural areas, with for example 2 of the 6 younger men stating that SSS should be given "*one teaspoon thrice daily.*"

In contrast to the Kanuris, all Bura participants in FGDs, except 2 of the 6 urban younger men who described incorrect measurements of water, correctly described the preparation of SSS, including levelling of the teaspoon measurements of salt and sugar. Overall, knowledge of administration of SSS was also more appropriate among the Buras. The Bura women noted that SSS was to be given

"*whenever the baby vomits or passes watery stools or feels thirsty.*" However, an urban younger man said that SSS was given "*3 to 4 times a day.*"

In the survey, about one-quarter of the ORT/SSS-aware respondents from both ethnic groups either described an incorrect method of SSS preparation or stated that they did not know its preparation (Table V); there was no significant difference between the two ethnic groups in the proportion of the ORT/SSS-aware respondents who described an incorrect method. Among those who gave incorrect descriptions, about half to two-thirds (depending on the ethnic group) missed several steps (Table V). The incorrect steps among the Kanuris involved lack of hand washing in three cases, and incorrect measurements of water in 12 cases, of salt in 17 cases, and of sugar in 26 cases (multiple responses were allowed). Among the Buras, the incorrect steps involved lack of hand washing in two cases, lack of tasting of the solution in one case, and incorrect measurement of water in 10 cases, salt in 22 cases, and sugar in 33 cases. One hundred fifty-eight (79%) of the 199 ORT/SSS-aware Kanuri mothers and 170 (78%) of the 218 ORT/SSS-aware Bura mothers ($c^2$=0.12; p=0.725) said that a fresh solution was to be made every day, while others stated longer periods of use.

Table III. Reasons given by ORT/SSS-aware mothers who had reported experience with SSS as to why it was thought to be effective when used

| Reasons | Kanuris (n=151) | Burns (n=218) | p* |
|---|---|---|---|
| Diarrhoea stopped | 96 (64%) | 41 (28%) | <0.00001 |
| Child gained energy | 21 (14%) | 31 (22%) | 0.12 |
| Diarrhoea stopped and child gained energy | 6 (4%) | 12 (8%) | 0.19 |
| Child got better | 0 | 14 (5%) | <0.0003 |
| Child maintained its weight | 0 | 7 (5%) | 0.006 |
| None given | 28 (19%) | 39 (27%) | 0.11 |

*Probability: chi-squared test per row (Yates corrected; degrees of freedom = 1) or, when appropriate, Fisher's exact test (two-sided)

Table IV. Complaints about SSS by ORT/SSS-aware mothers with reported experience with SSS

| Complaints | Kanuris (n=179) | Buras (n=184) | p* |
|---|---|---|---|
| None | 141 (79%) | 148 (80%) | 0.79 |
| Taste/smell disliked by children | 38 (21%) | 43 (23%) | 0.72 |
| Needed to be prepared frequently | 0 | 19 (10%) | <0.0001 |

Total responses larger than sample sizes due to multiple responses
*Probability: chi-squared test per row (Yates corrected; degrees of freedom = 1)

The availability of materials and ingredients for SSS preparation in the ORT/SSS-aware households is described in Table VI. Among the Kanuri and Bura mothers, 76% and 79%, respectively, ($c^2$=0.38; p=0.537), were able to present the materials for inspection, while the remainder lacked one or more items (Table VI). Among the Kanuris, 26 mothers lacked bottles, four lacked bowls, 19 lacked teaspoons, 28 lacked sugar, and 13 lacked salt. Among the Buras, 22 mothers lacked bottles, 8 lacked bowls, 16 lacked teaspoons, 13 lacked sugar, and 7 lacked salt.

## DISCUSSION

In the wake of the nation-wide launching of the Nigerian ORT programme in 1986 (8), ORT/SSS-awareness achieved in the region we studied has apparently been sustained. However, awareness has not been translated into an expected level of use (2) and knowledge of purpose of ORT/SSS is largely

inaccurate. Also, there is a potential for those having unrealistic expectations of SSS to discontinue its use or use it with antidiarrhoeal drugs. In addition, SSS preparation abilities need to be improved.

**Table V.** SSS preparation abilities of ORT/SSS-aware Kanuri and Bura mothers in survery

| | Kanuri (n=199) | Buras (n=218) | p* |
|---|---|---|---|
| Description of SSS preparation | | | |
| Correct | 147 (74%) | 156 (72%) | 0.68 |
| Incorrect | 38 (19%) | 40 (18%) | 0.94 |
| Don't know | 14 (7%) | 22 (10%) | 0.35 |
| Number of incorrect steps among mothers who gave incorrect descriptions | | | |
| One | 20 (10%) | 13 (6%) | 0.17 |
| Two | 9 (5%) | 22 (10%) | 0.049 |
| >Two | 9 (5%) | 5 (2%) | 0.32 |

* Probability: chi-squared test per row (Yates corrected; degrees of freedom = 1)

**Table VI.** Availability of materials and ingredients for SSS preparation in households of ORT/SSS-aware mothers

| | Kanuris (n=199) | Buras (n=218) | p* |
|---|---|---|---|
| Had all items lacked one or more items | 152 (76%) | 172 (79%) | 0.62 |
| One | 23 (12%) | 29 (13%) | 0.70 |
| Two | 10 (5%) | 9 (4%) | 0.84 |
| >Two | 14 (7%) | 8 (4%) | 0.19 |

*Probability: chi-squared test per row (Yates corrected; degrees of freedom = 1)

The gap between mothers' knowledge and SSS preparation abilities may be related to gaps in health education techniques, especially those that do not give mothers the opportunity to prepare and mix ORT themselves (4) or those that do not provide for reinforcement of skills after training. One Kanuri participant in an FGD indicated that "*it has been a long time that the officials taught us how to prepare SSS*".

Opportunities to reinforce skills in making SSS/ORT are needed, either through a network of village health workers or through training of community leaders/ members or voluntary bodies, such as church groups and market-women organisations in the village.

Because most people learn of ORT/SSS at the health facility, one approach might be to train community representatives in SSS preparation. These representatives could train in the communities in collaboration with hospital staff, and subsequently practise as trainers for their communities under periodic supervision. The results of a recently concluded study in Borno State, Nigeria (13), suggest that this approach is a very useful way of promoting ORT/SSS use and knowledge in rural communities.

It would appear from the results of this study that a significant proportion of the Kanuri and Bura populations is using SSS. However, how these populations are using SSS (what quantities are given to children having diarrhoea, whether SSS is commenced at the first sign of diarrhoea or after several days, how long it is used during the diarrhoeal illness, etc.) is not known. Further studies are needed to address these questions.

Reported ORT use in our study was similar to use reported in Pakistan (14), and in rural South Africa after health education campaigns (15). Reported rates of use in our study population may be greater than the actual rates. Indeed, courtesy, to please the interviewer, might introduce considerable bias. Still, the rates are believable because of governmental and non-governmental agencies' involvement in health education and ORT/SSS campaigns in north-eastern Nigeria. Perhaps, ORT promotion campaigns should shift focus from simply promoting awareness to addressing specific aspects of this awareness, such as knowledge of function of ORT/SSS, appropriateness of use, readiness for use, etc. For example,

more than one-quarter of the respondents in the survey could not correctly describe the preparation of SSS and were not SSS "ready" which means that the home management of diarrhoea would be largely inappropriate in many homes in the study area. Similar conclusions have been reached in other areas of Nigeria (7,16). We join other authors (4,17) in urging national and regional ministries of health to provide standardised cups for the measurement of water, salt and sugar to all homes country-wide to avoid the dangers that can result from the use of incorrectly prepared SSS. At current prices, it would require about US$0.25 (20 Naira) to provide a household with a measuring cup and spoon. For the Nigerian population, currently estimated at about 100 million, there would be about 1.7 million households, assuming an average of 6 persons per household (but the actual number could be considerably lower, because polygamy is common and the number of children per household tends to be much higher than 4). Thus, about US$425,000 (34 million Naira) would be required to provide measuring materials to every household. This amount is arguably not prohibitive, considering the potential savings in cost of hospitalisation, caretakers' time, etc. that could be made if the appropriate home management of diarrhoea were widely practised.

Lack of knowledge of its purpose can be an impediment to ORT use (1,4,5). It is alarming that approximately 40 to 80% of the respondents in the survey either had no idea of ORT or have an inappropriate perception of the function of ORT. Inappropriate expectations of ORT as an antidiarrhoeal drug leads to misuse of, and disappointments about oral rehydration (1,5).

A large proportion of mothers seeks treatment for diarrhoea in formal health facilities. Not surprisingly, it is there that many learned about ORT. The high user rate of health facilities in the study areas may be attributed to the erstwhile provision of free health services. In spite of extensive use of these facilities, many respondents lacked adequate SSS preparation skills, and readiness, and had inappropriate perceptions of the function of ORT. The ready recourse to the use of formal health facilities in the management of diarrhoea should be encouraged, because it is an important occasion for imparting ORT knowledge and preparation skills, but the methods of instruction appear to need improvement.

A few other observations in this study require to be commented on. The differences between the two ethnic groups in aspects of ORT/SSS-awareness and knowledge can be explained by the inter-ethnic differences in literacy level (Western education) and length of contact with Western medicine (9). We have no ready explanation as to why older women/grandmothers in FGDs among the Kanuris had a better knowledge of ORT/SSS than the younger women. Perhaps it is because of lesser restriction of the movement and interaction of the former compared to the latter which would consequently expose them to more sources of information.

The proportion of mothers who said that they would use SSS again was unexpectedly greater than the proportion that found it effective in a previous occasion. Perhaps this is because the reluctance from personal experience is balanced by the urge from promotional messages and campaigns; the introduction of bias from the respondents trying to please the interviewers may also be a factor.

Although one of the ideas behind the promotion of SSS was the need to discourage expectations of the ORS package as a drug (5), it is obvious that users also sometimes consider SSS as a drug. The problem with this perception is that it may lead to inappropriate use as indicated by one FGD participant who described the administration of SSS as "*one teaspoon thrice daily.*" This aspect would also need more attention in health education programmes.

In summary, ORT awareness is high in the north-eastern region of Nigeria. Yet, knowledge of the purpose of ORT is grossly inadequate in all the study locations. SSS preparation abilities and "readiness" are also inadequate in an important proportion of the study population. We recommend re-evaluation of health education techniques and messages relating to ORT/SSS use, to incorporate knowledge of ORT function, details of preparation and appropriateness of use. There is also the need to emphasise with more vigour the need of adequate SSS preparation abilities and of keeping the required ingredients at hand.

**ACKNOWLEDGEMENTS**

Financial support for this research was provided by the Applied Diarrheal Disease Research Project (ADDR) at Harvard University by means of a Co-operative Agreement with the U.S. Agency for International Development. The critical review of the manuscript by Dr. Fitzroy J Henry and Dr. Johannes Sommerfeld, both of ADDR, is gratefully acknowledged. Ms Elizabeth Joseph, University of Maiduguri, provided secretarial assistance.

**REFERENCES**

1. Levine NE. The determinants of correct use of home-based oral rehydration therapy: a critical review. Prepared for Diarrhoeal Disease Control Programme of WHO, Geneva, 1990.
2. Nigeria. Federal Ministry of Health, In collaboration with WHO, UNICEF, USAID/CCCD. Lagos: Nigeria Control of Diarrhoeal Diseases (CDD) Programme, 1991-1995.
3. Grange AO, Okeahialam TC, Seriki O, *et al*. Standardization of home-made salt sugar solution for the treatment of acute diarrhoeal diseases of childhood in Nigeria. *Nig J Paediatr* 1985;12:41-7.
4. Muninjaya AA, Tangking W, Soetjiningsih. Home treatment of acute diarrhoea in Bali, Indonesia. *J Diarrhoeal Dis Res* 1991;9:200-3.
5. MacCormack C, Draper A. Cultural meanings of oral rehydration salts in Jamaica. *In*: van der Geest S, Whyte SR, editors. The context of medicines in developing countries. Amsterdam: Kluwer, 1988:277-88.
6. Levine MM, Hughes TP, Black RE. Variability of sodium and sucrose levels of simple sugar/salt oral rehydration solutions prepared under optimal and field conditions. *J Pediatr* 1980;97:324-7.
7. Ekanem EE, Akitoye CO, Adedeji OT, Salako QA. A quantitative assessment of the Nigerian mother's ability to prepare salt-sugar solution for the home management of diarrhoea. *J Roy Soc Health* 1993;113:243-6.
8. Omotara BA, Padonu MKO. Oral rehydration therapy in a rural community of Gongola State: response of mothers. *Nig Med J* 1987;225-8.
9. Igene JO, Akpede GO, Omotara BA, Webb GD. Comparative study of the perception and treatment of diarrhea among the Kanuris and Buras of Borno State, north-eastern Nigeria. Research proposal of the Applied Diarrheal Disease Research Project (Grant # 129).
10. Nigeria. Federal Ministry of Health. In-depth programme review of EPI/CDD, Oct/Nov 1989:21.
11. Dean AG, Dean JA, Burton A, Dicker RC. Epi Info, Version 5: a word processing, database, and statistics program for epidemiology on microcomputers. Georgia: USD, 1990.
12. Akpede GO, Omotara BA, Webb GD, Bassi AP, Shettima AG. The Kanuris and Buras of north-eastern Nigeria: implications of differences in perception and treatment of diarrhoea. *Nig J Paediatr* 1994;21(Suppl):66-79.
13. Akpede GO, Omotara BA. Oral rehydration at home - with a little help from friends. *World Health Forum* 1997;18:75-9.
14. Ahmed A, Malik IA, Iqbal M, *et al*. The use of ORS (Nimkol) in management of childhood diarrhoea by mothers in the suburbs of Rawalpindi-Islamabad. *J Pak Med Assoc* 1990;40:178-82.
15. De-Swardt R, Ijsselmuiden CB. Knowledge and practice of oral rehydration therapy in a village in Gazankulu after the introduction of the Morley spoon. *S Afr Med J* 1989;76:506-8.
16. Nwoye LO, Uwagboe PE, Madubuko GU. Evaluation of home-made salt-sugar oral rehydration solution in a rural Nigerian population. *J Trop Med Hyg* 1988;91:23-7.
17. El-Mougi M, Santosham M, Hirschhorn N, *et al*. Accuracy of mixing oral rehydration solution at home by Egyptian mothers. *J Diarrhoeal Dis Res* 1984;2:159-161.

# Enteric Bacterial Pathogens in Stools of Residents of Urban and Rural Regions in Nigeria: A Comparison of Patients with and without Diarrhoea and Controls without Diarrhoea

**Chikwelu L Obi[1], Akintoye O Coker[2], James Epoke[3], and Roland N Ndip[1]**

*[1]Department of Microbiology, Edo State University, Ekpoma, Nigeria; [2]Department of Medical Microbiology and Parasitology, [3]College of Medicine, Universities of Lagos and Calabar, Nigeria*

## ABSTRACT

A total of 2,400 stool samples comprising 1,200 from patients with diarrhoea (600 each from urban and rural area) and 1,200 similarly divided controls were obtained from school children and clinic attendants of government and private clinics around three designated study centres of Edo, Lagos and Cross River states, Nigeria. These were screened for the prevalence of bacteria that could cause diarrhoea. Diarrhoea cases in urban areas had a high prevalence rate for *Campylobacter* spp. (28%), followed by enteropathogenic *Escherichia coli* (22%), *Salmonella* spp. (17%), *Shigella* spp. (14%), *Aeromonas* spp. (5%), and *Yersinia enterocolitica* (4%), whereas in rural areas *E. coli* was the most frequently encountered pathogen (18%), followed by *Salmonella* spp. (16%), *Aeromonas* spp. (15%), *Shigella* spp. (9%), *Campylobacter* spp. (8%), and *Plesiomonas shigelloides* (8%). A similar distribution but with lower rates was noted for controls in both urban and rural areas, however, no *P. shigelloides* was isolated. Results highlight a possible difference between the prevalence of enteric bacteria in rural and urban areas and reveals the strong association of *Aeromonas* and *Plesiomonas* species with cases of diarrhoea in Nigeria.

***Key words:*** Diarrhoea; Bacteria; *Aeromonas; Plesiomonas*

## INTRODUCTION

Diarrhoea is responsible for high morbidity and mortality in children and adults in developing countries (1,2). Globally, an estimated four million children and adults die annually of infectious diarrhoeas (3). In Nigeria, diarrhoea is the second major cause of morbidity among the notifiable diseases (4). According to the Federal Statistic Bulletin (5), more than 300 children die every day as a result of dehydration and malnutrition caused by diarrhoea. These statistics are likely to be underestimated as many patients do not have access to the limited number of hospitals and health centres that are available (6). Enteric bacteria account for a substantial proportion of diarrhoea episodes worldwide (29). However, studies on the spread of these enteric bacteria in urban and rural areas have received only cursory attention in Nigeria.

Recent reports from different parts of the world show varying frequencies of bacterial agents of diarrhoea, such as *Salmonella, Shigella, Campylobacter, Aeromonas, Escherichia coli,* and *Yersinia* spp. (2-14). In China (8) and India (10), pathogenic *Escherichia coli* strains were the most frequently detected pathogens in diarrhoea cases, whereas in one study in Egypt (9) *Campylobacter* was the leading pathogen. In Nigeria, studies on bacterial aetiology of diarrhoea have either not been updated within the last ten years (2) or are restricted to only urban areas (2,6). We, therefore, decided to conduct a cross-sectional study

to determine which Gram-negative bacteria were present in stool samples obtained from patients with diarrhoea and controls without diarrhoea in urban and rural regions of Nigeria.

Correspondence and reprint requests should be addressed to: Dr. CL Obi, Department of Medical Microbiology, Medical School, University of Zimbabwe, P.O. Box A178, Avondale, Harare, Zimbabwe

## SUBJECTS AND METHODS

### Patient population and controls

Stool samples from 2,400 consecutive patients were collected for the study. Of this number, 1,200 were from patients with diarrhoea (600 each from urban and rural areas) and another 1,200 were obtained from patients without diarrhoea (controls) also equally divided between urban and rural areas. The main occupation of the villagers (rural people) is farming (cash crop or subsistence) and occasionally fishing. The stool samples were collected from school children and clinic attendees (ages: 1 to 45 years) of government and private clinics around the designated study areas of Lagos, Edo and Cross River states. Specimens were then submitted to the screening centres at the Departments of Medical Microbiology, Universities of Edo State, Lagos, and Calabar, Nigeria. Diarrhoea in this study was defined as passage of watery stools, three times or more daily. Patients who were attending the clinic for routine examinations or for evaluations of non-diarrhoeal illness were selected as controls if they had had no diarrhoea three weeks prior to the collection of specimens. The patients and controls and the rural and urban group were examined over a 12-month period (February 1993 to February 1994). The age distribution of the two groups (controls and patients) was similar.

### Specimen transport

Stool samples from patients and controls from rural and urban areas of the three designated study centres in Edo, Lagos, and Cross River states were put in Cary-Blair transport medium (manufactured within 5 to 10 hours) and cultured for *Salmonella* spp., *Aeromonas* spp., *Shigella* spp., *Campylobacter* spp., *Plesiomonas* spp., *Vibrio* spp., *E. coli, and Yersinia* spp., at the following specific institutions: Microbiology Laboratory, Department of Microbiology, Edo State University, Ekpoma, Department of Medical Microbiology and Parasitology, College of Medicine, University of Lagos, Lagos State, and Microbiology Laboratory, Department of Medical Microbiology, University of Calabar Teaching Hospital, Calabar, Cross River State, Nigeria.

### Culture media

Primary isolation media comprised Campy blood agar (CBA), Skirrow's medium (SM), MacConkey's agar (MCA), xylose desoxycholate citrate agar (XDCA) and sheep blood agar containing ampicillin, 15 m g/mL (ABA). Others were thiocitrate bile salts-sucrose agar (TCBS) and Kligler's iron (KIA). These media are recommended for the isolation of specific diarrhoeagenic agents from specimens (11,12). The media (MCA, CBA, XDCA, KIA) were prepared from commercial bases (Difco Laboratories Detroit, Michigan, USA) according to the manufacturer's instructions. ABA was prepared as previously described (15). Also, appropriate enrichment broths and alkaline peptone water (pH 8.6) were used for enhancing the isolation of the organisms (16).

## Microbiological methods

For the isolation of *Campylobacter jejuni* from stools, Skirrow's and Butzler's media were used as previously described (16,17). Briefly, the plates were incubated at 42 ° C under microaerophilic conditions for 72 hours in a candle jar. Organisms were considered to be *Campylobacter* if they were S-shaped Gram-negative bacteria, motile, oxidase-positive, grew at 42 ° C, but not at 25 ° C and were sensitive to nalidixic acid. For the isolation of *Aeromonas* and *Plesiomonas* spp., specimens were plated onto XDCA and ABA, incubated at 37 ° C for 24 hours. Non-xylose-fermenting colonies on XDCA and all colonies on ABA were screened for oxidase production (16,18). Oxidase-positive colonies were further confirmed as belonging to *Aeromonas* or *Plesiomonas shigelloides* using an established protocol (19). For the isolation of other enteropathogens, the methods fully described by Ogunsanya *et al.* (6) and Alabi and Odugbemi (16) were employed to allow for some degree of comparability with their data. In brief, inoculations of faecal specimens were on appropriate media, such as MCA, DCA, and TCBS. Specimens were also inoculated into enrichment broths, such as selenite-F broth, to enhance the isolation of *Salmonella* and *Shigella* spp., whereas alkanine peptone water (APW) pH 8.6 was employed for the enrichment of *Vibrio cholerae, Plesiomonas* and *Aeromonas* spp. (6). Cold enrichment was used for *Yersinia* spp. in Dulbecco's phosphate buffered saline (PBS) at 4 ° C and subcultured onto MacConkey agar and DCA after 10 days and incubated at room temperature (6). The APW was subcultured onto TCBS agar, and XDCA and selenite broth cultures were subcultured onto DCA and SS agar as previously reported (6). All inoculated media (enrichment and subculture) were incubated at 37 ° C for 24 hours. Biochemical tests previously described (10) were employed for definitive identification. Slide agglutination with specific antisera (Wellcome Reagents Ltd., Wellcome Research Laboratories, Beckenham) were used for serological diagnosis (16). Strains of enteropathogenic *E. coli* (EPEC) were identified as previously described (16). Strains of enterotoxigenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC), and enteroadherent *E. coli (EAEC)* were not sought.

## Statistical methods

Data on demographic characteristics of patients and controls and results of stool bacteriology were coded and entered into a database in Epi Info. Differences in the proportion of urban and rural subjects with the various types of bacteria cultured from stool were compared using the chi-square test. Since the same subjects were compared multiple times, the probability of obtaining a significance by chance was decreased to 0.0064 (p=0.05/number of comparisons) (21).

# RESULTS

The frequencies with which potentially pathogenic bacteria were isolated from individuals with and without diarrhoea, in urban and rural areas of Nigeria, are presented in Table I. There were higher rates of isolation of all the diarrhoeagenic agents studies from patients with diarrhoea than in controls ($p<0.0063$). For patients with diarrhoea, *Campylobacter* spp., *Y. enterocolitica,* and *Shigella* spp. were significantly more frequently isolated from urban patients than rural patients, while *P. shigelloides* and *Aeromonas* spp. were more frequently isolated from rural patients. In controls, a similar pattern in their rates of occurrence was observed in urban and rural areas except that no *P. shigelloides* or *V. cholerae* were isolated from controls in either urban or rural areas. Single pathogens were isolated from most stool samples positive for diarrhoea as well as from controls in both urban and rural areas. Some patients and controls were infected with more than one pathogen, but data on these are not shown, because the pathogens were recorded independently of other co-existing pathogens in such a way that isolates, whether multiple or single, were recorded separately. The age and sex distribution for all enteric flora in patients with diarrhoea are shown in Table II.

## DISCUSSION

Our results indicate a changing pattern in the potential bacterial pathogens that may be associated with diarrhoea in urban areas of Nigeria as compared with previous studies (2,6,22). The trend reported in this study implicates *Campylobacter* spp. as the most frequently encountered enteropathogen associated with diarrhoea cases in urban areas. This was followed in decreasing order by *Escherichia coli, Salmonella* spp. and least for *V. cholerae.* These potential pathogens occurred more frequently in patients with diarrhoea than in controls ($p<0.0063$) and this agrees with previous reports (2,6-14,22). However, our data on the trend of these potential agents of diarrhoea contradict previous reports (2,22) in Nigeria. Those studies did not use methods to detect *Aeromonas* and *Plesiomonas* species, whereas our results now show a potential pathogenic role for both organisms in cases of diarrhoea at a frequency comparable to other established bacterial agents of diarrhoea. Dosunmu-Ogunbi *et al.* (2) reported *Shigella* spp. as the most predominant enteropathogen associated with acute enteric infections in Lagos, Nigeria (41%). This was closely followed by *E. coli* (24%), while *V. cholerae* accounted for only 2% of the isolates. In a related study in Benin City, Nigeria (22), *Salmonella* spp. accounted for up to 47% of the enteric pathogens isolated, while *Staphylococcus aureus* (1%) was the least encountered pathogen. In China, Kain *et al.* (8) reported ETEC as the most frequently detected pathogen in individuals with diarrhoea, accounting for 20% of the cases, while *Salmonella, Shigella, Campylobacter* and *Vibrio* spp. accounted for 12%, 3%, 2%, and 0.5% respectively.

**Table I.** Frequency of isolation of Gram-negative bacteria from stool specimens of patients with and without diarrhoea in urban and rural areas of Nigeria; numbers of isolates % of row totals

| Bacteria | Location | | | p value** |
|---|---|---|---|---|
| | Urban (n=1200) | Rural (n=1200) | p value* | |
| ***Campylobacter* spp.** | | | | 0.0000 |
| Diarrhoea (n=600) | 170 (28%) | 50 (8%) | 0.0000 | |
| No diarrhoea (n=600) | 32 (5%) | 8 (1%) | 0.0002 | |
| ***Escherichia coli (EPEC)*** | | | | NS |
| Diarrhoea (n=600) | 130 (22%) | 110 (18%) | NS | |
| No diarrhoea (n=600) | 23 (4%) | 9 (2%) | NS | |
| ***Salmonella* spp.** | | | | NS |
| Diarrhoea (n=600) | 140 (17%) | 96 (16%) | NS | |
| No diarrhoea (n=600) | 9 (2%) | 11 (2%) | NS | |
| ***Shigella* spp.** | | | | 0.006 |
| Diarrhoea (n=600) | 86 (14%) | 54 (9%) | 0.005 | |
| No diarrhoea (n=600) | 22 (4%) | 18 (3%) | NS | |
| ***Aeromonas* spp.** | | | | 0.0000 |
| Diarrhoea (n=600) | 30 (5%) | 89 (15%) | 0.0000 | |
| No diarrhoea (n=600) | 8 (1%) | 18 (3%) | NS | |
| ***Yersinia enterocolitica*** | | | | NS |
| Diarrhoea (n=600) | 22 (4%) | 13 (2%) | NS | |
| No diarrhoea (n=600) | 8 (1%) | 2 (0.3%) | NS | |
| ***Plesiomonas shigelloides*** | | | | 0.00004 |
| Diarrhoea (n=600) | 14 (2%) | 46 (8%) | 0.00004 | |
| No diarrhoea (n=600) | 0 | 0 | NS | |
| ***Vibrio cholerae*** | | | | NS |
| Diarrhoea (n=600) | 9 (2%) | 21 (4%) | NS | |
| No diarrhoea (n=600) | 0 | 0 | NS | |

* Separate comparisons of bacteria isolated from urban and rural patients with and without diarrhoea.
**Stratified analysis, comparing bacteria isolated from patients with and without diarrhoea, controlling for location (urban vs. rural).
Note: p value for significance=0.0063

In rural areas, we identified EPEC as the leading bacterial agent of diarrhoea, accounting for 18% of the total isolates. This was followed in decreasing frequency by *Salmonella* spp., *Aeromonas* spp., and *Y. enterocolitica.* We are unaware of any report on the distribution of these enteric organisms among diarrhoea cases in rural areas of Nigeria. Our data suggest divergence in the distribution patterns of enteric bacterial flora in both urban and rural areas and clearly reveal the possible association of the potential pathogens *Aeromonas* and *Plesiomonas* spp. with diarrhoea in rural settings. These seemingly high isolation rates observed for *Aeromonas* spp. and *P. shigelloides* in rural areas could be related to environmentally acquired isolates from steams, lakes, ponds, wells or consumption of contaminated water, as previously suggested (23). In Nigeria, contact with these sources is more frequent in rural areas. Holmberg *et al.* (24) found a clear association between the drinking of untreated water and the occurrence of chronic gastroenteritis in adults and acute gastroenteritis in children due to *Aeromonas* spp.

**Table II.** Age and sex distribution of enteric flora isolated from diarrhoea patients in urban and rural areas (with 600 patients in each area)

| Organisms | Sex | | Age group | | | | |
|---|---|---|---|---|---|---|---|
| | Male | Female | 0-5 | 6-10 | 11-20 | 21-30 | >30 |
| **a. Urban areas** | | | | | | | |
| *Campylobacter* spp. (n=170) | 92 (54) | 78 (46) | 79 (46) | 46 (27) | 20 (12) | 13 (8) | 12 (7) |
| *Escherichia coli* (EPEC) (n=130) | 63 (48) | 67 (52) | 44 (34) | 38 (29) | 18 (14) | 19 (15) | 11 (9) |
| *Salmonella* spp. (n=104) | 55 (53) | 49 (47) | 32 (30) | 26 (25) | 12 (12) | 18 (17) | 16 (15) |
| *Shigella* spp. (n=86) | 44 (51) | 42 (49) | 22 (26) | 24 (28) | 18 (21) | 10 (12) | 12 (14) |
| *Aeromonas* spp. (n=30) | 11 (37) | 19 (63) | 6 (20) | 4 (13) | 2 (7) | 5 (17) | 13 (43) |
| *Yersinia* spp. (n=22) | 14 (64) | 8 (36) | 6 (27) | 9 (41) | 3 (14) | 2 (9) | 2 (9) |
| *Plesiomonas shigelloides* (n=14) | 5 (36) | 9 (64) | 2 (14) | 3 (21) | 1 (7) | 4 (29) | 4 (29) |
| *Vibrio* spp. (n=9) | 5 (56) | 4 (44) | 2 (22) | 2 (22) | 0 (0) | 2 (22) | 3 (33) |
| TOTAL (n=565) | 289 (51) | 276 (44) | 193 (34) | 152 (27) | 74 (13) | 73 (13) | 73 (13) |
| **b. Rural areas** | | | | | | | |
| *Campylobacter* spp. (n=50) | 26 (52) | 24 (58) | 17 (34) | 14 (28) | 6 (12) | 5 (10) | 8 (16) |
| *Escherichia coli* (EPEC) (n=110) | 60 (55) | 50 (45) | 30 (27) | 32 (29) | 14 (13) | 21 (19) | 13 (12) |
| *Salmonella* spp. (n=96) | 50 (52) | 46 (48) | 21 (22) | 19 (20) | 24 (25) | 14 (15) | 18 (19) |
| *Shigella* spp. (n=54) | 25 (46) | 29 (54) | 12 (22) | 13 (24) | 12 (22) | 9 (17) | 8 (15) |
| *Aeromonas* spp. (n=89) | 28 (31) | 61 (69) | 20 (22) | 9 (10) | 8 (9) | 18 (20) | 34 (38) |
| *Yersinia* spp. (n=13) | 6 (46) | 7 (54) | 3 (23) | 4 (31) | 2 (15) | 2 (15) | 2 (15) |
| *Plesiomonas shigelloides* (n=46) | 15 (33) | 31 (67) | 8 (17) | 4 (7) | 8 (17) | 12 (26) | 14 (30) |
| *Vibrio* spp. (n=21) | 9 (43) | 12 (57) | 4 (19) | 7 (33) | 5 (24) | 3 (14) | 2 (10) |
| TOTAL (n=479) | 219 (46) | 260 (54) | 115 (24) | 102 (21) | 79 (16) | 84 (18) | 99 (21) |

Note: Figures in parentheses indicate percentages

For the control subjects screened in this study, a similar pattern in the rates of occurrence of these enteric bacteria was noted in both urban and rural areas. However, we isolated *Salmonella* spp., *Shigella* spp., and *Y. enterocolitica* from controls in both urban and rural areas, whereas Eko and Utsalo (25) failed to isolate these enteric bacteria from controls in their study. These divergent results may, however, be related to the season of collection and the media used for isolation. In agreement with previous studies in urban areas (16,25), we also found a 0% prevalence rate for *P. shigelloides* and *V. cholerae* among control patients in both urban and rural areas. However, Arai *et al.* (23) found *P. shigelloides* in the faeces of only 3 (0.01%) of 38,454 normal subjects in Japan. Echeverria *et al.* (26) and Pitarangsi *et al.* (27) found no difference in the isolation of *P. shigelloides* from normal controls and patients with diarrhoea in Thailand. As in previous studies (2,8,12,25,29), it was equally interesting to note that the isolation rates of all the pathogens encountered in this study were observed to be independent of age in both urban and rural areas. It must be mentioned that the big difference in the isolation rates between urban (565/600) and rural (479/600) may not be truly biological; a chi-square as high as 54.49 ($p<10^{-6}$) evokes other factors. A longer transportation time for the rural specimens may partly explain this, and this might have influenced the isolation rate of one species more than that of another one. For example, *Campylobacter* species may show poor growth after a longer transportation time.

We conclude that the frequency of isolation of Gram negative potential pathogens, which could be a cause of diarrhoea in urban areas, is changing and that in both urban and rural areas another group of such potential bacterial pathogens has emerged. We, therefore, recommend further study of types of media for transportation and isolation; improvement of isolation techniques to determine the extent of involvement of *Aeromonas* and *Plesiomonas* spp. as potential pathogens; and a continuing search for other possible potential bacterial agents of diarrhoea that have not been previously reported in Nigeria.

**ACKNOWLEDGEMENTS**

Financial and technical support for this research was provided by the Applied Diarrheal Disease Research (ADDR) Project at Harvard University through a Co-operative Agreement with the U.S. Agency for International Development. We are profoundly grateful to Drs. Sharon Huttly, Edward Cooper, and Mark Nichter for their comments on the manuscript. Dr. Fitzroy J. Henry is thanked for assistance with the execution of the Project and preparation of the manuscript.

**REFERENCES**

1. Rhode JE, Northrup A. Acute diarrhoea in childhood. North Holland: Elsevier Excerpta Medica, 1976:339. (Oba Symposium)
2. Dosunmu-Ogunbi O, Coker AO, Agbonlahor DE, Solanke SO, Uzoma KC. Local pattern of acute enteric bacterial infections in man, Lagos, Nigeria. *Develop Biol Standard* 1983;3:277-83.
3. Synder JD, Merson MH. The magnitude of the problem of acute diarrhoeal disease: a review on active surveillance data. *Bull WHO* 1980;60:605-13.
4. Barua D. The WHO global diarrhoeal disease control programme. *In:* Emejuaiwe SO, Ogunbi O, Sanni SO, editors. Global impacts of applied microbiology (Sixth International Conference), London: Academic Press, 1981:405-13.
5. Federal Ministry of Health Bulletin. Lagos statistics. Lagos: Government Press, 1987:20-5.
6. Ogunsanya TI, Rotimi VO and Adenuga A. A study of the aetiological agents of childhood diarrhoea in Lagos, Nigeria. *J Med Microbiol* 1994;40:10-4.
7. Blum D, Dosunmu-Ogunbi O. *In:* Abstracts of the 3rd International Epidemiology Association 1986:36.
8. Kain KC, Berttluk RL Kelly MT, Xin H, Hua GD, Yuan G, Procter EM, Byrne S, Stinger G. Etiology of childhood diarrhoea in Beijing, China. *J Clin Microbiol* 1987;29:90-5.
9. Pazzaglia G, Bourgeois AL, Araby I, Mikhail I, Podgore JK, Mourad A, *et al. Campylobacter* associated diarrhoea in Egyptian infants: Epidemiology and clinical symptoms of disease and high frequency of concomitant infections. *J Diarrhoeal Dis Res* 1993;11:6-13.
10. Joseph T. The isolation rates of pathogenic bacteria from stools of gastroenteritis patients. *Ind Microbiol* 1987;25:189-93.
11. Moyer NP. Clinical significance of *Aeromonas* species isolated from patients with diarrhoea. *J Clin Microbiol* 1987;25:189-93.
12. Millership SE, Chattopadhay B. Methods for the isolation of *Aeromonas hydrophila* and *Plesiomonas shigelloides* from faeces. *J Hyg Camb* 1992;92:145-52.
13. Mitra AK, Kabir I, Hossain MA. Pivmecillinam resistant *Shigella dysenteriae* type 1 infection in Bangladesh. *Lancet* 1990;335:1461-2.
14. Demol P, Bosmans E. *Campylobacter* enteritis in Central Africa. *Lancet* 1978;1:604.
15. Gracey M, Burke V, Robinson J. *Aeromonas*-associated gastroenteritis. *Lancet* 1982;1:1304-05.
16. Alabi SA, Odugbemi TO. Occurrence of *Aeromonas* species and *Plesiomonas shigelloides* in patients with and without diarrhoea in Lagos, Nigeria. *J Med Microbiol* 1990;32:45-8.
17. Coker AO, Dosunmu-Ogunbi O. Isolation of *Campylobacter jejuni* in Lagos, Nigeria. A new bacterial agent of diarrhoea. *East Afr Med J* 1984;61:52-5.
18. Koracs N. Identification of *Pseudomonas pyocyanea* by oxidase reaction. *Nature* 1956;178:703.
19. Von Graevenitz A. *Aeromonas* and *Plesiomonas. In:* Lenneth EH, Balows A, Hausler WJ, Shadomy HJ, editors. Manual of clinical microbiology. Washington, DC: American Society of Microbiology, 1985:278-82.

20. Gwan ST. Cowan and Steel's Manual for the identification of medical bacteria. Cambridge: Cambridge University Press, 1980.
21. Dawson-Saunders B, Trapp RG. Basic and clinical biostatistics 2d ed. Stamford, CT: Appleton and Lange, 1994;133-4.
22. Offor E, Wemambu SNC. Bacterial profile of diarrhoeal disease in Benin city. *Publ Health Lond* 1982;96:211-5.
23. Arai T, Ikejima N, Poh T, Sakai S, Shimada T, Sakazaki T. A survey of *Plesiomonas shigelloides* from aquatic environments, domestic animals, pets and humans. *J Hyg* 1980;84:203-11.
24. Holmberg SD, Schell WL, Fanning GR, Wachsmith LK, Hickmanbrenner FW, Blaker PA, *et al. Aeromonas* intestinal infection in the United States. *Ann Intern Med* 1986;105:683-9.
25. Eko FO, Utsalo SJ. Comparative study of the prevalence and clinical profiles of diarrhoea due to *Aeromonas* and other enteric pathogens. *J Hyg Epidemiol Microbiol Immunol* 1990;34:183-9.
26. Echeverria P, Blackwell NR, Sanford LB and Cukor GC. Travellers' diarrhoea among American Peace Corps volunteers in rural Thailand. *J Infect Dis* 1981;143:767-771.
27. Pitarangsi C, Echeverria P, Whitmire R, Tarapai C, Formal S, *et al.* Enteropathogenicity of *Aeromonas hydrophila* in individuals with and without diarrhoea in Thailand. *Infect Immun* 1982; 35:666-73.
28. Burke V, Gracey M, Robinson J, Peck D, Beaman J, Bundell C. The microbiology of childhood gastroenteritis: *Aeromonas* species and other infective agents. *J Infect Dis* 1983;148:68-74.
29. Paukova C, Fukalova A. Occurrence of *Aeromonas hydrophila* and *Aeromonas shigelloides* in faeces. *Zentralblatt fur Bakteriol.* I. *Abt Orig* 1968;206:212-6.

LETTER TO THE EDITOR

# Mechanism of Purgative Effect of Magnesium Sulphate on Mouse Colon

Sir:

Magnesium sulphate belongs to the class of saline laxatives that act by simple osmotic extraction of fluid into the gut lumen (1). Recently, a role for nitric oxide in the mechanism of action of magnesium sulphate has been postulated. Inhibitors of nitric oxide synthesis seem to block the laxative action of this compound (2). There is evidence to show that even castor oil acts through the release of nitric oxide (3). We have demonstrated that methylene blue, a guanylate cyclase inhibitor, blocks the effect of castor oil indicating the involvement of the enzyme in the chain of actions that finally lead to diarrhoea (4). The question asked here is whether guanylate cyclase is also involved in the action of magnesium sulphate.

To answer this question, experiments were conducted on mice implanted with intra-caecal cannulae. Male Swiss albino mice weighing 25 to 30 g were used for this study. Details of the method of cannulation and induction of diarrhoea have been described earlier (5). Briefly, one end of a thin polyethylene tube (PE 50, Clay Adams, ID 0.58 mm and OD 0.95 mm), measuring 6 cm in length, was introduced into the tip of the caecum brought out through a small incision on the ventral abdominal wall on the left side. The caecum was pushed back into the abdominal cavity, and the wound was closed. The other end of the tube was brought out through the nape of the neck after traversing the distance subcutaneously.

A week after recovery, six animals were used for each experiment. Introduction of 0.2 mL of a 30% solution of magnesium sulphate into the caecum produced copious diarrhoea within ten minutes. We compared this effect with that obtained after prior administration of indomethacin (a cyclooxygenase inhibitor), methylene blue (a guanylate cyclase inhibitor), 5-aminosalicylic acid (a lipoxygenase inhibitor), and anthracene-9-carboxylic acid (a chloride channel blocker). After having received the different compounds, the animals were placed in individual cages with floor lined by blotting paper. The time taken for the onset of passage of stools, in minutes, was noted in each animal. On the basis of stool consistency, a numerical score was assigned as follows: 1 = normal stool, 2 = semi-solid stool, 3 = watery stool. The faecal output index (FOI) is defined as the sum of the consistency scores of all the motions passed within the observation period of 3 hours. An animal, passing a stool of score 2 at least once, is counted as suffering from diarrhoea.

**Table I. Effect of pre-treatments on magnesium sulphate-induced diarrhoea**

| Sl. No. | Pre-treatment | Time of onset (min) | FOI |
|---|---|---|---|
| 1 | Control | No stools | No stools |
| 2 | Saline | 9± 1 | 33± 2 |
| 3 | Indomethacin | 17± 1 | 40± 3 |
| 4 | Methylene blue | 8± 1 | 32± 2 |
| 5 | 5-aminosalicyclic acid | 8± 1 | 41± 3 |
| 6 | Anthracene-9-carboxylic acid | 8± 1 | 20± 1 |

All values are means ± SEM of 6 experiments. All agents were given through the intracaecal route, 30 minutes prior to the administration of magnesium sulphate (0.2 mL of a 30% solution). The following dosages were given in a volume of 0.2 mL: indomethacin 120 m M/kg; methylene blue 0.65 m M/kg; 5- aminosalicylic acid 280 m M/kg; anthracene 9-carboxylic acid 6.75 m moles/kg. The control group received saline instead of the secretagogue. Only anthracene-9-carboxylic acid caused a significant reduction in FOI ($p<0.01$) when administered prior to magnesium sulphate.

The Table indicates that except anthracene-9-carboxylic acid none of the agents employed was able to significantly decrease the diarrhoeal response induced by administration of magnesium sulphate. Methylene blue, when given in similar concentration and by the same route of administration, was successful in curtailing diarrhoea caused by castor oil (4) or acidified sodium nitrite (5). Release of nitric oxide and activation of guanylate cyclase (2,4,5) are thought to be involved in the mechanism of these actions. Failure of methylene blue to reduce diarrhoea induced by magnesium sulphate indicates that the enzyme guanylate cyclase may not be involved in the action of magnesium sulphate on mouse colon. Indomethacin and 5-aminosalicylic acid were also ineffective in controlling the laxative response to magnesium sulphate. Hence the products of cyclooxygenase and lipoxygenase are probably not involved in the mediation of action of magnesium sulphate.

The ability of anthracene-9-carboxylic acid to significantly decrease diarrhoea induced by magnesium sulphate indicates that at least a part of the diarrhoeal response is due to the opening of chloride channels. In combination with glucose, anthracene-9-carboxylic acid was found to be effective in controlling the secretory response of small intestine induced by dibutyryl cyclic AMP (6).

It is possible that the osmotic changes in the colon caused by the introduction of magnesium sulphate may induce volume changes in the colonic crypt cells. Such volume changes are known to cause opening of the chloride channels in other cells (7,8). However, further experimentation is needed to investigate if similar changes occur in colonic epithelium on exposure to magnesium sulphate.

## REFERENCES


1. Gaginella TS, Bas P. Laxatives: an update on the mechanism of action. *Life Sci* 1978;23:100-10.
2. Izzo AA, Gaginella TS, Mascolo N, Capasso F. Nitric oxide as a mediator of the laxative action of magnesium sulphate. *Br J Pharmacol* 1994;113:22-3.
3. Mascolo N. Izzo AA, Barbato F, Capasso F. Inhibition of nitric oxide synthetase prevents castor oil induced diarrhoea in the rat. *Br J Pharmacol* 1993;108:861-64.
4. Robert FSM, Prokasa Rao J. Evidence for possible involvement of guanylate cyclaso in diarrhoea induced by castor oil in mice. *J Diarrhoeal Dis Res* 1996;14:218-9.

5. Robert FSM, Prakasa Rao J. Does nitric oxide induce diarrhoea in mice? *J Diarrhoeal Dis Res* 1997;15:25-7.
6. Rabbani GH, Oong Bao LU, Horvath K, Lebenthal E. Short chain glucose polymer and anthracene-9-Carboxylic acid inhibit water and electrolyte secretion induced by dibutyryl cyclic AMP in the small intestine. *Gastroenterology* 1991;101:1046-53.
7. Lewis RS, Ross PE, Cahalan MD. Channels activated by osmotic stress in T lymphocytes. *J Gen Physiol* 1993;101:801-26.
8. Kubo M, Okado Y. Volume regulatory chloride channel currents in cultured human epithelial cells. *J Physiol* 1992;456:351-71.

**F Stanley Mangalakumar Robert and**
**J Prakasa Rao**
*Department of Physiology,Christian Medical College, Vellore 632 002, India*

**LETTER TO THE EDITOR**

# Comparison of Main Features in Children with Cholera O1 and O139 in Yangon, Myanmar, 1996

**Dear Sir:**

*Vibrio cholerae* O139 strains were first isolated in our community in February 1994 (1). In endemic areas, older individuals are usually partly or completely immune to *V. cholerae* O1, as a result of repeated exposure. Children and infants are more vulnerable (2). On the contrary, in Bangladeshi patients with *V. cholerae* O139 infections there was a higher proportion of adults (3). This study on cholera O1 and O139 in children in Yangon, Myanmar, was carried out in 1996.

**Table.** Numbers (%) of patients with cholera and main features

| **Numbers (%) and main features** | ***V. cholerae* serotypes** | | **c 2** | **Probability** | **Relative risk 95% CL*** |
|---|---|---|---|---|---|
| | **O1** | **O139** | | | |
| Total number of patients | 475 (100%) | 319 (100%) | | | |
| Number of patients < 10 years | 101 (21%) (100%) | 26 (8%) (100%) | 24.2 | <10-6 | 0.26 – 0.33 – 0.58 |
| Reported number of stools<br>< 1<br>1 to 5<br>6 to 10 | <br>36 (36%)<br>52 (52%)<br>13 (13%) | <br>0<br>17 (67%)<br>9 (33%) | 11.2 | 0.0008 | 1.34 – 1.55 – 1.80 |
| Vomiting | 85 (84%) | 26 (100%) | - | 0.041** | 1.09 – 1.19 – 1.29 |
| Abdominal pain | 52 (52%) | 17 (67%) | 1.6 | 0.20 | 0.91 – 1.27 – 1.78 |
| Fever | 12 (12%) | 11 (42%) | - | 0.014** | 1.38 – 2.91 – 6.16 |
| Dehydration<br><br>Mild to moderate<br>Severe | <br><br>88 (88%)<br>13 (12%) | <br><br>11 (42%)<br>15 (58%) | <br><br>24.2<br>12.9 | <br><br><10-6<br>0.0004 | <br><br>0.31 – 0.49 – 0.77<br>1.61 – 2.65 – 4.34 |

*95% confidence limits - the relative risks apply to the V. cholerae 0139 patients compared with the 01 ones
**Two-tailed Fisher's exact probability test

Rectal swab samples were collected from 2534 patients passing rice-water stools and admitted for treatment to the Infectious Diseases Hospital, Yangon, during January to July 1996. Among them *V. cholerae* was isolated from 794 (31%) of which 475 (60%) were *V. cholerae* O1 and 319 (40%), *V. cholerae* O139. There were 101 *V. cholerae* O1 and 26 *V. cholerae* O139 cases in children under 10 years. These are the subjects of this study.

We compared differences in proportions with the c 2 test or the Fisher exact probability test. Fever was defined as a body temperature >36.8° C.

The table shows the numbers of patients and the frequency of the main signs and symptoms in children with cholera O139 compared with O1 patients.

In our community, the proportion of children less than 10 years old was much lower in patients with *V. cholerae* O139 than in those with *V. cholerae* O1. This confirms the poor herd immunity of adults against the recently emerged germ. On the other hand, children with the new *V. cholerae* strain had significantly higher reported purging rates and a higher frequency of abdominal pain, fever and severe dehydration. Our findings are in keeping with data from Bangladesh showing that adult *V. cholerae* O1 patients were more often hypothermic (oral temp. <36 degree Celsius) ($p<0.001$) and less often had abdominal pain or cramps ($p=0.035$) than did patients infected with *V. cholerae* O139 [4]. We found no significant difference in the frequency of vomiting. Overall, cholera O139 was the more severe of the two diseases.

All of the isolated strains of *V. cholerae* O1 and O139 were susceptible to chloramphenicol, gentamicin, tetracycline, and co-trimoxazole, and resistant to ampicillin. Thus tetracycline and co-trimoxazole were still the drugs of choice in treating cholera patients in this study.

## REFERENCES


1. Oo Kn, Than WM, Win T, Thida M, Aung KS. *V. cholerae* non-O1 as a causal pathogen in diarrhoeal patients in Yangon, Myanmar. *J Diarrhoeal Dis Res* 1995;3:130.
2. Swerdlow DL, Ries AA. *V. cholerae* non-O1 the eighth pandemic? *Lancet* 1993;342:382-3.
3. Hoch SPF, Khan A, Haq IU, Khan MA, Mintz EO. *V. cholerae* O139 in Karachi, Pakistan. *Lancet* 1993;342:1422-3.
4. Dhar U, Bennish ML, Khan WA, Sear C, Khan EH, Albert MJ, Salam MA. Clinical features, antimicrobial susceptibility and toxin production in *V. cholerae* O139 infection: comparison with *V. cholerae* O1 infection. *Trans R Soc Trop Med Hyg* 1996;90:402-405.



**Khin Nwe Oo[1], Myat Thida[2], and Ni Ni Aye[2]**

[1]Immunology and [2]Bacteriology Research Division,
Department of Medical Research, No. 5, Ziwaka Road,
Yangon 11191, Union of Myanmar

**LETTER-TO-THE-EDITOR**

# Glucose-based and Rice-based ORS

Sir:

The authors of the paper "Acceptability of Rice-based or Flavoured Glucose-based Oral Rehydration Solutions: A Randomized Controlled Trial" (1) conclude that there was "no clear advantage of rice-based or flavoured solutions over standard glucose ORS. This observation lends support to the continued recommendation of glucose ORS as standard therapy." The study was well designed and, within the limits of variability of four comparison groups with a n=76 to 105 in the different cells no differences were noted between the several ORT preparations investigated with respect to the variables that were measured. It is gratifying to note that the solutions were prepared accurately and that ORT was used by 79 to 87% of mothers with feeding occurring in the majority of children.

However, the conclusion that "rice-based ORS shows no clear advantage of rice-based over standard glucose ORS lends support to the continued recommendation of glucose ORS as standard therapy" is overly broad and extends well beyond the scope of the data gathered. The two most important effects of ORS are first to save lives threatened by volume depletion and second to improve growth and development with consistent use in all episodes of diarrhoea. In this paper, the population studied was not dehydrated or at risk of dying from volume depletion, and no nutritional follow-up was included in the design. The only valid conclusion from this study would then be only that in children with mild diarrhoea and little or no dehydration there was no distinguishable difference between rice and glucose-based ORS. In this four-way comparison of relatively small groups, the limits of error should also have been stated as these would be substantial, and differences of clinical importance could have been obscured by variation.

Rice-based ORS has been shown to decrease volume loss in severe dehydrating diarrhoea (2) and shortens the course of both watery and dysenteric diarrhoea (3). Furthermore, with consistent use cereal-based ORS has performed better that glucose ORS in promoting improved growth and development (4,5).

There should not be a bias against improved ORS solutions on the basis of a lack of advantage over standard glucose ORS in mild disease. Preventing death and/or hospitalization and promoting growth are surely more important goals.

## REFERENCES


1. Saniel MC, Zimicki S, Carlos CC, Maria A CS, Balis AC, Malacad CC. Acceptability of rice-based and flavoured glucose-based oral rehydration solutions: a randomized controlled trial. J Diarrhoeal Dis Res 1997;15:47-52.
2. Molla AM, Ahmed SM, Greenough WB, III. Rice-based oral rehydration solution decreases the stool volume in acute diarrhoea. *Bull WHO* 1985;63:751-6.
3. Molla AM, Bari A, Greenough WB, III. Rice oral rehydration solution hastens recovery from dysentery. *J Diarrhoeal Dis Res* 1995;13:8-11.
4. Molla AM, Bari A, Greenough WB, III. Food-based oral rehydration therapy for improved management of diarrhoeal diseases. *Indian J Pediatr* 1991;58:745-55.

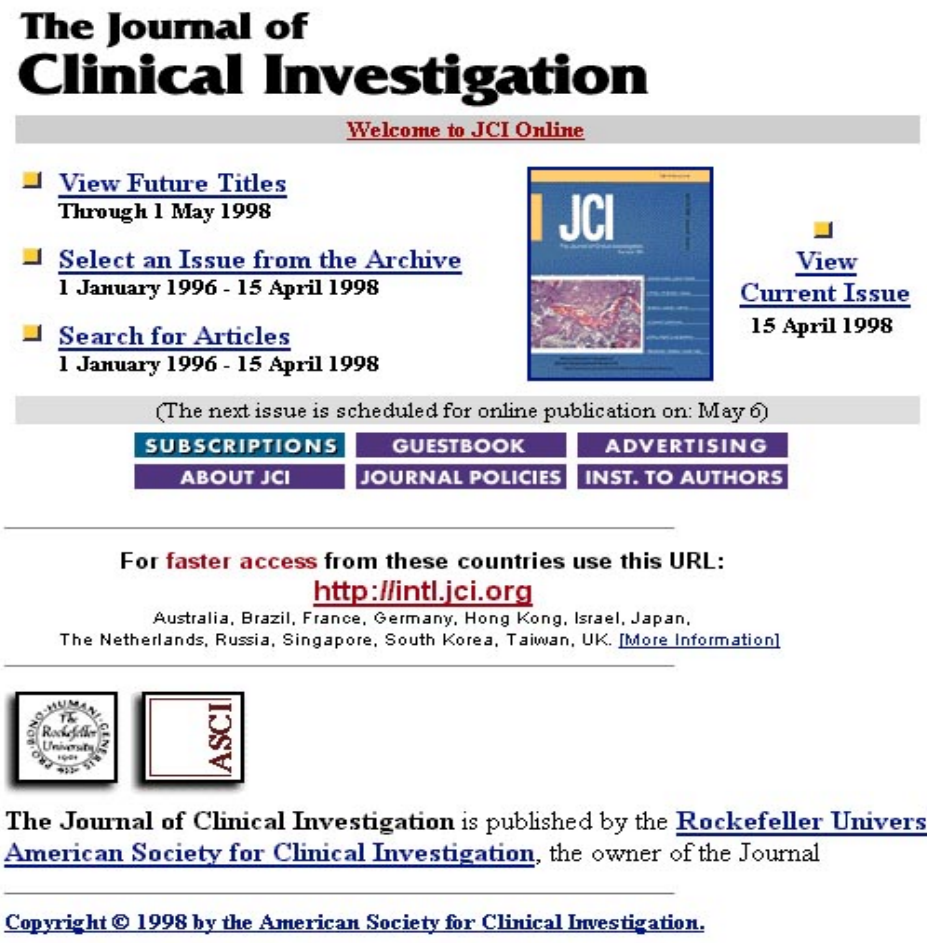
5. Murphy HH, Bari A, Molla AM, Zaidi A, Hirschhorn N. A field trial of wheat-based oral rehydration solutions among Afghan refugee children. *Acta Pediatr* 1996;85:151-7.

**William B Greenough, III**
*Division of Geriatric Medicine,*
*Johns Hopkins Geriatrics Center,*
*5505 Hopkins Bayview Circle, Baltimore,*
*MD 21224, USA*

# From the Internet . . . . . .

*Medical journals on the Internet*

A growing number of medical journals are appearing on the Internet. The differences between the printed and electronic versions are also considerably increasing rapidly.




The online version of the prestigious *Journal of Clinical Investigation* illustrates the features that make some, but not all, electronic journals superior to their printed copies (see box on the next page).

***eBMJ and The Lancet Interactive***

These two prestigious journals have upgraded their Internet sites in April 1998. The electronic *British Medical Journal* (*eBMJ*)' has features similar to those of the *JCI online*. This is not surprising: both journals (as many other ones) have sought assistance from Stanford University High Wire Press. All articles are available in their full-text version. Clicking on the "Search" field of the *eBMJ* opens a search-and-browse panel, similar to that of the *JCI online*. It is shown here.

| | |
|---|---|
| **Immediate availability.** | The *JCI online* appears on the Internet before it can arrive by postal mail. This is the more true the greater the distance between the subscriber and the printer. The difference can be weeks or, for developing countries scientists, even months. |
| **Free of charges.** | Access to the *JCI* is free; no payment, no registration and no username or password are required. |
| **Full contents** | The full contents are available, with the choice between summary or full-text of each article. The latter includes tables and figures. |
| **PDF version** | PDF files (portable document format) can be downloaded. They make it possible to print an exact copy of what appears in the paper copy of the *JCI*. |
| **PubMed citation and related articles** | Not only does a hypertext link leads to the Medline summaries of the articles (with the option of two formats), but one click suffices to see on the screen a printable list of the Medline references to papers on the same topic as the original one. Each of these references leads again to related articles. |
| **References with hypertext** | Hypertext links again allow seeing more details about each reference. The possibilities are, depending on the source journal, summary or full text or Medline citation. |
| **Download to Citation Manager** | Those who keep their references in a database can download each paper in the *JCI online* to specialised reference libraries, including Citattion Manager, and Endnote. |
| **Search Medline** | Medline indexed articles by any of the authors of the article can be directly accessed via Medline using a hypertext link. |
| **Archive and Search** | Earlier issues of the *JCI* are accesible online, and there JCI articles can be searched for by author's name or keyword. |
| **TOC** | One can ask to receive the TOC, or tables of contents, by e-mail. |

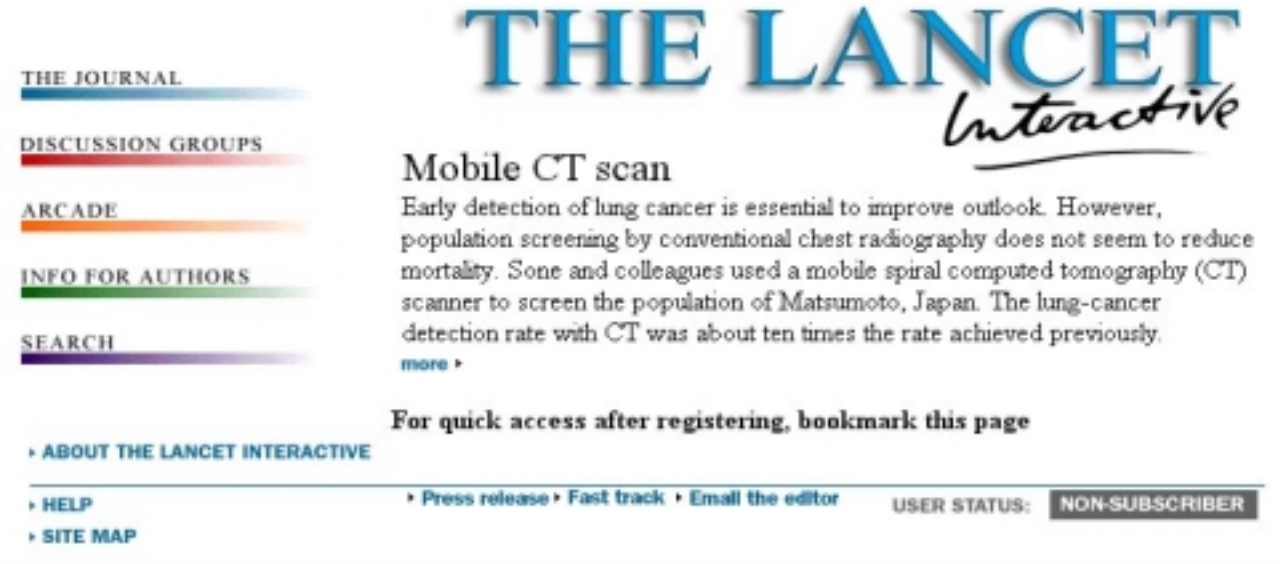
It is a powerful and flexible search engine, with full text search-and-retrieval capacity. Still, it requires some care. For example, a simple search with "breast-feeding" yields zero articles. Suggestions are offered for , but with suggestions about how to improve the search. The reason is that, while "breast-feeding" is spelled with a hyphen in all main British and American dictionaries, the *BMJ* spells it as two separate words. Searching the *eBMJ* for "breast feeding" on April 24, 1998, retrieved no less than 241 references of all kinds: full papers, editorials, letters, news features. One can check all references of interestone wants to have a better look at, and get a list of all checked abstracts. Another nice feature is that one can repeat one's search in a broad selection of other journals (but I guess spelling might cause problems). It is truly impressive. The *eBMJ* home page gives also direct access to Medline. As the *JCI online*, it has little boxes that invite you to have a look at other online medical journals such as *Applied and Environmental Microbiology*, *Infection and Immunity*, and *Science*. The list is probably very long and these "invitations" might well change frequently – and free access to them might only be temporary. For the time being, access to the *eBMJ* is free of charge. I hope it will remain so.

*The Lancet Interactive* has an renewed (?? upgraded???) website. It is also more commercial: only subscribers to the printed edition get access to the full-text version. For others there is limited access. A username and password is required in both cases.


Full access allows to see the **Journal** (current issue, supplements, back issues from December 6, 1997, and press releases), **Discussion Groups** (a forum to debate material published in *The Lancet,* limited to subscribers), and to use **Search** (including the possibility to download complete indexes of the printed journal since July 1995). Accessible to everyone is the **Information for Authors**. TheseIt includes the useful Uniform Requirements of the International Committee of Medical Journal Editors, and the also useful Consort Guidelines (for preparing reports on randomised clinical trials).

*The Lancet* editors, like many others, expect much from electronic publishing. In a short commentary they say: "As a medium for academic publishing the Internet now offers opportunities unimaginable in paper. *The Lancet Interactive* website (http://www.thelancet.com) is a step forward in our challenge…Rapidly

updated readers' comments form an integral part of the site, and we look forward to publishing research supported by video and sound." To have all the letters to the editors grouped together is certainly easier than having to search through a unknown number of printed issues of a journal. Whether readers and contributors will consider it important to have sound and moving pictures as parts of a scientific article is a matter for discussion. Unfettered access to electronic journals might be more important.




**The "knows" and the "know-nots"**

In his Prosperity and Upheaval – The World Economy 1945-1980, Herman Van der Wee writes: "Even if a redistribution of income, based on a satisfactory compromise with the principle of efficiency, should prove able to eliminate or considerably reduce the conflict between the 'haves' and the 'have-nots', problems will still remain. In the complex economy of the late twentieth century a new conflict is growing, that between the 'knows' and the 'know-nots'. This is a new source of frustration and alienation, one that cannot be resolved by a better distribution of material gains." The Internet may well fulfil its promises for the easier and faster distribution of medical knowledge. If so, it might further increase the stark differences between health professionals in developing countries and their colleagues in the industrialised world in the means to access that knowledge, and hence to contribute to it. The *BMJ* of April 11, 1998 addresses this question in one of its News features. Professor Subbiah Arunachalam from the Indian Institute of Technology in Madras and the M S Swaminathan Foundation is quoted as saying: "The internet may eventually be a great equaliser for research scientists around the world, but in the early days it will widen the gap".

This is an ominous prediction; all those involved in the further growth of medical information on the Internet should do all they can to prove it wrong.

R. Eeckels

## BIBLIOGRAPHY ON DIARRHOEAL DISEASES

**333. Abe A, Kenny B, Stein M, Finlay BB*. Characterization of two virulence proteins secreted by rabbit enteropathogenic *Escherichia coli*, EspA and EspB, whose maximal expression is sensitive to host body temperature. Infect Immun 1997 Sep;65(9):3547-55. 40 ref, Eng.** *Biotechnology Laboratory, University of British Columbia, Room 237, Wesbrook Blvd., 6174 University Building, Vancouver, BC, Canada V6T 1Z3

**334. Ackers M, Pagaduan R, Hart G, Greene KD, Abbott S, Mintz E, Tauxe RV. Cholera and sliced fruit: probable secondary transmission from an asymptomatic carrier in the United States. Int J Infect Dis 1997 Apr;1(4):212-4. 22 ref, Eng.** Foodborne and Diarrheal Diseases Branch, Mailstop A-38, Centers for Disease Control and Prevention, 1600 Clifton Road, Atlanta, GA 30333, USA

**335. Adah MI, Rohwedder A, Olaleye OD, Durojaiye OA, Werchau H. Further characterization of field strains of rotavirus from Nigeria VP4 genotype P6 most frequently identified among symptomatically infected children. J Trop Pediatr 1997 Oct;43(5):267-74. 45 ref, Eng.** Institute of Medical Microbiology and Virology, Ruhr-Universität, Universitätsstr 150, 44801 Bochum, Germany

**336. Adams LM, Simmons CP, Rezmann L, Strugnell RA, Robins-Browne RM*. Identification and characterization of a K88- and CS31A-like operon of a rabbit enteropathogenic *Escherichia coli* strain which encodes fimbriae involved in the colonization of rabbit intestine. Infect Immun 1997 Dec;65(12):5222-30. 50 ref, Eng.** *Department of Microbiology and Infectious Diseases, Royal Children's Hospital, Parkville, Victoria 3052, Australia

**337. Adeyemo AA, Sodeinde O*, Gbadegesin RA, Ademowo OG. Breastfeeding and bloody diarrhoea in young children in Ibadan, Nigeria [brief report]. J Trop Pediatr 1997 Aug;43(4):235-6. 7 ref, Eng.** *Department of Paediatrics, University College Hospital, Ibadan, Nigeria

**338. Al-Ali FM, Hossain MM*, Pugh RNH. The associations between feeding modes and diarrhoea among urban children in a newly developed country. Public Health 1997 Jul;111(4):239-43. 15 ref, Eng.** *Department of Community Medicine, Faculty of Medicine and Health Sciences, United Arab Emirates University, PO Box 17666, Al-Ain, United Arab Emirates

**339. Alam M, Yiyoshi S-I*, Tomochika K-I, Shinoda S. *Vibrio mimicus* attaches to the intestinal mucosa by outer membrane hemagglutinins specific to polypeptide moieties of glycoproteins. Infect Immun 1997 Sep;65(9):3662-5. 25 ref, Eng.** *Faculty of Pharmaceutical Sciences, Okayama University, Tsushima naka-1-1-1, Okayama 700, Japan

**340. Albert MJ, Bhuiyan NA, Talukder KA, Faruque ASG, Nahar S, Faruque SM, Ansaruzzaman M, Rahman M. Phenotypic and genotypic changes in *Vibrio cholerae* O139 Bengal. J Clin Microbiol 1997 Oct;35(10):2588-92. 42 ref, Eng.** International Centre for Diarrhoeal Disease Research, Bangladesh, GPO Box 128, Dhaka 1000, Bangladesh

"To find reasons for the recent decline of *Vibrio cholerae* O139 Bengal cholera in Bangladesh, phenotypic and genotypic changes in O139 isolates obtained from patients with cholera from 1993 to 1996 were studied. The isolates were tested for the presence of *ctx* and *tcpA* genes,

hemagglutinin/ protease (HA/P), capsule, D-mannose-sensitive hemagglutinin (MSHA), L-fucose-sensitive hemagglutinin (FSHA), tube test (tube) and CAMP test (CAMP) hemolytic activities, resistance to 2,4-diamino-6,7-diisopropyl pteridine (0/129) and trimethoprim-sulfamethoxazole (TMP-SMX), and genotype by pulsed-field gel electrophoresis (PFGE). All isolates possessed *ctx* and *tcpA* genes, HA/P, and a capsule. Most isolates were negative for FSHA, but although the majority of the isolates were positive for MSHA, no discernible trend in the activity was found during the study period. All early isolates were CAMP hemolysin positive and resistant to the vibriostatic compound 0/129 and TMP-SMX, the two properties that could be used for the presumptive diagnosis of O139 cholera. However, subsequently, isolates that were CAMP hemolysin negative and susceptible to TMP-SMX and 0/129 were increasingly encountered, with all the 1996 isolates being so, which suggested that these properties can no longer be used for the presumptive diagnosis of O139 cholera. *V. cholerae* O139 isolates that were CAMP hemolysin positive and resitant to 0/129 and TMP-SMX produced a disease of greater severity than that caused by the CAMP hemolysin-negative and susceptible isolates on the basis of the lengths of stay of the hospitalized patients. The study period witnessed the evolution of four different genotypes by PFGE. All of these data suggested that the *V. cholerae* O139 isolates have undergone changes in some properties. However, how these changes influenced their prevalence relative to that of *V. cholerae* O1 in human infection is not clear. Studies of the environmental factors will provide the key for an understanding of the relative abundance of these vibrios."

**341. Albert MJ, Neira M, Motarjemic Y. The role of food in the epidemiology of cholera. World Health Stat Q 1997;50(1/2):111-8. 59 ref, Eng.** International Centre for Diarrhoeal Disease Research, Bangladesh, GPO Box 128, Dhaka 1000, Bangladesh

**342. Arias MM, Alcaraz GM, Bernal C, Gonzalez G[*]. Oral rehydration with a plantain flour-based solution in children dehydrated by acute diarrhea: a clinical trial. Acta Paediatr 1997 Oct;86(10):1047-51. 20 ref, Eng.** [*]Departamento de Pediatria, Universidad de Antioquia, Apartado Aereo 1226, Medellin, Colombia

"A clinical trial was conducted in order to prove the efficacy of a solution containing 50 g/l of plantain flour and 3.5 g/l of sodium chloride (NaCl) for the rehydration of children with acute diarrheal diseases. 121 children were given WHO-ORS (Group A) and 117 a plantain flour-based solution (Group B). Rehydration was successful in 85.9% in Group A and 88.0% in Group B (p=0.634). Rehydration was completed in 5.28 h (SD 1.99) in Group A and in 4.88 h (SD 2.11) in Group B (p=0.132). The average solution intake for rehydration was 24.56 ml/kg/h (SD 10.12) in Group A and 21.17 ml/kg/h (SD 9.35) in Group B (p=0.00782). The mean stool output during rehydration was 8.45 g/kg/h (SD 9.72) in Group A and 4.69 g/kg/h (SD 4.98) in Group B (p=0.00053). Decrease in blood levels of sodium and potassium occurred in some children in group B. The plantain flour-based solution proved effective for the treatment of dehydration due to acute diarrheal diseases and should be considered as an alternative when standard WHO-ORS is not available."

**343. Baig LA, Thaver I. Does training affect quality of diarrhoea case management. J Pak Med Assoc 1997 Aug;47(8):204-7. 11 ref, Eng.** Faculty of Health Sciences, Baqai University, 51, Deh Tor Plaza, National Highway, Karachi, Pakistan

**344. Baig LA, Thaver I. Supervision for diarrhoea case management at the oral rehydration therapy corners and diarrhoea training units in Sindh. J Pak Med Assoc 1997 Aug;47(8):208-10. 7 ref, Eng.** Faculty of Health Sciences, Baqai University, 51, Deh Tor Plaza, National Highway, Karachi, Pakistan

**345. Barnes GL, Lund JS, Adams L, Mora A, Mitchell SV, Caples A, Bishop RF. Phase 1 trial of a candidate rotavirus vaccine (RV3) derived from a human neonate. J Paediatr Child Health 1997 Aug;33(4):300-4. 18 ref, Eng.** Department of Gastroenterology and Clinical Nutrition, Royal Children's Hospital, Parkville, Vic. 3052, Australia

**346. Benitez JA, Spelbrink RG, Silva A, Phillips TE, Stanley CM, Boesman-Finkelstein M, Finkelstein RA*. Adherence of *Vibrio cholerae* to cultured differentiated human intestinal cells: an in vitro colonization model (note). Infect Immun 1997 Aug;65(8):3474-7. 27 ref, Eng.*** Nikon Biomedical Confocal Systems, Melville, NY 91747, USA

**347. Bhattacharya MK, Teka T, Faruque ASG, Fuchs GJ. *Cryptosporidium* infection in children in urban Bangladesh. J Trop Pediatr 1997 Oct;43(5):282-6. 27 ref, Eng.** International Centre for Diarrhoeal Disease Research, Bangladesh, GPO Box 128, Dhaka 1000, Bangladesh

"We reviewed data during 1991-94 from a systematic 4 per cent subsample of all patients who presented with diarrhoea to our facility, in which there were 1949 cases of acute diarrhoea in children between the ages of birth to 59 months. Cryptosporidia oocysts were detected in the stools of 68 (3.5 per cent) of these children. A case-control study was designed using surveillance data which included the 68 children with stool positive for *Cryptosporidium* as cases. Two hundred and four children who did not have *Cryptosporidium* were randomly selected to serve as controls. The most common presentations were watery diarrhoea (91 per cent), dehydration (81 per cent), and vomiting (71 per cent), and *Cryptosoprodium* was detected throughout the year, but was most frequently isolated during April to October. Lowest rates of detection were observed in the months of November, December, and January. Age below 2 years, non-breastfeeding, and stunting were significantly associated with *Cryptosporidium* infection. In multivariate analysis of our study we found that only stunted (P=0.031) and non-breastfed children (P=0.022) had a greater risk of having *Cryptosporidium* infection."

**348. Bhutta ZA, Nizami SQ, Thobani S, Issani Z. Factors determining recovery during nutritional therapy of persistent diarrhoea: the impact of diarrhoea severity and intercurrent infections. Acta Paediatr 1997 Aug;86(8):796-802. 28 ref, Eng.** Department of Paediatrics, The Aga Khan University Medical Center, PO Box 3500, Stadium Road, Karachi 74800, Pakistan

"The recovery pattern and outcome were analysed in 261 consecutive children (age 6-36 months) with persistent diarrhoea who underwent inpatient nutritional rehabilitation with a rice-lentil (Khitchri) and yoghurt-based diet. Overall, 217 (83%) recovered successfully, as judged by a reduction in stool output and weight gain for a consecutive 3 d. Failures were more commonly febrile at admission [odds ratio (OR) 2.3. 95% confidence interval (Cl) 1.1-4.8] and a greater number had culture-proven sepsis (Fisher's exact test, $p<0.001$). Logistic regression analysis identified significantly increased risk of treatment failure with several admission characteristics, including stool frequency >5 $d^{-1}$ (OR 2.9, 95% Cl 1.6-5.2), vomiting (OR 2.5, 95% Cl 1.1-5.7) and sepsis (OR 2.8, 95% Cl 1.1-7.5). Survival analysis revealed significantly longer time-to-recovery among children with stool frequency >5 $d^{-1}$ at admission ($p<0.001$), suspected sepsis necessitating intravenous antibiotics ($p<0.001$) or oral candidiasis ($p<0.05$). These findings suggest that severity of diarrhoea and coexisting systemic infections key determinants of the response to nutritional therapy in children with persistent diarrhoea."

**349. Bondre VP, Srivastava R*, Sinha VB, Srivastava BS. Screening of Tn*pho*A mutants of *Vibrio cholerae* O139 for identification of antigens involved in colonisation. J Med Microbiol 1997 Dec;46(12):1007-11. 22 ref, Eng.*** Division of Microbiology, Central Drug Research Institute, Lucknow 226001, India

**350. Britten D, Wilson SM, McNerney R, Moody AH, Chiodini PL, Ackers JP*. An improved colorimetric PCR-based method for detection and differentiation of *Entamoeba histolytica* and *Entamoeba dispar* in feces. J Clin Microbiol 1997 May;35(5):1108-11. 23 ref, Eng.*** Department of Medical Parasitology, London School of Hygiene and Tropical Medicine, Keppel Street, London WC1E 7HT, UK

**351. Dalsgaard A, Tam NV, Cam PD. Cholera in Vietnam (note). Southeast Asian J Trop Med Public Health 1997 Mar;28(1):69-72. 11 ref, Eng.** Department of Veterinary Microbiology, The Royal Veterinary and Agricultural University, Bulowsvej 13, DK-1870 Frederiksberg C, Denmark

**352. Dalsgaard A, Skov MN, Serichantalergs O, Echeverria P, Meza R, Taylor DN. Molecular evolution of *Vibrio cholerae* O1 strains isolated in Lima, Peru, from 1991 to 1995. J Clin Microbiol 1997 May;35(5):1151-6. 33 ref, Eng.** Department of Veterinary Microbiology, The Royal Veterinary and Agricultural University, Bülowsvej 13, 1870 Frederiksberg C, Denmark

**353. Edet EE. Fluid intake and feeding practices during diarrhoea in Odukpani, Nigeria. East Afr Med J 1996 May;73(5):289-91. 24 ref, Eng.** Department of Community Health, University of Calabar, PMB 115, Calabar, Cross River State, Nigeria

**354. English RM, Badcock JC, Giay T, Ngu T, Waters A-M, Bennett SA. Effect of nutrition improvement project on morbidity from infectious diseases in preschool children in Vietnam: comparison with control commune. Br Med J 1997 Nov 1;315(7116):1122-5. 11 ref, Eng.** Department of Social and Preventive Medicine, Medical School, University of Queensland, Herston, Queensland 4006, Australia

"**Objective:** To evaluate the effect of a nutrition improvement project based on home garden production and nutrition education on morbidity from acute respiratory infection and diarrhoeal disease in preschool children. **Design:** The morbidity survey comprised five data collections undertaken by trained interviewers to ascertain the incidence and severity of respiratory infections and the incidence of diarrhoeal disease in children in two communes. **Setting:** A project commune and a control commune in Vietnam. **Subjects:** Preschool children to 6 years of age living in the project commune Khai Xuan (average 469 children) and the control commune Ching Cong (average 251 children). **Main outcome measures:** Differences between the two communes over time in the incidence and severity of respiratory infections and the incidence of diarrhoeal disease. **Results:** In Khai Xuan there was a significant reduction (P<0.0001) in the incidence of respiratory infections (from 49.5% to 11.2%) and diarrhoeal infections (18.3% to 5.1%); the incidence of pneumonia and severe pneumonia was also significantly reduced (P<0.0001). In Ching Cong there was no significant change in the incidence and severity of respiratory disease nor in the incidence of diarrhoeal disease. **Conclusions:** These findings emphasise the successful health outcome of a nutrition project based on household food production and nutrition education and the value of evaluating nutrition projects by reference to measurable health outcomes."

**355. Fallarino A, Mavrangelos C, Stroeher UH, Manning PA*. Identification of additional genes required for O-antigen biosynthesis in *Vibrio cholerae* O1. J Bacteriol 1997 Apr;179(7):2147-53. 50 ref, Eng.*** Microbial Pathogenesis Unit, Department of Microbiology and Immunology, The University of Adelaide, Adelaide, S.A., 5005 Australia

**356. Faruque SM, Ahmed KM, Siddique AK, Zaman K, Alim ARMA, Albert MJ. Molecular analysis of toxigenic *Vibrio cholerae* O139 Bengal strains isolated in Bangladesh between 1993 and 1996: evidence for emergence of a new clone of the Bengal vibrios. J Clin Microbiol 1997 Sep;35(9):2299-2306. 41 ref, Eng.** International Centre for Diarrhoeal Disease Research, Bangladesh, GPO Box 128, Dhaka 1000, Bangladesh

"*Vibrio cholerae* O139 Bengal emerged in 1992 and rapidly spread in an epidemic form, in which it replaced existing strains of *V. cholerae* O1 in Bangladesh during 1992 and 1993. The subsequent emergence of a new clone of *V. cholerae* O1 of the El Tor biotype that transiently displaced the O139 vibrios during 1994 to 1995 and the recent reemergence of *V. cholerae* O139 and its coexistence with the El Tor vibrios demonstrated temporal changes in the epidemiology of cholera in Bangladesh. We studied clonal diversity among *V. cholerae* O139 strains isolated from cholera patients and environmental surface water since their first appearance until their transient disappearance in 1994 as well as the O139 strains that reemerged during 1995 to 1996 and were isolated in the capital Dhaka and four rural districts of Bangladesh to investigate the origin of the reemerged strains. Analysis of restriction fragment length polymorphisms in genes for conserved rRNA and cholera toxin (CT) (*ctxA*) or in DNA sequences flanking these genes revealed four different ribotypes and four different *ctx* genotypes among the 93 strains of *V. cholerae* O139 studied. Ribotypes I and II and *ctx* genotypes A through C were shared by strains isolated from the epidemic outbreak during 1992 and 1993 in Bangladesh and India, ribotype III was represented by a single CT-negative O139 strain from Argentina, and 16 to 27 (59.2%) of the reemerged strains isolated during 1995 and 1996 belonged to a new ribotype of O139 vibrios designated ribotype IV. All 16 strains belonging to ribotype IV also belonged to a new *ctx* genotype (genotype 4). These results provide evidence for the emergence of a new clone of toxigenic *V. cholerae* O139 in Bangladesh. Further analysis of the *rfb* gene cluster by PCR revealed the absence of a large region of the O1-specific *rfb* operon and the presence of an O139-specific genomic region in all O139 strains. The PCR amplicon corresponding to the *rfaD* gene of a CT-negative O139 strain from Argentina was smaller in length than those of the toxigenic O139 strains but was identical to those of seven non-O1 and non-O139 strains. All O139 strains except the CT-negative strain carried structural and regulatory genes for CT and toxin-coregulated pili (*ctxA, tcpA, tcpI,* and *toxR*). These results suggest that the O139 Bengal strains possibly emerged from an El Tor strain but that the CT-negative non-Bengal O139 strain might have emerged from a non-O1, non-O139 strain. Thus, strains belonging to the 0139 serogroup may have emerged from similar serotype-specific genetic changes in more than one progenitor strain of *V. cholerae*".

**357. Felici A, Perilli M, Franceschini N, Rossolini GM, Galleni M, Frere J-M, Oratore A, Amicosante G[*]. Sensitivity of *Aeromonas hydrophila* carbapenemase to $^{3}$-cephems: comparative study with other metallo-ß-lactamases. Antimicrob Agent Chemother 1997 Apr;41(4):866-8. 10 ref, Eng.**[*]Dipartimento di Scienze e Tecnologie Biomediche Cattedra di Chimica Biologica e di Enzimologia, Universita degli Studi dell'Aquila, I-67010 Coppito (L`Aquila), Italy

**358. Fiederling F, Boury M, Petit C, Milon A[*]. Adhesive factor/rabbit 2, a new fimbrial adhesin and a virulence factor from *Escherichia coli* O103, a serogroup enteropathogenic for rabbits [note]. Infect Immun 1997 Feb;65(2):847-51. 23 ref, Eng.**[*]Unité Associée de Microbiologie Moléculaire, Institut National de la Recherche Agronomique/Ecole Nationale Vétérinaire, Ecole Nationale Vétérinaire, F-31076 Toulouse Cedex 3, France

**359. Fraser D, Dagan R, Naggan L, Greene V, El-On J, Abu-Rbiah Y, Deckelbaum RJ. Natural history of *Giardia lamblia* and *Cryptosporidium* infections in a cohort of Israeli Bedouin infants: a study of a population in transition. Am J Trop Med Hyg 1997**

**Nov;57(5):544-9. 39 ref, Eng.** Epidemiology Department, Faculty of Health Sciences, Ben-Gurion University of the Negev, PO Box 653, Beer-Sheva 84105, Israel

**360. Gajardo R, Vende P, Poncet D, Cohen J*. Two proline residues are essential in the calcium-binding activity of rotavirus VP7 outer capsid protein. J Viriol 1997 Mar;71(3):2211-6. 39 ref, Eng.*** Departament de Microbiologia, Facultat de Biologia, Universitat de Barcelona, 08028 Barcelona, Spain

**361. González R, Díaz C, Mariño M, Cloralt R, Pequeneze M, Perez-Schael I*. Age-specific prevalence of *Escherichia coli* with localized and aggregative adherence in Venezuelan infants with acute diarrhea. J Clin Microbiol 1997 May;35(5):1103-7. 40 ref, Eng.*** Instituto de Biomedicina, A.P. 4043, Carmelitas, Caracas 1010A, Venezuela

"To evaluate the epidemiological significance of HEp-2 cell-adherent *Escherichia coli* isolates in diarrheal disease, we performed a study with 513 Venezuelan infants with diarrhea and 241 age-matched controls to determine the prevalence of enteropathogenic *E. coli* (enteroadherent *E. coli*, enterotoxigenic *E. coli*, enteroinvasive *E. coli*, and enterohemorrhagic *E. coli*) and their correlation with O:H serotypes. *E. coli* isolates exhibiting localized and aggregative adherence in the HEp-2 cell assay were significantly more frequently isolated from the patients (8.5 and 26.9%, respectively) than from the controls (1.7 and 15%, respectively). This difference was significant for the group O to 2 months of age but not for older infants. Regardless of age, *E. coli* isolates with diffuse adherence were found at similar frequencies in both the patients and the controls. A striking correlation between classic O serogroups and localized adherence was also observed. These findings confirm the pathogenic role of *E. coli* with localized and aggregative adherence in diarrheal disease, as well as the epidemiological importance of O:H serotyping for characterizing localized-adhering *E. coli*."

**362. Grewal HMS, Valvatne H, Bhan MK, van Dijk L, Gaastra W, Sommerfelt H*. A new putative fimbrial colonization factor, CS19, of human enterotoxigenic *Escherichia coli*. Infect Immun 1997 Feb;65(2):507-13. 54 ref, Eng.*** Centre for International Health, University of Bergen, Armauer Hansen's Building, Bergen N-5021, Norway

**363. Gurgel RQ, Andrade JM, Machado-Neto P, dal Fabbro AL, Cuevas LE*. Diarrhoea mortality in Aracaju, Brazil. Ann Trop Paediatr 1997 Dec;17(4):361-5. 16 ref, Eng.*** Liverpool School of Tropical Medicine, Pembroke Place, Liverpool L3 5QA, UK

**364. Headley V, Hong M, Galko M, Payne SM*. Expression of aerobactin genes by *Shigella flexneri* during extracellular and intracellular growth [note]. Infect Immun 1997 Feb;65(2):818-21. 17 ref, Eng.*** Department of Microbiology, University of Texas, Austin, TX 78712, USA

**365. Helander A, Grewal HMS, Gaastra W, Svennerholm A-M*. Detection and characterization of the coli surface antigen 6 of enterotoxigenic *Escherichia coli* strains by using monoclonal antibodies. J Clin Microbiol 1997 Apr;35(4):867-72. 38 ref, Eng.*** Department of Medical Microbiology and Immunology, Göteborg University, Guldhedsgatan 10A, 413 46 Göteborg, Sweden

**366. Herias MV, Mattsby-Baltzer I, Cruz JR, Hanson LA. Antibodies to *Escherichia coli* and *Shigella flexneri* in milk from undernourished mothers: studies on sodium dodecyl sulfate-polyacrylamide gel electrophoresis-separated antigens. Pediatr Res 1997 Nov;42(5):644-50. 35 ref, Eng.** Department of Clinical Immunology, University of Göteborg, Guldhedsgatan 10, S-413 46 Göteborg, Sweden

"Analysis of IgA, IgM, and IgG antibodies against *Escherichia coli* O6, its lipopolysaccharide (LPS), and *Shigella flexneri* were performed in the milk of moderately undernourished Guatemalan women receiving either a low or a high calorie supplement, using SDS-PAGE. As expected, the immunostaining analysis of milk antibodies showed that IgA was the predominant isotype in both groups. Concerning the other Igs, antibodies against O6 LPS were mainly of the IgM isotype, whereas IgG antibodies were more prominent than IgM against the bacterial whole cell preparations. Seven to nine distinct bands, ranging in molecular mass from 13.5 to 109 kD were selected for each antigen to compare the milk antibodies between the two groups of women. After a 20-wk supplementation period, the IgA and IgG antibodies to the *E. coli*, O6 LPS, and *S. flexneri* were not found negatively affected by a low calorie intake. A significantly lower immunostaining intensity was, however, obtained for the low calorie intake group regarding the IgM antibody activity against four high molecular mass bands of the *E. coli* whole cell preparation. A decreased immunostaining intensity was also found in the same group for IgM antibodies against two bands of *E. coli* O6 LPS when analyzing paired samples collected at d O and wk 20. No differences were found for IgM antibodies against any of the *S. flexneri* antigens. In conclusion, the results suggest that low calorie intake does not significantly affect the production of milk IgA antibodies to *E. coli* and *S. flexneri* antigens in these women. Still, IgM antibodies against certain proteins and LPS molecules of *E. coli* may be decreased. IgG antibodies, although also present in milk, seemed to the directed mainly against bacterial proteins and not to LPS."

**367. Hinterleitner TA, Petritsch W, Dimsity G, Berard H, Lecomte J-M, Krejs GJ. Acetorphan prevents cholera-toxin-induced water and electrolyte secretion in the human jejunum. Eur J Gastroenterol Hepatol 1997 Sep;9(9):887-91. 38 ref, Eng.** Department of Medicine, Karl-Franzens University, Auenbruggerplatz 15, A-8036 Graz, Austria

"**Objectives**: Acetorphan is an orally administered inhibitor of enkephalinase in the wall of the digestive tract. It prevents inactivation of endogenous opioid peptides released by submucosal and myenteric neurons. The aim of this study was to examine the effect of acetorphan on jejunal water and electrolyte transport in healthy volunteers under basal conditions and in a state of intestinal secretion induced by a bacterial enterotoxin. **Design**: Ten volunteers in two groups were studied in an open trial. For the experimental design an intestinal perfusion technique was used. **Methods**: Cholera toxin was used to induce intestinal secretion in a model employing segmental perfusion of the human proximal jejunum. Acetorphan was given orally prior to intrajejunal administration of cholera toxin; its effect on intestinal transport was measured over a period of four hours after exposure to cholera toxin. Serum levels of methylthioether of thiorphan as the main metabolite were measured throughout three experiments to assure sufficient drug absorption. **Results**: Acetorphan had no influence on basal water and electrolyte absorption (133 vs. 140 ml/30 cm.h). In a control group with cholera toxin alone, significant water secretion was induced (131 ml/30 cm.h). Acetorphan completely prevented this secretion by leaving an absorption rate of 27 ml/30 cm.h. Intestinal electrolyte transport was also significantly changed towards absorption by acetorphan. **Conclusion**: Acetorphan can prevent jejunal water and electrolyte secretion induced by cholera toxin. Enkephalins may thus protect the small intestine from enterotoxin-induced secretion."

**368. Inward CD, Varagunam M, Adu D, Milford DV, Taylor CM*. Cytokines in haemolytic uraemic syndrome associated with verocytotoxin-producing *Escherichia coli* infection. Arch Dis Child 1997 Aug;77(2):145-7. 12 ref, Eng.** *Department of Nephrology, Birmingham Children's Hospital, Ladywood Middleway, Birmingham B16 8ET, UK

**369. Iredell JR, Manning PA[*]. Outer membrane translocation arrest of the TcpA pilin subunit in *rfb* mutants of *Vibrio cholerae* O1 strain 569B. J Bacteriol 1997 Mar;179(6):2038-46. 63 ref, Eng.** [*]Department of Medicine, QE2 Jubilee Hospital, Coopers Plains, Brisbane Qld. 4108, Australia

**370. Islam D, Veress B, Bardhan PK, Lindberg AA, Christensson B. In situ characterization of inflammatory responses in the rectal mucosae of patients with shigellosis. Infect Immun 1997 Feb;65(2):739-49. 71 ref, Eng.** International Centre for Diarrhoeal Disease Research, Bangladesh, GPO Box 128, Dhaka 1000, Bangladesh

"*Shigella* species cause bacillary dysentery in humans by invading epithelial cells of the colonic mucosa leading to colonic epithelial cell destruction and inflammation. For further analysis of local gut inflammation, morphological changes and the potential involvement of mediators in regulatory mechanisms of cell activation and cell proliferation were studied immunohistochemically in rectal mucosal biopsies taken from patients during the acute phase of shigellosis and at convalescence. Rectal biopsies from 25 *Shigella dysenteriae*-1 and 10 *Shigella flexneri*-infected patients and from 40 controls were studied. The frequencies of proliferative cells (Ki67-positive cells), p53-immunostaining cells, and cells coexpressing Ki67 with CD3 or with p53 were analyzed. Immunostaining for the inducible nitric oxide synthase (iNOS) and the endothelial NOS was assessed. In addition, the frequencies of apoptotic cells and $CD68^+$ cells that engulf apoptotic cells were assessed. By morphological grading, 20% of the patients had advanced inflammation (grade 3) in the acute phase; mild inflammation (grade 1) was seen in 37% of the patients at convalescence as well as in 10% of the controls. The findings in the present study suggest that in the acute phase of shigellosis inflammation is characterized by increased cell turnover in the lamina propria (LP) and the epithelium, increased iNOS expression in the surface epithelium, and apoptosis, which seems to be associated with LP macrophages. The findings also suggest that neither p53 nor iNOS are important factors for the induction of apoptosis in shigellosis. Expression or p53 may be related to early cell activation in crypt epithelium. Moreover, there is an indication of an active, low-level inflammatory process at convalescence. The results thus indicate that *Shigella*-induced inflammation is associated with a complex series of cellular reactions in the rectal gut mucosa which persist long after clinical symptoms have resolved."

**371. Islam D, Veress B, Bardhan PK, Lindberg AA, Christensson B. Quantitative assessment of IgG and IgA subclass producing cells in rectal mucosa during shigellosis. J Clin Pathol 1997 Jun;50(6):513-20. 43 ref, Eng.** International Centre for Diarrhoeal Disease Research, Bangladesh GPO Box 128, Dhaka 1000, Bangladesh

"*Aims*-To assess quantitatively both the morphological changes in the rectal mucosa and the changes in the relative frequency of IgA and IgG subclass producing cells found in the rectal mucosa during the acute phase of shigellosis and at convalescence. *Methods*-Rectal biopsies from 25 *Shigella dysenteriae* 1 infected patients, 10 *Shigella flexneri* infected patients, and 40 uninfected controls were studied. Morphological changes in the mucosa were graded. The frequency of IgA and IgG subclass producing cells was assessed. In addition, immunostaining for secretory component in epithelial cells was analysed. *Results*-Using morphological grading, 20% of the 35 patients studied had advanced inflammation (grade 3) in the acute phase of the disease. At convalescence, grade 1 inflammation was seen in 37% of the patients and in 10% of the controls. In the acute phase, as well as at convalescence, the number of IgAi, IgA, and IgGi positive cells was significantly higher than in the controls. The results were related to the histopathological degree of inflammation. *Conclusions*-In shigellosis, there is evidence for a prolonged humoral response residing in the mucosa long after the clinical symptoms have resolved, suggesting that shigellosis induces persisting mucosal humoral immune and inflammatory responses, remaining at least until 30 days after the infection."

**372. Itoh Y, Nagano I, Kunishima M, Ezaki T. Laboratory investigation of enteroaggregative *Escherichia coli* O untypeable: H10 associated with a massive outbreak of gastrointestinal illness. J Clin Microbiol 1997 Oct;35(10):2546-50. 24 ref, Eng.** Department of Microbiology, Gifu University School of Medicine, Tsukasa-machi 40, Gifu 500, Japan

**373. Klee SR, Tzschaschel BD, Timmis KN, Guzman CA[*]. Influence of different *rol* gene products on the chain length of *Shigella dysenteriae* type 1 lipopolysaccharide O antigen expressed by *Shigella flexneri* carrier strains [note]. J Bacteriol 1997 pr;179(8):2421-5. 38 ref, Eng.** [*]Division of Microbiology, GBF-National Research Centre for Biotechnology, Mascheroder Weg 1, 38124 Braunschweig, Germany

**374. Kudva IT, Hatfield PG, Hovde CJ[*]. Characterization of *Escherichia coli* O157:H7 and other shiga toxin-producing *E. coli* serotypes isolated from sheep. J Clin Microbiol 1997 Apr;35(4):892-9. 47 ref, Eng.** [*]Department of Microbiology, Molecular Biology, and Biochemistry, University of Idaho, Moscow, ID 83843, USA

**375. Lima AAM, Sidrim JJC, Lima NL, Titlow W, Evans ME, Greenberg RN[*]. Molecular epidemiology of multiply antibiotic-resistant *Shigella flexneri* in Fortaleza, Brazil. J Clin Microbiol 1997 May;35(5):1061-5. 20 ref, Eng.** [*]MN-68A, Chandler Medical Center, 800 Rose St., Lexington, KY 40536-0084, USA

"In northeastern Brazil, strains of *Shigella flexneri* resistant to multiple antibiotics are often found in patients in both urban areas and community hospitals. This study used pulsed-field gel electrophoresis (PFGE) and plasmid analysis to further analyze the molecular epidemiology of *Shigella flexneri* strains isolated from hospitals and an urban community in Fortaleza, Brazil. Twenty-six strains of *S. flexneri* from three distinct areas in the city of Fortaleza, Brazil, were examined: 14 strains from people with diarrhea who lived in an urban community of 2,000 persons, 5 strains from patients in the university hospital, and 7 strains from children in a pediatric hospital. PFGE identified six unique groups of *S. flexneri* circulating among patients during the 45-month study. Seven strains were further studied for antibiotic resistance plasmid profiles. Three unique antibiotic resistance plasmid profiles were found. Strains collected from the hospitalized patients demonstrated the variety of PFGE and antibiotic resistance patterns in the area. Strains collected from the patients living in the urban community setting demonstrated the persistence of certain PFGE patterns as well as the acquisition of multiple antibiotic resistance plasmids. Effective interventional strategies for such geographic locations as Fortaleza, Brazil, will be more complex than those for single-strain outbreak situations."

**376. Lima AAM, Silva TMJ, Gifoni AMR, Barrett LJ, McAuliffe IT, Bao Y, Fox JW, Fedorko DP, Guerrant RL. Mucosal injury and disruption of intestinal barrier function in HIV-infected individuals with and without diarrhea and cryptosporidiosis in northeast Brazil. Am J Gastroenterol 1997 Oct;92(10):1861-6. 46 ref, Eng.** Clinical Research Unit, Federal University of Ceara, P.O. Box 3229, Porangabussu, CEP 60.436-160, Fortaleza, CE, Brazil

"Objectives: To determine the relative effects of AIDS-related diarrhea with or without cryptosporidiosis and microsporidiosis on intestinal function and injury. *Methods:* We studied 40 HIV-infected patients (20 with and 20 without diarrhea) and 13 healthy volunteers, using the differential urinary excretion of ingested lactulose and mannitol as respective markers of barrier disruption and overall villous surface area. We also examined them for fecal leukocytes, lactoferrin, and $a_1$-antitrypsin. Fasting subjects drank test solution containing lactulose (5 g) and mannitol (1 g). Urine was collected for 5 h and tested for sugars by higher-performance liquid chromatography with pulsed amperometric detection. *Results:* HIV-positive patients with diarrhea had a 2.8-fold higher lactulose:mannitol excretion ratio (L:M) than HIV-positive patients without diarrhea (p=0.01) and 10.4-fold higher than healthy volunteers (p=0.004). This was accounted for

by a 1.5- to 3.1-fold higher rate of lactulose excretion by HIV patients with diarrhea than by those without diarrhea or by healthy volunteers. Mannitol excretion was 32-55% less in patients with diarrhea than in those without diarrhea or in healthy volunteers. Patients with cryptosporidial diarrhea had a nearly 6-fold higher L:M ratio than those without diarrhea ($P<0.001$) and nearly 3-fold higher than those with non-cryptosporidial diarrhea ($p=0.02$). One patient with microsporidial infection had a nearly 3-fold higher L:M ratio than controls without diarrhea. $a_1$-antitrypsin was positive in 40% of HIV-positive patients with cryptosporidial infections and none of 12 HIV-positive patients with non-cryptosporidial diarrhea. Fecal lactoferrin or leukocytes were increased in all HIV patients with diarrhea. *Conclusion:* HIV infection is associated with intestinal dysfunction and injury, even in patients who do not have diarrhea. However, those with diarrhea, especially with cryptosporidiosis or microsporidiosis, have even greater disruption of intestinal barrier function with potentially important nutritional consequences."

**377. Linton D, Lawson AJ, Owen RJ, Stanley J*. PCR detection, identification to species level, and fingerprinting of *Campylobacter jejuni* and *Campylobacter coli* direct from diarrheic samples. J Clin Microbiol 1997 Oct;35(10):2568-72. 28 ref, Eng.*** *Molecular Biology Unit, Virus Reference Division, Central Public Health Laboratory, 61 Colindale Ave., London NW9 5HT, UK

**378. Loubiala PJ, Jaakkola N, Ruotsalainen R, Jaakkola JJK. Day-care centers and diarrhea: a public health perspective. J Pediatr 1997 Sep;131(3):476-9. 29 ref, Eng.** Department of Public Health, University of Helsinki, PO Box 41, 00014, Finland

**379. Mache A, Mengistu Y, Cowley S. *Shigella* serogroups identified from adult diarrhoeal out-patients in Addis Ababa, Ethiopia: antibiotic resistance and plasmid profile analysis. East Afr Med J 1997 Mar;74(3):179-82. 22 ref, Eng.** Department of Medical Microbiology, Jimma Institute of Health Sciences, PO Box 378, Jimma, Ethiopia

**380. Malakooti MA, Alaii J, Shanks GD, Phillips-Howard PA. Epidemic dysentery in western Kenya. Trans R Soc Trop Med Hyg 1997 Sep-Oct;91(5):541-3. 14 ref, Eng.** Department of Preventive Medicine and Biometrics, Uniformed Services University of the Health Sciences, A1040N, 4301 Jones Bridge Road, Bethesda, MD 20814, USA

**381. Mamun KZ, Tabassum S, Hussain MA, Shears P*. Antimicrobial susceptibility of *Shigella* from a rural community in Bangladesh.** *Ann Trop Med Parasitol* **1997 Sep;91(6):643-7. 16 ref, Eng.** *Department of Microbiology, Institute of Postgraduate Medicine and Research, Shahbagh, Dhaka, Bangladesh

**382. Manjarrez-Hernandez A, Gavilanes-Parra S, Chavez-Berrocal ME, Molina-Lopez J, Cravioto A*. Binding of diarrheagenic *Escherichia coli* to 32- to 33-kilodalton human intestinal brush border proteins. Infect Immun 1997 Nov;65(11):4494-501. 39 ref, Eng.*** Department of Public Health, Faculty of Medicine, National Autonomous University, Mexico City 04510, Mexico

**383. Materu SF, Lema OE, Mukunza HM, Adhiambo CG, Carter JY. Antibiotic resistance pattern of *Vibrio cholerae* and *Shigella* causing diarrhoea outbreaks in the Eastern Africa region: 1994-1996. East Afr Med J 1997 Mar;74(3):193-7. 12 ref, Eng.** African Medical and Research Foundation, PO Box 30125, Nairobi, Kenya

"Between March 1994 and December 1996, 1797 rectal swabs were transported to the AMREF laboratory from sites in six countries in the eastern Africa region: 1749 were cultured for *Vibrio cholerae* and 48 for *Shigella/Salmonella*. Culture, isolation, identification and antibiotic susceptibility testing were performed using standardized techniques. The isolates were categorized as sensitive or resistant based on

standardized zones of inhibition. The rate of isolation of *V. cholerae* from rectal swabs increased progressively from less than 20% to more than 45% between 1994 and 1996. 80-100% of isolates of *V. cholerae* from Kenya and south Sudan, and 65-90% from Somalia were sensitive to tetracycline, although in 1995 isolates from Mogadishu showed only 44% sensitivity. All isolates from Tanzania and Rwanda were 100% resistant to tetracycline. In Kenya and Somalia, the percentage of isolates sensitive to chloramphenicol and cotrimoxazole reduced markedly from 85% in 1994 to <10 in 1996. 100% of isolates from Rwanda and Tanzania were resistant to chloramphenicol and cotrimoxazole while in south Sudan >70% of isolates were sensitive. Nalidixic acid and erythromycin retained >75% sensitivity in all areas. *Shigella dysenteriae* and *Shigella flexneri* were recovered from dysentery specimens in northern Kenya. Both species showed similar antibiotic sensitivity patterns and were sensitive only to nalidixic acid and furazolidone. Due to variations of resistance patterns within countries in the region, antibiotic sensitivity testing should be performed at the start of an outbreak, and antibiotic use should be restricted to severe cases of *V. cholerae* and *Shigella* infection."

**384. Mermel LA, Josephson SL, Dempsey J, Parenteau S, Perry C, Magill N. Outbreak of *Shigella sonnei* in a clinical microbiology laboratory (note).** *J Clin Microbiol* **1997 Dec;35(12):3163-5. 30 ref, Eng.** Division of Infectious Diseases, Rhode Island Hospital, 593 Eddy St. Providence, RI 02903, USA

**385. Mills M, Payne SM*. Identification of *shuA*, the gene encoding the heme receptor of *Shigella dysenteriae*, and analysis of invasion and intracellular multiplication of a *shuA* mutant (note). Infect Immun 1997 Dec;65(12):5358-63. 32 ref, Eng.*** Department of Microbiology and Immunology, Uniformed Services University of the Health Sciences, Bethesda, MD 20814-4799, USA

**386. Mirza NM, Caulfield LE*, Black RE, Macharia WM. Risk factors for diarrheal duration. Am J Epidemiol 1997 Nov 1;146(9):776-85. 50 ref, Eng.*** Department of International Health, The Johns Hopkins University School of Hygiene and Public Health, 615 North Wolfe Street, Baltimore, MD 21205, USA

"To identify child feeding behavior and household hygiene practices that are risk factors for prolonged diarrheal illness, a longitudinal community study was conducted over a 14-month period among 920 children aged 3-37 months who lived in an urban slum settlement in Nairobi, Kenya. Morbidity surveillance was done by home visits every third day in the absence of diarrhea and daily during diarrheal illness until termination of the episode. In-home observations were made to characterize maternal hygiene, cooking, and child feeding practices. Overall, 1,496 episodes of diarrhea were detected. The average diarrheal incidence was 3.5 episodes/child-year, and the incidence of diarrhea >14 days was 3 episodes/100 child-years. Cox regression was used to examine the independent effects of convariates on time to recovery from a diarrheal episode. Adjusted behavioral factors that were observed to influence recovery from diarrhea included: uncovered water containers (rate ratio (RR)=0.77, 95% confidence interval (CI) 0.64-0.94); giving no fluids (as opposed to oral rehydration solutions (ORS)/sugar salt solutions (SSS) (RR=1.42, 95% CI 1.14-1.77); and administration of diluted cow's milk during the first 3 days of an episode (RR=1.23, 95% CI 1.00-1.52). These associations remained significant after adjusting for diarrheal severity. The authors recommend, among other measures, improvement of water storage and promotion of continued feeding with cereal-milk mix during diarrhea."

**387. Mochizuki M, Nakagomi T, Nakagomi O*. Isolation from diarrheal and asymptomatic kittens of three rotavirus strains that belong to the AU-1 genogroup of human rotaviruses [note]. J Clin Microbiol 1997 May;35(5):1272-5. 20 ref, Eng.** *Department of Microbiology, Akita University School of Medicine, 1-1-1 Hondo, Akita 010, Japan

**388. Mounier J, Bahrani FK, Sansonetti PJ*. Secretion of *Shigella flexneri* Ipa invasins on contact with epithelial cells and subsequent entry of the bacterium into cells are growth stage dependent. Infect Immun 1997 Feb;65(2):774-82. 55 ref, Eng.*** Unité de Pathogénie Microbienne Moléculaire and Unité INSERM U389, Institut Pasteur, 28 rue de Dr. Roux, F-75724 Paris Cédex 15, France

**389. Munshi MMH, Morshed MG, Ansaruzzaman M, Alam K, Kay A, Aziz KMS, Rahaman MM. Evaluation of Teknaf enteric agar (TEA): a modified MacConkey's agar for the isolation of *Shigella dysenteriae* type 1 and *Shigella flexneri*. J Trop Pediatr 1997 Oct;43(5):307-10. 20 ref, Eng.** International Centre for Diarrhoeal Disease Research, Bangladesh, GPO Box 128, Dhaka 1000, Bangladesh

"To develop a better and selective medium for the isolation of *Shigella* spp., MacConkey's Agar (MAC) was modified by adding position tellurite ($K_2TeO_3$) at a concentration of 1 µg/ml. The formulation designated Teknaf Enteric Agar (TEA) was studied for the inhibitory effect of potassium tellurite on the growth of different enteric bacteria, and as a medium for isolating *Shigella* spp. from clinical stool samples (n=3125). We observed that the growth of *E. coli* was effectively inhibited on TEA with no effect on the growth of *S. dysenteriae* type 1 and *S. flexneri*. A total of 2019 shigellae were isolated through the combined use of TEA, MAC, and *Salmonella-Shigella* agar (SS). On TEA, 1921 *S. dysenteriae* type 1 and *S. flexneri* were isolated as compared to 1765 from the combined use of MAC and SS. A total of 194 of *S. dysenteriae* type 1 and *S. flexneri* were exclusively isolated from TEA as compared to 38 which were only made from MAC and SS. We conclude that TEA significantly increased the overall isolation rate of *Shigella* spp. as compared to the combined use of MAC and SS ($P<0.0001$), although it is not suitable for the isolation of *S. sonnei*."

**390. Musa HA, Hasan HS, Shears P. Occurrence in Sudan of *Shigella dysenteriae* type 1 with transferable antimicrobial resistance. Ann Trop Med Parasitol 1997;91(6):669-71. 15 ref, Eng.** Department of Medical Microbiology and Parasitology, University of Khartoum, P.O. Box 102, Khartoum, Sudan

**391. 272 Nitta H, Holt SC, Ebersole JL*. Purification and characterization of *Campylobacter rectus* surface layer proteins. Infect Immun 1997 Feb;65(2):478-83. 42 ref, Eng.*** Department of Periodontics, University of Texas Health Science Center at San Antonio, 7703 Floyd Curl Dr., San Antonio, TX 78284, USA

**392. Nurko S, Garcia-Aranda JA, Fisbbein E, Perez-Zuniga MI. Successful use of a chicken-based diet for the treatment of severely malnourished children with persistent diarrhea: a prospective, randomized study. J Pediatr 1997 Sep;131(3):405-12. 29 ref, Eng.** Pediatric Gastroenterology, Children's Hospital, 300 Longwood Ave., Boston, MA 02115, USA

**393. Okuda J, Fukumoto M, Takeda Y, Nishibuchi M*. Examination of diarrheagenicity of cytolethal distending toxin: suckling mouse response to the products of the *cdtABC* genes of *Shigella dysenteriae*. Infect Immun 1997 Feb;65(2):428-33. 23 ref, Eng.*** The Center for Southeast Asian Studies, Kyoto University, 46 Shimoadachi-cho, Yoshida, Sakyo-ku, Kyoto 606-01, Japan

**394. O'Neal CM, Crawford SE, Estes MK, Conner ME*. Rotavirus virus-like particles administered mucosally induce protective immunity. J Virol 1997 Nov;71(11):8707-17. 72 ref, Eng.*** Division of Molecular Virology, Baylor College of Medicine, Houston, TX 77030, USA

**395. Oni GA. Infant feeding practices, socio-economic conditions and diarrhoeal disease in a traditional area of urban Ilorin, Nigeria. East Afr Med J 1996 May;73(5):283-8. 14 ref,**

**Eng.** King Fahad Hospital, National Guard Health Affairs, Primary Health Care Directorate, PO Box 22490, Riyadh 11426, Kingdom of Saudi Arabia

"A cross-sectional study involving 771 children under the age of one year, was carried out in a traditional area of urban Ilorin, Nigeria, to determine how socio-economic conditions and feeding practices relate to diarrhoeal disease among infants. After adjustment has been made (through logistic regression) for covariates, five factors had significant association with diarrhoeal disease. These are the age of the child, parity, mother's education, availability of household kitchen and the feeding of semi-solid food to the infants. The lowest diarrhoeal rate occurred in infants aged 0-3 months while the highest rate occurred among infants seven to nine months old (Odds Ratio=4.2). Children who were of the fifth or higher birth order had significantly higher risk of diarrhoea compared with children of illiterates (OR=1.9;P<0.05). Households that had no kitchen had significantly higher risk of infantile diarrhoea than households with kitchen facilities (P<0.01). Finally, infants receiving semi-solid food had higher risk of diarrhoea compared to those children not receiving semi-solid food (P<0.05). Diarrhoeal disease awareness campaign to educate mothers on the dangers of childhood diarrhoea and how to prevent it, through proper hygiene, especially, food hygiene, is advocated."

**396. Orrett FA. Drug resistance and plasmid profile of *Shigella* organisms from different outbreaks in Trinidad and Tobago in 1994. East Afr Med J 1997 Mar;74(3):43-6. 23 ref, Eng.** Department of Microbiology, Faculty of Medical Sciences, University of the West Indies, St. Augustine, Trinidad, West Indies

"The plasmid profiles of 16 *Shigella* species isolated from cases of bacillary dysentery from different areas of Trinidad and Tobago during one calendar year, and their resistance pattern to eight antibiotics were analysed. All isolates except one *S. sonnei* strain, were multiple resistant. Two strains (both *S. sonnei*) had none plasmid bands which were the maximum found. There were several plasmid bands common among resistant strains with multiple or single resistant profiles. Resistance to ampicillin and sulphonamide were the most common (93.8%), followed by trimethroprim (25.0%), and tetracycline (6.25%). The results indicate that the outbreaks may not be related."

**397. Osawa RO, Okitsu T, Sata S, Yamai S. Rapid screening method for identification of cholera toxin-producing *Vibrio cholerae* O1 and O139. J Clin Microbiol 1997 Apr;35(4):951-3. 15 ref, Eng.** Department of Bacteriology and Pathology, Kanagawa Prefectural Public Health Laboratory, Nakao-cho 52, Yokohama 241, Japan

**398. Pai M, Kang G[*], Ramakrishna BS, Venkataraman A, Muliyil J. An epidemic of diarrhoea in South India caused by enteroaggregative *Escherichia coli*. Indian J Med Res 1997 Jul;106:7-12. 20 ref, Eng.** [*]Department of Gastrointestinal Sciences, Christian Medical College, Vellore 632004, India

"A diarrhoeal epidemic in a village close to Vellore was investigated in January 1996. Faecal samples were obtained from 20 subjects with diarrhoea and from 11 individuals without diarrhoea (controls) and were examined for bacterial, viral and parasitic enteropathogens. Water samples from all sources in the village were analysed. The epidemic affected all age groups (overall attack rate 15%). The mean duration of diarrhoea was 11 days. Individuals who consumed water exclusively from a borewell had a lower relative risk (RR) of disease (0.14, 95% Cl 0.02-1.01) compared to users of two open wells (RR 6.93, Cl 0.99-48.66 and RR 7.81, Cl 1.02-59.79, respectively). No conventional bacterial enteropathogens were isolated from the stool samples enteroaggregative *Escherichia coli* (EAggEC) were identified in the stool of 11 of 20 subjects with diarrhoea, and in 1 of 11 control samples (P=0.02). All the EAggEC isolates from the patients had identical antibiotic sensitivity patterns and produced a toxin in

ussing chamber studies. Serotyping indicated that all the EAggEC from individuals with diarrhoea belonged to one or other of two serotypes. All water samples had high coliform counts and *E. coli* were cultured from the two open wells but not from the borewell. The evidence suggests that EAggEC was responsible for this outbreak of diarrhoea. EAggEC should be considered as a possible pathogen in unexplained diarrhoeal outbreaks in developing countries."

**399. 280 Paton AW, Voss E, Manning PA, Paton JC[*]. Shiga toxin-producing *Escherichia coli* isolates from cases of human disease show enhanced adherence to intestinal epithelial (Henle 407) cells. Infect Immun 1997 Sep;65(9):3799-3805. 27 ref, Eng.**[*]Molecular Microbiology Unit, Women's and Children's Hospital, North Adelaide, S.A. 5006, Australia

**400. Perez-Schael I, Guntinas MJ, Perez M, Pagone V, Rojas AM, Gonzalez R, Cunto W, Hoshino Y, Kapikian AZ. Efficacy of the rhesus rotavirus-based quadrivalent vaccine in infants and young children in Venezuela. N Engl J Med 1997 Oct 23;337(17):1181-7. 40 ref, Eng.** Instituto de Biomedicina - Fuvesin, A.P. 4043, Carmelitas, Caracas 1010A, Venezuela

**401. 282 Porter ME, Dorman CJ. Positive regulation of *Shigella flexneri* virulence genes by integration host factor. J Bacteriol 1997 Nov;179(21):6537-50. 55 ref, Eng.** Department of Microbiology, Moyne Institute of Preventive Medicine, University of Dublin, Trinity College, Dublin 2, Republic of Ireland

**402. Qadri F, Wenneras C, Albert MJ, Hossain J, Mannoor K, Begum YA, Mohi G, Salam MA, Sack RB, Svennerholm A-M. Comparison of immune responses in patients infected with *Vibrio cholerae* O139 and O1. Infect Immun 1997 Sep;65(9):3571-6. 42 ref, Eng.** International Centre for Diarrhoeal Disease Research, Bangladesh, GPO Box 128, Dhaka 1000, Bangladesh

"*Vibrio cholerae* O139 has recently emerged as the second etiologic agent of cholera in Asia. A study was carried out to evaluate the induction of specific immune responses to the organism in *V. cholerae* O139-infected patients. The immune responses to *V. cholerae* O139 Bengal were studied in patients by measuring antibody-secreting cells (ASC), as well as vibriocidal and antitoxic antibodies in the circulation. These responses were compared with those in patients with *V. cholerae* O1 disease. Strong immunoglobulin A (IgA) and IgM ASC responses were seen against the homologous lipopolysaccharide or serogroup of *V. cholerae*. The magnitude and isotype of the responses were similar in O139- and O1-infected patients. Vibriocidal antibody responses were seen against bacteria of the homologous but not heterologous serogroup, and these responses reflect the lack of cross-protection between the infections caused by the two serogroups. The two groups of patients showed comparable cholera toxin-specific ASC responses, with the IgG isotype dominating over the IgA isotype, as well as comparable antitoxic immune responses in plasma. These results suggest that despite having a polysaccharide capsule, *V. cholerae* O139 induces systemic and intestine-derived ASC responses in peripheral blood comparable to those seen in patients with *V. cholerae* O1 disease."

**403. Radwan SF, Gabr MK, El-Maraghi S, El-Saifi AF. Serotyping of group A rotaviruses in Egyptian neonates and infants less than 1 year old with acute diarrhea (note). J Clin Microbiol 1997 Nov;35(11):2996-8. 21 ref, Eng.** Department of Pediatrics, School of Medicine, Cairo University, Cairo, Egypt

**404. Rice BE, Rollins DM, Mallinson ET, Carr L, Joseph SW. *Campylobacter jejuni* in broiler chickens: colonization and humoral immunity following oral vaccination and experimental infection. Vaccine 1997 Dec;15(17/18):1922-32. 50 ref, Eng.** Enteric Diseases Program, Naval Medical Research Institute, Bethesda, MD 20889-5607, USA

**405. Rosenberg T, Kendall O, Blanchard J, Martel S, Wakelin C, Fast M. Shigellosis on Indian reserves in Manitoba, Canada: its relationship to crowded housing, lack of running water, and inadequate sewage disposal.** *Am J Public Health* **1997 Sep;87(9):1547-51. 24 ref, Eng.** Department of Community Health Sciences, Faculty of Medicine, University of Manitoba, Winnipeg, Canada V8R 1J8 (Present address: HW 106 Memorial Pavilion, 2335 Richmond Ave., Victoria, British Columbia, Canada V8R 1J8)

"***Objectives*.** This study compares incidence and hospitalization rates for shigellosis between Indians and the rest of the population in Manitoba, Canada. It examines the relationship between shigellosis and environmental conditions on reservices. *Methods*. Rates were calculated with surveillance data and a survey of environmental infrastructure was done. *Results*. Indians had shigellosis incidence and hospitalization rates that were 29 and 12 times as high, respectively, as those of the rest of the population. Household crowding, lack of piped water, and inadequate sewage disposal were significantly associated with an increased incidence of shigellosis on reserves. *Conclusions*. Many cases of shigellosis may be prevented by improving living conditions on Indian reserved."

**406. Ryan ET, Butterton JR, Zhang T, Baker MA, Stanley SL, Jr., Calderwood SB[*]. Oral immunization with attenuated vaccine strains of *Vibrio cholerae* expression a dodecapeptide repeat of the serine-rich *Entamoeba histolytica* protein fused to the cholera toxin B subunit induces systemic and mucosal antiamebic and anti-*V. cholerae* antibody responses in mice. Infect Immun 1997 Aug;65(8):3118-25. 43 ref, Eng.** [*]Infectious Disease Unit, Massachusetts General Hospital, Boston, MA 02114, USA

"*Entamoeba histolytica* is a significant cause of morbidity and mortality worldwide. The serine-rich *E. histolytica* protein (SREHP) is a surface-expressed trophozoite protein that includes multiple hydrophilic tandem repeats. A purified fusion protein between the dodecapeptide repeat of SREHP and cholera toxin B subunit (CTB) has previously been shown to be immunogenic in mice after oral inoculation when cholera toxin is coadministered as an immunoadjuvant. We engineered a live attenuated El Tor *Vibrio cholerae* vaccine strain, Peru2, to express the SREHP-12-CTB fusion protein to the supernatant from either a plasmid [Peru2 (pETR5.1)] or from a chromosomal insertion (ETR3). Vector strains were administered orally to germfree mice that were subsequently housed under nongermfree conditions; mice received one (day 0) or two (days 0 and 14) inoculations. No immunoadjuvant or cholera holotoxin was administered. Mice that received two inoculations of Peru2 (pETR5.1) had the most pronounced antiamebic systemic and mucosal immunologic responses. Less marked, but significant, anti-SREHP serum immunoglobulin G antibody responses were also induced in mice that received either one or two oral inoculations of strain ETR3. Anti-*V. cholerae* responses were also induced, as measured by the induction of serum vibriocidal antibodies and by serum and mucosal anti-CTB antibody responses. These results suggest that *V. cholerae* vector strains can be successful delivery vehicles for the SREHP-12-CTB fusion protein, to induce mucosal and systemic antiamebic and anti-*V. cholerae* immune responses. The magnitude of these responses is proportional to the amount of SREHP-12-CTB produced by the vector strain."

**407. Saha PK, Nair GB[*]. Production of monoclonal antibodies to the non-membrane-damaging cytotoxin (NMDCY) purified from *Vibrio cholerae* O26 and distribution of NMDCY among strains of *Vibrio cholerae* and other enteric bacteria determined by monoclonal-polyclonal sandwich enzyme-linked immunosorbent assay [note]. Infect Immun 1997 Feb;65(2):801-5. 17 ref, Eng.** [*]National Institute of Cholera & Enteric Diseases, P-33, CIT Road, Scheme XM, Beliaghata, Calcutta 700010, India

**408. Saidi SM, Iijima Y, Sang WK, Mwangudza AK, Oundo JO, Taga K, Aihara M, Nagayama K, Yamamoto H, Waiyaki PG, Honda T*. Epidemiological study on infectious diarrheal diseases in children in a coastal rural area of Kenya. Microbiol Immunol 1997;41(10):773-8. 18 ref, Eng.** *Department of Bacterial Infections, Research Institute for Microbial Diseases, Osaka University, 3-1 Yamadaoka, Suita, Osaka 565, Japan

**409. Salcedo J, Keates S, Pothoulakis C, Warny M, Castagliuolo I, LaMont JT, Kelly CP*. Intravenous immunoglobulin therapy for severe *Clostridium difficile* colitis. Gut 1997 Sep;41(3):366-70. 41 ref, Eng.** *Dana 601, Gastroenterology, Beth Israel Deaconess Medical Center, 330 Brookline Avenue, Boston, MA 02215, USA

**410. Saliki JT, Fulton RW, Hull SR, Dubovi EJ. Microtiter virus isolation and enzyme immunoassays for detection of bovine viral diarrhea virus in cattle serum. J Clin Microbiol 1997 Apr;35(4):803-7. 30 ref, Eng.** Oklahoma Animal Disease Diagnostic Laboratory, Oklahoma State University, Stillwater, OK 74078, USA

**411. Sang WK, Oundo JO, Mwituria JK, Waiyaki PG, Yoh M, Iida T, Honda T. Multidrug-resistant enteroaggregative *Escherichia coli* associated with persistent diarrhea in Kenyan children (note). Emerg Infect Dis 1997 Jul-Sep;3(3):373-4. 12 ref, Eng.** Kenya Medical Research Institute, Nairobi, Kenya

**412. Sarker RI, Ogawa W, Shimamoto T, Shimamoto T, Tsuchiya T*. Primary structure and properties of the $Na^+$/glucose symporter (Sg1S) of *Vibrio parahaemolyticus* [note]. J Bacteriol 1997 Mar;179(5):1805-8. 35 ref, Eng.** *Department of Microbiology, Faculty of Pharmaceutical Sciences, Okayama University, Tsushima, Okayama 700, Japan

**413. Sato M, Nakagomi T, Tajima K, Ezura K, Akashi H, Nakagomi O*. Isolation of serotype G8, P6[1] bovine rotavirus from adult cattle with diarrhea [note]. J Clin Microbiol 1997 May;35(5):1266-8. 29 ref, Eng.** *Department of Microbiology, Akita University School of Medicine, 1-1-1 Hondo, Akita 010, Japan

**414. Schroeder DG, Torun B, Bartlett AV, Miracle-McMahil H. Dietary management of acute diarrhea with local foods in a Guatemalan rural community. Acta Paediatr 1997 Nov;86(11):1155-61. 29 ref, Eng.** Department of International Health, Rollins School of Public Health at Emory University, Atlanta, GA 30322, USA

"A community-based, randomized trial was conducted to evaluate a locally available diet for the management of acute diarrhea (n=99 episodes) in 90 Guatemalan children, 4-42 months of age. The Test Diet (TD), a combination of a semi-solid pap (maize flour, black bean, oil) and a liquid gruel, *Incaparina* (maize flour, cotton seed flour, sugar), in addition to breast-milk and other home foods (group TD, n=45 episodes) was offered for 14 d and compared to usual home feeding (group HF, n=54 episodes). Diarrhea episodes after admission were significantly shorter for group TD (median 2.0 d) than group HF (median 4.4 d, p=0.003) after adjusting for potential confounders. Weight gains did not differ significantly between groups. We conclude that community-based dietary management of acute childhood diarrhea using energy-dense, locally available foods is feasible and may shorten diarrhea duration. This may encourage mothers to follow recommendations for continued feeding during diarrhea in developing country environments."

**415. Schuch R, Maurelli AT*. Virulence plasmid instability in *Shigella flexneri* 2a is induced by virulence gene expression. Infect Immun 1997 Sep;65(9):3686-92. 42 ref, Eng.** *Department of Microbiology and Immunology, F. Edward Hebert School of Medicine, Uniformed Services University of the Health Sciences, 4301 Jones Bridge Road, Bethesda, MD 20814-4799, USA

**416. Schultsz C, Moussa M, van Ketel R, Tytgat GNJ, Dankert J. Frequency of pathogenic and enteroadherent *Escherichia coli* in patients with inflammatory bowel disease and controls. J Clin Pathol 1997 Jul;50(7):573-9. 21 ref, Eng.** Department of Medical Microbiology, Academic Medical Centre, Meibergdreef 9, 1105 AZ Amsterdam, The Netherlands

**417. 298 Sharma C, Maiti S, Mukhopadhyay AK, Basu A, Basu I, Nair GB, Mukhopadhyaya R, Das B, Kar S, Ghosh RK, Ghosh A*. Unique organization of the CTX genetic element in *Vibrio cholerae* O139 strains which reemerged in Calcutta, India, in September 1996 (note). J Clin Microbiol 1997 Dec;35(12):3348-50. 21 ref, Eng.*** Institute of Microbial Technology, Sector 39A, Chandigarh 160036, India

**418. 299 Shimodori S, Iida K, Kojima F, Takade A, Ehara M, Amako K. Morphological features of a filamentous phage of *Vibrio cholerae* O139 bengal. Microbiol Immunol 1997;41(10):757-63. 17 ref, Eng.** Department of Bacteriology, School of Health Sciences, Kyushu University, 3-1-1 Maidashi, Higashi-ku, Fukuoka, Fukuoka 812, Japan

**419. Siu LK, Ho PL*, Yuen KY, Wong SSY, Chau PY. Transferable hyperproduction of TEM-1 ß-lactamase in *Shigella flexneri* due to a point mutation in the pribnow box. Antimicrob Agent Chemother 1997 Feb;41(2):468-70. 21 ref, Eng.*** Department of Microbiology, The University of Hong Kong, Queen Mary Hospital Compound, Pokfulam Road, Hong Kong

**420. Smit TK, Steele AD, Peenze I, Jiang XI, Estes MK*. Study of Norwalk virus and Mexico virus infections at Ga-Rankuwa Hospital, Ga-Rankuwa, South Africa. J Clin Microbiol 1997 Sep;35(9):2381-5. 38 ref, Eng.*** Department of Virology, Medical University of Southern Africa, Medunsa, South Africa 0204

**421. Stroeher UH, Parasivam G, Dredge BK, Manning PA*. Novel *Vibrio cholerae* O139 genes involved in lipopolysaccharide biosynthesis. J Bacteriol 1997 Apr;179(8):2740-47. 58 ref, Eng.*** Microbial Pathogenesis Unit, Department of Microbiology and Immunology, University of Adelaide, South Australia 5005, Australia

**422. Takeshi K, Ikeda T, Kubo A, Fujinaga Y, Makino S, Oguma K, Isogai E, Yoshida S-i, Sunagawa H, Ohyama T, Kimura H. Direct detection by PCR of *Escherichia coli* O157 and enteropathogens in patients with bloody diarrhea. Microbiol Immunol 1997;41(10):819-22. 17 ref, Eng.** Hokkaido Institute of Public Health, North 19, West 12, Chuo-ku, Saporo, Hokkaido 060, Japan

**423. Taylor DN, Tacket CO, Losonsky G, Castro O, Gutierrez J, Meza R, Nataro JP, Kaper JB, Wasserman SS, Edelman R, Levine MM, Cryz SJ. Evaluation of a bivalent (CVD 103-HgR/CVD 111) live oral cholera vaccine in adult volunteers from the United States and Peru. Infect Immun 1997 Sep;65(9):3852-6. 22 ref, Eng.** Center for Vaccine Development, University of Maryland School of Medicine, Baltimore, Maryland 2120, USA

"To provide optimum protection against classical and El Tor biotypes of *Vibrio cholerae* O1, a single-dose, oral cholera vaccine was developed by combining two live, attenuated vaccine strains, CVD 103-HgR (classical, Inaba) and CVD 111 (El Tor, Ogawa). The vaccines were formulated in a double-chamber sachet; one chamber contained lyophilized bacteria, and the other contained buffer. In the first study, 23 U.S. adult volunteers received CVD 103-HgR at $10^8$ CFU plus CVD 111 at $10^8$, $10^7$,or $10^8$, $10^7$, or $10^6$ CFU, CVD 111 alone at $10^7$ CFU, or placebo. In the second study, 275 Peruvian adults were randomized to received CVD 103-HgR at $10^9$ CFU plus CVD 111 at $10^9$ or $10^8$ CFU, CVD 111 alone at $10^9$ CFU, CVD 103-HgR alone at $10^9$ CFU, or placebo. Three of 15 U.S. volunteers who received CVD 111 at $10^7$ or $10^8$ CFU developed mild diarrhea, compared to none of 4 who received CVD 111 at $10^6$ CFU and 1 of 4 who received placebo. Twelve (63%) of 19 vaccine recipients shed the El Tor vaccine strain. All but one volunteer developed significant Ogawa and Inaba vibriocidal antibody titers.

Volunteers who received CVD 111 at $10^7$ CFU had geometric mean Ogawa titers four to five times higher than those of volunteers who received the lower dose. In the second study, all dosage regimens were well tolerated in Peruvians. About 20% of volunteers who received CVD 111 at the high dose excreted the El Tor organism, compared to 7% in the low-dose group. CVD 111 was detected in the stools of two placebo recipients, neither of whom had symptoms or seroconverted. In all vaccine groups, 69 to 76% developed fourfold rises in Inaba vibriocidal antibodies. Among those who received the bivalent vaccine, 53 to 75% also developed significant rises in Ogawa vibriocidal antibodies. We conclude that it is feasible to produce a single-dose, oral bivalent vaccine that is safe and immunogenic against both biotypes (El Tor and classical) and both serotypes (Inaba and Ogawa) of cholera for populations in both developed and developing parts of the world."

**424. Thirumalai K, Kim K-S, Zychlinksky A*. IpaB, a *Shigella flexneri* invasin, colocalizes with interleukin-1ß-converting enzyme in the cytoplasm of macrophages [note]. Infect Immun 1997 Feb;65(2):787-93. 23 ref, Eng.*** The Skirball Institute, New York University School of Medicine, 540 First Avenue, New York, NY 10016, USA

**425. Vicente ACP, Coelho AM, Salles CA. Detection of *Vibrio cholerae* and *V. mimicus* heat-stable toxin gene sequence by PCR. J Med Microbiol 1997 May;46(5):398-402. 19 ref, Eng.** Department of Genetics, Institute Oswaldo Cruz, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil

**426. Vila J, Vargas M, Ruiz J, Gallardo F, de Anta MTJ, Gascon J. Isolation of verotoxin-producing *Escherichia coli* O-rough:K1:H7 from two patients with traveler's diarrhea. J Clin Microbiol 1997 Sep;35(9):2279-82. 28 ref, Eng.** Laboratori de Microbiologia, Hospital Clinic, Universitat de Barcelona, Villarroel 170, 08036 Barcelona, Spain

**427. Vinje J, Altena SA, Koopmans MPG. The incidence and genetic variability of small round-structured viruses in outbreaks of gastroenteritis in the Netherlands. J Infect Dis 1997 Nov;176(5):1374-8. 15 ref, Eng.** Research Laboratory for Infectious Diseases, National Institute of Public Health and the Environment (RIVM), PO Box 1,3720 BA Bilthoven, The Netherlands

**428. Walker JC, Verma NK. Cloning and characterisation of the *aroA* and *aroD* genes of *Shigella dysenteriae* type 1. Microbiol Immunol 1997;41(10):809-13. 27 ref, Eng.** Division of Biochemistry and Molecular Biology, Faculty of Science, School of Life Sciences, The Australian National University, Canberra ACT 0200, Australia

**429. Ward RL, Knowlton DR, Zito ET, Davidson BL, Rappaport R, Mack ME. Serologic correlates of immunity in a tetravalent reassortant rotavirus vaccine trial. J Infect Dis 1997 Sep;176(3):570-7. 26 ref, Eng.** Division of Infectious Diseases, Children's Hospital Medical Center, 3333 Burnet Ave., CH-1, Room 1331, Cincinnati, OH 45229-3039, USA

**430. Wolf MK. Occurrence, distribution, and associations of O and H serogroups, colonization factor antigens, and toxins of enterotoxigenic *Escherichia coli*. Clin Microbiol Rev 1997 Oct;10(4):569-84. 75 ref, Eng.** Department of Gastroenterology, Division of Medicine, Walter Reed Army Institute of Research, Washington, DC 20307-5100, USA

**431. Yamamoto T, Wakisaka N, Nakae T. A novel cryohemagglutinin associated with adherence of enteroaggregative *Escherichia coli*. Infect Immun 1997 Aug;65(8):3478-84. 35 ref, Eng.** Department of Infectious Diseases and Tropical Medicine, Research Institute, International Medical Center of Japan, 1-21-2 Toyama, Shinjuku-ku, Tokyo, Japan

**432. Zaman K, Baqui AH, Yunus M, Sack RB, Chowdhury HR, Black RE. Malnutrition, cell-mediated immune deficiency and acute upper respiratory infections in rural Bangladeshi children. Acta Paediatr 1997 Sep;86(9):923-7. 30 ref, Eng.** International Centre for Diarrhoeal Disease Research, Bangladesh, GPO Box 128, Dhaka 1000, Bangladesh

"A community-based longitudinal study conducted in rural Bangladesh investigated the association between nutritional status, cell-mediated immune status and acute upper respiratory infections (URI). A total of 696 children aged 0-59 months was followed prospectively for 1 y yielding 183865 child-days' observation. Trained field workers visited each child every 4th d and collected morbidity data on symptoms suggesting URI (cough, fever, nasal discharge) for the preceding 3 d by recall. On the day of visit they examined each child reporting cough and/or fever to record the temperature, presence of nasal discharge, rate of respiration and presence of chest indrawing. Anthropometry for all children was conducted monthly. Cell-mediated immune competence was assessed by a multiple antigen skin test at baseline and thereafter every 3 months. The incidence of URI was 5.3 episodes per child-year observed. Approximately three-quarters of the study children were below - 2 Z-score weight for age and height for age, and a quarter below - 2 Z-score weight for height. During different test periods 9-21% of the study children did not respond to any of the test antigens. In a regression model children < -2 Z-score for weight for height had 16% [odds ratio (OR) 1.16, 95% confidence interval (Cl) 1.03-1.31, p-0.01] higher risk of developing URI. Anergic children had 20% higher risk (OR 1.20, Cl 1.05-1.38, p=0.009) of URI than immunocompetent children. The study demonstrated that wasting and depressed cell-mediated immunity (CMI), but not stunting, were associated with the incidence of URI among rural Bangladeshi children."

**433. Yii K-C, Yang T-I, Lee K-K*. Isolation and characterization of *Vibrio carchariae*, a causative agent of gastroenteritis in the groupers, *Epinephelus coioides*. Curr Microbiol 1997 Aug;35(2):109-15. 31 ref, Eng.*** Department of Aquaculture, National Taiwan Ocean University, 2, Pei-Ning Road, Keelung, Taiwan 202, Republic of China

"An outbreak of serious mortality among the cultured groupers *Epinephelus coioides*, characterized by a swollen intestine containing yellow fluid, occurred in the summer of 1993 in Taiwan. A motile strain EmI82KL was isolated from the intestinal yellow fluid of the moribound groupers with tryptic soy agar supplemented with 2% NaCl and/or thiosulfate citrate bile salt sucrose agar. This strain was characterized and identified as *Vibrio carchariae* and was susceptible to chloramphenicol, doxycycline-HCl, nalidixic acid, oxolinic acid, oxytetracycline, and sulfonamide while resistant to ampicillin and penicillin G. In addition, the strain was neither auto-agglutinating nor hemagglutinating, but it was hemolytic against erythrocytes from sheep, rabbit, tilapia, and grouper. The bacteria could be reisolated from kidney, liver, and the transparent yellow fluid of swollen intestine of moribound groupers after bacterial challenge and re-identified as the same species. The $LD_{50}$ value was $2.53 \times 10^7$ colony forming units/g grouper body weight."

JOURNAL OF DIARRHOEAL DISEASES RESEARCH

Volume 15 Number 4 December 1997

CONTENTS



Printed by BRAC Printers Dhaka Bangladesh