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Oral Rehydration Therapy Programme in India : Standard Case Management of Acute Watery Diarrhoea

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THE Diarrhoeal Diseases Control Programme in this country was started in 1978 with the objective of reducing mortality and morbidity due to diarrhoeal diseases. In 1985-86, with the inception of the National Oral Rehydration Therapy (ORT) Programme the focus shifted to strengthening case management of diarrhoea for children under the age of 5 years and improving maternal knowledge related to the use of home available fluids, use of oral rehydration salts (ORS) solution and continued feeding. Ensuring availability of ORS packets at health facilities and in the community is an important aspect of the programme. Since 1992-93, the programme has become part of the Child Survival and Safe Motherhood (CSSM) Programme. All programme activities are now integrated with those of the CSSM Programme.

SITUATION IN 1985:

A nationwide study in 1978 by the Registrar General of India[1] identified diarrhoea as a major killer and cause of illness among children. In a study carried out in 1985[2], in urban and rural areas in eleven States, the median diarrhoea incidence among children under 5 years, unadjusted for seasonality, varied from 1.5 episodes/child/year (Ep/Ch/Yr) in urban areas to 4.7 in rural areas. Based on the findings of this survey it was estimated that on an average each child suffered 3 attacks of diarrhoea per year thus totalling to an estimated 300 million episodes of diarrhoea in the 100 million children under the age of 5 years[3,4]. Assuming that one in 200 episodes was fatal, the data obtained from field investigations[5] suggested that an estimated 1.5 million children under 5 years died due to diarrhoeal diseases each year. Nearly one third of all causes mortality among these children[2] was due to diarrhoea associated causes. These findings, along with the fact that two-thirds of these deaths occurred due to dehydration and could be reliably averted with effective oral rehydration, formed the basis of the action plan for the 'National Programme of Oral Rehydration Therapy for Children under 5 Years'[6] launched in 1985-86.

CURRENT SITUATION:

In subsequent surveys in 1991 and 1992[7,8] incidence of diarrhoea varied from 1.2 Ep/Ch/Yr in Bihar to 1.9 in J&K and Tamil Nadu. In the slum areas around major towns an incidence of 10.5 Ep/Ch/Yr was reported. Although though there are pockets where the incidence of diarrhoea continues to be high, surveys indicate a declining trend in the average number of episodes per child per year compared to 1985.

The surveys carried out in 1992 also revealed that the proportion of deaths due to diarrhoea compared to total deaths in children under 5 years of age to be 19.2% and 11.9% respectively for Maharashtra and Orissa. The overall child mortality (0-4) rate in the country which was 41.2 in 1981 has also declined to 26.5 in 1991. As a result of this drop an estimated 16.8 lakh lives are being saved annually. Based on this and the estimated decline in diarrhoea associated mortality it is estimated that deaths due to diarrhoea have declined from an estimated 1.5 million in 1985 to about 0.6-0.7 million in 1991.

MALNUTRITION EMERGING AS AN IMPORTANT ISSUE:

Of all infectious diseases, the diarrhoeal diseases (along with measles) have the greatest adverse effects on growth of young children. This is the result of factors such as malabsorption of nutrients caused by the infectious process in the small bowel and reduced dietary intake resulting from anorexia and food withdrawn during diarrhoea as a consequence of traditional practices and improper advice from health workers. Malnourished children in turn have more severe diarrhoea, setting up a vicious circle which often leads to persistent form of diarrhoea, with high case-fatality rates and long lasting effects on the quality of life[9].

Feed back from hospitals and results of the National Family Health Survey (NFHS) 1992-93[10] indicate that it is the younger children in the age group 6-11 months who contribute significantly to morbidity and mortality due to diarrhoea. Malnutrition is being increasingly recognised as a major contributor to these deaths. Recent studies also suggest that the proportion of deaths due to persistent diarrhoea is also increasing. NFHS has also

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known that only a little more than half of the infants under 4 months are exclusively breastfed and less than a third of children 6-9 months, receive timely complementary feeding. Improvement of nutritional status of younger children is emerging as a major area of focus in the programme. The past emphasis on continued feeding in the management of diarrhoea has to be expanded to incorporate advise to mothers of young infants on exclusive breastfeeding and appropriate weaning and infant feeding practices. All contacts with the health providers must be utilised for this purpose.

RATIONALE FOR STANDARD CASE MANAGEMENT :

Studies conducted during the past two decades have conclusively demonstrated that establishing an aetiological diagnosis is not necessary to provide correct treatment for diarrhoea cases[9]. Majority of these cases can be successfully managed by following simple principles of standard case management strategy which comprises prevention of dehydration through proper management at home using home available fluids and ORS; treatment of cases with dehydration due to diarrhoea using oral rehydration salts (ORS) solution; continued and appropriate feeding during and after diarrhoea; and selective use of intravenous fluids for severely dehydrated cases and of antibiotics for suspected cases of cholera and dysentery. This case management approach applies to the treatment of acute watery diarrhoea, dysentery and persistent diarrhoea.

Standard case management forms the basis of the programme of control of diarrhoeal diseases in the country. This approach applies to the treatment of all types of diarrhoea : Acute watery diarrhoea, dysentery and persistent diarrhoea. In this article the standard case management of acute watery diarrhoea is discussed.

STANDARD CASE MANAGEMENT OF ACUTE WATERY DIARRHOEA

Diarrhoea is the passage of loose liquid or watery stools. These liquid stools are usually passed more than 3 times a day. However, it is the recent change in consistency and character of stools rather than the number of stools that is more important. Passage of even one large watery stool in a young child may constitute diarrhoea.

DEFINITION OF DIARRHOEA

- *Three or more loose or watery stools in a day*
- *Change in consistency and character of stools*

Passage of frequent formed stools, pasty stools in breastfed infants, stools during or immediately after feeding, loose greenish yellow stools on the 3rd to 7th day of life (transitional stools) may at times be erroneously considered to be diarrhoea. Infants during initial 3-4 months of life may pass 3-6 formed or semi-formed stools daily; this is also not diarrhoea[11], if they are gaining adequate weight. In most cases the mother knows what is an abnormal stool for her child.

TYPES OF DIARRHOEA :

CLASSIFICATION OF DIARRHOEA
(by clinical syndromes)

- *Acute watery diarrhoea*
- *Dysentery (blood in the stools)*
- *Persistent diarrhoea*

Acute watery diarrhoea — It starts suddenly and is characterised by passage of loose watery motions. Most episodes of acute diarrhoea recover within 3 to 7 days. More than three-fourths of all diarrhoeal episodes in the community are acute watery episodes.

It is important to understand that cholera is a form of acute watery diarrhoea. Recognising cholera early is important in order to start treatment promptly. If treatment is delayed or is inadequate, death may occur quickly from dehydration and circulatory collapse. Cholera should be suspected when a patient older than 5 years of age develops severe dehydration from acute watery diarrhoea (usually with vomiting) and in any patient who comes with symptoms of acute watery diarrhoea from an area where there is an outbreak of cholera.

Dysentery — This is diarrhoea with visible blood in faeces. The presence of blood may also be accompanied with abdominal cramps, fever, anorexia and rapid weight loss.

Persistent diarrhoea — An acute episode of watery diarrhoea or dysentery may last up to 14 days. If it persists longer, it is classified as persistent diarrhoea. These cases require careful management.

The relative contribution to diarrhoea-associated mortality in children under 5 years of age by each type of diarrhoea is shown in Fig 1.

ASSESSMENT OF A CHILD WITH DIARRHOEA :

Clinical assessment is sufficient to initiate appropriate therapy[9,11]. Routine determination of the aetiology of diarrhoea in a laboratory is neither necessary nor practical. Microscopy and stool culture is of little value in the routine management of diarrhoea. The management of a case of diarrhoea is based on the clinical features of the disease.

A careful history must be taken to determine whether the child has diarrhoea or not, type of diarrhoea (whether it is acute watery, dysentery or persistent diarrhoea). Did the child vomit during the preceding 6-8 hours ? History of feeding is important and it must be ascertained if

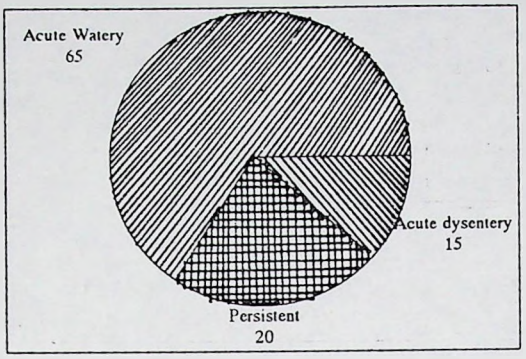

Fig 1 — Showing Mortality by Type of Diarrhoea

feeding has been reduced or modified in a way that the child is receiving reduced quantity of energy intake. Child's immunisation status especially as regards measles immunisation must also be determined[12].

History should be followed by physical examination of the child to assess the degree of dehydration, nutritional status of the child and presence of infections like pneumonia, otitis media or other associated infections.

The assessment of the degree of dehydration helps to classify the cases into those with no signs of dehydration, those with signs of dehydration and those with signs of severe dehydration. The classification, based on the presence of physical signs, is done with the help of the simple chart developed for this purpose and being extensively used in the National Programme.

SIGNS OF DEHYDRATION
(two or more signs including at least one marked*)

- *Restlessness, irritability**
- *Increased thirst**
- *Decreased skin turgor**
- *Dry mouth and tongue*
- *Tears absent*
- *Sunken eyes*

SIGNS OF SEVERE DEHYDRATION

- *Lethargic or unconscious, floppy*
- *Unable to drink*

PRINCIPLES OF THE MANAGEMENT OF ACUTE DIARRHOEA:

Fluid therapy for prevention and treatment of dehydration, feeding, rational use of drugs and appropriate advice to mothers form the basis of management of a child with diarrhoea.

Fluid therapy — Fluid and electrolyte replacement is necessary for all types of diarrhoea and is the mainstay of the treatment of a case with diarrhoea. Oral rehydration therapy (ORT) for replacement of fluid losses is a simple, cost-effective, easily accessible and scientifically appropriate remedy for dehydration due to diarrhoea[13].

The physiological basis of ORT is that glucose linked sodium transport is largely intact in diarrhoeas of varied aetiology. Thus any solution, that provides glucose (or starch which release glucose after hydrolysis) and salt, would promote absorption of sodium and water. Further some amino acids or dipeptides derived from dietary protein, eg, l-glycine, l-alanine and others also help in sodium co-transport similar to glucose[11].

ORS is the best available solution for fluid and electrolyte replacement. In the early stages of diarrhoea or when ORS packets are not immediately available, home available fluids and water can be used.

PURPOSE OF FLUID THERAPY

Prevention of dehydration for cases with no signs of dehydration.

- *Provide for normal daily intake*
- *Replace on-going losses*

Treatment (rehydration) of cases with signs of dehydration

- *Correct existing water and electrolyte deficit*
- *Provide for normal daily intake*
- *Replace on-going losses*

HOME AVAILABLE FLUIDS (HAF) :

Home available fluids (HAF) are appropriate for a child who does not show any clinical signs of dehydration and should be given frequently and also after each loose stool to prevent dehydration. A good HAF is one which is safe when used in large quantities. Some of these fluids which are commonly used in the homes and have a sound physiological basis are : Food based solutions like, rice water, dal or dal water with salt, and butter milk (lassi) with salt. Other fluids which can be used are lemon water, soups, coconut water and plain water. Water is a good home available fluid if taken with food[11].

These fluids are generally more effective when given along with food (ie, feeding should be continued), thus providing for starch (ultimately glucose) and protein (ultimately amino acid), to promote absorption of luminal sodium. These fluids together with food provide ORT. In younger infants breastfeeding is an ideal fluid and should be frequently given.

Soft drinks, sweetened fruit juices and sweetened tea should not be used. These have a high osmolarity and can lead to worsening of dehydration.

ORAL REHYDRATION SALTS (ORS) :

ORS is a balanced mixture of glucose and electrolytes for prevention and treatment of dehydration.

The presence of potassium in the mixture is important in view of the large potassium losses associated with

acute diarrhoea[11], especially in malnourished infants. Malnourished children also have a chronic potassium deficit. The function of citrate or bicarbonate in ORS solution is to correct acidosis. Glucose in ORS helps in transporting sodium and water across the intestinal membrane during diarrhoea.

COMPOSITION OF ORS (per litre of ORS solution)	
Sodium chloride	*3.5g*
Potassium chloride	*1.5g*
Sodium citrate	*2.9g*
Or,	
*Sodium bicarbonate**	*2.5g*
Glucose anhydrous IP	*20.0g*

*Less preferred. In the National Programme ORS containing sodium citrate is used

ORS conforming to the above composition (WHO-formulation) has been used all over the world during the last 25 years and its efficacy and safety has been proven beyond all doubts by its large scale use. A number of preparations of ORS with different concentrations of glucose and sodium are available in the market. It is the responsibility of treating physicians to ensure that only those conforming to the recommended formulation are prescribed. Higher concentration of glucose (more than 20g/l increases the risk of osmotic diarrhoea thus increasing the risk of dehydration and should never be used. Concentrations of sodium <3.5 g/l (90 mEq/l) may reduce its rate of intestinal abosrption and clinical efficacy particularly in the presence of high purge rates. The ORS packets available in the market also carry varying instructions for mixing and use. As with all drugs, ORS solution has to be used according to prescribed guidelines. It is therefore important not only to use the rational formulation of ORS but also to properly advise mothers on mixing of ORS from a one litre packet and on the quantities of the solution to be given. Rice based ORS formulations which are available in the market have been found to be good in cases of adult cholera but do not offer substantial clinical benefits to children.

ORS in newborns — Several studies have shown[11] that ORS solution can be used to treat dehydration in neonates who can drink or who accept oral fluids. ORS can be safely used in this situation when amounts of ORS appropriate for the degree of dehydration and plain water is offered in a (2 : 1) ratio of ORS and water. Breastfeeding must be continued.

WHO recommended formulation of ORS is the most scientific practical and appropriate choice for doctors and health care providers managing patients with diarrhoea. Preparations of ORS containing more than 20g/l of glucose should NEVER be used as these can lead to osmotic diarrhoea and worsen the condition of the child. Instructions to mothers on proper mixing and ensuring that adequate quantities of ORS solution are given to the child with diarrhoea are the most important aspects of standard case management.

CASES WITH NO SIGNS OF DEHYDRATION :

In early stages, when fluid loss is less than 5% of the body weight, children may not show any clinical signs of dehydration, but may still have sub-clinical or incipient dehydration. The purpose of fluid therapy in such cases is to prevent dehydration by giving HAF or ORS. ORS should invariably be advised to all cases who are brought to the health facilities/clinics. Mothers must be advised on how to prepare ORS solution and on how much ORS should be given. The quantities of fluids to be given are shown in Table 1.

Table 1 — *Showing Amount of ORS to be Given for Replacement of Ongoing Stool Losses to Prevent Dehydration (Maintenance Therapy)*

Age	After each liquid stool
≤6 months	Quarter glass (50 ml)
7 months - 2 years	Quarter to half glass (50 to 100 ml)
>2 to 5 years	Half to one glass (100-200 ml)

It is important to advise mothers to continue giving additional quantities of HAF or ORS solution as long as diarrhoea lasts. Breastfeeding must be continued in infants. In exclusively breastfed babies frequent breastfeeding is usually enough to prevent dehydration. If milk other than the mother's milk is being consumed, this should not be diluted. Food normally taken by the child should be continued.

CASES WITH SIGNS OF DEHYDRATION :

The purpose of fluid therapy in such cases is to correct fluid deficit and to provide for ongoing losses and normal daily requirement. Children who have dehydration should be kept under observation in the health facility/clinic for a few hours and given a prepared ORS solution during this period. During the first 4 hours, the child should receive 100 ml per kg of body weight of ORS. The quantities of ORS solution to be given by age and weight of the child is shown in Table 2.

If the child vomits, wait for 10 minutes and then continue to give ORS, but more slowly. If the child develops puffiness of the eyelids, stop ORS, continue to give plain water (or breastmilk) till puffiness disappears. After 4-6 hours of treatment the child should be reassessed for signs of dehydration. If the child has improved (as will happen in most cases), put the child on maintenance therapy as indicated in Table 1.

If the child still has some signs of dehydration after 4 hours of fluid therapy with ORS, continue therapy

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Table 2 — *Showing the Approximate Amount of ORS Solution to be Given in the First 4 Hours according to Age and Weight of the Child (Rehydration Therapy)*

Age	<4 months	4-11 months	12-23 months	2-4 years	5-14 years
Weight (kg)	<5	5-8	8-11	11-16	16-30
ORS (ml)	200-400	400-600	600-800	800-1200	1200-2200
Measure (glass)	1-2	2-3	3-4	4-6	6-11

*If the child wants more ORS than shown, give more

**Inform mother to give the child breastmilk in between the feeds of ORS

***For infants who are not breastfed, also give 100-200 ml clean water during this period

with ORS solution for another 4 hours. Inform mother to give the child breastmilk in between the feeds of ORS. If the child is not breastfed, give 100-200 ml of water, before starting the therapy. After rehydration has been achieved, ORS must be continued for replacement of ongoing losses (maintenance therapy) as indicated in Table 1.

If the condition of the child improves with thearpy but the mother has to leave before the child is fully recovered, she must be given enough quantity of prepared ORS solution for the next 4 hours. She should be advised to continue ORS till diarrhoea stops and also to continue feeding the child. Mothers must also be advised on how to prepare ORS solution and on how much ORS should be given.

HOW TO PREPARE ORS SOLUTION FROM A ONE LITRE PACKET

Mother must be taught how to measure one litre of water. It is important that a measure which is commonly available in the houses is identified and the mothers told the exact number of such measures that will make one litre. There is no need to boil the water for preparing the ORS solution. Clean water which the household normally uses for drinking purposes can be used.

Hands must be washed with soap before making the ORS solution.

Full packet of ORS must be used. Generally the mothers will tend to save a part of the packet in order to use it later. It is important to emphasise that the whole packet is to be mixed in one litre of water.

The container should be kept covered. The solution can be used for 24 hours and should be discarded if not consumed within this period. Fresh solution should be prepared, if required.

SHOW THE MOTHER HOW TO GIVE ORS SOLUTION

Give at least one tea spoonful every 1-2 minutes. Give frequent sips from a cup for an older child.

If the child vomits, wait for 10 minutes, then give the solution more slowly.

If diarrhoea continues after ORS packets are used up tell the mother to give home available fluids and get more ORS packets.

CASES WITH SIGNS OF SEVERE DEHYDRATION :

Children with signs of severe dehydration must be admitted for inpatient care. These signs appear when the fluid and electrolyte losses are excessive. The condition of the child in such cases is critical and it is essential that the child is rehydrated quickly by using intravenous (IV) infusions. IV therapy may also be required in cases where ORT has proved to be ineffective in correcting dehydration.

A number of IV solutions are available in the market. However, most of them do not contain the optimal amounts of the electrolytes required to correct the deficits associated with acute diarrhoea. The IV infusions recommended for use during acute diarrhoea are :

Preferred solutions : Ringers Lactate solution (also called Hartman's solution for injection).

Acceptable solutions : Normal saline and half normal saline with 5% dextrose.

Unsuitable solutions : Plain glucose (dextrose).

The rate of administration of IV fluids is important and is indicated in Table 3. After signs of severe dehydration disappear and the child is able to drink, further therapy should be continued with ORS. The child should be kept under observation for at least 6 hours after discontinuation of IV therapy. Before the mother leaves the hospital enough packets of ORS should be given and the mother told how to use these packet.

Table 3 — *Showing Rates and Quantities of IV Infusion for Correcting Severe Dehydration*

Age	First give 30ml/kg in	Then give 70 ml/kg in	Total 100 ml/kg
Infants	First hour	Next 5 hours	6 hours
Older children	First 30 minutes	Next $2\frac{1}{2}$ hours	3 hours

During IV therapy, the patient must be reassessed every 1-2 hours. If hydration is not improving, give IV infusion more rapidly. If the patient can drink, administration of ORS solution must also be started along with the IV infusion. The patient must be evaluated after the completion of the therapy (100 ml/kg body weight). If signs of severe dehydration have disappeared, continue treatment with oral therapy. If signs of severe dehydration persist, IV fluid therapy must be continued. If it is not possible to start the IV infusion for some reason, the patient can be given ORS solution through a nasogastric tube.

FEEDING IN DIARRHOEA:

Feeding during and after diarrhoea is the other essential component of standard case management. Diar-

Feeding is physiologically sound and prevents or minimises the deterioration of nutritional status that normally accompanies diarrhoea. It is the responsibility of treating physicians to utilise the contact with diarrhoea cases as opportunities for educating mothers on correct feeding practices.

rhoea is known to worsen nutritional status of the child because of the decreased food intake due to anorexia, withholding of food by mothers and intestinal malabsorption of nutrients. The traditional concept of resting the bowel in the belief that children fed during diarrhoea will have increased volume and frequency of stools has been challenged by the findings of recent studies. It has been clearly shown that feeding does not worsen diarrhoea or increase risk of dehydration and that malabsorption itself is corrected by feeding since it facilitates mucosal recovery[9,14].

Feeding prevents or minimises the deterioration of nutritional status that normally accompanies diarrhoea. At least two-thirds of calories given during convalescence get absorbed[15]. Early and adequate feeding during diarrhoea enables children to gain weight and grow normally in spite of frequent diarrhoeal episodes[9,11,15].

Energy dense foods with least bulk, which are routinely available in the households should be given to the child in small quantities but frequently, at least once every 2-3 hours interval. To make foods energy dense these can be enriched with fats and oil or sugar, eg, khichri with oil, rice with milk or curd and sugar, mashed banana with milk or curd, mashed potatoes with oil and lentils.

Breastfeeding should be continued uninterrupted even during rehydration with ORS. Milk should not be diluted with water during any phase of acute diarrhoea[11,16]. Milk can also be given as milk cereal mixtures, eg, dalia, sago, milk-rice mixture. This reduces the lactose load while preserving energy density. Routine lactose free feeding viz, soy formula is not recommended during acute diarrhoea even when reducing substances are detected in the stools.

If the child is more than 4 months of age, mother should be advised about breastfeeding and proper weaning. After the child recovers and normal appetite reappears, the child may be given more food than normal to regain lost weight.

RATIONAL USE OF DRUGS:

ORS is the drug of choice for all cases of diarrhoea. It is life saving when used timely and in adequate quantities. Clinical studies have shown that antibiotic therapy does not offer any significant clinical benefit in acute diarrhoea except in cases of suspected cholera. Use of specific antibiotics in cholera have been found to diminish the severity and duration of diarrhoea and shorten the duration of excretion of pathogens. The other indications for use of antibiotics are dysentery and associated non-gastro-intestinal infections, if present (Table 4). Amoebiasis and giardiasis are rare in children and drug therapy should be started only when trophozoite of *Ent histolytica* or *Giardia lamblia* are seen in the feaces[17].

Antidiarrhoeal drugs (antimotility drugs, binding agents, stimulants, steroids and other drugs) did not provide any clinical benefits and were found to be potentially dangerous when used in children. Their marketing has been banned in India.

DIARRHOEA ASSOCIATED WITH OTHER ILLNESSES:

Children with diarrhoea may also have other potentially serious illnesses. Some of the common illnesses associated with diarrhoea are measles, pneumonia and fever. The management of diarrhoea following measles is same as described earlier. The child should be examined for signs of vitamin A deficiency. If such signs are present, treatment should be started with therapeutic doses of vitamin A. If the child has pneumonia, the recommended treatment for pneumonia should be started along with rehydration. Fever is frequent in patients with diarrhoea. Treat fever with paracetamol. These patients should be carefully evaluated for any associated infection and treated promptly.

ADVICE TO THE MOTHER:

Mother plays a key role in the treatment of the child. She is responsible for feeding and caring for the child. She must be told that use of ORS and home available fluids in increased quantities along with continued feeding are crucial for saving her child's life. It is extremely

Table 4 — *Showing Antimicrobial Used in the Treatment of Specific Causes of Acute Diarrhoea in Children*

Causes	Drug (s) of choice	Alternative
Cholera	Doxycycline 6 mg/kg/day in a single dose x 3 days or, tetracycline 50 mg/kg/day in 4 doses daily x 3 days	Erythromycin 30 mg/kg/day in 3 doses x 3 days
Shigella dysentery	Paediatric co-trimoxazole 1 tablet twice a day x 5 days (under 2 months) 2 tablets twice a day x 5 days (2-12 months) 3 tablets twice a day x 5 days (>1-5 years of age)	Nalidixic acid 55 mg/kg/day in 4 doses x 5 days
Acute intestinal amoebiasis	Metronidazole* 30 mg/kg/day in 3 divided doses x 5-10 days	
Acute giardiasis	Metronidazole* 15 mg/kg/day in 3 divided doses x 5 days	

*Tinidazole can also be used

important that she knows how to prepare ORS solution and how much of ORS to give to the child. She must be told that ORS will prevent and treat dehydration and that diarrhoea will take some time to recover and that ORS should be continued as long as diarrhoea lasts.

Presence of blood in stools, many watery stools, repeated vomiting, marked thirst, eating or drinking poorly, fever, floppiness and difficulty to wake or unconsciousness are danger signs which the mother must know to enable her to seek immediate treatment. It is the duty of the treating physicians to ensure that mothers are educated about these signs.

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MATERNAL BEHAVIOUR AND FEEDING PRACTICES AS DETERMINANTS OF CHILDHOOD DIARRHOEA : SOME OBSERVATIONS AMONGST RURAL BENGALEE MOTHERS

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Introduction :

Diarrhoeal disease is one of the important cause of childhood morbidity and mortality specially amongst children below five years of age[1]. Estimated incidence of diarrhoea was found to be virtually same by Bern *et al* in 1992 (2.6 episodes per child per year) as that estimated by Synder and Merson in 1982 although diarrhoeal mortality was found to be lower[2,3]. A community based longitudinal study revealed that a large number of children do not suffer from diarrhoea within a year living in more or less same environment as that of diarrhoeal children[4]. It has been observed that certain behavioral pattern of family e.g. storage of water and handwashing practices with soap are major determinants for incidence and transmission of diarrhoeal disease[5,6]. We describe in this communication some demographic and epidemiological variables and behavioral pattern of diarrhoeal families as compared to non-diarrhoeal families.

Materials and method :

A total of 980 rural families near Calcutta having children below 3 years were longitudinally followed for one year for occurrence of diarrhoea. These families were kept under twice-a-week active surveillance by local resident surveillance workers. They were trained to deliver ORT services for prevention, management of dehydration and prompt referral of intractable cases to the nearest health facility. Initial demographic and epidemiological information about the study families were collected by house to house visit, entered in the computer and were analyzed using case control method. Families were divided into two groups on the basis of occurrence of diarrhoea. Families having a diarrhoeal child were regarded as 'case families' where as families having study children without diarrhoea were considered as 'control families'. At the end of one year, 570 (58.2%) of 980 families had diarrhoea cases and 410(42.8%) families had no study children with diarrhoea.

As soon as a diarrhoea case occurred amongst study families, we undertook an observational study to observe mother's/ family behaviour for 6 hours in the morning and afternoon. An age matched

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neighbourhood child and his family was similarly observed as control. However, if the control child developed diarrhoea, the family was eliminated from analysis as control family and included as case family. In these cases observational study was repeated and change(s), if any, from previous observation, was incorporated in the schedule. Initially we studied 76 case families and equal number of control families. However 29 control families where subsequently diarrhoea developed in a study child were analysed as case families. We could, therefore study prospectively the mothers behavior in 105 diarrhoeal families and compare with 47 non-diarrhoeal families. The variables studied were personal and domestic hygiene, food hygiene, sanitation and water storage habits.

Results and discussion :

The summary results of demographic and epidemiological variables of study and control families have been presented in table I. It would be evident that Kuchcha housing, lower income (<Rs.500/- per month), illiteracy of mother were more prevalent in diarrhoeal families as compared to control group. Presence of soap for washing hands and sanitary latrine were observed more frequently in non-diarrhoeal families. Birth spacing >=4 years) appeared to play a significant contributory role in preventing diarrhoea.

Mothers behaviour in respect of certain hygienic habits have been compared between diarrhoeal and control families in table II. More mothers were found to use soap or detergent for washing feeding container, maintained better personal hygiene for themselves and their children in non-diarrhoeal families compared to mothers in diarrhoeal families. Leftover food and wide-mouth containers for storing drinking water were less used in non-diarrhoeal families than their counterparts. Those mothers who used left over

Table - I

Some demographic and epidemiological variables of study families

Variables	Diarrhoeal families (n=570)	Non-diarrhoeal families (n=410)	P-value
Housing (kuchcha)	255 (44.7)	139 (33.9)	0.0006
Income (< 500/-)	252 (44.2)	150 (36.6)	0.016
Education			
illiterate father	131 (23.1)	80 (19.5)	NS
illiterate mother	304 (53.5)	186 (45.4)	0.013
Birth spacing (4 yrs. or more)	84 (14.7)	84 (20.5)	0.018
Presence of sanitary latrine	288 (50.5)	262 (63.9)	0.000031
Soap used			
a. for ablution	82 (14.4)	94 (22.9)	0.0005
b.before food handling	17 (03.0)	29 (07.1)	0.0046
feeding children	14 (02.5)	26 (06.3)	0.0041
Presence of animal	185 (32.5)	116 (28.3)	NS

Table - II

Significant difference of mothers behavior in Diarrhoeal & non-diarrhoeal families

Mothers behavior	Diarrhoeal families (n=105)	Non-Diarrhoeal families (n=47)	P-value
Feeding Container washed with soap	32 (30.4)	23 (48.9)	0.03
Use of left. over food in next feed	40 (38)	9 (19.1)	0.02
Body and clothes not clean	42 (40.0)	9 (19.1)	0.01
Indiscriminate stool disposal	77 (73.7)	26 (55.3)	0.02
Sharing common latrine	38 (36.2)	7 (15.9)	0.008
Storage of dr. water in wide mouth container	89 (84.8)	31 (66.0)	0.008

Figures in parenthesis indicate percentages

food for next feeding of their children or disposed children's stool indiscriminately also showed a significantly higher prevalence of diarrhoea.

In this study we have identified certain demographic and epidemiological variables which appeared to have a direct relationship with causation of diarrhoea. It is generally well understood from various studies that poor feeding techniques and sanitary practices can lead to contamination of weaning foods and it's ill effects eg. diarrhoea and malnutrition[7,8].

In our study we also observed significant contribution of feeding practices, washing of feeding container, use of left over food and status of personal hygiene in causation of diarrhoea amongst children. It appears that poverty, ignorance, illiteracy, unavailability and sharing of latrine and food hygiene all contribute towards occurrence of diarrhoea. Similar observation was made by other investigators from Srilanka[9]. However we feel that further welldesigned anthropological studies of observational nature are required to determine mother's beliefs and attitude, facilities available in and around home behind high risk behaviors in the family which need to be intervened.

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OBITUARY

It is a matter of great distress and sorrow to announce that DR. RADHAKRISHNA BANERJEA, founder member and Fellow of Indian Public Health Association (FIPHA, 1971) has passed away on 23 April, 1994. Dr. Banerjea started his career as Asst. Clinical Pathologist in Medical College, Calcutta; then as Demonstrator of Pathology and Bacteriology, in National Medical Institute; as Asst. Professor, Public Health Laboratory and also as Officer-in-Charge, Rural Public Health Laboratory (Singur) of All India Institute of Hygiene and Public Health, Calcutta. He was a close associate of Dr. U.N. Brahmachari in research on Kalaazar. In death of Dr. Banerjea, the country has lost a capable public health bacteriologist.

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Etiology of acute diarrhoea among children in developing countries: a multicentre study in five countries*

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A 1-year etiological survey of acute diarrhoea in children aged 0–35 months who were attending treatment facilities was carried out using a standardized protocol in five hospitals in China, India, Mexico, Myanmar, and Pakistan. A total of 3640 cases of diarrhoea and 3279 age- and sex-matched controls were studied; about 60% of the patients were aged less than 1 year and 60% were male. An enteric pathogen was identified in 68% of the cases and in 30% of the controls. In all the study centres, the pathogens most frequently associated with disease were rotavirus (16% of cases, 2% of controls), Shigella *spp. (11% of cases, 1% of controls) and enterotoxigenic* Escherichia coli *(16% of cases, 5% of controls). Rotavirus was commonest among 6–11-month-olds, accounting for 20% of all cases in this age group; 71% of all rotavirus episodes occurred during the first year of life.* Shigella *spp. were commonest among those aged 12–23 months and 24–35 months, accounting for 22% and 27% of the cases, respectively. The proportion of cases that yielded no pathogen was inversely related to age, being highest (41%) among infants below 6 months of age and lowest (19%) among those aged 24–35 months. These results suggest that microbe-specific intervention strategies for the control of childhood diarrhoeal diseases in developing countries should focus on rotavirus,* Shigella *spp. and enterotoxigenic* E. coli.

Introduction

Knowledge of the etiology of acute diarrhoea is relevant for planning diarrhoeal disease control strategies, especially vaccine development. The possibilities for the etiological diagnosis of diarrhoea were greatly enhanced in the 1970s through a series of advances, the most important of which were the discovery of rotaviruses and *Campylobacter jejuni*, the discovery and development of diagnostic tests for enterotoxigenic *Escherichia coli* (ETEC), and the detection by electron microscopy of several noncultivable enteric viruses. With improved diagnostic methods, it became possible to detect an etiological agent in 70–75% of acute cases of diarrhoea in children treated at hospitals in developed countries (*4*, *13*). In comparable etiological surveys in developing countries, the rate of positive identification of microorganisms has been slightly lower, and compared with viruses the role of bacterial agents has been greater (*1–3*, *5–12*). Moreover, in developing countries it has been recognized that enteric pathogens can frequently be encountered also in healthy children, making it more difficult to determine their true etiological role in causing diarrhoea (*5*, *6*). Furthermore, in developing countries it is not uncommon to isolate more than one enteric pathogen from the same child (*1*, *3*, *5*, *6–8*, *10*).

To define more carefully and compare the etiology of acute diarrhoea in young children in different areas of the world, the WHO Diarrhoeal Diseases Control (CDD) Programme initiated and supported a multicentre study in five developing countries. Standardized protocols for sampling patients and controls and for the laboratory procedures were used to allow comparison between sites

The following persons also contributed to this study: Shi Ou Ying, Xie Mei Wen, Duan Shu Cheng, Xia Lu Di, and Wang Shun Zhen (Shanghai); S.M. Pereira, T. Raghupathi, C. Kirubakaran, C. Balakaran, D.P. Rajan, R. Martin and V.I. Mathan (Vellore); A. Torres, E. Galindo, C. Soler, and M. del Carmen Bezualdo (Mexico); and Nyunt Nyunt Wai, Myo Khin, Thane Toe, Daw Tin Tin, Daw Mar Mar Nyein, Phyu Phyi Win, Mi Mi Khin, Kyaw Moe, Tin Khin, Soe Thein, May La Lin, Daw Khin, and Than Nu (Yangon).

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and permit more general conclusions to be drawn. This article summarizes the main results from the study.

Methods

The hospitals and laboratories outlined below participated in the study.

- Shanghai, China: Shanghai Hygiene and Anti-epidemic Centre, and the Shanghai Children's Hospital.
- Vellore, India: Wellcome Research Unit of the Christian Medical College Hospital.
- Mexico City, Mexico: Hospital Infantil de Mexico "Federico Gomez".
- Yangon, Myanmar: Department of Medical Research and the Yangon Children's Hospital.
- Islamabad and Rawalpindi, Pakistan: National Institute of Health (Islamabad), and the Rawalpindi General Hospital and Holy Family Hospital (Rawalpindi).

The standardized protocol, which is summarized below, was developed by the CDD Programme's Scientific Working Groups on Bacterial Enteric Infections and Viral Diarrhoea. All participating laboratories had prior experience in microbiological studies of enteric infections; in some instances assistance was provided to establish specific diagnostic tests at the study site. Although each study lasted 2 years, not all centres participated at the same time. The first study (in Myanmar) began in February 1982, and the last (in Pakistan) in October 1985.

Selection of cases

The patients were children aged 0–35 months who were seeking treatment for diarrhoea at the outpatient clinics of the participating hospitals. According to the original study protocol, children with a history of acute diarrhoea (an increase in the number or volume of stools) that lasted for 72 hours or less and clinical evidence of dehydration were eligible for the study. In practice, however, dehydration was not recorded, and most of the children probably were not clinically dehydrated. Also included were children with a history of blood or mucus in stools and a temperature of at least 38.5 °C. Children were excluded if they had an associated complicating illness or had received an antibiotic or antiparasitic drug within the preceding 10 days.

Cases were selected each week for 104 consecutive weeks. Based on records from previous years, a month-by-month sampling proportion was determined that took into account normal seasonal variations in disease incidence. The objective was to sample 5–12 cases per week throughout the study period, with the exact number varying according to the seasonal incidence of diarrhoea so that the proportion of total cases sampled each month remained nearly constant. Provided children met the case definition given above, their age, sex and type of diarrhoea (watery or dysenteric) were not considered when they were selected for the study; thus, the cases studied reflected the proportions actually seen for each age group, sex and type of diarrhoea.

Each child admitted to the study was matched with a control—a healthy child from the same geographical area and of the same sex, age (±30 days for those aged 1–11 months and ±60 days for others), socioeconomic status, and ethnic group, who had not had diarrhoea within the previous month.

A questionnaire was completed for each patient, giving details of the clinical history, pre-illness feeding practice, household environment, and demographic information (age, sex, and ethnic group). Most of the patients were from an urban/periurban environment, except in Vellore, where the study was carried out in a periurban/rural environment.

Laboratory procedures

The laboratory procedures have been described elsewhere.[a]

At least 5 ml (5 g) of faeces was collected from each patient within 1 hour of admission. Rectal swabs were used with dysenteric patients or controls if stools could not readily be obtained. Specimens were brought to the laboratory and divided for immediate culture and examination (2 g) and for virological studies (1 g diluted 1 : 5 in phosphate-buffered saline). A spare specimen (2 g) was stored frozen (−70 °C) for future use.

A portion of each stool was examined microscopically for trophozoites (*Entamoeba histolytica*) and cysts (*Giardia lamblia* and *E. histolytica*). *Cryptosporidium* spp. were not routinely sought, since the importance of these pathogens was not recognized when the protocol was developed.

For bacteriological examination the following media were inoculated: selenite broth (salmonella enrichment), alkaline peptone water (*Vibrio cholerae* enrichment), McConkey agar (22 °C for *Yersinia enterocolitica*; 37 °C for *E. coli*, *Shigella* spp. and *Salmonella* spp.), TCBS agar (*V. cholerae*), and Butzler's or Skirrow's medium (Campylobacter). Additional selective media for *Salmonella* spp. and *Shigella* spp. were inoculated as desired by the participating laboratory.

[a] *Manual for laboratory investigation of acute enteric infections*. WHO unpublished document CDD/83.3.Rev.1. Requests for single copies should be addressed to Diarrhoeal Diseases Control Programme, World Health Organization, 1211 Geneva 27, Switzerland.

Preliminary identification of suspicious colonies was carried out using standard biochemical tests. After preliminary identification of *E. coli* was completed, enterotoxin production was demonstrated using the Biken test or, in Mexico, using the Y-1 adrenal cell assay, for heat-labile toxin (LT), and the suckling mouse assay for heat-stable toxin (ST). Enteropathogenic *E. coli* (EPEC) were tentatively identified by serogrouping with O-antigen antisera[b] using both slide and tube agglutination techniques. Enteroinvasive *E. coli* (EIEC) were studied by testing two colonies (including atypical lactose-negative strains) for invasiveness using the Sereny test. Isolates of *V. cholerae* 01 were serotyped.

All stools were examined by electron microscopy for rotavirus, Norwalk-agent-like particles, and adenovirus. Initially, negatively-stained specimens were screened for all virus particles. This was followed by immune electron microscopy using pooled sera from indigenous donors. In addition, most samples were tested for rotavirus antigen using an enzyme-linked immunosorbent assay (ELISA) provided by the WHO Collaborating Centre for Human Rotavirus, Birmingham, England.

Results

A total of 3640 cases and 3279 controls were investigated; this represents approximately 10% of the total number of diarrhoea cases seen in the five centres during the 2-year study (Table 1). Throughout their respective study periods, no centre recognized unusual epidemics that would have distorted the findings.

Table 1: Distribution of cases of diarrhoea in the five study sites

Study site	No. of diarrhoea cases	No. admitted into the study	
		Cases	Controls
China	6192	594 (10)[a]	562
India	5862	916 (16)	587
Mexico	6376	559 (9)	559
Myanmar	NA[b]	813	813
Pakistan	9438	758 (8)	758

[a] Figures in parentheses are percentages of the total number of diarrhoea cases in each site.
[b] NA = not available.

The age distribution of cases was generally similar in all five centres (Table 2); however, the proportion of cases aged 0–5 months was greater in Mexico (40%) than Myanmar (16%), while the proportion of children aged 12–23 months was greatest in Myanmar (43%) and least in Mexico (22%). Overall, about 60% of the patients were aged less than 12 months, 30% were aged 12–23 months, and less than 10% were aged 24–35 months.

In all the study centres approximately 60% of all cases were boys (Table 2). Two centres (Shanghai and Vellore) determined the correlation of sex with the diarrhoeal etiological agent. In both instances, males predominated for all agents except isolates of *Shigella* spp. from Shanghai, which were detected equally frequently from males and females.

An enteric pathogen was detected in 68% of diarrhoea cases, with a bacterial agent being detected in 48%, a viral agent in 23%, and *G. lamblia* in 3% of cases; these included cases from which more than one pathogen was isolated. More than one enteric pathogen was found in at least 20% of diarrhoea episodes in Mexico City, Shanghai, and Vellore, but in only about 5% in Islamabad and Yangon. An enteric pathogen was detected in approximately 30% of healthy controls, the highest prevalence being 49% in Vellore.

The only agents that were consistently and substantially more prevalent in cases than in controls were rotavirus, *Shigella* spp. and ETEC (Table 3 and Table 4). The prevalence of EPEC was relatively high in controls, and, with the exception of Islamabad, nearly equal to that in cases. *Salmonellae* spp. were more frequently detected in cases than controls in Islamabad and Shanghai, but not at the other study sites. *C. jejuni* was clearly more prevalent in cases than controls in Islamabad, Mexico and Shanghai, but not in Vellore or Yangon. Mixed infections were detected approximately three times more frequently in cases than controls. *G. lamblia* and *E. histolytica* were seldom found in the study cases (Table 4).

Table 2: Distribution of diarrhoea cases, by age and sex in the study sites

Study site	% of cases in age group (months):				Sex distribution (all ages)	
	0–5	6–11	12–23	24–35	Males (%)	Females (%)
China[a]	28	31	32	6	63	37
India	33	28	30	9	61	39
Mexico	40	35	22	3	57	43
Myanmar	16	31	43	10	59	41
Pakistan	27	38	28	7	56	44
All sites	28	32	32	7	60	40

[a] Age was not reported for 2% of cases.

[b] Difco Laboratories, Detroit, MI, USA.

Table 3: **Distribution of bacterial agents detected in children aged under 3 years with diarrhoea and in healthy controls in the five study sites[a]**

	% of individuals from whom the agent shown was detected:											
	Escherichia coli (ETEC)		*E. coli* (EPEC)		*Salmonella* spp.		*Shigella* spp.		*Vibrio cholerae* 01		*Campylobacter jejuni*	
Study site	Cases	Controls	Cases	Controls	Cases	Controls	Cases	Controls	Cases	Controls	Cases	Controls
China	6	2	3	3	5	2	18	0.4	0	0	17	2
India	14	7	9	7	4	6	20	3	2	0.2	15	14
Mexico	17	7	10	9	4	3	11	1	0	0	15	10
Myanmar	26	7	8	5	1	1	3	1	0.5	0	2	2
Pakistan	17	4	14	8	3	0.3	6	1	2	0.8	10	5
All sites	16	5	9	6	3	2	11	1	1	0.2	11	7

[a] In some instances two or more agents were isolated from the same individual. ETEC = enterotoxigenic *E. coli*; EPEC = enteropathogenic *E. coli*.

Table 4: **Distribution of viral and parasitic agents detected in children aged under 3 years with diarrhoea and in healthy controls in the five study sites[a]**

	% of individuals from whom the agent shown was detected:									
	Rotavirus		Adenovirus		Norwalk-like agent		*Giardia lamblia*		*Entamoeba histolytica*	
Study site	Cases	Controls	Cases	Controls	Cases	Controls	Cases	Controls	Cases	Controls
China	13	1	2	1	3	4	0.2	0	0	0
India	18	1	6	2	3	2	7	7	0.1	0.2
Mexico	13	2	3	1	5	2	5	1	0.7	0
Myanmar	22	1	—[b]	—[b]	—[b]	—[b]	0.1	0.1	0	0
Pakistan	14	4	6	3	2	1	3	3	0.6	0.4
All sites	16	2	4	2	3	2	3	3	0.3	0.1

[a] In some instances two or more agents were isolated from the same individual.
[b] Not stated.

The effect of age on the prevalence of specific pathogens was evaluated by determining the rate of isolation of a specific pathogen (or no pathogen) from children within defined age groups, e.g., the proportion of diarrhoeic children aged 6–11 months who were positive for rotavirus; and by determining the distribution by age group of all isolates of specific agents, e.g., the proportion of all rotavirus isolates from diarrhoeic children aged 6–11 months. Table 5 summarizes these data from four of the study centres for children aged 0–5, 6–11, 12–23, and 24–35 months; no data are shown for the fifth centre, Yangon, which reported age-specific data on etiology for only 0–11-month-olds and 12–35-month-olds. Nevertheless, the general pattern in Yangon was similar to that in the other centres. The three commonest enteropathogens isolated from 0–5-month-olds were rotavirus, ETEC, and *C. jejuni*. The data for 0–6-month-olds from these study sites were further analysed by monthly age groups (Table 6).

Table 5: **Age-specific isolation rates for the major enteropathogens in four of the study sites[a]**

Entero-pathogen	% of cases that were positive for specific pathogens in different age groups (months)			
	0–5	6–11	12–23	24–35
Rotavirus	13 (28)[b]	20 (43)	12 (25)	12 (5)
ETEC[c]	11 (26)	14 (34)	15 (31)	18 (9)
Campylobacter jejuni	11 (26)	14 (34)	16 (33)	17 (8)
Shigella spp.	5 (12)	10 (26)	22 (49)	27 (14)
None detected	41 (44)	25 (28)	25 (24)	19 (4)

[a] Yangon was excluded, as noted in the text.
[b] Figures in parentheses show the % of all isolates of specific pathogens, by age group.
[c] Enterotoxigenic *Escherichia coli*.

The main results from the analysis of the age-specific data are summarized below.

- Rotavirus was the most frequently detected pathogen in diarrhoea episodes during the first year of life and most episodes that were rotavirus-positive (71%

Table 6: Combined data for four of the study sites on the detection of rotavirus, ETEC and campylobacter from cases and controls in the first 6 months of life[a]

	Data from cases in age group (days):						
	0–29	30–59	60–89	90–119	120–149	150–179	Total
Rotavirus							
No. of isolates	5 (3)[b]	10 (4)	24 (6)	20 (4)	26 (2)	33 (1)	118 (20)
% of cases in age group 0–5 months	4	9	20	17	22	28	100
ETEC							
No. of isolates	7 (3)	7 (5)	16 (16)	22 (8)	21 (14)	27 (10)	100 (56)
% of cases in age group 0–5 months	7	7	16	22	21	27	100
Campylobacter							
No. of isolates	5 (2)	13 (5)	25 (9)	13 (7)	25 (10)	24 (11)	105 (44)
% of cases in age group 0–5 months	5	12	24	12	24	29	100

[a] ETEC = enterotoxigenic *Escherichia coli*.
[b] Figures in parentheses refer to data from controls.

also occurred during the first year of life (Table 5). The highest incidence of rotavirus was among 6–11-month-olds, for whom it was detected in 20% of all diarrhoea cases. A total of 28% of all rotavirus-associated cases occurred among under-6-month-olds (Table 5), 87% of these being in infants aged 90–179 days.

Most isolates of *Shigella* spp. were from cases aged 6–23 months and these were the most frequently isolated pathogens in diarrhoeic children aged 12–35 months (Table 5). These findings were observed in all study centres and were most striking where the proportion of cases with shigellosis was highest—Shanghai, Vellore, and Mexico. *Shigella* spp. were rarely isolated from controls (29 isolations from over 1000 healthy control children).

ETECs were detected at a relatively constant rate throughout the first 3 years of life. In three of the five centres they were, with *Shigella* spp., the agent most frequently isolated from children over 1 year of age. A total of 76% of ETEC strains from cases that were typed produced only ST, 23% only LT, and 10% both ST and LT. In less than 5% of cases, strains that produced only ST and only LT were found in the same patient. A predominance of strains producing only ST was observed in all centres. Among controls, 36% of ETEC produced only LT and 57% only ST; and 7% produced both toxins. Altogether, 70% of cases with ETEC were infants aged less than 6 months; however, ETEC was also common among control infants in this age group.

C. jejuni was isolated at a relatively constant rate throughout the first 3 years of life. The rate of isolation in Yangon (2% of cases) was much lower than that in the other centres. A total of 26% of *C. jejuni* isolates from cases were from infants aged 0–5 months (data from four centres excluding Yangon). The difference in the isolation rates of *C. jejuni* between cases and controls was greater for this age group than that for older children.

Table 5 also shows the age distribution of diarrhoea cases whose etiology was undetermined. The proportion of such cases was highest among the youngest children, i.e., those aged less than 6 months, and this occurred in all the study centres. For this age group, 41% of all cases were of undetermined etiology, whereas for those aged 12–35 months only 19% of cases did not yield a potential pathogen.

Information on clinical findings was not consistently collated for each case in terms of specific etiology or the age of the child. However, the observations outlined below could be made about the clinical features of cases.

—Only 1.8% of cases presented with severe dehydration, and these were due mostly to rotavirus, *V. cholerae* 01, or ETEC.

—Stools from more than 80% of the patients were described as "loose"; watery diarrhoea occurred in less than 20% of cases. Watery diarrhoea was recorded in 8% of cases in Myanmar, 13% in Pakistan, and 32% in Mexico City. Apart from the few cases of cholera, watery diarrhoea was most frequently associated with rotavirus (27–36% of cases), ETEC (25–37%), and campylobacter (24–29%).

—Overall, visible faecal blood was reported in approximately 20% of cases, ranging from 4% in Myanmar to 29% in Mexico. As expected, it was most frequently associated with illness caused by *Shigella* spp. (45–67% of cases) and campylobacter (35–37%). Blood was rarely observed for illness caused by rotavirus, ETEC, or *V. cholerae* 01.

—Vomiting was reported as "relatively rare" among Indian cases, but was recorded for 43% of cases in Mexico and 51% in Myanmar. In Pakistan and Mexico, vomiting was reported in 61% and 69% of rotavirus-associated cases, respectively.

Discussion

We have focused on the clinical features and etiology of acute diarrhoea in young children seen at the five participating centres, and have attempted to draw broad conclusions where possible. This approach appears justified because the data were collected from a large number of patients using the same study protocol. The findings at each centre were similar with regard to the age and sex distribution of the diarrhoea cases and the overall etiological pattern. While some regional differences in the etiology of diarrhoea occurred, they were minor relative to the general pattern found and also with respect to other comparable studies in developing countries (*1–3, 5–12*). On the other hand, it should be recognized that this multicentre study was geographically limited, particularly because no centre from Africa or South America was included.

The results show that by using appropriate methodology it is possible to detect a potential microbial cause in nearly 70% of children with acute diarrhoea or dysentery who attend a treatment centre in developing countries; this proportion is similar to that in developed countries, although the distribution of individual agents is different (*4, 13*). It should nevertheless be borne in mind that 30% of cases could not be attributed to a specific microbial agent. The highest proportion of such cases at all sites involved infants aged less than 6 months; further studies are required to more fully determine the causes of acute diarrhoea in this age group.

In all the participating centres the high rate of isolation of identifiable enteric pathogens from healthy control children emphasizes the need for caution in interpreting the results of stool cultures for individual cases. The agents with the highest case: control ratios for isolation rates were rotavirus and *Shigella* spp., followed by ETEC; on the basis of this study, only these three could unequivocally be regarded as important causes of childhood diarrhoea in all five centres. Campylobacter was associated with diarrhoea in some study sites, but mainly among 0–5-month-olds.

Rotavirus was detected in 16% of the cases; this is a lower proportion than that found in surveys of hospitalized children in developing countries (median, 35%), but higher than that in community-based longitudinal surveys in developing countries (8–10%) (*15*). The higher proportion of rotavirus cases in hospital-based studies is consistent with the finding, confirmed by the present study, that rotavirus diarrhoea is of above-average severity. The lower proportion of rotavirus cases found in this study compared with other hospital-based surveys probably reflects the relative mildness of diarrhoea among the participating children, many of whom were not hospitalized, but treated as outpatients.

The present study also confirmed that in developing countries rotavirus is a significant pathogen among infants aged 2–5 months; in contrast, in developed countries few episodes of rotavirus diarrhoea involve this age group (*15*). This finding clearly has implications for research towards a rotavirus vaccine: a candidate rotavirus vaccine for use in developing countries should be efficacious in infants aged 1–2 months. This requirement has been considered in designing trials of such vaccines supported by the WHO Diarrhoeal Diseases Control Programme (*14*).

The second most important etiological agent in this study (*Shigella* spp.) was the most common pathogen in children over 1 year of age. Accordingly, *Shigella* spp. (particularly the *S. dysenteriae* and *S. flexneri* serotypes) should also be regarded as a priority target for vaccine development, especially since dysenteric illness is not treated primarily with oral rehydration salts, but usually requires antimicrobial therapy. ETEC may also be regarded as a candidate for vaccine development on the basis of this study. Campylobacter emerged as a significant pathogen mainly among under-6-month-olds. Further studies are needed to assess the role of novel diarrhoeal viruses in developing countries; the present study, however, failed to demonstrate any significant role for viruses (other than rotavirus) that were detectable by electron microscopy. *G. lamblia* and *E. histolytica* were not important causes of parasitic diarrhoea among young children in the study; the role of *Cryptosporidium* spp. was not investigated.

In summary, the major findings of this large multicentre study were as follows:

—over 60% of cases of acute diarrhoea involving children under 3 years of age were among 0–11-month-olds;

—about 60% of the patients were male; and

—rotavirus, *Shigella* spp. and ETEC were the most important causative agents.

While *V. cholerae* was an uncommon etiological agent, it is an important cause of severe dehydration in endemic areas. As targets for vaccine development, priority should be given to rotavirus, *Shigella* spp., ETEC and *V. cholerae*. For the greatest benefit, candidate vaccines should be efficacious in young infants.

Résumé

Etiologie de la diarrhée aiguë de l'enfant dans les pays en développement: une étude multicentrique dans cinq pays

On a mené pendant 2 ans, au moyen d'un protocole normalisé, une enquête sur l'étiologie de la diarrhée aiguë chez des enfants âgés de 0 à 35 mois amenés pour consultation dans cinq hôpitaux situés en Chine, en Inde, au Mexique, au Myanmar et au Pakistan. Au total, 3640 cas de diarrhée et 3279 témoins appariés selon l'âge et le sexe ont été étudiés; environ 60% des malades avaient moins d'un an et 60% étaient des garçons. Un germe entérique a été décelé chez 68% des malades et chez 30% des témoins. Les germes les plus souvent associés à la diarrhée aiguë dans tous les centres étaient les rotavirus (16% des cas, 2% des témoins), *Shigella* sp. (11% des cas, 1% des témoins) et *Escherichia coli* entérotoxinogène (16% des cas, 5% des témoins). Chez les 6–11 mois, ce sont les rotavirus qui étaient les plus courants, représentant 20% de tous les cas dans ce groupe d'âge; 71% de tous les épisodes de diarrhée à rotavirus sont survenus chez les moins d'un an. Chez les 12–23 mois et les 24–35 mois, *Shigella* sp. était le germe le plus courant, représentant respectivement 22% et 27% des cas. Le nombre de cas dans lesquels aucun germe n'a été décelé est inversement proportionnel à l'âge: maximal (41%) chez les nourrissons de moins de 6 mois, il devient minimal (19%) chez les 24–35 mois. Ces résultats indiquent que pour lutter contre les diarrhées infantiles dans les pays en développement, les stratégies d'intervention devraient être axées sur les rotavirus, *Shigella* sp. et *Escherichia coli* entérotoxinogène.

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CM136.4

Rotavirus and other viral diarrhoeas*

WHO Scientific Working Group[1]

Recent evidence indicates that viruses are an important cause of acute diarrhoea in infants and young children in both developed and developing countries. This article reviews the available information on the epidemiology, clinical features, and laboratory diagnosis of acute diarrhoea due to two of the more important and recently discovered viruses, namely rotaviruses and the Norwalk and Norwalk-like agents, or to other viral agents. Research priorities are also recommended that will help to elucidate the epidemiology, pathophysiology, and means of preventing viral diarrhoeas. Foremost among these research priorities is the development of a rotavirus vaccine for use in man.

The problem of viral diarrhoea is a particularly important topic since recent evidence has indicated that viruses are responsible for the majority of diarrhoeal episodes in infants and young children in both developed and developing countries and may account to a considerable extent for malnutrition owing to associated malabsorption. Identification and characterization of these viruses and a clear understanding of their epidemiology are necessary in order to be able to prevent their transmission and, especially, to develop suitable vaccines.

ROTAVIRUS DIARRHOEA

Rotavirus diarrhoea in man

The virus

Rotavirus was first detected in man in Australia in 1973 by thin-section electron microscopic examination of duodenal biopsies obtained from children with acute diarrhoea, and soon afterwards in Australia, Canada, the United Kingdom, and the United States of America by electron microscopic examination of diarrhoeal stool specimens. The virus is 70 nm in size, contains RNA, and has an inner and outer capsid. It derives its name from the Latin word "rota", meaning wheel, which it resembles in appearance. Initially, it was also referred to as "orbivirus", "duovirus", "reovirus-like agent", and "infantile gastroenteritis virus".

Human rotaviruses are morphologically similar and share certain common antigens with animal rotaviruses. By complement fixation (CF) the human rotavirus has been shown to be closely related to at least 4 animal rotaviruses: Nebraska Calf Diarrhoea Virus (NCDV), Epizootic Diarrhoea of Infant Mouse virus (EDIM), Simian Agent (SA)-11, and the offal (O) agent of sheep and calves. In fact, these four animal antigens can be used as CF antigens for detecting serological evidence of rotavirus infection in man; this has been useful for epidemiological studies since the supply of human rotavirus is limited because it has not been propagated efficiently in cell culture. (The relationship between animal and human rotaviruses is discussed further on pages 186-188.)

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[1] This article is based on the Report of a Sub-group of the Scientific Working Group on Epidemiology and Etiology, CDD Programme, held in Washington, DC, in March 1979. The participants in the Sub-group are listed on page 198. A French translation will be published in a future edition of the *Bulletin*.

for. Most cases are children 6–24 months old with a peak incidence at 9–12 months. In a number of hospital-based studies carried out in infants and young children in developed and developing countries, rotavirus has been detected in approximately 50% of diarrhoea cases, sometimes with seasonal variation (see below). In some studies the number of male cases has been up to 20% higher than that of female cases, but whether this is due to a greater susceptibility or exposure of male children, or to a higher likelihood of their being brought for medical care is not known. Data from community-based studies are much more limited, but one carried out in Guatemala and one in Bangladesh suggest that rotavirus accounts for approximately 10–20% of all community diarrhoea cases.

A number of serosurveys have been made to determine the frequency of rotavirus infection in early life. In a study of persons living in the Washington, DC, area, rotavirus antibody was detected by CF and immunofluorescence in over 90% of children by the third year of life. In Melbourne, Australia, 40% of infants 2 months of age or less possessed rotavirus CF antibody; by 3–5 months of age the percentage with antibody had increased and approached 70% by the age of 3 years. Approximately 70% of adults were also shown to have antibody. Workers in Toronto have reported similar results. In a serosurvey done in Vellore, India, in which rotavirus antibody was measured by counter-immunoelectrophoresis, 85% of newborns were shown to have antibody; by the fifth month 30% and by the third year of life nearly 90% had evidence of prior infection. Using the more sensitive enzyme-linked immunosorbent assay (ELISA), rotavirus antibody prevalence was found to be 100% in European and Aboriginal populations 2–60 years of age living in the same area in Australia and in adults and children living in an area of southern India. Other surveys using a variety of serological techniques have been carried out in other geographical areas and have shown similar results, namely that the prevalence of rotavirus antibody is high in newborns due to transfer of passive antibody from the mother, then diminishes in the first 6 months of life, is again very high by the age of 2–3 years, and is maintained throughout life. This pattern of rapid acquisition of antibody is comparable to that observed with respiratory syncytial and parainfluenza type 3 viruses.

The high prevalence of antibody in later childhood and adult life can be explained by the results of family studies from Canada, Norway, and the USA, in which up to 55% of older siblings and household contacts of paediatric patients with rotavirus gastroenteritis showed evidence of infection on the basis of serological studies. Most of the cases are asymptomatic and probably represent reinfection. It is not known whether it is the infected younger child or the usually asymptomatic adult member who is most likely to introduce rotavirus into the family unit.

Recent evidence has demonstrated the existence of more than one serotype of human rotavirus. Workers in Belgium using a CF assay and immune electron microscopy, in England using neutralization of immunofluorescent foci, and in the USA using ELISA have defined two distinct serotypes. These serotypes appear to be widely distributed geographically. In the Washington, DC, metropolitan area, the sera of most children aged 2 years contained antibodies to both serotypes, and in hospitalized patients type 2 rotavirus was seen more frequently. In addition, studies of patients who had experienced sequential infections revealed that illness caused by one serotype did not provide protection against illness caused by the other serotype. It is not certain whether other serotypes exist; workers in England using a fluorescent neutralization test have claimed to have found two other serotypes.

Seasonality

It has been clearly demonstrated in studies in Australia, England, and North America, that in temperate climates rotavirus disease is much more prevalent in the colder season. One exception may be infection in newborns; in Sydney, Australia, no seasonal variation

Clinical features

The incubation period of rotavirus enteritis ranges from 1 to 7 days, but is usually less than 48 hours. In one volunteer study the onset of diarrhoea occurred 2–4 days after oral administration of the rotavirus-containing inoculum. In the usual case of rotavirus enteritis, vomiting is a prominent early symptom and in many cases precedes the onset of watery diarrhoea. Mucus is found in the stool in up to 25% of cases but blood is rare. Mild temperature elevation occurs in about 30–50% of cases. Respiratory symptoms have been reported but there has been no evidence to indicate that they were caused by the virus. As in other enteric infections, the severity of symptoms varies. The average duration of illness is 5–7 days and virus shedding continues not infrequently for up to 10 days. In the more severe cases seen at treatment centres, severe dehydration and electrolyte imbalance have been observed. Deaths from rotavirus diarrhoea have been reported, although the impact of rotavirus disease on infant mortality in developing countries has yet to be determined.

In adults infected with rotavirus, mild diarrhoea or, more commonly, subclinical infection occurs, probably because of active immunity (see page 185). For reasons that are unknown, newborns infected with rotavirus are also often asymptomatic or have only mild disease.

Treatment of rotavirus disease generally requires standard rehydration therapy. This can be administered orally in all but the most severely dehydrated patients who are unable to drink, or in patients with intractable vomiting. The glucose–electrolyte oral rehydration solution (ORS) developed originally for the treatment of cholera has been used successfully for the rehydration of rotavirus diarrhoea cases in a number of developing countries.[a]

An understanding of the pathophysiology of rotavirus diarrhoea has been derived from a sequential biopsy study of gnotobiotic colostrum-deprived calves challenged with human rotavirus. This study revealed a sequence of events in the small intestine consisting of infection of the absorptive villous epithelial cells, replacement of the tall columnar villous epithelial cells with cuboidal cells, shortening of the villi, lymphocytic infiltration of the villous lamina propria, and repair. Such changes appeared in a cephalocaudal direction and suggest that much of the diarrhoea may be related to a loss of absorptive capacity in the small intestine. Similar morphological findings were observed in small intestinal biopsy studies in infants.

Human rotavirus can also induce diarrhoeal illness in other newborn, colostrum-deprived animals, including gnotobiotic and conventional piglets, rhesus monkeys, and gnotobiotic lambs. It can also infect but not induce illness in newborn puppies. This finding that only newborn animals are susceptible to human rotavirus has not been explained. One hypothesis is that this predilection may be the result of higher lactase concentrations in the infant animal, the lactase acting as a receptor and an uncoating enzyme for the rotavirus.

A question of considerable interest is whether rotavirus could be responsible for cases of chronic diarrhoea, but no studies have been done to test this hypothesis. Patients with acute rotavirus diarrhoea usually have increased reducing substances in their stool, reflecting defects of absorption and of digestion of carbohydrates. This is not surprising given the pathophysiology of the disease, but these abnormalities do not prevent hydration with glucose–electrolyte oral solutions.

Incidence

Rotavirus enteritis is generally a disease of infants and young children and appears to have a worldwide distribution. It has been the most frequently observed virus in the stool of infants and young children with diarrhoea in almost all areas where it has been looked

[a] Report of the Scientific Working Group on Clinical Management of Acute Diarrhoea, 1978, unpublished document WHO/DDC/79.3.

was found when rotavirus infection was studied in nurseries for the newborn. Whether this seasonal pattern occurs in developing countries with tropical climates is unclear. In studies from Costa Rica and Venezuela little or no seasonal variation in occurrence has been observed; however, in studies in Bangladesh and in Calicut and Vellore, India, rotaviruses have been found most frequently in stool samples collected from diarrhoea cases between November and March, which are the coolest months of the year. In the Vellore study, rotaviruses were present in the neonatal nursery throughout the year, as was observed in the Australian study cited above. In one small study conducted in Mexico City, where there is almost no seasonal difference in temperature, there was a peak of cases in the autumn months.

Transmission

All evidence to date indicates that rotavirus infection spreads by faecal–oral transmission; this has been confirmed by volunteer and animal experiments. There is no evidence to suggest that rotavirus multiplies with production of infectious particles anywhere else than in small bowel enterocytes.

Although IgA specific antibody has been found in the colostrum and breast milk of lactating mothers in a number of countries, it is not clear what role breast milk plays in protection against rotavirus disease, especially in developing countries where breast feeding frequently continues past the sixth month of life, when rotavirus disease is common.

Outbreaks of rotavirus diarrhoea have been documented in nurseries, especially those providing special care, and in a number of paediatric hospital wards. This problem of nosocomial transmission of rotavirus has been a difficult one to control.

Role of rotavirus in disease other than acute enteritis

Rotavirus infection has been described in children with intussusception and children with gastroenteritis and self-limited gastrointestinal bleeding. Clinical findings compatible with Henoch-Schoenlein purpura developed in one child in the latter group. In another report, a child with rotavirus-associated gastroenteritis had a severe central nervous system manifestation and developed fatal Reye's syndrome. In another child, encephalitis was reported. Several children with rotavirus infection have developed the haemolytic uraemic syndrome or disseminated intravascular coagulation. Elevated SGPT and SGOT levels have also been reported in some cases. In addition, there are reports that rotavirus has been isolated from intestinal tissue obtained from patients with Crohn's disease. One such report could not be confirmed in another laboratory, in which a contaminating, fastidious *Mycoplasma* was detected from such a patient. The isolation in cell culture of a rotavirus from extracts of mesenteric lymph nodes from a patient with Crohn's disease has been described recently. The role of rotavirus in the above conditions is unclear and needs further study.

Rotavirus diarrhoea in animals

Epidemiological features

Rotavirus has been shown to be a common intestinal infection in animals and has been demonstrated as a cause of diarrhoea in mice, calves, piglets, foals, young rabbits, deer, pronghorn antelope, chickens, turkeys, goats, kittens, a chimpanzee, and a gorilla. There is also evidence that it can infect other animals, as shown by virus isolation studies in monkeys and dogs, and serological studies in goats, dogs, and guinea pigs. From virological and serological studies it is evident that infection occurs in early life in 90–100% of pigs and calves, as is the case in man, but at a lower level (38% in one study) in sheep. This low infection rate in sheep may reflect the different systems of husbandry; the sheep studi

lived under less crowded conditions which may have reduced the probability of transmission. Laboratory colonies of rabbits and guinea pigs show a wide variation in the number of animals with antibody to rotavirus (0–100%). However, under natural conditions of husbandry, where populations have an urban distribution or where many young are congregated, the incidence of rotavirus infection in these species frequently reaches 100%.

In pigs and calves the severity of rotavirus infection, as judged by mortality, is usually less than that associated with *Escherichia coli* or coronavirus infections, although epizootic disease has had a reported mortality of up to 90%. However, it should be borne in mind that whereas *E. coli* and coronavirus infections can be diagnosed efficiently by staining of intestinal tissue obtained at autopsy, rotavirus infection cannot be so diagnosed and often requires electron microscopy for its detection. Some veterinarians have observed an apparent association of other agents, including bacteria and viruses, and also environmental stress with increasing severity of disease in naturally occurring epizootics.

The epidemiology of rotavirus infection in animals is not well understood. Although the disease usually occurs in infants or neonates, diarrhoea has been seen in adult animals in whom the rotavirus serum antibody titres are zero or low; often both the dam and her offspring have concurrent illness. It is probable that intestinal immunity of a level sufficient to prevent reinfection of the gut lasts not more than 6 months following infection, and thus the virus may be circulating constantly in the adolescent and adult population. In addition, the great stability of the rotavirus in faeces and its resistance to pH changes (pH 2–9.5), temperature (60°C), and a number of commonly used disinfectants, probably result in prolonged survival of the virus in contaminated buildings and water supplies.

It has been suggested that rotavirus may cross the placenta and infect the fetus, which would then be born either with infection or with actively acquired immunity. This hypothesis was based on reports, largely unpublished, that antibody to rotavirus was found in fetuses and that calves were frequently ill within 48 hours after birth. However, of the many hundreds of calves and pigs obtained by Caesarean section for rotavirus research from herds in which rotavirus infection was common, none has been shown to possess serum antibody, to be immune to experimental infection, or to be actively excreting virus. Since the incubation period for rotavirus infection is short (1–2 days), it is more probable that most infections in early life occur as a result of postpartum contact with an infected person or environment.

Immunological considerations

Preliminary data suggest that, although all rotaviruses have a common antigen, a wide range of antigenic subtypes can be differentiated by laboratory tests (see page 190). At present, these are recognized mainly among isolates obtained from different mammalian species, although at least two subtypes have been reported among human rotaviruses (see page 185). Most rotavirus strains tested under experimental conditions show cross-infection between mammalian species (e.g., human, bovine, equine, porcine, and ovine rotaviruses can infect piglets; human rotaviruses can infect monkeys; human and equine rotaviruses can infect calves). Some, but not all, of these cross-infections cause diarrhoea (see page 184). There has been no evidence to prove that rotaviruses cross species barriers under natural conditions. However, in England one human rotavirus isolated from a child was found to be serologically close to a bovine strain, and one isolate from a pig was serologically more closely related to a bovine than to a porcine strain. Thus it appears that different rotavirus strains exist with different antigenic specificities. Whether the different antigenic specifications would be likely to cause problems in a vaccination programme is not known. However, with a closely related virus, blue tongue of sheep, there are at least

22 strains which, although they share a common antigen, show relatively poor cross-protection. This virus infection is limited to sheep (virulent infection), to cattle (largely subclinical), and possibly to wild ungulates. In comparison, rotaviruses are more widely distributed in the animal kingdom and thus can be expected to show as wide, or wider, antigenic variations.

Cross-protection studies between different animal species in different laboratories have given conflicting results. In one study, 3 gnotobiotic calves were inoculated with foal rotavirus and 3 with human rotavirus within 3 days after birth and none in either group developed a diarrhoeal illness; however, on challenge with bovine rotavirus 21 days after the initial inoculation, 2 of 3 animals in each group developed a diarrhoeal illness, suggesting that only limited, if any, protection was induced by the initial inoculum. In two more recent studies in pigs, bovine rotavirus and human rotavirus were ineffective in protecting piglets from later challenge with porcine virulent rotavirus. In another study of the relationship between human, ovine, equine, and porcine rotaviruses in pigs, all of which are separable by a neutralization test, there was poor evidence of cross-protection. In contrast to these studies, other workers have reported good evidence of cross-protection, including the demonstration that (*a*) bovine colostrum can protect pigs against challenge with a porcine isolate of rotavirus, (*b*) bovine rotavirus vaccine can induce protection against human type 2 rotavirus in calves, and (*c*) pigs infected with human rotavirus type 2 are protected against later challenge with porcine rotavirus. In none of these studies were the isolates used of the same origin, nor was the ID_{50} of the challenge virus determined.

It is thus evident that further studies are required to determine the amount of cross-protection between rotavirus strains of different species. Even if cross-protection between strains is demonstrated experimentally, the efficiency of the protection may be less under field conditions as most reports of experimental studies indicate a lower mortality from rotavirus infections than that seen in natural outbreaks, except in newborn piglets.

In contrast to the conflicting data on cross-protection, there is good experimental evidence that animals can be effectively immunized against disease induced by rotavirus of their own species. Although limited, all the work published to date has shown that animals that are inoculated with rotavirus and allowed to recover are, on challenge 3 weeks later, immune to rotavirus illness and shedding. Similarly, calves vaccinated with an attenuated live rotavirus are immune to challenge with the parent virulent virus or with another bovine virulent rotavirus of close antigenic relationship. Also, virulent virus in the presence of colostral antibody in the intestine induces a subclinical infection that immunizes the animals against illness and rotavirus shedding for at least a limited period. It can be concluded from the available evidence that rotavirus infection in mammals results in the development of immunity to illness and rotavirus shedding, and it is most likely that for vaccines to be effective they will need to protect against each serotype that can infect the particular mammalian species under consideration.

Laboratory diagnosis

Methods for the detection of rotavirus in stools

In human infections large numbers of rotavirus particles are excreted in the stool. The optimal period for virus detection is within the first 3–5 days after onset of symptoms. The virus particles can usually be seen easily by negative-stain electron microscopy (EM) following differential centrifugation; in many instances they may be seen without centrifugation. The size and the characteristic double-capsid structure of the particles can be readily recognized. In stools that contain few virus particles the use of immune electron microscopy (IEM), i.e., the addition of specific antiserum to the stool extract to aggregate the particles may occasionally make them easier to identify.

Use of electron microscopy is an efficient way of detecting rotavirus infection, but it is costly in time and equipment, and most developing countries do not have such facilities. Thus, other methods to detect virus particles have been explored.

One of these is immunoelectro-osmophoresis; this method is rapid and cheap but its sensitivity depends on the quality of the antiviral serum used. Although some workers find it more sensitive than EM, most have found it either as good or somewhat less sensitive.

The use of a special modified complement-fixation test (CF) to detect rotavirus in stool suspension has also been reported and has been found to be almost as sensitive as EM. One major problem is that many stool extracts are anti-complementary, although the addition of fetal calf serum may overcome this difficulty. With this method, high-titred human sera were found to be better than rabbit sera for the detection of human rotavirus.

Radioimmunoassay (RIA) and enzyme-linked immunosorbent assay (ELISA) have been used successfully for the detection of human and calf rotaviruses. The methods are claimed to be at least as sensitive as EM and probably more sensitive. With ELISA, the specificity of positive specimens has to be established with appropriate "blocking" reagents. The [illegible]nts with RIA are that sophisticated equipment is required and radioactive material may be hazardous. In this respect, ELISA shows more promise, especially because it can be used under field conditions to examine a large number of stool specimens.

Immunofluorescence (IF) has been used to stain cells or cell debris excreted in stools from infected calves. The problem of non-specific fluorescence has prevented its widespread use on human stools, but this can be overcome if the virus antigen is first separated by immune precipitation prior to staining. However, this method is laborious and time-consuming when done on a large scale. A simpler method is needed to make the use of IF more acceptable.

A new test termed solid-phase aggregation of coated erythrocytes (SPACE) has been described, which combines some of the features of ELISA and of solid-phase radioimmunoassay. This method involves the coating of microtitration U-plates with specific antiviral antibody and the addition of 10% faecal suspensions. The absorbed viral antigens are then detected by the addition of erythrocytes coated with specific antiviral IgG. The results are read in the same manner as the conventional haemagglutination test. This test has been shown to give very good agreement with both EM and IF, but appears to be limited in application because of the need for frequent preparation of coated erythrocytes.

As mentioned previously, one of the most urgent needs is to find a simple method for the efficient propagation of human rotavirus in cell culture; this would undoubtedly also improve diagnostic tests. Enhancement of virus growth in cell cultures by adding proteolytic enzymes to the culture medium has been reported. A cell culture method that is capable of demonstrating the presence of human rotavirus antigen, usually in a single passage, has been employed successfully; this involves centrifugation of stool specimens on to a guinea-pig kidney cell line followed by IF staining. This is a relatively simple way of diagnosing rotavirus infection, titrating virus infectivity, and estimating neutralizing antibodies in serum. Serial propagation of human rotavirus by this technique alone has not been reported.

Methods for the measurement of rotavirus antibody

A wide variety of tests have been developed for the measurement of rotavirus antibody. EM was used in some earlier studies on human sera; however, for general serological surveys or for serodiagnosis a simpler and quicker method was needed and the complement-fixation test (CF) was shown to meet this requirement. Initially the CF antigen used was human rotavirus obtained from stool extracts, but it was found that this could be replaced by the more readily available bovine rotavirus grown in cell culture, or even more efficiently by the O agent (see page 183). One limitation of the CF test is that it does not

distinguish between antibodies of different species origin. Another simple and cheap method for detection of group-specific rotavirus antibody is radial immunodiffusion (ID). With ultracentrifuged concentrates of stools as antigen, a single strong line common to all the rotaviruses can be seen against antisera from convalescent children and animals. The ID test has been used to measure serum antibody titres against human rotavirus. Antibodies have also been quantitatively detected by RIA and ELISA using the "double-antibody sandwich" technique. These tests are very sensitive and have the added advantage of being adaptable to measure specific immunoglobulin classes of antibody. Anti-rotaviral IgA has also been measured by IF.

Methods for the identification of rotavirus strains and serotypes

CF, IF, ID, and IEM techniques have shown that rotaviruses share a common group antigen that is associated with the inner capsid of the virus particle. However, human and animal rotaviruses can be distinguished by neutralization of immunofluorescent foci and ELISA blocking techniques. A one-way serological relationship between human and simian rotaviruses has been reported as a result of IEM studies; a one-way ELISA blocking test showed these viruses to be distinct.

The existence of two rotavirus serotypes in man has been demonstrated by neutralization tests, CF, and ELISA. Two additional serotypes have been described by workers using the neutralization technique (see page 185).

Electrophoretic migration of viral nucleic acid has been useful for distinguishing viruses and is based on differences in the migration of RNA segments on polyacrylamide gels. RNA analyses can be carried out directly on most stool samples in which rotavirus particles are visible by EM. However, a correlation between serotypes and "electrophoretypes" has not been established and a difference detected by this technique may not necessarily reflect an antigenic difference.

DIARRHOEA DUE TO NORWALK AND NORWALK-LIKE AGENTS

Description and characteristics of the Norwalk-like agents

Since 1972, a new group of agents, of which the 27-nm Norwalk particle is a prototype, has been discovered and found to be associated with outbreaks of generally mild gastroenteritis occurring in school, community, and family settings. In antibody prevalence studies employing a newly developed immune-adherence haemagglutination assay (IAHA), it has been found, in the Washington, DC, metropolitan area, that antibody to the Norwalk agent is acquired gradually in childhood and more rapidly in adult life, so that antibody is present in 50% of the population in the fifth decade of life. This pattern of antibody acquisition contrasts markedly with that of rotavirus infection and suggests that the Norwalk agent and probably other morphologically similar agents are not a major cause of gastroenteritis in infants and young children but are more likely to be primarily associated with illness in older children and adults.

The Norwalk agent was initially discovered in an outbreak of gastroenteritis that occurred in Norwalk, Ohio, in which 50% of the pupils and teachers in an elementary school and 32% of the family contacts became ill. A filtrate made from a rectal swab obtained from a secondary case was administered orally to three adult volunteers

produced disease in two; it was subsequently successfully passaged serially. The agent could not be propagated conclusively in any cell or organ culture system and was first visualized by immune electron microscopy (IEM) in an inoculated volunteer's infectious stool filtrate. It has recently also been detected in vomitus from 4 of 5 volunteers who developed gastroenteritis after administration of a 2% filtrate of the agent.

Particles resembling the Norwalk agent morphologically and in density were subsequently discovered by IEM in individuals in two family outbreaks of gastroenteritis, one in Hawaii (Hawaii agent) and the other in Montgomery County, Maryland (MC agent). By IEM and cross-challenge studies, the Hawaii and Norwalk agents were found to be distinct, the Norwalk and MC agents to be related, and the relationship between the Hawaii and MC agents was inconclusive. Viruses resembling the Norwalk agent in morphology and density were later observed in faecal specimens from: (*a*) a gastroenteritis outbreak in a primary school in Ditchling, England; (*b*) a volunteer who became ill after administration of the "W" agent that had been obtained from a boy who developed gastroenteritis during an outbreak in a boarding school; and (*c*) individuals developing gastroenteritis after eating cockles. By IEM, the Ditchling and "W" agents are related but are distinct from the Hawaii and Norwalk agents, whereas the cockle agent appears to be distinct from the Norwalk agent. However, the relationship of the cockle agent to the other agents is inconclusive. Thus, besides the cockle agent, there appear at present to be three serotypes of this group of agents.

These agents have a diameter (shortest diameter) of 25–27 nm. They are not perfectly round, the Norwalk agent, for example, averaging 27 nm in its shortest diameter and 32 nm in its longest; they are similar morphologically to the picorna or parvoviruses and the hepatitis A virus. A clear-cut substructure is not visible by electron microscopy (EM). They have not yet been propagated definitively *in vitro* and therefore must be detected by EM, IEM or, in addition, for the Norwalk agent, by the recently developed radioimmunoassay (RIA) or IAHA.

These agents have a buoyant density in caesium chloride of 1.37–1.41 kg/litre as determined by ultracentrifugation and IEM or EM. Their nucleic acid and protein contents are unknown. The Norwalk agent is stable after exposure to pH 2.7 for 3 hours at room temperature, as determined from infectivity studies in volunteers. Similar studies have shown that the Norwalk and "W" agents are stable after exposure to 20% ether at 4°C for 24 and 18 hours, respectively. The Norwalk agent is relatively heat-stable, retaining infectivity in volunteers after heating at 60°C for 30 minutes.

These agents have not yet been classified. However, since the Norwalk agent shares certain characteristics with the parvoviruses, such as morphology, density, and ether, acid, and relative heat resistance, it appears to be parvovirus-like. It should be emphasized however, that none of this group of agents has had its nucleic acid content identified; thus they cannot be classified into any group.

The Norwalk agent has been administered to mice, guinea pigs, rabbits, kittens, calves, baboons, chimpanzees, and rhesus, marmoset, owl, patas, and cebus monkeys; none of the animals developed illness. When paired sera from monkeys, baboons, and chimpanzees were examined for a Norwalk serological response, only the chimpanzee developed such a response. Chimpanzees were also found to be shedding Norwalk antigen when examined by IEM after challenge.

Clinical and epidemiological characteristics

Of the 604 primary and secondary cases in the original Norwalk outbreak, 85% had nausea, 84% vomiting, 52% abdominal cramps, 57% lethargy, 44% diarrhoea, 32% fever,

and 5% chills. Symptoms lasted 12–24 hours in the majority of cases and seldom longer than 48 hours; none of the patients was hospitalized. The average incubation period was 4 hours. The signs and symptoms of illness observed in 32 of 55 volunteers who developed illness following the administration of 2% filtrates of stool containing Norwalk agent were very similar to those observed under natural conditions: incubation period 10–51 hours, fever (37.4°C) in 16 (50%), diarrhoea in 27 (84%), vomiting in 20 (63%), abdominal discomfort in 23 (72%), anorexia in 30 (94%), headache in 27 (84%), and myalgias or malaise in 20 (63%). The clinical manifestations usually lasted for 24–48 hours. The illness was generally mild and self-limited, although a volunteer who vomited approximately 20 times within a 24-hour period required parenteral administration of fluid. Shedding of the Norwalk particle in volunteers, as determined by IEM, was at a maximum during the first 72 hours after onset of clinical illness and occurred only infrequently afterwards. The signs and symptoms of illness observed in volunteers following administration of the Hawaii, MC, and "W" agents have been similar to those observed with the Norwalk agent.

In volunteer studies with the Norwalk agent short-term and long-term immunity have been observed. It appears that mechanisms other than that mediated by serum antibody are of primary importance in immunity to Norwalk gastroenteritis.

In volunteers challenged with the Norwalk or Hawaii agents, characteristically, there is broadening and blunting of the villi of the jejunal mucosa; the mucosa itself is histologically intact. Moderate amounts of mononuclear cell infiltrates and cytoplasmic vacuolization of epithelial cells have also been observed. Examination of thin sections of the jejunal mucosa by transmission electron microscopy has revealed intact epithelial cells with shortening of microvilli, dilatation of rough and smooth endoplasmic reticulum, swelling of mitochondria, and an increase in lysozymes; virus particles were not seen inside these cells. Biopsies taken 6 or more weeks after infection have been normal. During Norwalk illness the gastric mucosa appeared normal histologically. Small intestinal brush-border enzymes (including alkaline phosphatase, sucrase, and trehalase) were decreased; significantly, adenylate cyclase activity in the jejunal mucosa during Norwalk or Hawaii illness was found not to be elevated.

The role of the Norwalk agent in outbreaks of gastroenteritis has recently been investigated employing the newly developed Norwalk RIA and radioimmuno-blocking assay. Six of 23 outbreaks were found to be associated with the Norwalk agent; of these, two occurred on college campuses in the USA, one in a primary school in Japan, two on cruise ships, and one in two members of a family, one of whom was involved in an elementary school outbreak while the other, a secondary case, developed a serological response and shed morphologically Norwalk-like particle. If one includes the original Norwalk outbreak as well as the serologically related outbreak in Montgomery County, both of which were associated with Norwalk-like particles as determined by IEM, 8 separate outbreaks of gastroenteritis (32%) have been identified among 25 studied. If suitable serological tests had been available for Hawaii and "W" agents, it is possible that the majority of these outbreaks would have had an assignable etiology. In addition, a recent study in Australia suggests that at least part of another outbreak of gastroenteritis related to the eating of oysters was associated with the Norwalk agent.

Laboratory diagnosis

Methods for direct detection in stool of Norwalk and Norwalk-like agents

Immune electron microscopy (IEM) was used for the initial detection of the Norwalk agent. To detect the Norwalk agent in stool material, a stool suspension or filtrate

incubated with a convalescent Norwalk serum or immunoglobulin. The mixture is then centrifuged, the supernatant poured off, and the pellet or sediment reconstituted with distilled water and examined with a negative stain by electron microscopy.

The Norwalk group of agents cannot be identified directly by electron microscopy (EM) because they do not have a sufficiently distinct morphology. However, the Ditchling and "W" (and cockle) agents have been examined by EM after concentration by ultracentrifugation, followed by density gradient centrifugation in caesium chloride: for EM, a drop of fluid from a fraction was placed on a slide covered with 0.9% agarose, a grid was subsequently placed on the drop for 30 minutes to permit the caesium chloride to diffuse into the agarose, before examining the grid with a negative stain.

A radioimmunoassay (RIA) has recently been developed for detection of Norwalk agent in faecal specimens and appears to be even more efficient than IEM. The assay is based on a differential binding of stool suspensions containing Norwalk particles to a microtitration well coated with convalescent and another coated with preinfection anti-Norwalk antibody. By this method, non-specific interaction can be separated from specific Norwalk binding. The assay can detect soluble viral antigen as well as particulate material. Unfortunately, some stool specimens (less than 5%) have high non-specific activity that masks specific Norwalk binding. The success of this technique is dependent on the availability of specific reagents, and the only available, satisfactory antisera to the Norwalk agent have been obtained from human subjects or chimpanzees infected with the Norwalk agent. High-titred convalescent and antibody-negative preinfection sera from the same person or chimpanzee must be used. At present, these sera are not available commercially.

Methods for measurement of antibody to Norwalk and Norwalk-like agents

Until recently IEM was the only method for detecting a serological response to the Norwalk agent. Since IEM is employed for the detection and recognition of the Norwalk agent, an IEM serological assay with appropriate paired sera is essential to determine the significance of a particle visualized as a result of reaction with convalescent serum or immune serum globulin.

More recently, an immune adherence haemagglutination assay (IAHA) has been developed. In this technique, the Norwalk antigen is prepared from human stool containing the Norwalk particle using various extractions and concentration procedures. The test employs several reagents and human O erythrocytes, and has similarities to both a complement-fixation (CF) and a haemagglutination test. It has been quite specific for detecting serological responses as it has a high degree of concordance with IEM and RIA-BL (see below), but IEM and RIA-BL are slightly more efficient. In addition, IAHA cannot be used to detect Norwalk antigen in stool specimens. A major drawback with the use of IAHA for serological studies is the lack of an adequate supply of Norwalk antigen. Although IAHA requires considerably less antigen than CF, it still uses considerably more than the RIA-BL test. In addition, not all human O erythrocytes react comparably in the IAHA and it may be necessary to screen numerous donors' erythrocytes to find a donor that reacts with enough sensitivity to a known antigen.

The radioimmunoassay for detecting Norwalk antigen has been modified to identify Norwalk antibody in the form of a radioimmunoassay blocking test (RIA-BL). The assay is based on the ability of a test serum to block the binding of ^{125}I IgG antiNorwalk to Norwalk antigen; it is highly sensitive and specific and was found to be slightly more efficient than IEM in detecting serological responses in volunteers challenged with Norwalk agent. The RIA-BL test is suitable for large-scale studies and can theoretically be used to detect antibody in body fluids other than serum (i.e., intestinal secretions or milk).

OTHER VIRAL DIARRHOEAS

A number of viral agents have been identified whose role as etiological agents of acute diarrhoea is not clear. These can be divided into two groups. One includes those viruses such as adenoviruses, coronaviruses, and enteroviruses that are universally recognized mammalian viruses; the other includes particles or agents that have been described by a number of investigators and termed astrovirus, calicivirus, and mini-rotavirus. The available knowledge on these agents is summarized below.

Adenovirus

Adenoviruses are well established as respiratory viruses (33 serotypes have been recognized in cell culture) and the common serotypes can be isolated from stools in cell culture. Recently, a number of workers have reported visualizing by electron microscopy (EM) morphologically indistinguishable viruses in stool which could not be grown subsequently in cell culture. The reason for this phenomenon is unknown, but it has prevented more than tentative identification of such strains. Adenoviruses have been isolated from many animal species but none has been clearly implicated as a cause of diarrhoea.

In several studies adenoviruses have been found by EM in stools from 5–8% of normal children; in some, prolonged excretion was common. No one serotype has been associated convincingly with diarrhoea. In one recent outbreak the virus could be identified only by immune electron microscopy (IEM) and could not be grown. The role of adenoviruses, especially those that do not grow or grow poorly in cell cultures, as etiological agents of diarrhoea needs further study. In volunteer studies, which have been confined to well established strains, viruses were grown from faeces but gastrointestinal symptoms were not seen.

Astrovirus

Astrovirus was first described in Scotland in 1975 in the diarrhoeal faeces of infants examined by EM and has subsequently been observed elsewhere in the United Kingdom. Very similar, but possibly not identical, particles have been reported from Australia and Canada. These particles cannot be grown in routine cell cultures but limited growth has occurred in human embryo kidney (HEK) cells; growth was detected by immunofluorescence using an antiserum prepared in guinea pigs. No evidence has yet been found of the existence of more than one serotype.

The role of astrovirus as an etiological agent is unclear. In one study, they were found more frequently in the stools of infants with diarrhoea than in controls; they have also been implicated in two outbreaks in the United Kingdom. Postinfection increases in antibody have been detected by IEM. In adult volunteers challenged with astrovirus, infection was common and was accompanied by excretion of large amounts of virus and by seroconversion, but illness occurred in only a small number of individuals.

Morphologically similar viruses have been found in the faeces of lambs and calves. The lamb virus has been shown to cause diarrhoea in gnotobiotic lambs, but the calf virus induced only seroconversion in gnotobiotic calves. Astroviruses from man, lambs, and calves have been reported to be serologically distinct.

Calicivirus

This agent was first described in a community survey in Scotland in 1976, where it was detected by EM in the faeces of infants, and similar viruses have been reported by two

laboratories in the United Kingdom. Apparently similar particles have been reported by two laboratories in Canada, but in both cases they were labelled astroviruses and thus the situation is somewhat confused. No growth of this agent in cell cultures of human or feline origin has been reported, and different serotypes have not been described.

In one report, calicivirus particles were detected in the faeces of children involved in a school outbreak of "winter vomiting". The particle was found only in the faeces of patients and not in contacts. One adult teacher was also affected, but did not seroconvert. Other reports have indicated a less definite association between calicivirus and gastroenteritis. No volunteer studies have been reported. Morphologically similar viruses have been isolated from pigs, cats, and sea-lions, though none of these agents has been shown to cause diarrhoea in the natural host. It is not yet clear whether there is more than one serotype of the feline virus.

Coronavirus

The importance of coronavirus as a cause of diarrhoea remains unknown. In one report coronaviruses were detected by EM in stools obtained from 3 outbreaks of gastroenteritis in adults and the particles in a stool from one of the outbreaks were propagated in organ and cell cultures. No volunteer studies with the virus have been done. Coronaviruses, however, naturally infect many animals (chickens, mice, pigs, calves, dogs, rats, and cats) in which they cause a variety of clinical symptoms including diarrhoea in certain animals.

Enterovirus

Sixty-eight enteroviruses have been identified, all of which, with the possible exception of 2, can be isolated from faeces. Electron microscopy showed them to be small (25-nm) featureless spheres indistinguishable from one another. Techniques for isolating enteroviruses have been available for over 10 years and numerous investigations have attempted to implicate them as causes of diarrhoea. In general, no convincing evidence has accrued, though from time to time outbreaks apparently involving well-characterized types have been reported (for example, echovirus types 11, 14, 18, and 19). Whether such outbreaks were due to the enterovirus or to an undetected agent is not known. There is no evidence that the well known pathogenic enteroviruses like the polioviruses and coxsackieviruses cause diarrhoea.

The absence of any clear association between enteroviruses and diarrhoea has stimulated few volunteer studies. Some studies have been performed using strains of echoviruses and coxsackie A viruses isolated from the throat, but these caused little or no gastrointestinal illness.

Enteroviruses are found in a number of animal species but there is no evidence that they cause diarrhoea in their natural host.

Other small, round, virus-like objects (SRVs)

Some stool extracts have been shown to contain other virus-like particles 25–35 nm in diameter which to some investigators are clearly different from the background materials; they have not been grown in cell cultures. These SRVs have been reported mostly in association with outbreaks of gastroenteritis in communities or institutions. Within each outbreak the SRVs were consistent in size and appearance, but they differed between one outbreak and another. Several investigators have reported SRVs in the faeces of patients with sporadic diarrhoea (e.g., the "mini-rotaviruses" described in Canada, which were

morphologically similar to the viruses observed in an outbreak in Scotland) and in the absence of associated outbreaks it has been difficult to obtain enough material for the detailed investigation. Similar SRVs have been seen in the faeces of dogs and pigs with diarrhoea, but their relationship to human SRVs, if any, is unknown.

RECOMMENDATIONS FOR RESEARCH

Rotaviruses have emerged as the most important viral agents associated etiologically with severe diarrhoeal disease in infants and young children, and simple techniques are available for their identification. Therefore, priority should be given to research aimed at elucidating the epidemiology, pathophysiology, and means of prevention of illnesses caused by rotaviruses. Norwalk and Norwalk-like agents have also been recognized as a cause of diarrhoeal diseases, predominantly in older children and adults.

The following recommendations are made for further research:

Rotavirus diarrhoea

1. Studies are needed to define more completely the mortality and morbidity attributable to rotavirus diarrhoea in different geographical areas. Any factors, either host or environmental, that affect the severity of the disease need to be identified.

2. The epidemiological characteristics of rotavirus diarrhoea in different geographical areas also need elucidation. Studies should seek to define the following: relative incidence of the two recognized serotypes; seasonal occurrence of the disease; its modes of transmission; natural history of the disease; reinfection rate; incidence of asymptomatic infection; the relationship between nutritional status and the incidence of disease; possible association of the virus with chronic diarrhoea; the natural reservoir of human rotaviruses; factors influencing the survival of rotaviruses in the environment and the physicochemical stability of the virus.

3. Studies are needed to determine why human neonates infected with rotavirus often experience mild disease or subclinical infection. The relative infrequency of severe disease in human neonates with rotavirus infection is in direct contrast to the natural history of such infection in older infants and in young children; in the animal world also, the newborn experience the most severe rotavirus illness. These studies should consider such factors as immune status, virulence of the virus, diet, and influence of other faecal organisms. Information is also needed to learn whether human neonates with initial subclinical infections are susceptible to reinfection and/or disease from the same or another serotype later in life, or whether they are rendered immune by the initial infection.

4. Further studies are needed to determine the pathophysiology of rotavirus diarrhoea.

5. The effect of feeding during diarrhoea on the clinical course of rotavirus diarrhoea needs to be determined.

6. The role, if any, of rotavirus disease in malabsorption and subsequent nutritional deficiencies should be ascertained.

7. More research should be undertaken on the serotyping of rotaviruses isolated from animals and humans, to better define possible reservoirs of infection. Under experimental conditions, rotavirus virulent for one mammalian species may infect another species subclinically. Thus, under natural conditions, it is possible that human contacts may represent a source of infection for animals and that animal contacts are a source of infection for humans.

8. Research is needed to find methods for determining the virulence of rotavirus in animals and humans, both as a means of comparing different isolates and also as a method for determining whether a causal relationship exists between a virus present in the faeces and the clinical syndrome.

9. Since human rotaviruses do not grow to sufficiently high titre to allow them to be used directly in a vaccine development effort, a high priority should be given to studies of methods for more efficient propagation of these agents.

10. Although intestinal IgA rotavirus antibody is known to be of prime importance in preventing rotavirus illness, additional studies are needed to establish the duration of, and means of enhancing, the immune response. Studies of rotavirus antibody levels in secretions such as saliva and breast milk are required to determine whether they reflect antibody content in small-intestinal fluid.

11. Studies should be carried out to determine the influence of breast feeding on the natural history of rotavirus infection. Epidemiological, immunological, and social factors should be investigated. Knowledge gained from such studies would help to determine whether a rotavirus vaccine should be administered to women of child-bearing age.

12. Since passive administration of rotavirus antibody by the alimentary route has resulted in resistance to rotavirus challenge in various animal models, studies in humans on the effect of oral administration of human rotavirus antibody might be considered. Another approach might be the oral administration of purified separated fractions of cow's "immune milk" (containing antibody to rotavirus).

13. An experimental animal in which disease could be induced beyond the early period of life should be sought. This is important for studying the safety and efficacy of candidate rotavirus vaccines and for studies of virulence.

14. The group recognizes the need to support studies aiming at the development of a rotavirus vaccine. In that regard it endorses the recommendations of the Scientific Working Group on Immunity and Vaccine Development.[1]

15. There is a need for further development and evaluation of simple and reliable techniques for the detection of rotavirus and for the identification of serotypes.

16. In order to conduct most of these studies, a supply of high-quality reference reagents must be available. WHO should immediately assume the preparation and distribution of the following standard reagents:

(*a*) Pre-immunization and hyperimmune serum to rotavirus, prepared in goats and guinea pigs.

(*b*) Pre-infection and convalescent (3–4 weeks) antiserum to rotavirus prepared in gnotobiotic calves.

(*c*) Rotavirus antigen for use in ELISA and other immunological tests. To standardize diagnostic tests worldwide, a single antigen should be used in the preparation of (*a*) and (*b*). Such a reagent is in the process of preparation under contract to the National Institutes of Health, USA, and the Pan American Health Organization, and every effort should be made to distribute it globally as a WHO reference reagent. At a later date, when techniques are available, specific hyperimmune antiserum to the two human rotavirus serotypes should be developed and made available through WHO.

17. Training materials should be developed and workshops conducted for training laboratory workers to perform diagnostic tests.

Diarrhoea due to Norwalk and Norwalk-like agents

1. Since the Norwalk group of agents do not grow in a cell culture system, efforts should be made to find a way of propagating these agents.

[1] See *Bulletin of the World Health Organization*, 57 (5): 719-734 (1979).

2. Attempts should be made to find additional, serologically distinct 27 nm-like agents associated with viral gastroenteritis.

3. Practical detection methods and serological assays for other Norwalk-like agents such as Hawaii, "W" or Ditchling agents, have to be developed so that their natural history can be further elucidated.

4. Additional efforts should be made to discover animal models for the study of illness caused by the Norwalk group of agents.

5. The observed absence of long-term immunity to Norwalk agent in one group of volunteers in contrast to another which had such immunity has raised important questions in connection with intestinal immunity. The reasons for this lack of immunity in one group need elucidation.

Other viral diarrhoeas

Research should be carried out to determine the significance of astroviruses, caliciviruses, coronaviruses, enteric adenoviruses (untyped), and other virus-like particles detected in stools from acute cases of human diarrhoea. This should include longitudinal and case-control epidemiological studies, and laboratory studies to find a way to propagate them *in vitro* for their further identification. Such studies should include an exchange of specimens between laboratories in order to agree on the identification of these different morphological entities.

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Epidemiology & management of persistent diarrhoea in children of developing countries

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Diarrhoea that begins acutely but lasts longer than two weeks is defined to be persistent. Revised estimates in developing countries including India showed that acute diarrhoea accounts for 35 per cent, dysentery 20 per cent and non-dysenteric persistent diarrhoea (PD) for 45 per cent of total diarrhoeal deaths. PD also often changes marginal malnutrition to more severe forms. Factors that increase the risk of acute diarrhoea becoming persistent have been identified in India and other developing countries. These include antecedent malnutrition, micronutrient deficiency particularly for zinc and vitamin A, transient impairment in cell mediated immunity, infection with entero aggregative *Escherichia coli* and cryptosporidium, sequential infection with different pathogens and lack of exclusive breast feeding during the initial four months of life particularly use of bovine milk. Several issues regarding the management of persistent diarrhoea in hospitalized children in India have been resolved. Diets providing modest amounts of milk mixed with cereals are well tolerated. In those who fail on such diets providing carbohydrate as a mixture of cereals and glucose or sucrose hasten recovery. The role of antimicrobial agents and individual micronutrients in PD is currently being investigated. A management algorithm appropriate for India and other developing countries has been developed and found to substantially reduce case fatality in hospital settings to about 2-3 per cent. Recent epidemiological and clinical research related to persistent diarrhoea is also reviewed.

Key words Impaired immunity - incidence - lactose intolerance - mortality micronutrient deficiency - persistent diarrhoea

Although the majority of episodes of acute diarrhoea are self limiting, a small proportion last several weeks[1]. These episodes persistent diarrhoea are important because of a substantially higher case fatality rate than in acute diarrhoea, impact on nutritional status and the fact that these adverse outcomes may not be prevented by oral rehydration therapy alone[1-7].

The distribution of the duration of diarrhoeal episodes is a continuum[8]. As such, delineating a subgroup of diarrhoeal episodes as persistent by any cut-off is arbitrary. The World Health Organization (WHO) has now defined persistent diarrhoea as an episode that begins acutely and lasts for at least 14 days[1]. The use of 14 days cut-off to partition acute and persistent episodes seems justified. In India for instance, in a longitudinal study the case fatality rates were similar for diarrhoeal episodes of 1 and 2 wk duration (0.61 and 0.8% respectively) and this increased to 14 per cent as the duration exceeded two weeks[7]. The term persistent diarrhoea does not include specific disorders, such as hereditary syndromes, celiac disease or surgical conditions and these are not considered in this review[1].

Incidence of persistent diarrhoea

Estimates indicate that 3-20 per cent of episodes of acute diarrhoea become persistent[1]. The incidence of persistent diarrhoea obtained in several longitudinal studies varied considerably from 7-150 episodes per 100 child years[5,9-14]. In most settings the incidence of persistent diarrhoea peaked between 7 months and 2 yr of age; in countries with very high diarrhoea attack rates, the incidence was as high even in the first six months of life[12,14,15]. The disease incidence declined rapidly after the fourth year. This high variability in attack rates across countries may be due to differences in disease definitions and intensity of household surveillance, apart from true geographical differences.

Mortality and growth faltering related to persistent diarrhoea

Recent research has led to a revision of the earlier estimates of the contribution of different types of diarrhoea to diarrhoea related mortality. In an international study that compared clinical patterns of diarrhoea among diarrhoea related deaths in India, Bangladesh, Brazil and Senegal, a similar pattern was observed; acute diarrhoea accounted for 35 per cent (range 25 to 46%), dysentery 20 per cent (range 8 to 24%) and non-dysenteric persistent diarrhoea 45 per cent (range 23 to 62%) of the total diarrhoeal deaths[14,16]. The dysenteric deaths included those related to acute as well as persistent illness. In India the cause specific mortality rates (per 1,000) in children aged less than five years for acute watery diarrhoea[5,6], dysentery[4,9] and non dysenteric persistent diarrhoea[5,6] were similar (Table 1)[7]. While efforts to promote oral rehydration therapy and antibiotic treatment of dysentery must be sustained, persistent diarrhoea needs greater attention than at present.

Antecedent malnutrition, lack of breast feeding, and associated systemic infection increase the risk of death during persistent diarrhoea[2,7,14,17-19]. In a community based study in north India, nearly a third of fatal cases of persistent diarrhoea had associated pneumonia[7].

In Bangladesh, malnourished children had a 68 fold higher risk of death from persistent diarrhoea than those who were better nourished and residing in the same communities[17].

Table 1. Cause specific mortality rate* among children under five years of age[7]

Type of diarrhoea	No. of deaths	Cause specific mortality rate
Acute watery	8	5.6
Acute dysentery	3	2.1
Dysenteric PD	4	2.8
All dysentery	7	4.9
Non-dysentric PD	8	5.6
All persistent diarrhoea	12	8.5

* (number of deaths in a year/mid population) x 100
PD, Persistent diarrhoea
Superscript number indicates the reference no.

The contribution of dehydration to persistent diarrhoea associated deaths is uncertain but available data indicate that it is much less important than in acute watery diarrhoea[7,14,19].

Role of enteric pathogens in persistent diarrhoea

Several theoretical models for the role of microbial agents in the genesis of persistent diarrhoea can be conceived[8,20]. Firstly, an acute enteric infection may result in non infectious complications *e.g.*, lactose intolerance which leads to prolonged symptoms. Secondly, persistent diarrhoea may occur as a result of sequential acute infections with different pathogens. Lastly, prolongation of diarrhoea may result from persistence of intestinal infection; the failure to eliminate the organisms from the intestinal tract may be related to the characteristics of the pathogen, to host factors or to both.

Across various studies, one or more pathogens were detected in half to two-thirds of children with persistent diarrhoea during the initial few days of illness[2,5,13,14,21,22]. The interpretation of these isolations is difficult because the pathogens were also commonly excreted by asymptomatic children. In general, the pathogens excreted during persistent diarrhoea were the same as those reported in acute diarrhoeal episodes with the exception of rotavirus which was often detected in acute episodes, but rarely in those that became persistent. To identify enteric pathogens which may have a predilection for causing prolonged diarrhoea, the approach commonly adopted has been to compare their initial excretion rates in

episodes that last less than 14 days with those that last for a longer period (Table II). Among the many pathogens examined, enteroaggregative *Escherichia coli* and cryptosporidium were isolated with a significantly greater frequency in persistent episodes in some studies[14].

Enteroaggregative *E. coli* infection may be particularly important given a high rate of isolation in one-third to half the cases of persistent diarrhoea in India, Bangladesh and Mexico; these rates were higher than in acute diarrhoea[13,23-25]. However, this pathogen was excreted with a similar frequency in persistent and acute episodes in some studies[21,22]. Notably, enteropathogenic *E. coli*, diffusely adherent *E. coli*, shigella and *Giardia lamblia* were detected with similar frequency in acute and persistent diarrhoea in most reported studies. The importance of shigella infection in persistent diarrhoea may have been masked by the timely and appropriate antibiotic treatment of acute dysentery.

Studies in Bangladesh and Peru where sequential stool cultures were obtained, suggest that persistent infection with the same organism is an uncommon phenomenon[21,22]. A more frequent occurrence was the isolation of different pathogens at various phases of the persistent episode. In persistent diarrhoea associated with AIDS, *Cryptosporidium parvum, Isospora belli, Enterocytozoon bieniusi* and *Mycobacterium avium - intracellulare* are the most frequently identified pathogens[26]. The importance of these pathogens in persistent diarrhoea among HIV negative children has not been well investigated.

Nutritional status, immune mechanisms and persistent diarrhoea

An increased diarrhoeal incidence in malnourished children has not been consistently observed[27-31]. On the other hand studies have consistently shown that malnutrition is associated with a substantial increase in the average duration of diarrhoea and the incidence of persistent diarrhoea[32-34].

Table II : Per cent of enteric pathogens identified in acute or persistent diarrhoea during either the first or third week of illness[14]

Pathogen	Persistent episode culture <7 days				Persistent episode culture ≥ 14 days		
	India (n=43)	Bangladesh (n=251)	Bangladesh (n=184)	Peru (n=161)	Bangladesh (n=153)	Bangladesh (n=170)	Peru (n=35)
Rotavirus	2.3	2.8	1.6	0.7	5.9	0.6	2.9
Aeromonas sp.	0	4.8	3.3	9.0	2.6	1.8	20.0
Campylobacter sp.	4.7	7.1	12.0	32.2	8.2	11.2	22.9
EAEC-AA	34.9	27.4	18.6	12.0	24.5	17.0	
EAEC-DA	0	9.6	16.4	12.8	11.3	20.8	
EAEC-LA	2.3	0	4.5	4.3	0	3.8	
ETEC	9.3	4.8	14.6	24.2	2.6	12.5	25.7
Salmonella sp.	4.7	0.4		0	0.7		0
Shigella sp.	2.3	5.6	5.4	7.6	5.2	5.9	5.7
Vibrio sp.	0	0.4	1.1	2.1	0	1.2	2.9
Cryptosporidium			5.6	0.8		0	0
Entamoeba histolytica	2.3	0	0	0	1.0	0	0
Giardia lamblia	2.3	1.6	1.2	22.9	4.1	4.2	42.9

EAEC, enteroadherent *Escherichia coli:* AA, aggregative; DA, diffuse adhering; LA, localized adhering; ETEC, enterotoxigenic *Escherichia coli.* Superscript no. indicates the reference no.

Malnourished children are more likely to have subclinical deficiency of individual micronutrients and the deficiency of some of these may explain, at least partly, the increased risk of persistent diarrhoea in the undernourished. In a recent Indian study, children 6-36 months of age attending a community clinic with acute diarrhoea were supplemented with zinc gluconate or placebo during the enrollment episode and for a 6 months period thereafter; zinc supplementation resulted in a significant reduction in the average duration of the acute enrollment episodes and in the proportion that became prolonged[35]. Over the ensuing 6 months the incidence of persistent diarrhoea was reduced by 49 per cent in zinc supplemented children older than 11 months but there was no such effect in those below this age[36]. Zinc supplementation was previously shown to reduce the duration and severity of acute diarrhoea by others[37,38].

A positive association between vitamin A deficiency and diarrhoeal morbidity has been reported in only some studies[39,40]. In Brazil, routine administration of vitamin A (200,000 IU) at 4 monthly intervals to children aged 1-5 yr reduced the incidence of severe diarrhoea by 20 per cent and diarrhoea prevalence by 23 per cent; the incidence of persistent diarrhoea was not reported[41]. Micronutrients such as zinc and possibly vitamin A reduce the severity and duration of diarrhoea either by a rapid and effective repair of the intestinal epithelium following an acute enteric infection due to its role in the regulation of cell division or by enhancing the immune response[42,43].

Immuno incompetence, particularly decreased cell mediated immunity is one of the likely underlying mechanisms for the increased incidence of persistent diarrhoea among malnourished children[31,34,40,44]. In Peru and Bangladesh, however, reduced delayed hypersensitivity responses (DHR) to several skin antigens were found to be associated with an increased risk of persistent diarrhoea, even after controlling for nutritional status[31,34]. The impairment in DHR was often transient. Transient cell mediated immunosuppression may be the result of acute viral infections[45,46]. Anergy has also been reported following bacterial infections such as tuberculosis and pneumonia[47,48]. Diminished cell mediated immunity could also be related to decline in micronutrient status[42].

Recent morbidity and persistent diarrhoea

It has been seen that children are at an increase risk of persistent diarrhoea following an episode measles or acute diarrhoea but not acute lower re piratory tract infection[49,50]. The basis for an increase risk of persistent diarrhoea over the 2 to 3 month period following an acute diarrhoeal episode is u clear. A possible explanation may be that childre who develop persistent diarrhoea are a subgroup wit a high overall diarrhoea burden[51]. Alternatively, a acute diarrhoeal episode may induce alterations the intestinal epithelium or in immune responsive ness that renders the subsequent diarrhoeal episode to be more severe or prolonged. Acute diarrhoea characterized by excessive faecal zinc losses[42]. It ha been postulated that the impaired epithelial cell re newal and immuno incompetence associated wit subclinical zinc deficiency may be the basis for th increased susceptability to persistent diarrhoea fo lowing acute diarrhoea. These hypotheses need to b examined in future studies.

Type of feeding and persistent diarrhoea

It is important to identify feeding practices th increase the risk of persistent diarrhoea as these a potentially modifiable.

Factors that increase the overall risk of diarrhoe are also likely to cause more persistent diarrhoea.

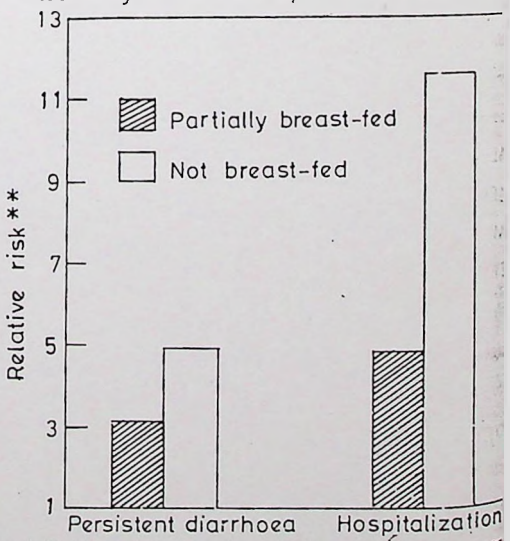

Fig. 1. Relative risk of persistent and severe diarrhoea amon infants under 3 months of age by feeding mode* (Ref 18) *at the a of 1 wk; **reference group : exclusively breast-fed.

Brazil, breast feeding was protective against persistent diarrhoea even after controlling for socio-economic status[18]. In early infancy, use of milk supplements in addition to breast feeding increased the risk of persistent diarrhoea by three fold. The risk was even greater among the non breast-fed (Fig. 1).

Once an acute diarrhoeal illness has occurred, the mode of feeding during the acute phase may influence the severity and duration of symptoms. Breast feeding during acute diarrhoea reduces the severity and duration of the episode[52].

A highly contentious issue is whether the use of bovine milk or infant formula during acute diarrhoea increases the episode duration and severity. Several factors may influence the response to milk feeding during acute diarrhoea: the source of milk, amount, type of processing, other foods consumed during the illness, severity of the infection and factors related to the host[53]. The lack of consistency in the findings on this issue across studies is therefore understandable. There is evidence that feeding of non human milk as the sole or predominant nutrient source during acute diarrhoea may increase the episode severity and duration[54]. In many of the studies where milk intake during acute diarrhoea was moderate, or when milk was offered as a part of a mixed diet containing cereals, a significant increase in the episode duration or severity was not observed[55,56]. Lactose malabsorption is dose dependent and it is likely that milk in moderate amounts particularly when mixed with other foods is well tolerated by most children with acute diarrhoea.

In an Indian study, the risk of persistence of acute diarrhoea was more with bovine milk than with an infant formula[57]. This seems plausible as bovine milk has a higher lactose content and osmolarity and the sensitization capability of milk protein in the infant formula is reduced due to spray drying during the manufacturing process.

Fermented products of milk such as yoghurt have been shown to be better accepted by lactose intolerant subjects and by children with persistent diarrhoea[58]. In a recently completed trial however, the feeding of yoghurt instead of milk to malnourished children during acute diarrhoea was not associated with a reduction in the episode duration or the risk of persistent diarrhoea (Singh *et al* unpublished data).

When viewed together, the reported data indicate that the use of mixed diets including low to moderate amounts of milk during acute diarrhoea promote weight gain without increasing the episode duration.

Type of oral rehydration salts (ORS) solution and persistent diarrhoea

Rice-based ORS was shown to substantially reduce the stool output in cholera in comparison to standard ORS, but its effect on stool output and episode duration in non cholera acute diarrhoea has been relatively small[60]. In a study from Bangladesh, the use of rice ORS during acute diarrhoea was reported to have reduced the proportion of episodes that became persistent[61].

In recent clinical trials, where feeding was carefully standardized, there was no significant difference in the outcome of episodes treated with rice-based ORS than with standard ORS[61]. Thus, in the presence of optimal feeding, rice-based ORS during acute diarrhoea is unlikely to substantially reduce the risk of development of persistent diarrhoea.

In a recent multi-centre WHO sponsored clinical trial including India, the effect of a reduced osmolarity ORS (224 mmol/litre) on the stool output and episode duration was examined in comparison to the standard WHO ORS (311 mmol/litre). Stool output was reduced by 30 per cent and the average episode duration by 22 per cent in the children treated with reduced osmolarity ORS[62]. Data on the proportion of episodes that became persistent were not reported.

There are few community-based reports on the risk of development of persistent diarrhoea in relation to antibiotic use during the first week of diarrhoeal illness. In a study from India, antibiotic use during the initial days of illness was equally common in episodes that were eventually classified as acute or persistent[5]. In another study, initial antibiotic treatment was significantly less common in the episodes that became persistent, raising the possibility of a beneficial effect[12]. Nevertheless it is important to recognize that unwarranted antibiotic treatment for acute diarrhoea could lead to *Clostridium difficile* associated pseudo membranous colitis and hasten the emergence of antibiotic resistant pathogens.

Specific causes and their role in the pathogenesis

The common causes of persistent diarrhoea as seen in developing countries are persistent infection

with one or more enteric pathogens, sequential enteric infection, disaccharide and rarely monosaccharide malabsorption and dietary protein intolerance[5,14,22,63-70]. The hallmark of the disorder is persistent mucosal damage which may result from failure to eliminate the causative agent or delayed and ineffective mucosal restoration. Protein energy malnutrition and micronutrient deficiency in humans are known to be associated with abnormalities in intestinal structure and function. Delayed regeneration of the epithelium with reduced crypt cell multiplication and ineffective maturation of cells during their migration up the villi following an enteric infection has been demonstrated in experimental malnutrition[67]. Pathogenic bacteria cause mucosal damage and diarrhoea through mucosal effacement or invasion and action of enterotoxins or cytotoxins; malnutrition in the host prolongs the healing of the injured mucosa, the state of malabsorption and diarrhoea. In a proportion of cases, an immunological response to luminal, bacterial or dietary antigens has also been proposed as the basis for gut mucosal damage in persistent diarrhoea but the data are still inconclusive[68].

The role of dietary protein intolerance in persistent diarrhoea is highly contentious. Based on studies in India and other developing countries, it is unlikely to be of significance in the pathogenesis of persistent diarrhoea[63-65,69,70]. Although, increased intestinal uptake of intact proteins has been demonstrated following acute gastroenteritis in early infancy, the clinical significance of this phenomenon is not established[70].

There is a substantial impairment of fat and protein absorption during persistent diarrhoea; carbohydrate malabsorption is only moderately affected on diets that are predominantly cereal based[71]. Secondary malabsorption of sugars, particularly of lactose is common. In a proportion of cases, disaccharides other than lactose are also malabsorbed to a clinically significant degree.

Clinically significant monosaccharide malabsorption is fortunately infrequent[62,63,66]. The causes of carbohydrate malabsorption are acute or persistent enteric infection and malnutrition by decreasing brush border enzymes. Unabsorbed sugars are osmotically active and induce water and electrolyte secretion into the intestinal lumen.

Faltered growth in persistent diarrhoea is not only the result of nutrient malabsorption; inadequate dietary intake due to anorexia, a continuation of faulty pre-illness feeding practices or as a response to diarrhoeal illness itself by the family or the physicians, are all important.

Management

The initial step is to determine the appropriate place for care. Patients with persistent diarrhoea require hospitalization in the presence of dehydration, associated systemic infection requiring intravenous antibiotics or when anorexia is severe. Intravenous fluid therapy may be required initially when dehydration is severe, to correct major electrolyte abnormalities or acidosis, and in extremely cachexic or systemically infected infants who accept oral fluids poorly.

Children with some but not severe dehydration can be effectively treated with oral rehydration salt solutions. It is important to provide additional potassium supplements to those severely malnourished.

When clinical dehydration is not associated, home-available fluids are appropriate for replacement of ongoing stool losses.

Basis for dietary management

Several general principles have been established through recent studies in persistent diarrhoea.

In developing countries, the need to use total intravenous nutrition arises very rarely. Optimal oral feeding, based on an appropriately constituted diet is well tolerated and achieves recovery and catch up growth in the vast majority of these patients. Although there is some malabsorption of nutrients in persistent diarrhoea, about 80-90 per cent of carbohydrates and 70-75 per cent of fats and proteins are actually absorbed from mixed diets based on locally available ingredients.

Breast feeding is safe and well tolerated during diarrhoea. Although, a few predominantly breast-fed infants with acute diarrhoea may continue to pass stools with more than the usual frequency or stools of somewhat liquid consistency for more than two weeks, physical growth is well maintained.

Should milk be withdrawn in persistent diarrhoea?

In the non breast-fed babies, an important issue is whether milk should be totally eliminated or simply reduced in amount during persistent diarrhoea.

Brown and colleagues reported increased stool weights on diets predominantly based on whole milk as compared to lactose hydrolysed milk[72]. The milk intakes were equivalent to about 6 g/kg/day or more of lactose; few children in Indian communities take such large quantities of milk. The issue of whether lower intakes equivalent to 2.5 g/kg lactose load per day would also be poorly tolerated has been recently examined in a clinical trial: preliminary analysis shows significantly greater weight gain in the group receiving a mixture of cereals with milk in which the latter provided 35 per cent of the total calories than in the other group consuming isocaloric cereal-based diet without milk. There was only a modest 15 per cent increase in the stool output in the milk group but the treatment failure rates were similar (Table III)[73].

Similarly, Bhutta *et al*[74] reported lower stool output and greater weight gain in persistent diarrhoea with curds cereal mixtures than with lactose free soy based diets. Together, these studies make a case for reduction rather than total elimination of milk as the initial step. A modest amount of milk in cereal diets improves their protein quality, trace elements and mineral content. Further, the consistency and palatability ensures higher intakes of these diets than with purely cereal-based diets. The possibility of occasional milk protein allergy is out weighed by the benefits offered by adding modest amounts of milk to cereal based diets.

Specific recommendations for the initial diet

Once a child is ready for oral feeding after few hours of stabilization, the choice of an initial diet would be milk rice mixtures with added oil, yielding an energy density of about 85-95 kcal/100g with 30-35 per cent calories from milk. The diet provides the ideal minimal 10 per cent energy from a protein source. The composition of one such diet is given in Table IV.

In a small proportion of patients with very severe diarrhoea where some clinicians feel reluctant to use milk even in small quantities, rice sugar oil based diets are appropriate. Egg is well tolerated and provides useful animal protein in such diets.

Table III. Comparison of milk-based and milk-free diets in persistent diarrhoea[73]

	Milk cereal (n=47)	Milk free cereal (n=46)	Difference in median (95% CI)
Median stool weight in males*(g/kg/h)			
0-48 h	1.7 (0.9, 2.5)	1.5 (0.6, 2.4)	0.23 (-0.26, 0.73)
0-120 h	1.6 (0.9, 2.7)	1.3 (0.61, 2.39)	0.26 (-0.19, 0.74)
% change in weight at 120 h*	2.8 (0.8, 5.9)	2.3 (-0.1, 4.3)	0.91 (-0.48, 2.4)
Number with :			
Stool output >200g/kg in any 24 h period	3	1	
Reappearance of dehydration during study	4		
Stool output >60g/kg on day-7	2	7	
Weight on day-7 < rehydration weight	1		
Treatment failures by any of the above (%)	17.2	23.6	0.6** (0.24, 1.8**)

* Median (range)
** Odds ratio (95% CI)
Superscript no. indicate the reference no.

Table IV. Composition of diet A

Ingredients	Amount (g
Puffed rice	12.5
Milk	40
Sugar	2.25
Oil	2.0
Water to make	100
Energy density (cal/100g)	96
Per cent protein	10.0
Per cent carbohydrate	55.87
Per cent lactose	1.73
Per cent fat	33.9
Amino acid score	1.0

Nearly 25 per cent of hospitalized patients show a poor response to diet A. Useful criteria for defining treatment failure are reappearance of dehydration at any time, passage of 7 or more liquid stools in a day at the end of 7 days treatment and weight loss or poor weight gain despite an oral intake of at least 100 cals/kg/day over the previous three days. Poor oral intake as a result of systemic infection is more often the cause of weight loss than true dietary failure in hospitalized children. In milder cases that are managed at the household level, a common reason for poor weight gain is the offering of only small quantities of thin foods to the child by the family.

The factors related to treatment failure on low lactose (milk) cereal based diets are systemic infection, severe carbohydrate intolerance involving not only lactose but also other disaccharides and starch. Therefore, dietary modification should be made only after effective treatment of associated systemic infection. The second line diet should be milk free with substitution of part of the starch by sucrose or glucose (diet B; Table V). This mixture of sugars achieves the right balance between dietary osmolarity, digestibility and energy density. In such a diet, egg or chicken is a suitable protein source. Monosaccharides as the only carbohydrates in the diet should be used for the few patients who are treatment failures on diet B as it is difficult to provide sufficient energy density with the permissible 2-3 per cent glucose concentration; at higher concentrations osmotic diarrhoea may develop.

Until their role in the management of persistent diarrhoea is well established, generous but safe amounts of micronutrients equivalent to 2 times the RDA should be provided. These may include vitamin A, zinc, iron, folate and when feasible others. Severely malnourished children should receive magnesium 1-1.5 ml/kg body weight of a 50 per cent solution, given IM for 2-3 days. Patients on a milk free diet should also receive calcium supplementation.

Several commercial diets are also available. For reasons that are not fully explained, home based low lactose or lactose free diets perform much better than commercial soy based formulations[74]. Semi elemental diets like Nutramigen or Progestimil are useful but expensive. They usually contain protein hydrolysate or calcium caseinate, mixture of disaccharides and oligosaccharides and part or whole of the fats a medium chain triglycerides. Micronutrients and vita mins are already added. The diet B is based o similar principles and is at least as effective. It advantage is that the concentration of the individua sugars can be tailored to each individual child, it i cheap and can be easily prepared by mothers at hom and in small hospitals, the disadvantage is that vita mins and minerals need to be supplemented.

Table V. Composition of diet B

Ingredients	Amount (g)
Puffed rice	13.50
Egg	11.0
Glucose or Sucrose	3.50
Oil	3.50
Water to make	100
Energy density (cal/100g)	95.22
Per cent protein	9.51
Per cent carbohydrate	56.9
Per cent fat	33.29
Amino acid score	1.00

The role of antimicrobial agents against enter pathogens in terms of improved nutrient absorptio decreased stool output or shortened illness duratio is undecided. In a recent Indian study large doses o nonabsorbable, broad spectrum antibiotic were ad ministered based on the hypothesis that it woul eradicate aerobic bacterial overgrowth. There was n improvement in purge rates or weight gain with mas sive doses of oral gentamicin as compare to a pl cebo, despite clearance of stool pathogenic orga isms including adherent *E. coli*[75]. It was conceivab that systemic antibiotics may be of greater benefit persistent diarrhoea. However, a recently conclud double blind field trial done to evaluate the effica of metronidazole given alone or in combination wi nalidixic acid in comparison to a placebo has sho no significant clinical benefit (Behl *et al*, unpu lished data). Metronidazole was evaluated for action against anaerobes. Other controlled trials w co-trimoxazole have shown similar results. It a pears that when the mucosa is already severely da aged, whatever the initiating factors, nutritional su port is the key to its rapid repair.

An effective antishigella agent should be used in the presence of blood or numerous pus cells in the stools. Treatment for giardiasis and amoebiasis is indicated when a stool examination reveals trophozoites. Currently, there is no suitable treatment for cryptosporidium infection.

Cholestyramine, which binds unconjugated bile salts or bacterial toxins, has not proved to be useful. There is also little evidence of clinical benefit when lactobacilli are administered to replace intestinal microflora.

Systemic antibiotics are required to treat associ-

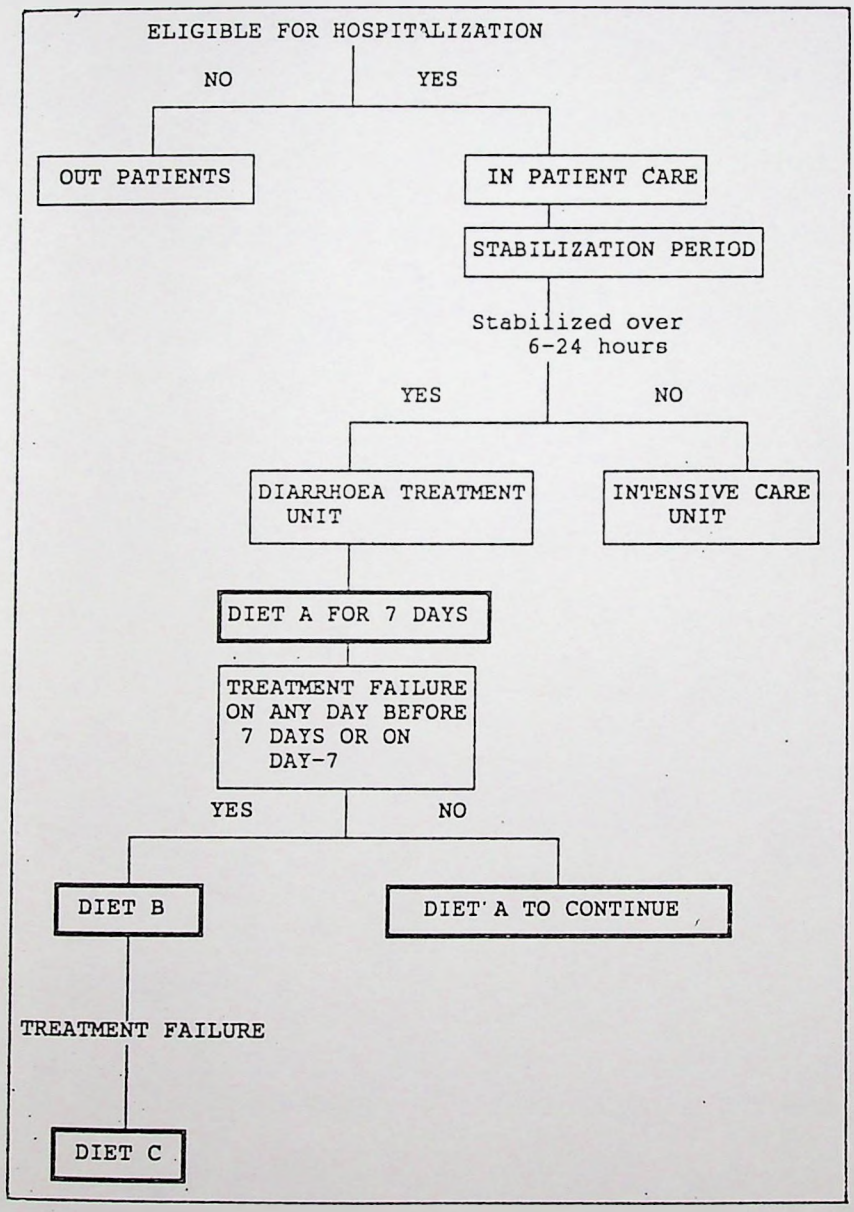

Fig. 2. Practical algorithm for the treatment of persistent diarrhoea.

ated pneumonia, septicemia, meningitis or urinary tract infection in patients with persistent diarrhoea. These infections are detected in nearly 60 per cent of patients with associated severe malnutrition usually seen in a hospital setting, they are much less common when malnutrition is only of moderate severity, as is likely with those being treated as outpatients. The search for such infections should be vigorous, even in the absence of fever; useful indicators are persistent anorexia, refusal of liquids and breast milk and dehydration despite modest stool losses.

These recommendations are summarised in a treatment algorithm (Fig. 2). This algorithm was evaluated in an international study including the group at the All India Institute of Medical Sciences (AIIMS), New Delhi. The success rate for the evaluated 460 children with persistent diarrhoea while on diet A was 70 per cent (95% CI 65%, 75%) and it was 84 per cent (95%CI 76%, 93%) for those evaluated while on diet B. Weight gain was achieved in over 90 per cent and associated illnesses requiring antibiotics were found in 61 per cent of the children. The children at greatest risk were the youngest, those severely malnourished, with highest initial purging rates and associated infection.

As diarrhoea is common in children residing in poor communities where family feeding habits contribute to malnutrition, an interaction with health care providers during the illness offers a good opportunity to improve the nutrient intake through purposeful nutritional counselling. Mixtures of milk and cereals or of cereals and legumes fortified with oil are well tolerated during acute and persistent diarrhoea. They have the required energy density and palatability. About 30-40 per cent of calories can be derived from fat sources without any deleterious effect. Mothers must receive nutritional counselling from health care providers that is practical and takes into account the family views and realities and includes clear instructions on the frequency of meals, the amounts to be fed at each and the solutions to problems of the individual child and family. This is currently the weakest link in the sick child - health care provider interaction.

The vast majority of patients with persistent diarrhoea are unable to avail of hospital care due to physical and situational constraints of the family. Therefore, it is the outpatient care of patients with persistent diarrhoea that needs strengthening instead of the current over focus on sophisticated treatment of the few patients who actually get to hospitals.

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CH36·1

Brief Reports

Diarrhea Management in Some Jhuggi Clusters of Delhi

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Oral rehydration therapy (ORT) is the basis of the Diarrheal Diseases Control Programme in India. It aims at reduction in 70% diarrhea deaths among the under-fives(1). It is felt that the continuing high mortality due to diarrhea is to a large extent because of low ORT use, lack of knowledge for correct preparation of oral rehydration solution (ORS), traditional misbeliefs and practices among mothers(2). Antimicrobial agents have only a limited role and anti-diarrheals have no role in the treatment of acute diarrhea(3), yet due to deficient clinical training of doctors and expectation of mothers there is tendency to lay stress on drugs than oral rehydration(4,5).

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The present study was undertaken to obtain information on the action taken at home and health facility, in case of acute diarrhea and assesses the knowledge of the households in preparation of ORS and sugar salt solution.

Subjects and Methods

The study was conducted in three large J.J. clusters, *viz.*, Sanjay Amar Colony, Hathi Park, and Jai Prakash Colony situated in the vicinity of Maulana Azad Medical College, New Delhi. A total of 6285 persons residing in 1090 households were studied. A responsible person, mostly a housewife, present in the household at the time of visit, was interviewed by the interns with the help of pre-structured and pre-tested questionnaire. Enquiry was made on occurrence of diarrhea in the household in previous two weeks. In households where a case of diarrhea had occurred, detailed information was obtained regarding action taken by the household and nature of treatment given by private/government health agencies. Respondents were also asked to show the preparation of sugar salt solution and ORS using household measures.

Results

Amongst 6285 persons surveyed, 183 (2.9%) had diarrhea in the previous two weeks. Majority were under-fives (68.3%). Blood in the stool was associated in 24 (13.1%) cases. *Table I* shows the first action taken by the households when diarrhea occurred. One-fourth received ORT as ORS solution (3.3%), sugar salt solution (10.4%), dal water (5.5%) or shikanji

(5.5%). There was no significant difference in management between under and over-fives ($p > 0.05$).

The correct preparation of sugar salt solution by using finger pinch, scoop or spoon and glasses was known to 36.6% households. However, only 11.5% households could measure correctly the water required to prepare one litre of ORS.

The first action was often delayed upto the second (13.1%) or third day (18.0%). During the course of diarrhea, as first action or subsequently 102 (55.7%) cases were taken to private practitioners and 13 (7.1%) to a government health facility. The pattern of treatment provided by them is shown in *Table II*. All cases of dehydration were given ORT or intravenous fluids. However, to prevent dehydration, home available fluids (HAF) *e.g.*, dal water, shikanji, lassi or sugar salt solution were advised by government health facilities in 20.0% and private practitioners in 15.7% cases only.

Discussion

The continued poor use of ORT(2,6) calls for a fresh look at its implementation. The preferences for the private practitioners even though government health facilities were nearby, in this and other studies(6) emphasize the need for their involvement in the programme.

Use of ORT to prevent dehydration needs more emphasis as this was often not advised. The excessive use of drugs including antimicrobials and antidiarrheals by private practitioners(81.4%) and government health facilities (61.5%), even though antimicrobials were indicated only in the 13.1% cases of dysentery, is alarm-

TABLE I–*First Action Taken by Households in Diarrhea*

Action taken*	≤ 5 Years (n = 125)	> 5 Years (n = 58)	Total (n = 183)
Home available fluids/ORS	33 (26.4)	12 (20.6)	45 (24.6)
Stopped food/fluids	10 (8.0)	4 (6.8)	14 (7.6)
Household remedies	13 (10.4)	11 (18.9)	24 (13.1)
Visited private doctor	46 (36.8)	29 (50.0)	75 (40.9)
Visited government health facility	9 (7.2)	0 (0.0)	9 (4.9)

* In 14 (11.2%) under-fives and 2 (3.4%) over-fives no action was taken when diarrhea occurred.
Figures in parenthesis indicate percentages.

TABLE II–*Treatment Received From Health Agencies*

Treatment*	Private practitioner (n = 102)	Govt. health facility (n = 13)	Total (n = 115
Drug	83 (81.4)	8 (61.5)	91 (79.1
ORS/Home available fluids	28 (27.4)	8 (61.5)	36 (31.3
Intravenous fluids	1 (0.9)	1 (7.7)	2 (1.7

* Responses not mutually exclusive.
Figures in parenthesis indicate percentages.

ing. The misuse of drugs leads to adverse reactions, resistant organisms, and increase in the cost of treatment. It also delays the initiation of appropriate treatment and complicates the condition of the patients.

Our results emphasize the need for frequent re-orientation training of private practitioners and in-service doctors on appropriate case management and rational use of drugs in acute diarrhea. The families also need to be informed and demonstrated the correct preparation and use of ORT for prevention and treatment of diarrhea and restriction on the use of drugs.

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Calf Circumference as a Predictor of Low Birth Weight Babies

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The prevalence of low birth weight (LBW) in India ranges from 30-40%. The perinatal mortality among LBW babies (188.8/1000) is about 8 times higher than that in the infants weighing more than 2500 g(1). Birth weight is used as a measure of LBW because of its correlation with gestation and ease of recording in hospital setting. However, in our country 70-90% deliveries are conducted at home by traditional birth attendants and untrained relatives and weight recording is a problem. The present study was conducted with an aim to find an alternate, cheap and reliable predictor of LBW babies that can be used by a trained or untrained person.

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Subjects and Methods

The study was conducted on 1600 newborns in the Department of Pediatrics, G.S.V.M. Medical College, Kanpur. The birth weight, crown-heel length and midarm, head, chest, thigh and calf circumferences were measured by standard techniques(2). Weight of the nude baby was recorded in a beam type weighing

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INCIDENCE OF DIARRHOEA AND SOME RELATED EVNIRONMENTAL AND BEHAVIOURAL FACTORS IN JHUGGIS OF DELHI

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Summary

A total of 6285 persons residing in 1090 households in three Jhuggi clusters of Delhi were studied for incidence of diarrhoea by 2 weeks recall method and enviornmental and behavioural factors affecting it. Overall incidence of Diarrhoea was 29.1 per thousand persons, and was selectively predominant among under fives (60.2 per thousand). This low incidence of diarrhoea could be attibuted to safe drinking water availability and common practice of handwashing by most of the people. But unsafe storage of drinking water at household level (70.5%) and peridomestic open air defaecation by children (22.9%) are potential threat for transmission of the disease.

Introduction

Diarrhoea is a leading cause of childhood morbidity and mortality[1]. Extensive promotion of Oral rehydration therapy has resulted in preventing over one million deaths each year[2]. However, globally one billion cases of diarrhoea and more than 3 million deaths due to diarrhoea among underfives are still occurring every year[1]. In India diarrhoea is responsible for 23-33% of all deaths below five years of age[3].

Incidence of diarrhoea is maximum among jhuggi clusters[4] as these have inadequate basic civic amenities. Delhi has 1080 Jhuggi Clusters having about 4.8 lakhs jhuggi's[4]. The present study was undertaken in three jhuggi clusters to study the incidence of diarrhoea and some environmental and behavioural factors affecting it.

Materials & Methods

The present study was conducted in three jhuggi clusters of Delhi, viz. Sanjay Amar Colony, Haathi Park and Jai prakash Colony situated within 2 km. from Maulana Azad Medical College, New Delhi. All the jhuggis were covered except those found locked, or where no adult member was present at the time of visit. A total of 6285 persons residing in 1090 households were surveyed. A responsible person, mostly a housewife present in the household was interviewed with the help of pretested questionnaire. Information was obtained for occurrence of diarrhoea in the household in previous two week. In case of occurrence information regarding source of water, storage of water, practice of handwashing and defaecation was obtained.

Acute diarrhoea was defined as the pas-

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sage of 3 or more loose/watery stools in a 24 hour period. Episodes that lasted more than 14 days were defined as persistent. Dysentry was diagnosed when stool mixed with blood was reported[5].

Results

The overall incidence of diarrhoea was 29.1 per thousand persons. It affected children underfive years of age (60.2 per thousand) more than the older subjects (13.8 per thousand). Persistent diarrhoea was found to 7.3 per thousand in the underfives whereas none of the overfives had persistent diarrhoea. Dysentry was observed in 13.1%.

The various sources of drinking water were community tap (53.0%), municipal water tankers (34.4%) and hand pumps (12.6%).

Water was stored unhygienically in majority of the household (70.5%) in the open buckets kept on floor without a laddle to draw it.

Most of the adults were using public (68.9%) or their own (8.2%) latrines whereas, 22.9% adults were still practicing openfield defaccation. Contrary to this majority of the children were defaecation either into the drains just outside their jhuggi (44.3%) or open field (26.8%) and only 20.8% children used private/public latrine.

Most of the household members (96.2%) washed their hands after defaecation. Various materials used for hand washing were soap (56.8%), plain water (24.1%), rakh (10.9%) and mud (6.6%). Handwashing before meals was practised by 76.5% household.

Discussions

The incidence of diarrhoea among underfive (60.2 per thousand) was lower than observed in other studies (87-467 per thousand)[6,11,12]. Main reason for this seems to be the use of safe water supply for drinking by; the majority of the households (87.4%) which was either from municipal taps or tankers.

Use of tap water instead of shallow hand pumps is associated with decrease in diarrhoea incidence[6]. Though storage of water was in unhygienic manner, probably it did not affect the consumers due to short period of storage (2-3 hours) required during the study period and action of residual chlorine in municipal water supplies[7]. However, during summer months when water supplies are cut of for long periods, storage of water under the existing conditions, as well as use of unsafe sources like shallow hand pumps poses a threat of outbreak of diarrhoea.

Season is another factor that would have contributed to lower incidence as the current study was done in the month of March, just prior to seasonal increase in diarrhoea; similar observations have been made by others[8,9].

Practice of handwashing after defaccation and before meals by most of the households is also a contributory factor.

Lower incidence of diarrhoea and no case of persistent diarrhoea among overfives is consequent to developing some degree of immunity & consequent a symptomatic or less severe infections[7].

The proportion of persistent diarrhoea to that of acute diarrhoea was within the known range[10], while proportion of dysentery was higher than others[8].

Lower incidence of diarrhoea observed among the residents of some jhuggi clusters of Delhi is a positive finding. This i

attributed to the use of safe drinking water, practice of hand-washing after defaecation and before meals and seasonal variation.

However, unsafe storage of water and peridomestic defaecation by children holds potential threat of an outbreak during summer months when there is shortage of tap water supply. These finding have implications for the contents of health education.

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