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Intestinal Amoebiasis: Antibody-secreting Cells and Humoral Antibodies

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ABSTRACT

Splenic plasma cell response and systemic antibody response to intestinal amoebiasis were studied in C$_3$H/HeJ mice from 5 to 60 days post-inoculation with *Entamoeba histolytica*. At various time intervals specific antibody-secreting cells (ASC) in the spleen were measured in infected mice and non-infected control mice by enzyme-linked immunospot (ELISPOT) assay. Serum antibodies were measured by enzyme-linked immunosorbent assay (ELISA). The infected animals showed high IgA ASC from 30 to 50 days post-inoculation as compared to IgM and IgG ASC. However, class-specific serum antibody showed high IgG titre from 30 to 60 days post-inoculation as compared to IgM and IgA serum titres. Our results suggest that *E. histolytica* trophozoites can induce a plasma cell response in the spleen that is different from anti-amoebic antibody response in serum.

Key words: Entamoeba histolytica; Amoebiasis; Immune response; Enzyme-linked immunosorbent assay

INTRODUCTION

Amoebiasis is one of the most common health problems in the world. It is estimated that annually about 480 million people develop clinical amoebiasis and at least 40,000 die (1). Invasive amoebiasis generally induces a systemic humoral immune response demonstrable about 1 week after the onset of symptoms (2,3). Anti-amoebic antibodies persist even after invasive amoebiasis has healed or after subclinical amoebic infection has disappeared (4). The major amoebic serum antibodies belong to the IgG class (5). However, IgA (6) and IgM (7) antibodies are also present. *In vitro* studies showed that specific antibodies may inhibit the growth (8) or neutralise the virulence (9) of *Entamoeba histolytica*.

It is known that mucosal cells act as the first line of defence against pathogens trying to invade the human body. Local immune mechanisms, particularly secretory antibodies, are thought to have a central role in this defence. After contact with the antigen, activated lymphocytes in Peyer's patches migrate to local lymph nodes to mature and latter return to the gut via lymphatic and blood vessels (10-12). However, after contact with *E. histolytica* trophozoites at the mucosal surface, the response of class-specific antibody-secreting cells (ASC) in the spleen is unknown. In the present study of experimental intestinal amoebiasis in mice we describe ASC response in the spleen and compare this with the class-specific circulating antibody response.

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MATERIALS AND METHODS

Preparation of *E. histolytica* antigen

E. histolytica strain HM1:lMSS were grown axenically in TYI-S-33 medium (13). Amoebae were harvested as described elsewhere (14) and lysed by 3 cycles of freeze-thawing. Protein concentrations were determined by the method of Bradford (15) using bovine serum albumin (BSA) as a standard. Amoebic antigens were kept in aliquots at -70º C until used.

Monoxenic cultures of *E. histolytica*

Axenic *E. histolytica* strain HM1:IMSS were maintained in Robinson's medium (16) associated with *Escherichia coli* as described elsewhere (17).

Animals

We used C3H/HeJ mice of 3 to 5 weeks of age, weighing 20 to 25 g. Mice were maintained at the "Instituto de Investigaciones Biomedicas, Universidad Nacional Autonoma de Mexico." The animals were free of parasites as shown by repeated stool examination. They were given sterile Purina pellets (Purina de Mexico) and water ad libitum.

Intracaecal inoculation of mice

Mice were inoculated with monoxenic *E. histolytica* culture as described before (17). Briefly, mice underwent laparotomy under light ether anaesthesia and then about 5×10^5 trophozoites in 0.15 mL culture medium were inoculated intracaecally. Control mice were inoculated in the same manner with Robinson's culture medium (16) containing *Escherichia coli*, but free of *E. histolytica*. Mice were sacrificed at regular intervals, and positive diagnosis for caecal infections was done, based on the presence of amoebae in the caecal contents or in caecal wall scrapings. The presence of amoebae was confirmed by inoculating caecum fragments of infected mice in Robinson's medium (16). Growth of trophozoites occurred after 48 to 72 hours of culture.

Scoring of the caecal lesions

The conditions of the caecal wall and contents were graded according to the scoring system introduced by Neal (18). This is as follows: [i] caecal wall: Normal, 0; Slight thickening, 1; Marked local thickening and contraction, 2; Extensive thickening and contraction, 3; Caecum shapeless, extensive ulceration with abscess formation, 4; [ii] Caecal contents: Normal, 0; Slightly less solid than normal, 1; Slightly mucoid, 2; Mucoid, some solid matter present, 3; No solid matter, white or yellow mucus only, 4.

Preparation of splenic lymphoid cell suspensions

Mice were euthanized at specified intervals after intracaecal infection with *E. histolytica*. Spleen cell suspensions were prepared separately from infected and non-infected control mice as described elsewhere (14). Cell viability was determined by trypan blue exclusion and cell numbers by a count in a haemocytometer. The numbers of cells were adjusted to 2.5×10^5 viable cells per mL. Cell viability was usually >95%.

Anti-*E. histolytica* cell assay

The immunoglobulin-specific antibody-secreting cell response to *E. histolytica* infected and non-infected control mice was studied on days 5, 10, 15, 20, 25, 30, 40, 50, and 60 post-inoculation. The enzyme-linked immunospot (ELISPOT) assay was adapted to detect immunoglobulin (Ig) secreting B lymphocytes according to Czerkinsky *et al.* (19). *E. histolytica*-specific spot-forming cells (SFCs) were enumerated in 96-well nitrocellulose-based plates (Millititer HA, Millipore Co. Bed. Ford, MA). Each well was coated with 10 µg of *E. histolytica* antigen in 100 µl of PBS, pH 7.2 at 4ºC in a humidified chamber. After thorough washings with PBS-Tween, the reaction was blocked with 1% BSA. The plates were washed and 100 mL splenic cell suspensions were added, adjusted to 2.5×10^5 cells/mL in RPMI 1640 medium supplemented with 10% foetal calf serum, 100 U penicillin, 100 µg streptomycin per mL, 2 mM L-glutamine, 1 mM sodium pyruvate and 0.1 mM non-essential amino acids adjusted to pH 7.2 with 7.5% sodium bicarbonate (Gibco Laboratories, Grand Island, NY). Cells were incubated for 3 hours at 37ºC in 5% CO_2 and 100% humidity. After washing, conjugate peroxidase-labelled antibodies goat anti-mouse IgM, IgG or IgA (Sigma) were incubated in quadruplicate for 2 hours at room temperature. Following wash, colour development was made with 3.3'-diaminobenzidine, nickel chloride, cobalt

chloride, hydrogen peroxide in PBS. The reaction was stopped by adding distilled water and definite dark black coloured circular spots were counted under low magnification (x16). Artifactual, faintly spread undefined dots were not counted, as recommended by Czerkinsky *et al.* (20). Results are reported as medians, interquartile ranges and the upper and lower values of each group.

Serological tests

The infected and non-infected control mice were bled through the orbital plexus before being sacrificed. Antibody titres were determined by enzyme-linked immunosorbent assay (ELISA) (21). Briefly, 96-well plates (Immulon II, Dynatech Laboratories Alexandria, VA) were coated with 5 µg amoebic protein per well in 100 µL carbonate-bicarbonate buffer (0.1 M, pH 9.6) and incubated at 4°C. Plates were washed with PBS-Tween and blocked with BSA. Plates were incubated with 100 µL of serum samples at a dilution of 1:100 for 1 hour in duplicate. Plates were thoroughly washed and 100 µL of peroxidase- labelled antibodies goat anti-mouse IgM, IgG or IgA (Sigma) were added to each well. Colour was developed by adding O-phenylenediamine and H_2O_2 in 0.1 M citrate buffer, pH 4.5. The reaction was stopped by adding 100 µL of 1 M H_2SO_4 to each well. The plates were then read at 495 nm in an ELISA processor M (Behring Marburg, Germany).

Histological studies

The caecum was removed from the infected and non-infected control mice and fixed in 10% formalin in phosphate-buffered saline (PBS, pH 7.2). The tissues were dehydrated and embedded in paraffin and sectioned at 4 µm. Sections were stained with Harris' haematoxylin and eosin (22).

Statistical analysis

Because of the non-normal distribution of ASC counts, their medians, interquartile ranges and upper and lower values (shown as box-and-whisker plots) were used for summarising the data, with the Mann-Whitney U-test to evaluate differences between groups (23). Specific anti-amoebic antibodies are shown as means (SD).

RESULTS

Course of *E. histolytica* infection in mice

The *E. histolytica*-infected and non-infected control mice were sacrificed on days 5, 10, 15, 20, 25, 30, 40, 50 and 60 post-inoculation, and their abdomen was opened. The gross pathological changes observed in the caecum were graded as indicated above. At 5 day post-inoculation, the caecum of most mice appeared to be contracted. The caecal contents of the infected animals consisted of mucoid yellowish green exudate. At 10 and 15 days post-inoculation the caecum was more contracted with ulcerations of the mucosa and abundant white or yellow mucoid material. On 20 day post-inoculation the caecum was shapeless and usually attached to the abdominal wall; it contained yellowish or whitish mucoid material, and ulcerations of the mucosa were common. On days 25 to 60 post-inoculation atrophy and contraction of the caecum were less pronounced. Figure 1 shows the combined results for the 12 infected mice in each group. The control mice had no lesions in their caecum.

Table. Determination of anti-amoebic antibody response in the sera of infected mice by ELISA

Days after infection	Mean OD value at 495 nm		
	IgM SD	IgG (SD)	IgA (SD)
5	1.66 (0.07)	0.18 (0.06)	0.08 (0.03)
10	1.74 (0.06)	0.55 (0.25)	0.21 (0.11)
15	1.23 (0.29)	0.85 (0.23)	0.14 (0.07)
20	1.63 (0.02)	1.67 (0.36)	0.21 (0.03)
25	1.22 (0.13)	1.68 (0.41)	0.55 (0.23)
30	1.66 (0.17)	2.43 (0.42)	1.39 (0.08)
40	1.52 (0.15)	2.48 (0.43)	1.28 (0.33)
50	1.54 (0.32)	2.09 (0.31)	1.51 (0.05)
60	1.55 (0.31)	2.53 (0.46)	1.50 (0.07)
Non-infected controls	0.17 (0.03)	0.14 (0.05)	0.12 (0.04)

Total IgM, IgG and IgA anti-amoebic antibodies were detected by ELISA as described under Materials and Methods. The sera were diluted 1:100 for IgM, IgG and IgA. Results are reported as means (SD) of 12 mice.
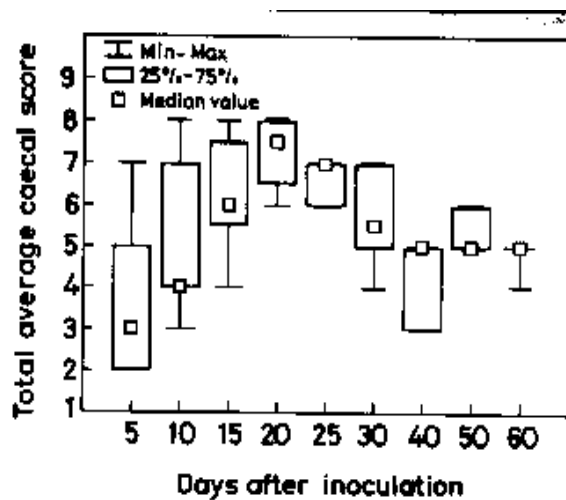
Fig. 1. Box-and-Whisker plot with the median, interquartile ranges and the upper and lower values of group of 12 mice after combining the score of caecal wall and contents showing course of *E. histolytica* infection in intracaecally inoculated mice C3H/HeJ; grading was done according to Neal (1951).
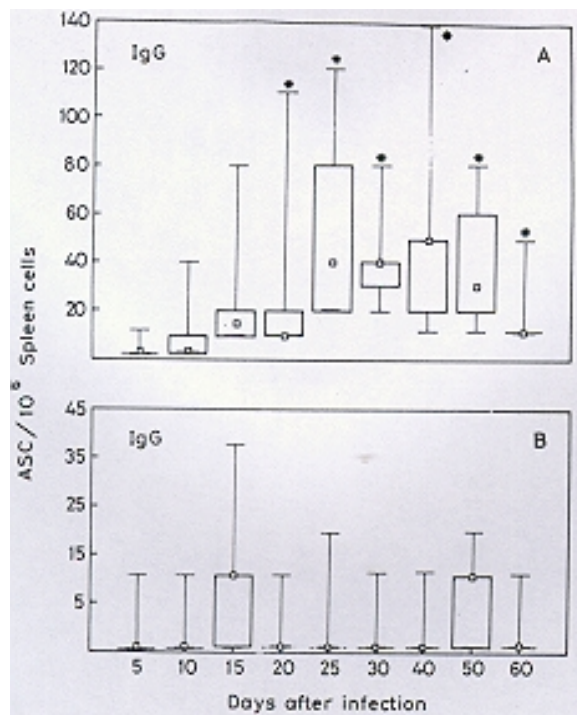
Fig. 2. Splenic anti-amoebic antibody secreting cells (ASC). Box-and-Whisker plots show medians interquartile ranges and upper and lower values in groups of 12 mice. Mice were sacrificed and spleen cells obtained separately from either *E. histolytica* infected (A) or non-infected control (B) mice. *Probability of the null hypothesis of no difference between A and B (<0.05).

Measurement of antibodies

As shown in the Table, specific serum IgM titres were high from 5 till 60 days post-inoculation. IgG and IgA titres gradually increased to 17-fold and 11-fold to the initial values, respectively, on day 30 post-infection. Both remained high till day 60 compared to the non-infected control mice.

Relation of ASC and antibodies in serum

IgM ASC numbers and serum IgM titre were high from day 5 till day 60 post-inoculation. On the contrary, IgG ASC and serum IgG titre increased only on day 25 and 30 post-inoculation respectively. IgA ASC and serum IgA titre increased on day 30 post-inoculation.

DISCUSSION

In this study, we observed that *E. histolytica* can induce specific IgM, IgG and IgA ASCs responses in experimental amoebiasis. We used a mouse model of intestinal amoebiasis that is comparable to the human disease (17). High numbers of specific IgA-secreting cells in the spleen were detected on days 30 to 50 post-inoculation. On day 60, IgA ASC response was high (p<0.0003), but it had decreased significantly as compared to day 50 post-inoculation (200 vs. 85, n=12, p<0.0002). On the other hand, specific serum IgA antibodies remained high from day 30 onward. Similarly, specific IgG-secreting cells in the spleen showed a maximum on day 25 post-inoculation and gradually decreased till day 60 post-inoculation, whereas IgG serum antibodies remained elevated. IgM ASCs showed a maximum on day 30 post-inoculation, but the IgM serum antibodies were elevated throughout the study period.

We observed persistence of IgM, IgG and IgA ASC response in spleen and anti-amoebic antibody serum titres even on days 30 to 60 post-inoculation when the degree of caecal infection had decreased. This might reflect persistence of antigens in tissues, which might have a bearing on post-infection sequels (2).

Moreno-Fierros *et al.* (24) reported that local or systemic immunisation of mice with glutaraldehyde fixed *E. histolytica* trophozoites by rectal, intragastric or intraperitoneal route elicited IgM, IgG and IgA

ASC response. They observed that splenic anti-amoebic IgM ASC response was higher than that of IgG and IgA ASC (25). In contrast, in our experimental system IgA ASC response was higher than the IgG and IgM ASC responses.

We observed different time-related patterns of class-specific antibody responses in spleen and in serum. This might be explained by lymphocytes being stimulated differently or, alternatively, because of different structures and action of the antigens involved (e.g. lipopolysaccharide, toxins, adherence factors, etc). The high IgA splenic ASC response observed in our study may reflect mucosal response. Earlier researchers reported IgA dominated ASC response after mucosal infections (26-28).

Pre-treatment of anti-amoeba antibodies *in vitro* exert a cytotoxic effect on *E. histolytica* trophozoites and such trophozoites do not produce liver abscess on intrahepatic inoculation in hamsters (8,29,30). Thus, the means through which antibodies limit parasite growth during parasitic infection are unclear (31). However, it has been observed that polyspecific rabbit anti-amoebic antibodies can induce surface redistribution of the bound antibody, leading to the formation of caps that are subsequently internalised or shed, protecting the parasite from the harmful action of antibodies and complement (32,33). Alternatively antibody bound to the parasite membrane may be digested in situ by non-specific proteases (34).

It is known that live organisms provide better immunogens than dead bacterial or viral antigens. We speculate that amoeba could be a promising approach for developing anti-amoebic vaccines as they might induce a better protection than purified antigens. Strober and Brown (31) reported that crude preparations of cholera organisms are more protective than the purified toxin. Moreover, it is considered that suitable vaccines are those that elicit a mixture of antibodies response and can act at several levels against the pathogens.

In conclusion, splenic plasma cell and serum antibody responses to *E. histolytica* were induced in C3H/HeJ mice by intracaecal infection. We observed that *E. histolytica* induces a splenic immune response different from the serum response. The measurement

of the ASC response offers a new means of assessing the immune response in intestinal amoebic infection that may be useful in future vaccine development against amoebiasis.

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Rotavirus G and P types in Children from Belém, Northern Brazil, as Determined by RT-PCR: Occurrence of Mixed P Type Infections

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ABSTRACT

Fifty-four group A rotavirus-positive stool samples, obtained from children aged less than three years during a longitudinal (December 1982 to March 1986) study in Belém, Brazil, were re-examined. The samples were tested by reverse-transcription and polymerase chain reaction to determine their G-type and P-type specificity. Only 17 (32%) of these rotavirus strains could be successfully G- and P-genotyped. While 10 (59%) of the 17 strains showed single G- and P-type specificity, the remaining belonged to single G- and mixed P-genotypes. Rotavirus strains P[8],G1 and P[4],G1 predominated, accounting for 29% and 18% of the typed strains respectively. Mixed P-type infections caused by rotaviruses classified as P[8]+P[4], G1 were identified in 23% of cases. All but 3 of the 54 rotavirus strains displayed long genomic profiles, as demonstrated by the analysis of RNA by polyacrylamide gel electrophoresis. Most (70%) rotavirus strains with single G- and P-type specificity were detected during the first year of life, whereas 5 (71%) of the seven mixed P-type infections occurred throughout the second or third year of age. Reinfections were noted in two children, both of them being infected with P[8]+P[4], G1 rotavirus strains when aged 20 months. The high proportion of untypeable rotavirus strains suggests that unusual types may be circulating in Belém. In addition, the occurrence of mixed P-type infections in our region indicates the potential for reassortment between different rotavirus genogroups. Monitoring of these rotavirus strains may have important implication in the context of future strategies of rotavirus vaccination in Brazil.

Key words: Rotavirus; Serotyping; Rotavirus infections;Diarrhoea, Infantile; Longitudinal studies

INTRODUCTION

Human rotaviruses constitute the single most important cause of severe gastroenteritis among infants and young children in both developing and industrialised countries (18). The significant rotavirus disease burden, mainly in the tropical regions of the world, makes the development of an effective

rotavirus vaccine a high priority for the World Health Organization (8,17). It is currently accepted from numerous vaccine trials throughout the world that an effective protection conferred by a rotavirus vaccine would be achieved if it contains the epidemiologically important types (3). Therefore, to determine future vaccination strategies a better knowledge on the distribution of rotavirus G and P types is required, particularly in tropical regions of the world. Indeed, neutralising antibodies to both viral proteins VP4 and VP7 have largely been associated with protective immunity (14,26).

Rotaviruses belong to the *Reoviridae* family and their genome consists of 11 discrete segments of double-stranded RNA surrounded by a triple-layered shell virion (9). The two outer capsid proteins VP4 and VP7 independently induce type-specific neutralising antibodies that form the basis for the current adoption of a dual serotyping classification system (14,26). G (of glycoprotein) serotype specificity is associated with VP7, whereas P (of protease-sensitive) refers to VP4. To date, 14 G serotypes and 12 P types have been found among rotavirus strains of both human and animal origin (5). While it had been established that nine G types (G1-4, G6, G8-10, and G12) infect humans, only four (G1-4) are of major importance in the aetiology of infantile diarrhoea worldwide (15,18). Both nucleotide and amino acid sequence analyses of gene 4 have identified six distinct rotavirus VP4 genotypes in humans, designated as P[8] (prototype strain Wa), P[4] (strain DS-1), P[6] (M37), P[9] (AU228), P[10] (69M) and P[12] (PA169) (11,33). On the basis of the currently proposed binary system for rotavirus characterisation, the majority of isolates from diarrhoeic children fall into four groups: P[8],G1, P[8],G3, P[8],G4 and P[4],G2 (10,29). Of these, P[8],G1 and P[4],G2 rotavirus strains are the most prevalent worldwide (7,27,28).

Studies conducted to date in Brazil indicate that the predominant strains are essentially those identified elsewhere (i.e. P[8],G1 and P[4],G2, accounting for two thirds of isolates) (11,21). Of interest, the occurrence of unusual genotypes has been largely recognised from surveys in several regions of Brazil. Thus, genotypes G2 or G4 with P[6], P[9],G3 and P[8],G5 have been characterised, the latter one accounting for nearly 13% of single infections (21).

Although the occurrence of mixed rotavirus G type infections have been reported by several authors (25,27,31,32), a limited number of surveys have identified co-infections involving different P types (19,35,36). In this context, studies conducted in Brazil have shown that over 20% of rotavirus-positive specimens had multiple G and/or P types, therefore providing evidence of infections with multiple rotavirus strains (11,21,34). These findings clearly indicate the potential for reassortment between rotavirus strains belonging to different genotypes (and even species), raising an important implication concerning future strategies of rotavirus vaccination in Brazil. The present report deals mainly with the occurrence of concurrent rotavirus G and/or P types among children who were followed up from birth to the age of three years in Northern Brazil.

MATERIALS AND METHODS

Patients and methods

We re-examined fifty-four stool specimens from children aged 0 to 3 years, obtained during a three-year (December 1982 to March 1986) longitudinal, community-based study carried out in Belém, Brazil (22,23). All these specimens had tested group A rotavirus-positive with the WHO ELISA kit provided by the Regional Virus Laboratory, East Birmingham Hospital, Birmingham, UK. This method has already been fully described (2). Also using ELISA, these specimens were reacted with G serotype-specific monoclonal antibodies according to a technique described by Beards (1). Electrophoresis of deproteinised rotavirus RNA was performed with a 5% polyacrylamide gel using discontinuous buffer system, as described by Laemmli (20). The reverse-transcription and polymerase chain reaction (RT-PCR) typing methods were performed in two steps, essentially as described before (12,36). Briefly, in the first amplification, a mixture of primers was used (Table I) as follows: (a) Beg9 and End9, which are 28

and 27 nucleotides long respectively, and represent full-length copies of gene 9 (or gene 8) from any group A rotavirus strain; and (b) P1A[+] and P1A[-], corresponding to nucleotide sequence numbers 11-32 (+ sense) and 1072-1094 (- sense) of the VP4 gene of strain KU, which are highly conserved among human rotavirus strains regardless of the difference in their P types or gene 4 alleles. The second amplification involved the use of: (a) for G genotyping a mixture of oligonucleotide primers specific to G1, G2, G3 and G4, which correspond to nucleotide locations 314-335, 411-435, 689-709 and 480-498 respectively; and (b) for identification of gene 4 alleles, a mixture of primers specific to P[8], P[4], P[6] and P[9], corresponding to nucleotide positions 487-508, 325-348, 733-755 and 900-921 respectively. All PCR products were subsequently subjected to electrophoresis on 1% agarose in TBE buffer containing ethidium bromide (1 μg/mL).

Table II. Correlation between VP7 (G) and VP4 (P) typing of 54 ELISA rotavirus-positive stool samples from children in Belém, Brazil

Type	Numbers (%) of G and P typeable strains*					Total
	G1	G2	G3	G4	G untypeable	
P[8]	5(29)					5
P[4]	3(18)					3
P[6]				2(12)		2
P[9]						
P[8]+P[4]	4<(23)	1<(6)			1<	6
P[8]+P[4]+P[6]	2(12)					2
Subtotal	14(82)	1(6)		2(12)		
P untypeable	9		1		26	36
Total	23	1	1	2	27	54

*All strains displayed long electropherotypes, except those indicated by < (1 < = 1 strain); the percentages in the box refer to the 17 samples in which both P- and P-types were identified.

Table I. Primer sequences and their location on both VP7 and VP4 genes

Primer	Sequence (5'- 3')	Position	Genotype
BEG9	GGCTTTAAAAGAGAGAATTTCCGTCTGG	1-28	G
END9	GGTCACATCATACAATTCTAATCTAAG	1036-1062	G
BT1	CAAGTACTCAAATCAATGATGG	314-335	G1
CT2	CAATGATATTAACACATTTTCTGTG	411-435	G2
ET3	CGTTTGAAGAAGTTGCAACAG	689-709	G3
DT4	CGTTTCTGGTGAGGAGTTG	480-498	G4
P1A+	TGGCTTCGTTCATTTATAGACA	11-32	P[8]
P1A-	CTAAATGCTTTTGAATCATCCCA	1072-1094	P[8]
P1A	ATATTCCTACGAGTTTAGTATC	487-508	P[8]
P1B	ACTAACATGTGGTTCAACTGCGAT	325-348	P[4]
P2	CTGAGCACGTTGATAAAGTCAC	733-755	P[6]
P3	CGTATATTGATAGTTCATGGG	900-921	P[9]

RESULTS

Table II shows that only 17 (32%) of the 54 tested rotavirus strains had both their G and P types, characterised by RT-PCR. Of these, 10 (59%) represented a combination of single G and P types, whereas the 7 remaining strains belonged to single G and mixed P types. Rotavirus strains P[8],G1 were found to predominate, accounting for almost a third of those which were both G[-] and P[-] typed. Of interest, rotaviruses bearing P[8]+P[4] and G1 genotype specificity were identified in 23% of the 17 samples. Taken separately, a G type could be assigned to 27 (50%) of the 54 strains, whereas 18 (33%) were P typed. G1 was largely predominant, corresponding to 85% of the G typed rotavirus strains. Among the VP4- genotyped strains, P[8], P[4] and P[6] types were found either as single (56%) or mixed (44%) infections. Table II also indicates that all but 3 rotavirus strains displayed a long electropherotype.

Figure 1 shows the age-distribution of the 17 rotavirus samples with both G and P types identified,

during the three-year study period, specifying whether infections were either symptomatic or asymptomatic. Seven of the 10 rotavirus infections bearing single G- and P- genotype specificity occurred during the first year of life. Among these, the four inapparent infections (2 P[8],G1 and 2 P[4],G1 strains) occurred within the first six months of life. Conversely, 5 of the seven infections involving rotavirus strains with mixed P- and single G-genotype specificity occurred throughout the second or third years of life. The two mixed P type infections during the first year of life were asymptomatic, whereas both apparent (n=3) and asymptomatic (n=2) infections were characterised among children aged 19 to 36 months. Two cases of reinfection were noted: one patient suffering first and second infections at one month (asymptomatic, P[8],G1) and 20 months of age (symptomatic, P[8]+P[4],G1) respec-tively; and a second child that had asymptomatic infections at 4 and 20 months of age by strains classified as P[8]+P[4]+P[6],G1 and P[8]+P[4],G1 respectively

specificity is in accordance with findings from several other investigations throughout the world (11). We were unable to identify those less common (unusual) human types, such as P[8],G5 and P[6],G9 rotavirus strains, since our RT-PCR was directed to the worldwide dominant G- and P- human types. While previous, multicentre studies in Brazil have shown that rotavirus bearing P[8],G5 type-specificity may account for up to 9% of cases of acute gastroenteritis among infants and young children (21,34), in India, P[6],G9 strains represent 13% of the isolates (7). Since a significant proportion (about 50%) of our strains could not be typed, it is likely that types other than those studied circulate among children living in Northern Brazil. On the other hand, it is also likely that high rates of untypeable rotavirus strains in the present study are caused by too low volumes of several stool samples left to perform RNA extraction. The use of phenol chloroform in our study may also
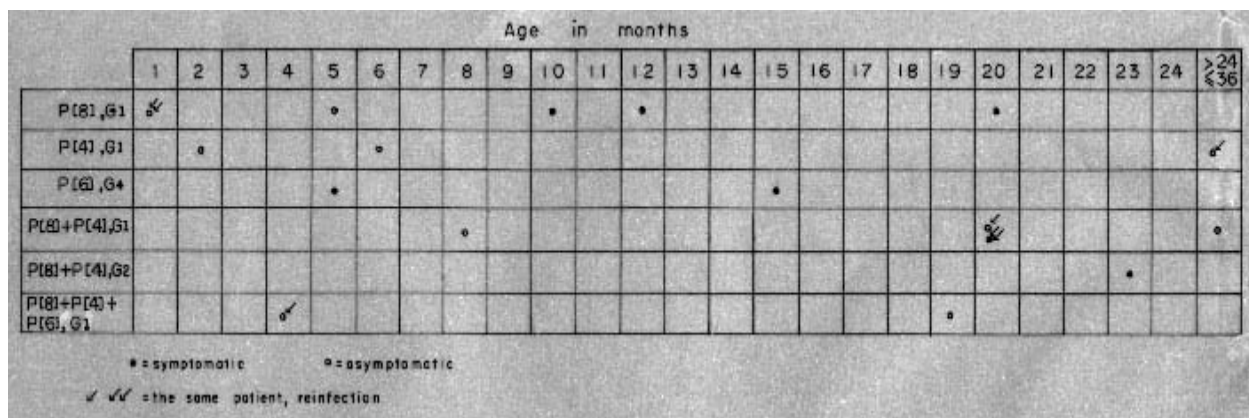
Figure 2 shows the G and P genotypes of rotavirus strains, as determined by RT-PCR. Single G- and P-genotypes were identified in 10 children, whereas single G- and P- mixed P-type strains were found in seven of them.

explain this large number of untypeable strains by inhibition of reverse transcriptase.

Our results reflect in general the usual correlation between the two G- and P- antigenic specificity for each typed rotavirus strains. All but one P[8] rotavirus isolate were classified as G1, whereas two P[6] strains correlated with G4 serotype (16). Of particular interest was the detection of reactivity to more than one VP4-type specific primers in about 40% of typed rotavirus strains: there were 5 dual infections by types P[8] and P[4] and two children were concurrently infected by genotypes P[8], P[4] and P[6]. This rate

DISCUSSION

Our results present the first G- and P-type characterisation of rotavirus strains in the Amazon region, as determined by RT-PCR. Among the typed strains, the predominance of those bearing P[8],G1

of mixed P-type infection is higher, for example, than the less than 5% reported in South African children (30). In addition, it was twice the rates of previously reported mixed P-type infections among Brazilian children from 9 States and the Federal District (21). The large proportion of mixed P-type infections found among children living in Belém may be due to the heavily contaminated environment where our study has been carried out. All children who participated in this study lived in the peripheral area of Belém, in general under poor sanitary conditions. Furthermore, most of the mixed P-type infections occurred during the second year of life, when children are more likely to be infected through either contaminated water or food, and are in closer contact with other children in their neighbourhood. Further, confirmatory tests, such as the use of probe hybridisation will be done to check the specificity of these findings. Interestingly, the occurrence of mixed infections clearly indicates the potential for reassortment between rotavirus strains belonging to different genogroups. This has important implication concerning future strategies of rotavirus vaccination in Brazil.

During the study period (December 1982 to March 1986), the G1, "long" electropherotype accounted for 85% of serotyped strains. This is in contrast with more recent findings in Belém, Brazil (November 1992 to November 1994) showing that rotavirus serotype G2, "short" electropherotype has largely prevailed (80%) over the G1, "long" electropherotype strains (13). Thus, these findings support the concept that the dominant serotypes/electropherotypes change over time (4).

Two cases of reinfection were recorded during the present study. These sequential rotavirus infections involved homologous G (G1) and P (P[8] and P[4]) types, suggesting that homotypic protection may be incomplete. Successive infections caused by the same G-types have previously been detected in Belém (23,24), however, the present study provides the first evidence from Northern Brazil on the occurrence of reinfections involving the same P types. Although numerous longitudinal surveillance studies indicate that reinfection is in general associated with mild
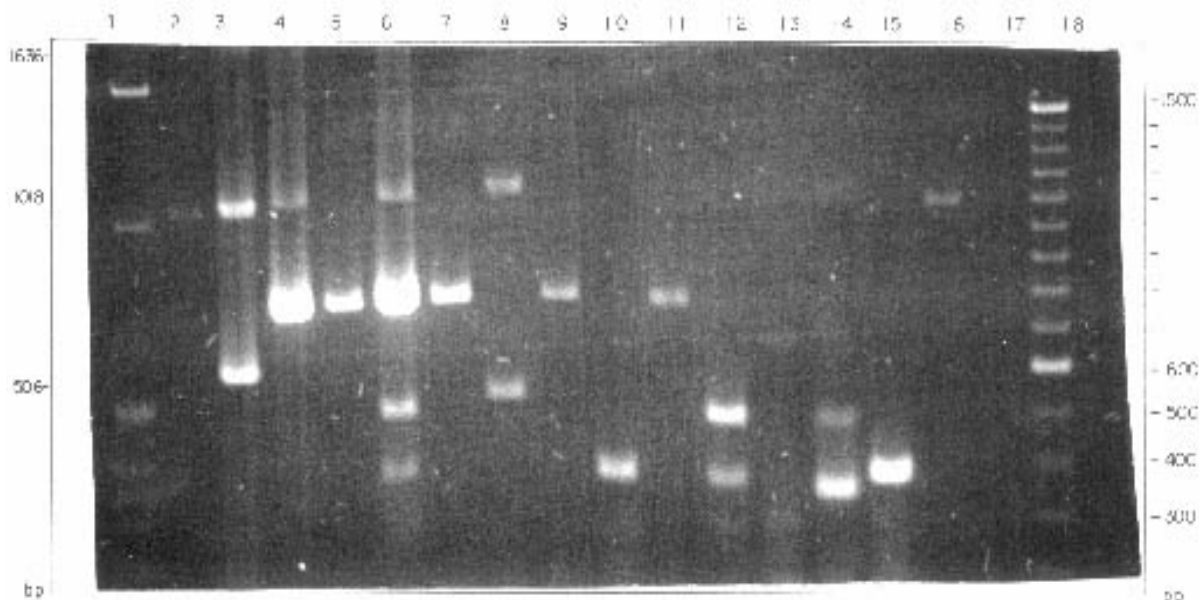
Fig. 2: G- and P-genotypes of rotavirus strains, as determined by RT-PCR. Lane 1: molecular weight markers (1 Kb ladder; Gibco BRL, Long Island, N.Y.); Lane 2: G-type (VP7 gene); Lanes 3 and 4: G4 and P[6] respectively (2 children shed rotaviruses with this G and P specificity), Lanes 5 and 6: G1 and P[8]+P[4]+P[6] respectively (2 children); Lanes 7 and 8: G1 and P[8] respectively (5 children); Lanes 9 and 10: G1 and P[4] respectively (3 children); Lanes 11 and 12: G1 and P[8]+P[4] respectively (4 children); Lanes 13 and 14: G2 and P[8]+P[4] respectively (1 child); Lane 15: AS11, control; Lane 16: P-type (VP4 gene); Lane 17: negative control; and Lane 18: 100-bp molecular weight markers (Gibco BRL).

symptoms (6,22), our data suggest that subsequent infections may give rise to an equally or even more severe illness on the second occasion. Also in contrast with our findings, some authors have claimed that severe symptoms during reinfection are associated with a P type differing from that responsible for the primary infection (4).

Our findings document the G- and P-types of rotavirus strains isolated from 1982 to 1986 and may not reflect the currently prevalent types in Belém, Brazil. To yield success in future rotavirus immunisation strategies in Brazil, it is strongly recommended that circulating G- and P-types rotavirus be routinely monitored throughout the country. This might be achieved, for example, through a strain surveillance including a hospital-based sentinel system in several regions of Brazil.

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LETTER TO THE EDITOR

A Simple Method for Measuring Caeco-colonic Transit Time in Mice

Sir,

Several methods have been described to measure the colonic transit time in animals and man. Many of these methods use labelled or unlabelled chemicals, beads and pellets. In methods using ^{51}Cr or charcoal, the animals have to be sacrificed after experimentation to determine the geometric centre -- point of maximum activity or concentration in order to measure the distance of transit (1,2). This is necessary because, while administered as a bolus the marker tends to spread over a segment of intestine. Barium studies are characterised by prolonged retention of the marker in the caecum and ascending colon (3), and the bead expulsion test is indicative of the distal colonic function only (4). Moreover, the methods employing labelled compounds require elaborate and costly equipment (5). We describe here a simple modification of the method of Yagi *et al.*(6) to determine caeco-colonic transit time in mice using olive oil instead of barium sulphate.

Albino mice weighing 25 to 30 g were used for the study. They were maintained in the laboratory with free access to water and food. Under nembutal anaesthesia, the abdomen was opened and a thin polyethylene tube (internal diameter 0.58 mm, external diameter 0.95 mm and length 6 cm) was inserted into the caecum and secured. The other end of the tube was passed subcutaneously and brought out through the skin at the nape of the neck. On the free end of the tube a cap was placed that could be removed for introduction of vehicles and drugs into the caecum. A collar on the distal end of the tube prevented its removal when animals tugged at the free end. All animals recovered well and passed normal stools within two days. They were used for experimentation after 3 days.

Different oils were introduced into the caecum in an effective volume of 0.2 mL by injection through the tube, making allowance for the dead space. The animals were kept in individual cages lined with filter paper. The time elapsed between the introduction of oil and the passage of oily stools was taken as the caeco-colonic transit time. The following drugs were generous gifts: loperamide hydrochloride from Citadel Fine Pharmaceuticals, Madras, India; indomethacin from Micro Labs Limited, Bangalore, India. The following drugs were purchased: atropine sulphate from Boehringer Ingelheim, Germany; anhydrous theophylline (Indian Pharmacopoeia) from Bakul Aromatics and Chemicals Limited, Bombay, India. Olive oil in the pure form was obtained from a local pharmacy. The polyethylene tubing, PE-50, used in this study, was acquired from Clay Adams, Parsippany, N.J., USA.

Preliminary experiments, using castor oil, coconut oil, sesame oil and olive oil, yielded similar caeco-colonic transit times ranging from 5 to 10 minutes. Further experimentation with olive oil resulted in a mean (SD) colonic transit time of 7.5 (0.21) minutes (n = 52). Olive oil was chosen because of its easy availability in a pure form from a local pharmacy.

Values obtained in the same animals after repeated administration at half-hourly intervals did not differ significantly. Figure 1 shows the dose-response relationships obtained with 4 compounds, each given by a different route of administration: atropine (intraperitoneally), theophylline (intravenously), loperamide (intracaecally), and indomethacin (orally). Computer graphs were obtained using the 'LOGISTIC' programme developed by Barlow (7). The Table gives the maximal responses and the median effective doses (ED50) derived by the computer programme. The four drugs used in these

Table I. Effect of various drugs on caeco-colonic transit time of olive oil

Drugs	Maximal response in minutes	ED 50 μmol/kg body wt.	ED 50 mg/kg body wt.
Atropine	71	0.4	0.3
Theophylline	98	26.4	4.8
Loperamide	230	4.3	2.2
Indomethacin	313	161.4	58.7

experiments considerably increased the caeco-colonic transit time. They all are known to act as costive agents, probably by relaxing gastrointestinal smooth muscle through different mechanisms of action (8). In our experiments, they prolong the caeco-colonic transit time in a dose-dependent manner. Our set-up is responsive to drugs administered by various routes. Because of its simplicity, this method can be used as a preliminary screening for compounds that are likely to slow caeco-colonic transit.
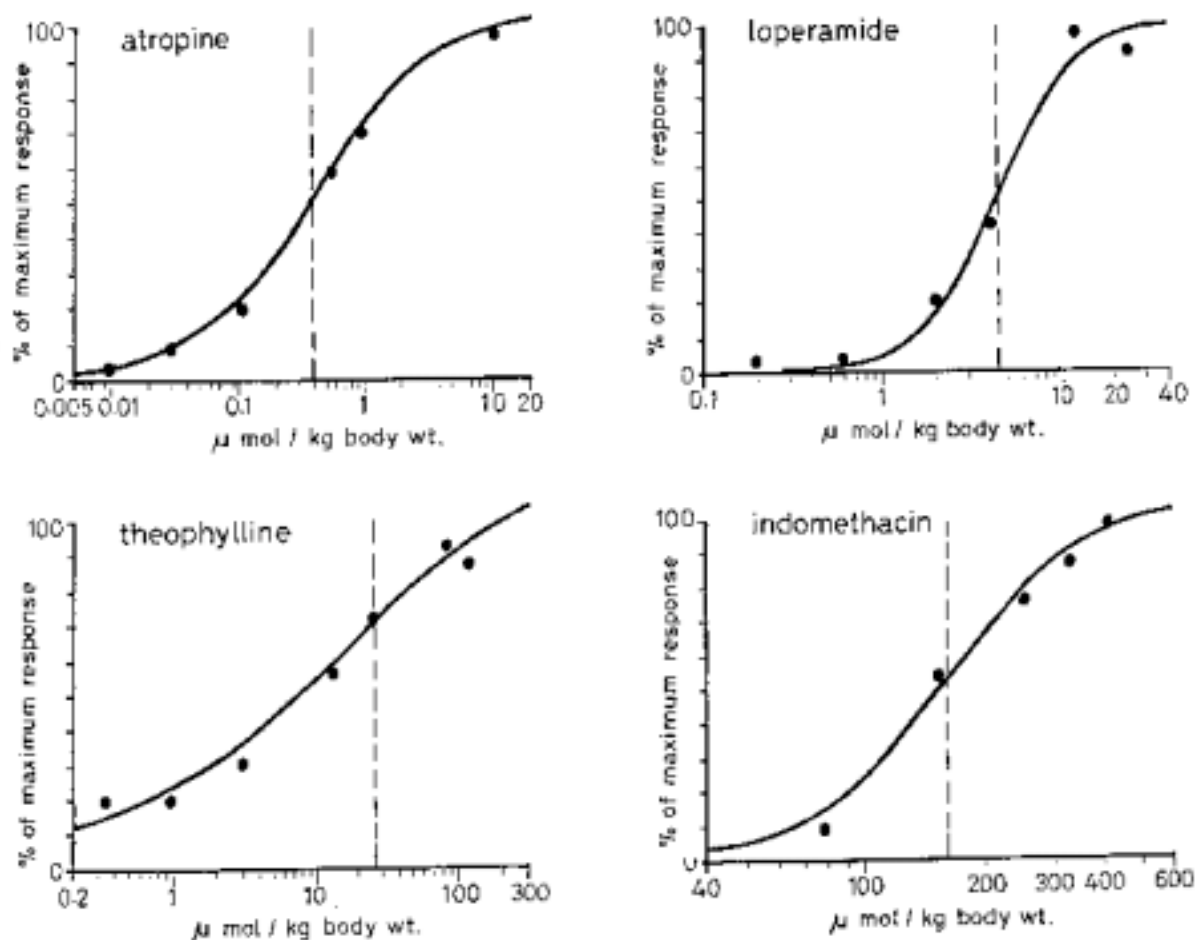
Fig. Dose-response relationships of four costive agents. The graphs represent the effects of the four following relaxants on caeco-colonic transit time in mice: atropine sulphate, (intraperitoneally, half an hour before experimentation), theophylline (intravenously, 10 minutes before experimentation), loperamide (intracaecally, half an hour before experimentation), and indomethacin (orally, one hour before experimentation). Each point represents the mean of the values obtained in 3 to 4 mice. The X axis shows the logarithm of the dose in micromoles per kilogram body weight; the Y axis shows percentages of the maximum response.

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Introduction

The Centre organizes a scientific conference every year to disseminate its research findings and share knowledge with several hundred researchers, health professionals, policy-makers, and communications experts from all over the world.

A recent change in the format of ASCON is the invitation to non-ICDDR,B researchers and policy-makers to present the results of their work. The participants interact on the issues of national and international importance to set future agenda for health and population research.

The Seventh Annual Scientific Conference (ASCON) adopted two themes: (i) Nutrition, and (ii) Emerging and Re-emerging Infectious Diseases. These themes were chosen because of their immediate relevance to child health and maternal health. A key aim of this year's Conference was to serve as an occasion to further explore some of the findings presented and to create an impetus for future research on nutrition and emerging and re-emerging infectious diseases.

Nutrition research has been a priority of ICDDR,B since its inception. Addressing nutrition issues is central to the mission of ICDDR,B and is a primary concern in Bangladesh which has a wide prevalence of nutritional deficiencies. Deficiencies of macronutrients and of micronutrients have an enormous impact on health and productivity due to primary effects and their effects as critical co-factors in gastrointestinal and other infections, low birth-weight, and psychomotor and cognitive development, among others. Questions relating to malnutrition, the nutritional status of children and the impact of nutritional status on maternal health have motivated research at ICDDR,B in areas such as management of diarrhoeal diseases, micronutrient deficiencies, nutrient metabolism, and body composition. Nutrition research conducted at the Centre has evolved over the years to expand from divisional research protocols to the creation of the Nutrition Working Group, which includes participation from the four scientific divisions of ICDDR,B: Clinical Sciences Division, Laboratory Sciences Division, Public Health Sciences Division, and Health and Population Extension Division. More recently, nutrition research has broadened in scope through our participation in the Bangladesh Integrated Nutrition Project, a collaborative effort with the Government of Bangladesh.

Research on emerging and re-emerging infectious diseases has global relevance as we enter an era where we witness the worldwide re-emergence of diseases thought to be under control, such as tuberculosis and malaria. As we continue to struggle to control cholera, new and emerging infectious diseases such as HIV, hemorrhagic *E.cli* and others have been identified. The widespread and often inappropriate use of antibiotics has led to the emergence of drug-resistant strains of infectious diseases. Consequently, once manageable diseases such as bacterial dysentery, malaria, tuberculosis, and STDs, such as gonococcus are becoming more difficult to treat and in some cases deadly due to their resistance to traditional therapies.

Research at ICDDR,B on diarrhoeal diseases through laboratory sciences, clinical studies and community-based trials is recognized globally. More recently, our research in infectious diseases has expanded in the important areas of reproductive health and acute respiratory infections. Our expanded capacity to conduct research on these health issues critical to Bangladesh and global populations in general, is due to the recent establishment of our STI/RTI laboratories, and the upgrading of our laboratories and diagnostic facilities designed to conduct necessary research on antimicrobial-resistant pathogens. The strength of our hospital surveillance system, overall epidemiological expertise, and the important inclusion of social and behavioural science in conducting field research enables us to identify disease patterns and prevalence among populations and create strategies designed to educate people and to implement preventive strategies designed to achieve sustainable results.

This year colleagues from the Government of Bangladesh, the research community, including NGOs, participated in the 7th ASCON. We continue the practice of opening ASCON to outside presenters. This underscores the recognition of important work done throughout the research community in Bangladesh on both nutrition and emerging and re-emerging infectious diseases. It also provided an opportunity and a forum for interaction with our colleagues and collaborators and to learn more about the research conducted in Bangladesh in these two important fields. We believe many of the results presented here will be shown to have implications for health policy and programmes in Bangladesh as well as for the global community. We would here like to also express our gratitude to UNICEF, WHO, Helen Keller International, and USAID/Washington for their assistance in co-sponsoring this event.

Prof. George Fuchs, MD
Chairperson, Organizing Committee
7th Annual Scientific Conference (ASCON)
and Interim Director, ICDDR,B

Public Health Nutrition

Birth Weight and Its Association with Maternal Nutrition and Socioeconomic Variables in Rural Bangladesh

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Objective: Examine the distribution of birth weight and its relationship with maternal nutrition and major socioeconomic variables.

Methodology: Between October 1995 and November 1996 at Matlab, 675 women, at 5-7 months' gestation period, were enrolled in a controlled diatary supplement intervention. Half of the women (n=341) received daily dietary supplement of 20 g soybean oil. Maternal nutrition status (weight, height, and MUAC) and socioeconomic variables (maternal education, parity, family size, household income, and land ownership) were measured at baseline, and infants' birth weights were taken within seven days of delivery. Gestational age was calculated from the last menstrual period. Analyses were conducted on 564 (83%) mother-infant pairs with complete data.

Results: Preliminary results showed no significant association between dietary supplement and mean birth weight or low birth weight. Mean (SD) birth weight for the entire sample was 2,520 (440) g, and the incidence of low birth weight was 47%. Maternal weight in mid- or late pregnancy, non-primiparity, and larger family size (>4) had significant positive association with birth weight. While short stature and primiparity were found to have significant negative association with low birth weight.

Conclusion: The findings suggest that small amount of calorie supplementation during pregnancy has no effect on birth weight in the study population. These results also suggest that poor maternal nutrition status, but not the socioeconomic status, is the major determinant of birth weight in rural Bangladesh.

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Determinants of Infant Growth in the Slums of Dhaka City: Size and Maturity at Birth and Breast-feeding

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Objective: Investigate the effect of low birth weight (LBW), intrauterine growth retardation (IUGR), prematurity, and breast-feeding on infant growth.

Methodology: The sample consisted of 1,654 infants born in selected slum areas of Dhaka city. They were enrolled at birth and followed up prospectively till their first birthday. Apart from repeated anthropometric measurements, the mothers were also interviewed for information on infant feeding and morbidity at each follow-up visit. Analytical techniques included correlation analysis and random effects regression for modelling infant growth.

Results: Correlation was high and stationary between repeated body weight measurements from 3 months onward. Correlation between weights before 3 months and later weights was lower and declined rapidly with increasing age gap, suggesting greater plasticity of growth in the first 3 months of life. After adjusting for other variables, the mean differences in body weight by birth weight, IUGR, and prematurity categories remained constant throughout infancy. For example, low- and normal-birth-weight infants differed by 556-603 g, while the differences between symmetric and asymmetric IUGR babies were 172-184 g. A positive impact of exclusive breast-feeding in the first 3-5 months on infant growth was detectable at 12 months of age (+95g). The overall growth in this sample was of the pattern that heavier babies grew even heavier. However, exclusive breast-feeding appeared to counteract this pattern by equally benefitting the lighter and heavier infants.

Conclusion: The study has demonstrated the important role of weight at birth and appropriate breast-feeding practices in determining nutritional status in infancy. Effective strategies for improving birth weight, till now a poorly-addressed issue in Bangladesh, are urgently needed. The sustained effect on growth and the beneficial effect on LBW infants are compelling reasons for increased and effective promotion of exclusive breast-feeding in early infancy.

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Mineral Status in Relation to Rickets in Chakaria, Bangladesh

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Objective: Explore the aetiology of rickets in Chakaria and identify opportunities within the local food system to prevent the disease. The rickets prevalent among children of the Chakaria region of Bangladesh is not usually associated with vitamin D deficiency. Therefore, Ca-deficiency would appear to be at least a predisposing factor in its aetiology. That rickets has emerged as a public health problem in Chakaria within the last two decades suggests that changes in food habits and/or environmental exposures may have contributed to the disease either by reducing Ca intakes (e.g. reduced access to Ca-rich foods) or use (e.g. increased exposure to such Ca-antagonistic factors as Al, Pb, Cd, F, Sr, Ba, low P, low-B). The Chakarian food system has indeed changed during this time: winter rice (requiring irrigation during the dry season) has been introduced; shrimp production in flooded paddy fields has increased; deep tubewells have been drilled to provide potable water.

Methodology: Mineral analyses were done on samples of whole blood and foods collected from the Chakaria region in October 1997. Blood was obtained from children aged 36-98 months identified by their families as either rachitic (n=11) or unaffected (n=8), who were each given physical and radiographic examinations (results reported separately). Samples of drinking water from tubewell, cooking water (pond), and cooked and uncooked rice were collected from three households, one of which had rachitic children. Samples of other foods likely to be sources of Ca and other limiting nutrients (mungbean, grasspea, chickpea, Indian chickpea, cowpea, lentil, black gram, amaranth, red chillies, taro, a sea-fish, churie, shrimp, and faishya) were purchased from the market at Chakaria. Water pH was measured at the point of sampling; samples were held frozen (blood) or at ambient temperature (water), or dried (food) prior to analysis. Samples were digested with nitric-perchloric acids and analyzed for 20 elements (Pb, Cd, Cu, Zn, Co, P, K, Na, Mg, Fe, B, Mo, Ni, V, As, U, Cr, Al, Sr, and Ba) by inductively coupled plasma emission spectrometry.

Results: The results of the study showed blood mineral values for rickets cases and controls to be similar with the exception of P (serum: cases, 43 mg/L vs. control, 52 mg/L, p>.05; whole blood: cases, 216 mg/L vs. control, 235 mg/L, p>.05). All values in both pond and well water samples were within normal limits. All elements in the rice samples were within safe limits reported for plant foods; rice was very low in Ca (86 mg/kg as eaten). All elements in the local foods were within the normal ranges reported for these elements with two notable exceptions: amaranth and shrimp, both containing high concentrations of almost all elements (amaranth, mg/kg dry weight: Ca, 26,947; Al, 1455; Pb, 1.5; Sr, 129; Ba, 32; Cr, 9.8; V, 3; As, 0.2; shrimp, mg/kg dry weight: Ca, 37,278; Al, 209; Pb, 0.3; Sr, 322; Ba, 34; V, 0.5; As, 4.3).

Conclusion: The results do not indicate wide exposure to antagonists of Ca use, but point to a food supply generally low in Ca.

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Consequences of Low Birth Weight on Infant Growth, Development, and Morbidity

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Objective: Observe the consequences of low birth weight (LBW) on growth, development, and morbidity of infants, and compare the feeding practices between infants with normal and low birth weight.

Methodology: BRAC and the London School of hygiene & Tropical Medicine jointly undertook a research project on maternal morbidity in three unions of Manikganj district (1991-1993). The present investigation forms a part of that study and was carried out in the same setting during 1993-1994. Six hundred fifty pregnant women were registered in their last trimester of pregnancy. Birth weights of 644 infants were taken within 48 hours of delivery. They received monthly follow-up visit up to one year of age. During each visit, information was collected using a structured questionnaire.

Results: Although the LBW infants showed catch-up growth (z-score>-2) at 1-6 month(s) of age, their growth started to falter earlier than the infants with normal birth weight. Within the same birth weight group, the growth of the male infants faltered more than the female infants. Factors, such as maternal weight, father's literacy, birth weight, floor condition of living room, absence of illness of infants, had a significant association with the total weight gain at the 12th month of age. A very small proportion of the LBW infants could cross each of the selected development milestones, such as head-holding, sitting, crawling, teething , walking, etc. Acute respiratory infection (ARI) and diarrhoea were the major causes of mortality and morbidity in both the groups. Seventy-three percent of the 34 infants who died before 12 months of age were the LBW babies. The mean episodes per year of the three major illnesses among the infants were: ARI 4, diarrhoea 2, and skin infection 1. The mean chest circumference and the mean body weight of the infants with LBW were lower than their counterparts at 1-12 month(s), but the differences were highly significant at 7th and 8th month. A significant higher proportion of the LBW infants received early supplementation compared to the normal infants which seems to be a dangerous practice.

Conclusion: All members of the community should be educated on the consequences of LBW and its prevention as well as proper care of the infants with LBW.

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Effect of BRAC's Rural Development Programme on Calorie Consumption

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Objective: Explore the level of calorie consumption, and determine the effect of BRAC membership on calorie consumption of the programme participants.

Methodology: The analysis was based on the data on food intake by 2,061 households of the first round survey of the BRAC-ICDDR,B Joint Research Project in Matlab. Data were collected during April-August 1995 using a four-cell study design. Socioeconomic and food consumption data were collected through home visits through a 7-day recall of major food items. Daily household calorie consumption was divided by adult food consumption unit to obtain per capita calorie consumption. Variables found significant through bivariate analysis were regressed against calorie consumption less than 1805 kcal/day to assess the relative effect of BRAC membership.

Results: Only 25% of the total households had adequate calorie intake (>2310 kcal/day). Per day calorie consumption was significantly higher among the BRAC member households compared to the BRAC non-members ($p < 0.05$). Calorie consumption was significantly associated with household size, household land holding, occupation, and literacy of the household head, per capita monthly food and non-food expenditure ($p < 0.001$). Controlling for those associated factors, the BRAC member households had 33% less possibilities to consume <1805 kcal/day compared to the non-member households ($p < 0.01$).

Conclusion: A vast rural population (75%) could not afford to meet the daily calorie requirement. It seems that an intervention, like that of BRAC, can have a significant impact in meeting the daily calorie requirement. In-depth analysis is needed to determine the pathways through which different BRAC inputs lead to an increased calorie consumption.

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Effect of Birth Weight, Intrauterine Growth Retardation and Prematurity on Infant Survival: a Prospective Study in the Slums of Dhaka City

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Objective: Study the effect of low birth weight (LBW), intrauterine growth retardation (IUGR), and prematurity on infant survival and on risk of deaths due to acute respiratory infections (ARI) and diarrhoea.

Methodology: A cohort of 1,677 infants born in a sample of slums in Dhaka city was enrolled and studied prospectively. One-hundred eighty deaths were reported to occur by 12 months of age. Cause of death was assigned based on data collected with a structured verbal autopsy questionnaire. Baseline data, and birth weights and lengths were measured at enrolment. Proportional hazards regression analysis was used for estimating the effect of the key explanatory variables while controlling for the effect of other variables.

Results: Overall, 21.7% of the deaths were due to ARI, 14.4% due to diarrhoea, and 5.6% due to both the causes. The proportional hazards regression analysis identified LBW, IUGR, and prematurity as important determinants of infant mortality. Preterm-IUGR infants were most at risk, especially for deaths due to ARI (RR=6.03). Diarrhoea deaths were 2.83 times more likely among the symmetric-IUGR infants compared to the non-IUGR infants. Compared to the normal-birth-weight infants, the LBW infants were at a greater risk of death due to all causes (RR=2.08), ARI (RR=2.52), and diarrhoea (RR=2.79). Although both prematurity and IUGR were associated with the increased risk of death, the timing of the effect varied with IUGR contributing to greater postneonatal mortality.

Conclusion: The results of the study show that size and maturity at birth are two important determinants of infant survival. The high prevalence of LBW and IUGR and the associated increased risk of deaths due to ARI and diarrhoea partially explain why these are still common causes of infant deaths in Bangladesh. Substantial improvements in infant survival, especially in the postneonatal period, can be expected with improved foetal growth and birth weight in this population.

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Comparison of Anthropometrical Indicators between Malnourished Children Admitted to a Nutrition Rehabilitation Unit and Their Counterparts in a Community

A de Francisco, J Chakraborty, and F Ahmed

Objective: Relate z-scores of malnourished children treated in a Nutrition Rehabilitation Unit (NRU) with that of their counterparts in a community.

Methodology: The Matlab MCH-FP Programme regularly measures mid-upper arm circumference (MUAC) to detect severe malnutrition in a community. An NRU was established to rehabilitate children detected as severely malnourished (MUAC <120 mm), and to involve mothers in the process. The z-score values (ANTHRO) of the admitted children were compared with those for their counterparts selected randomly in a community, using normal test comparisons for continuous variables.

Results: During a five-year period, 548 malnourished children aged less than five years from the intervention area were admitted for a mean (SD) of 19.1 (17.1) days. Admission was highly seasonal, particularly high during the pre-harvest period; 3.3% of the children were admitted twice during the study period; 32% of the admitted patients were infants, and 75% were aged less than two years. Mean weight gain was linear over time till the 5th week of admission when it reached a plateau. The weight-for-age and weight-for-height values, but not height-for-age, improved significantly among patients at the NRU. The admitted children were both stunted and wasted. Five hundred community children from a neighbouring non-intervention area were significantly less stunted and wasted than those admitted in the NRU. The malnourished comparison children had a higher weight-for-height score (z=-11.5, p<0.0001) and weight-for-age score (z=-9.5, p<0.0001) than those recorded for children from the intervention area when admitted to the NRU, and had also a significantly higher weight-for-age score than that recorded on children when discharged from the NRU (z=-4.8, p<0.001).

Conclusion: The results of the study showed that children rehabilitated in an NRU improved significantly in terms of their nutritional status, but their z-score values on discharge were still comparable to those of the malnourished children living in the community. Community-based rehabilitation programmes may be required to continue caring for the still fragile children at risk of morbidity and mortality.

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Birth Weight in Rural Bangladesh

Sadia A Chowdhury and Zeba Mahmud

Objective: Study the feasibility of recording birth weight in a rural community, and examine factors associated with the patterns of birth weight.

Methodology: Data were collected from a population of 60,000 throughout Bangladesh over a 3-year period, where BRAC provides health and nutrition education. In this study, 2,516 village women who had given birth to singleton liveborn infants were identified, registered, and followed up through their pregnancy. Information on their age, parity, occupation, education, receipt of antenatal care (ANC) services, etc. was obtained using a structured questionnaire administered by the members of the BRAC staff. The birth weights of their infants were recorded within 72 hours of birth. Both bivariate and multivariate analyses were performed to determine the risk factors of low birth weight (LBW). Odd ratios were calculated in each type of analysis to estimate the risk of an individual mother having a low-birth-weight infant.

Results: The mean birth weight (MBW) was 2601±409 g, and 22% of the infants were born with LBW. There was an increase in the percentage of infants with normal birth weight (i.e. >2.5 kg) from 64% to 80% during the study period. The MBW for the female infants was 2,559 g with 25% LBW, while the male infant had the MBW of 2,638 g and 20% LBW. The first-born infants had the highest LBW rate (32%) and weighed 200 g less than the infants with >3 birth order. Women aged less than 18 years were twice as likely to have LBW infants compared to the women aged 18-34 years. The risk of delivering an LBW infant decreased as the number of ANC visits increased. Maternal education and socioeconomic status were found to be inconsistent risk factors for LBW.

Conclusion: Multiple factors influence birth weight. Therefore, there is a need to address mothers' age, parity, non-formal education on nutrition and care during pregnancy to decrease the number of LBW infants. These issues should be addressed in all programmes for the improvement of maternal and child health.

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Community-based Nutrition Pilot Initiative of BRAC

Sadia A Chowdhury, Emily W Counts, and Zeba Mahmud

Objective: Ascertain the feasibility of implementing a community-based nutrition programme aimed at reducing endemic malnutrition in mothers and in their children aged less than two years.

Methodology: Since 1993, BRAC has been operating a community-based nutrition project in Muktagacha thana of Mymensingh district. In the programme area, each village is served by a full-time BRAC health functionary. As part of the programme, children aged less than two years receive monthly growth monitoring. Those identified with growth faltering or are severely malnourished are included in the supplementary feeding demonstration. At the feeding centre, the mothers also receive nutrition education from the community health workers regarding child feeding and caring practices. The food supplement is made from a mix of rice powder, pulse powder, molasses, and oil and is given for 90 days.

Results: 72% of the children in the programme exited with a weight gain greater than 500 g in 1993, which increased to 87% by the end of 1995. Although the exiting criterion was only a gain of 500 g, the mean weight gain was 700 g in 1993 and increased to 900 g in 1995. Males and females exhibited the same level of weight gain and success in exiting from the feeding programme. However, based on z-scores, girls routinely entered the programme with slightly better nutritional status. Children aged 13-18 months entered with the poorest nutritional status, but as to be expected, were benefited the most.

Conclusion: There has been a positive impact on the nutritional status of the children by providing nutrition education through feeding demonstration by the community health workers.

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Comparison of Nutritional Status among Pre-school Children Living in Rural, Slum and Urban Dhaka

Lynnda Kiess

Objective: Describe and compare the nutritional situation among pre-school children living in rural, urban and urban slum areas in Bangladesh.

Methodology: The Nutrition Surveillance Project conducts nutrition and health surveillance in partnership with NGOs in 4 urban slum working areas of Dhaka, Chittagong and Khulna and 41 sites throughout rural Bangladesh. In December 1996, the Helen Keller International (HKI) and the International Centre for Diarrhoeal Disease Research, Bangladesh collaborated on a cross-sectional health and nutrition study in Lalbagh, a non-slum area in Dhaka. Data from the December round of the NSP for slum areas (n=1,788) and the rural sites (n=16,140) were used with the non-slum site (n=1,392). Similar instruments and methodology were employed in all three areas. Descriptive analysis was undertaken to explore the differences in household socioeconomic, food expenditure and consumption patterns, and health and nutritional status among the pre-school children aged 6-59 months living in these areas. Logistic regression was used for estimating the odds of being malnourished. Various risk factors and the relative strength of the risk factors for malnutrition were also measured for the three different environments.

Results: The prevalence of stunting (%<-2 z-score) among the pre-school children was higher in the urban slums, followed by the rural and urban non-slum areas (66.2%, 61.1%, and 52.5%) respectively. Analysis of the household socioeconomic situation showed that there was also great disparity within each of these areas. Comparing nutritional status among the children from vulnerable households (landless and/or where the main earner is a casual worker) by area revealed the severity of underweight in the urban slums: 71.0% vs. 61.9% in the urban non-slums vs. 64.8% in the rural areas. Diarrhoea, vulnerability, and slum habitat were significantly associated with increased risk of being underweight.

Conclusion: Urban growth is currently estimated at over 9% per year in Bangladesh. While urbanization is associated with industrialization and economic development, it also results in an increase in slum and squatter settlements. As seen from this analysis, the pre-school children in the slum areas of Dhaka and other sites were at equal or greater risk of poor health and malnutrition than their counterparts in the rural and non-slum areas. While the malnutrition rates for children living in the areas are also high by international standards, the results of the study suggest that particular attention should be given to improve the situation for children living in the urban slums.

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Assessing the Impact of a Community-based Nutrition Project in Rural Bangladesh through Active Participation of Women

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Objective: Assess the impact of community-based nutrition activities of the Bangladesh Integrated Nutrition Project (BINP) in reducing the prevalence of malnutrition in children aged less than two years.

Methodology: The Bangladesh Integrated Nutrition Project (BINP) has been implemented in six rural thanas by the Government of Bangladesh since early 1996. Its main objective is to develop a national nutrition programme to bring about positive behavioural changes for the control of malnutrition among the children and women. Direct beneficiaries of the Project are children aged less than two years, pregnant and lactating mothers. Data for this study were collected through the monthly performance reports prepared at the Community Nutrition Centres (CNC) by the Community Nutrition Promoters (CNP) and were analyzed at the Project office. The study reports the progress of the community-based nutrition activities and the impact of the Project over an eight-month period since April 1997. Nine hundred seventy-two community nutrition centres were opened to bring about 60,000 children under regular monthly growth monitoring and promotion (GMP) to identify severely malnourished and growth-faltered children. Weights of the children were plotted on the growth-monitoring chart, and the nutritional status was determined by the CNP. Children having weight-for-age less than 60% of the median of the NCHS standard were considered severely malnourished as per above criteria and were targeted for educative/therapeutic supplementary feeding to improve their nutritional status.

Results: The GMP began with a coverage rate of 75.5% children in April 1997 and rose to 92.7% in November 1997. The severe malnutrition rate among the targeted children came down to 2.88% from 13.81% over an eight-month period. While the rate was compared with the data collected during the baseline survey at the beginning of the Project, it seemed that the severe malnutrition rate in the project thanas reduced significantly. The rate of severe malnutrition in the BINP thanas was also found remarkably low compared to the findings of the Child Nutrition Survey undertaken by the Bangladesh Bureau of Statistics (BBS) and the Bangladesh Demographic and Health Survey conducted in 1997.

Conclusion: Participation of the community people, especially women in interventions can reduce the prevalence of malnutrition in children within a short time.

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Aetiology of Anaemia in Bangladesh

Mohammad Mushtuq Husain[1] and SM Keramat Ali [2]

Objective: Find out the magnitude and aetiology of anaemia prevailing in Bangladesh.

Methodology: A sample of 7,764 people drawn from 14 villages of 14 districts was selected using the multistage cluster-sampling technique. Sociodemographic data were collected using an interview schedule. Blood samples were collected for Hb estimation by the cyanmethaemoglobin method. Anaemia was diagnosed as per WHO criteria. To find out the relationship between worm infestation and anaemia, stools of all subjects were examined in the field condition in saline and iodine preparation. To find out the relationship between food intake pattern and anaemia, the dietary intake pattern of the study population aged over two years was collected through 24-hour recall method and that of children aged less than two years was collected by interviewing mothers.

Results: Of the respondents, 44.4% were male, and 55.6% were female. Overall, 86.0% of the study population were detected to be anaemic [cut-off value: haemoglobin 12 g per 1 dl of blood]. Using WHO criteria, the prevalence of anaemia was found to be 66.5% in the male children and 71.3% in the female children [age group 0-5 years, cut-off value: Hb 11 mg/dl of blood for both sexes], 90.7% for boys and 90.6% for girls [age group 6-14 years, cut-off value: Hb 12 mg/dl of blood for both sexes], 89.1% for adult males [age group >14 years, cut-off value: Hb 13 mg/dl of blood] and 86.8% for adult females [age group >14 years, cut-off value: Hb 12 mg/dl of blood]. More females were moderately and mildly anaemic (7-11.9 g/dl). Males in comparison with their female counterparts in the same age groups had higher proportion with normal haemoglobin concentration of 12 g/dl or more. Most study subjects (76.5%) were moderately and mildly anaemic (Hb 7-11.99 g/dl). Of them, most anaemic people (59.6% of the total study population) had Hb 7-10.99 g/dl. The distribution of the prevalence of anaemia was almost the same all over the country. Anaemia was found to be positively associated with poor social status, larger family size, and socioeconomic status indicated by the type of house, the type of water supply, and possession of latrine. Anaemia was also found to be associated with poor nutritional status both for the children aged 0-120 months (z-score) and the adult population (BMI). Cooking food by water other than tubewell water was found risky for anaemia (odd ratio=1.67; c2=10.05; p<0.001). The latrine type or the mode of use (OR=1.25; c2=11.17; p<0.001) was associated with anaemia. The presence of anaemia was also related with the presence of intestinal parasites (OR=1.3; c2=15.61; p<0.0001). The presence of ova of AL was highly significant (OR=1.5; c2=15.61; p<0.0000001). The type of food (vegetarian and omnivorous population) had association (OR=1.5; c2=34.77; p<0.00000001) with anaemia. The children, who are not exclusively breastfed, but given any supplementary food, had relation (OR=3.45; c2=15.83; p<0001) with anaemia. Diagnosis of anaemia by clinical signs was not proved very sensitive. Using cut-off point for Hb of 10 mg/dl for anaemia, sensitivity and specificity were adequate. Morphological study showed that most anaemic subjects had iron deficiency.

Conclusion: In the study, anaemia was found to be associated with age, sex, social status, source of water, type of food, and infection. Anaemia, however, may be marked for nutritional, social and environmental factors rather than only these problems. It is, therefore, uncertain if the therapeutic treatment of anaemia alone can improve the situation.

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Micronutrients

Effects of Vitamin A and ß-carotene Supplementation to Lactating Mothers and Their Infants in Bangladesh

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Objective: Assess the efficacy of vitamin A and ß-carotene supplementation for the improvement of vitamin A status of women and their breastfed infants.

Methodology: Women delivering live infants in Matlab were randomized to receive either single-dose 200,000 IU of vitamin A at 2 weeks postpartum and daily placebos (n=74), daily dietary doses (n=73) of ß-carotene (7.6 mg=1 RDA) or daily placebos for 9 months postpartum (n=73). Breastmilk vitamin A concentrations were measured at baseline, 3, 6 and 9 months postpartum. Serum retinol concentrations and modified relative-dose response (MRDR) test ratios were measured in infants and a subsample of women.

Results: Vitamin A supplementation to lactating mothers improved the maternal vitamin A status and breastmilk concentrations at 3 months, but the effect was not sustained. Dietary ß-carotene significantly improved the maternal vitamin A status and breastmilk vitamin A concentrations only at 9 months. At 6 months postpartum, 25% of the women had low liver stores, and over 65% produced breastmilk with low concentrations of vitamin A. The status of 6-month old infants reflected the trend in breastmilk concentrations and was the highest in the vitamin A group, followed by the ß-carotene and placebo groups. Of the infants of the supplemented women, over 25% had serum retinol concentrations <0.70 mmol/L, and over 85% had low liver stores of vitamin A.

Conclusion: Both the interventions had beneficial effects on the maternal and infant vitamin A status. However, neither was sufficient to completely overcome the subclinical vitamin A status present in these women. Both vitamin A dose and dietary ß-carotene failed to build adequate vitamin A stores in their 6-month old infants. Mothers should be supplemented with 200,000 IU of retinol within 8 weeks postpartum, in line with the government recommendations. Additional ß-carotene supplementation may improve vitamin A levels in mothers and their infants.

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Subclinical Vitamin A Deficiency in Pre-school Children Living in Urban Slums of Dhaka City

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Objective: Assess the biochemical evidence of vitamin A deficiency in pre-school children living in the urban slums of Dhaka.

Methodology: To examine the separate and combined effects of low-dose ß-carotene supplementation and anti-helminthic therapy, 226 children aged 2-5 years from different slums in Mirpur thana were recruited for this study. All children were free from apparent illness, frank malnutrition, and sign of clinically evident vitamin A deficiency. Their serum ß-carotene and retinol levels were measured using high pressure liquid chromatography.

Results: The mean (range) serum ß-carotene and retinol levels were 5.67 (2.6-21.7) and 18.9 (5.6-37.3) mg/dl respectively. Fifty-seven percent of the children were vitamin A-deficient based on the serum retinol level (<20 mg/dl). About 20% of the children had no measurable ß-carotene in their serum. Neither serum retinol nor ß-carotene showed any relationship with age or nutritional status.

Conclusion: The results of the study indicate a high prevalence of vitamin A deficiency among the pre-school children in urban slums.

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Effect of Iron and Other Micronutrients on Haematological Indices and Nutritional Status of Urban Slum Children

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Objective: Compare the effect of iron and a micronutrient package (folic acid + vitamin C) on haematological indices and nutritional status of dewormed and non-dewormed children of Mirpur slum, Dhaka.

Methodology: Two hundred thirty-three anaemic (<11 g/dl) children aged 2-12 years were randomly assigned to 6 groups: (dewormed: A1, A2, and A3, and non-dewormed: B1, B2, and B3). A1 and B1 received ferrous fumerate, A2 and B2 micronutrients, and A3 and B3 placebo. Biweekly anthropometry and pre/post-interventional haematological profiles were determined examining 0.5 mL fingerprick blood. Outcome means were compared with the baseline data using the SPSS/PC+/anthropometry software.

Results: Children of A1 and B1 groups had an increased RBC count and higher Hb/PCV values [A1>A3 (p=0.000); B1>B2 (p=0.000), except Hb (p=0.07)] and B1>B3(p=0.000). Although no differences existed between A1 and A2 for RBC count (p=0.82), it was higher for Hb (p=0.000) and PCV (p=0.000) level. Children of both A1 and A2 had higher RBC count/Hb/PCV level than that of B2 [(p=0.000, except Hb (p=0.01)] and B3 [(p=0.000 for both)], but it did not differ from B1 [(p=0.23 and 0.27 for RBC), p=0.54 and 0.09 for Hb and p=0.26 and 0.006 for PCV respectively]. The RBC count was related with post-interventional weight and height gains (p=0.03 and 0.02 respectively). Although higher mid-upper arm circumference (MUAC) value was associated with Hb and PCV level (p=0.001 and 0.003 respectively, increased Hb and PCV values were associated with both dewormed (p=0.003 and 0.000 respectively) and non-dewormed iron-supplemented children (p=0.002 and 0.000 respectively).

Conclusion: Iron as supplement was better than micronutrients to boost up haematological indices, but had less impact on the nutritional status. Higher RBC count was associated with weight and height gains among the iron-supplemented children, while the Hb and PCV level was related to the higher MUAC values. Further study is needed.

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Determination of Zinc Status in Children Suffering from Acute Respiratory Tract Infection and ARI Associated with Severe Protein-Energy Malnutrition

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Objective: Determine zinc status in children suffering from acute respiratory tract infections (ARI), i.e. pneumonia and ARI associated with severe protein-energy malnutrition (PEM).

Methodology: A randomized case-control study was conducted on 137 children aged 6-60 months. Of them, 25 patients had ARI (pneumonia), 52 severe PEM (weight-for-age less than 60% of NCHS standard or the presence of nutritional oedema) without pneumonia or other significant clinical problems, and 25 patients severe PEM complicated by ARI. Thirty-five well-nourished children without significant clinical problem of the same age group were taken as control. Serum and corresponding hair zinc concentration were estimated by flame atomic absorption spectrophotometry. Results were expressed in ppm (particle per million).

Results: The results of the study showed that serum zinc was significantly lower (<0.05) in ARI ($1.26\pm.0.60$ ppm), PEM (1.31 ± 0.55 ppm), and in PEM complicated by ARI (0.90 ± 0.51 ppm) than control ($1.76\pm.98$ ppm). Serum zinc in ARI with PEM was also significantly lower ($p<0.05$) than ARI without PEM. In case of hair zinc, the control group (249 ± 154 ppm) had significantly higher zinc level ($p <0.05$) than the ARI group (158 ± 48 ppm), the PEM group (174 ± 75 ppm), and ARI with the PEM group (177 ± 70 ppm). However, unlike serum zinc, there was no significant difference between hair zinc in ARI, with that of ARI, and associated with severe PEM. In addition to bivariate analysis, multivariate linear logistic and regression model was used, which showed the significant negative association of serum zinc with ARI, PEM, and ARI with PEM, but hair zinc showing statistically insignificant (>0.05) negative association with ARI, PEM, and ARI with PEM.

Conclusion: The results of the study suggest that low zinc status is associated with ARI and PEM, and may further be decreased when ARI occurs in PEM. Interventions to improve zinc intake in children of developing countries, like Bangladesh, particularly to malnourished children will improve zinc status, and thereby may reduce ARI and PEM-related morbidity and mortality.

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Effectiveness of an Iron Supplementation Programme for Pregnant and Postpartum Women in Rural Bangladesh

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Objective: Compare the levels of anaemia in a population served by an iron supplementation programme with that in other rural populations in Bangladesh.

Methodology: Haemoglobin concentration was determined from a venous sample of blood using the HemoCue system for women during the postpartum period. Two hundred twenty women who delivered between June and August 1994 were examined: half of them at 2 weeks and 6 months after delivery, and the other half at 3 months and 9 months postpartum (mean of two values are presented). The Matlab MCH-FP intervention programme has been distributing iron/folic acid tablets to all pregnant and lactating women since 1974. Compliance was tested using a questionnaire. Laboratory results were compared with: (i) historical data: haematocrit levels taken in Matlab in 1975 from 2,445 women (converted to estimate haemoglobin levels by 0.33); and (ii) contemporary data: haemoglobin surveys conducted by the International Food Policy Research Institute (IFPRI) in 1996 from non-pregnant married women aged less than 50 years using the HemoCue method. Criteria for non-anaemia in non-pregnant women was 12 g/dl Hb.

Results: The mean Hb levels (95% confidence interval; percentage below 12.0 g/dl) were as follows: (1) Matlab 1994: 12.9 (12.7-13.1) g/dl (23%); (2) Matlab 1975: 11.7 (11.7-11.8) g/dl, (60%); (3) IFPRI Manikganj: 12.0 (11.8-12.1) (47%); (4) IFPRI Mymensingh: 11.3 (11.1-11.5), 59%; (5) IFPRI Jessore: 11.9 (11.8-12.1), 44%. There were no significant differences in height and weight among the study populations. The Matlab 1994 had a significantly higher proportion of women aged less than 30 years. Compliance with iron/folic acid in this population was very high. The iron/folic acid distribution programme may have had an impact varying between 47 and 61% of reduction in the prevalence of anaemia, with an increase of 0.9 to 2.1 g/dl Hb concentration.

Conclusion: The low postpartum anaemia levels may be associated with an intensive iron/folic acid supplementation programme in Matlab.

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Use of Nutritional Surveillance Project to Monitor Factors that Determine Vitamin A Capsule Distribution

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Objective: Describe the determinants of vitamin A capsule distribution during the national vitamin A (VA) week.

Methodology: In April 1990, the Helen Keller International (HKI) and several partner organizations set up a nutritional surveillance project (NSP) in rural areas and selected urban slums. Information has been collected bimonthly from 41 rural thanas and 4 urban slums on health, nutrition and socioeconomic status of approximately 18,000 children. Monitoring the vitamin A capsule (VAC) coverage (received and fed) is an important function of the NSP. In this analysis, data from round 44 of the NSP were used for assessing the coverage of the national VA week conducted in early June 1997. The sample consisted of 11,283 children aged 12-59 months.

Results: The overall VAC coverage in the rural area was 79.1%, but ranged by thana from 51.5% to 97.7%. Several reasons may have contributed to this wide range of coverage, including varying levels of organization, distribution, and the effects of the May 1991 cyclone. However, in most thanas, more than 75% of the children were reached based on the NSP finding. Preliminary analysis showed that there was no difference in the coverage due to gender or mother's education. However, a slight difference was observed for difference in the ownership of land. The NSP has also been successful in monitoring changes over time. One is the drop in coverage to 31% due to a VAC supply problem resulting from a change in donors supporting the programme. The second is the substantial increase to 87% in the rural VAC coverage following the linkage of capsule distribution to the second National Immunization Day (NID) campaign for polio eradication. The Government of Bangladesh has built on this success to continue with a centre-based national vitamin A week.

Conclusion: The centre-based distribution during the national vitamin A week is an effective method for VAC distribution. While the Government, through donor support, has sustained this effort for more than two years, there are still more children (15-25%) who are not being reached. There is need for an immediate action to reach these children as well as looking for more sustainable approaches to prevent vitamin A deficiency in Bangladesh.

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Zinc Supplementation During Pregnancy in Bangladeshi Women had no Effect on Birth Weight

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Objective: Study the effect of zinc supplementation during pregnancy on birth weight in Bangladeshi women.

Methodology: Observational studies suggest a positive association between zinc status during pregnancy and pregnancy outcome, but the results of controlled supplementation trials have been mixed. A prospective double-blind trial was conducted in a very poor urban Bangladesh community where low birth weight was highly prevalent. Five hundred and fifty-nine women were enrolled between 12 and 16 weeks gestation, stratified by parity and randomly assigned to two groups: 30 mg elemental zinc/day (n=269) or placebo (n=290). Supplementation continued until delivery, and compliance with supplementation was 86%. Serum zinc levels were estimated at baseline and at 7 months gestation, while anthropometrics and dietary intake of the women were assessed monthly and morbidity weekly. The newborns were measured by a physician within 72 hours after birth, and weights and gestational ages were known for 410 singleton infants. The overall incidence of low birth weight was 42.9% (45.9% in zinc-supplemented vs. 40.3% in placebo-supplemented; p=0.27). The incidence of prematurity by LMP was 21.2% (22.7% in zinc-supplemented vs. 19.9% in placebo-supplemented group; p=0.52). Means and distributions of birth weight, length at birth, and head circumference at birth were not significantly different between the two groups.

Conclusion: It is concluded that antenatal supplementation with daily 30 mg elemental zinc alone has no apparent impact on birth outcome in the study population.

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Infant Nutrition

Breastmilk Retinol Levels in Bangladeshi Mothers: Reflection on the Serum Retinol Level of Infants

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Objective: Examine the retinol levels in breastmilk and serum of 32 mothers and same from cord blood and serum of infants of urban middle class families.

Methodology: Colostrum and morning collection of full expression of breastmilk at 4, 12, and 20 weeks were obtained. Cord blood and infant's serum at 6, 14, and 22 weeks were taken. All samples were collected and stored at -20 ºC following standard procedures. These were analyzed by the high pressure liquid chromatography (HPLC) method. The fat content of breastmilk was estimated by creamatocrit method on the day of specimen collection.

Results: The retinol content of breastmilk (9%) and serum samples (17%) was below 10 µg/dl; the retinol content of all breastmilk (22%) and serum samples (59%) was below 20 µg/dl. Both median and mean retinol levels in mothers' milk were directly correlated with the amount of fat in the breastmilk samples. The median fat content of mothers' milk was 7% (range 3-20) with a lower median in the colostrum (6%). The colostrum retinol level was low (14.1 µg/dl±14.22); the breastmilk retinol level increased up to 12 weeks (61.9 µg/dl±43.72) and had a slight decrease at 20 weeks. The median fat and retinol contents were the lowest in the colostrum (7.2±2.4% and 14 µg/dl±14.2 respectively). The breastmilk retinol levels had a mean value of 72.67 µg/dl, 72.30 µg/dl, and 67.96 µg/dl at 4, 12, and 20 weeks' samples respectively. The median serum retinol level for cord blood samples was 16 µg/dl±2.7, and the values for the serum samples collected at 6,14, and 22 weeks were: 12.97 µg/dl, 21.5 µg/dl, and 19.79 µg/dl respectively.

Conclusion: Infant's serum appears to reflect only 20-40% of the consumed breastmilk in the previous two weeks. The results of the study indicate that maternal reserves of retinol must be improved to produce adequate vitamin A status in their infants.

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Initial Breast-feeding Practices of Urban Mothers Can be Influenced by Peer Counselling

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Objective: Assess the effect of peer counselling on early postpartum breast-feeding practices of urban mothers.

Methodology: Forty localities of similar size in Dhaka were randomized as intervention or control areas. From each of the 20 intervention areas, local women who had breastfed their babies and were motivated to help other mothers, were trained as peer counsellors. Three counselling sessions were provided (two visits before delivery in presence of influential family members, and one within 48 hours of delivery) to initiate early breast-feeding and to breastfeed exclusively for five months. Socioeconomic data and information on previous infant-feeding practices were collected in the last trimester of pregnancy by trained interviewers. On day 4, they collected post-delivery feeding practices.

Results: Mothers selected for the study (363 in each group) were of similar age and socioeconomic status. Significantly more mothers in the intervention group initiated breast-feeding within one hour (64% *vs*. 15%) and gave their babies colostrum as the first food (69% *vs*. 11%). Of the intervention mothers whose babies had received prelacteals, most reported that either the baby's grandmothers had administered the prelacteals contrary to their own wishes, or they had to accept the advice of local health facility staff and family members. In spite of these obstacles, on day 4, significantly more mothers were breastfeeding exclusively in the intervention group (84% *vs*. 30%).

Conclusion: Peer counsellors can improve early postpartum breast-feeding practices, but could be even more effective if health staff and family members do not give conflicting advice.

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Exclusive Breast-feeding Reduces ARI and Diarrhoea Deaths among Infants in Dhaka Slums

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Objective: Describe the breast-feeding practices and assess the effect of exclusive breast-feeding in early infancy on the risk of infant deaths, especially those due to acute respiratory infections (ARI) and diarrhoea.

Methodology: In a prospective study in the slums of Dhaka city, 1,677 infants were followed up from birth till 12 months of their age. Based on the baseline information at enrollment, the infants were visited 5 more times for anthropometric measurements and infant-feeding information. Verbal autopsy, based on a structured questionnaire, was used for assigning causes to the 180 reported deaths. Proportional hazards regression models were used for estimating the effect of breast-feeding practices, introduced as a time-varying variable, after accounting for other variables.

Results: The proportion of infants exclusively breastfed was only 6.2% at enrollment, increasing to 53.1% at 1 month and then gradually declining to 4.8% at 6 months of age. Predominant breast-feeding declined from 65.9% at enrollment to 4.1% at 12 months of age. Very few infants were not breastfed, while the proportion of partially breastfed infants increased with age. The breast-feeding practices did not differ between the low- and the normal birth-weight infants at any age. The overall infant mortality was 114 deaths per 1,000 live-births. Compared to exclusive breast-feeding in the first few months of life, partial or no breast-feeding was associated with 2.30-fold higher risk of infant deaths and 2.48- and 3.96-fold higher risk of deaths due to ARI and diarrhoea respectively.

Conclusion: The important role of appropriate breast-feeding practices in the survival of infants is clear from this analysis. The reduction in ARI deaths underscores the broad-based beneficial effect of exclusive breast-feeding beyond its role in reducing dietary contamination as evident here in the strong protection against deaths due to diarrhoea. Formulation of effective strategies for increasing the practice of exclusive breast-feeding in early infancy is recommended.

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BRAC's Rural Development Programme and Child Nutrition

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Objective: Explore the effect of the BRAC's rural credit programme on the nutritional status of children aged 6-72 months.

Methodology: Mid-upper arm circumference (MUAC) of 1,518 children aged 6-72 months were recorded from the first round survey of the BRAC-ICDDR,B Joint Research Project in Matlab. Data were collected during April-August 1995 using a four-cell study design. Measurements were taken using a TALC MUAC tape. Apart from intra-cell comparison, these children were also compared with similar data from a baseline survey done in 1992. Both bivariate and multivariate analyses of data were done.

Results: The prevalence of protein-energy malnutrition (PEM) was significantly lower among the children of the BRAC members compared to those of the non-members (p<0.01). During the pre-intervention period, the prevalence of severe PEM was found to be similar among these two groups. After three years of the BRAC intervention, it decreased from 15.4% to 8.7% among the children of the BRAC member households (p<0.05). However, among the non-member households, the prevalence remained unchanged (15.8%). In the bivariate analysis, children of the older members (25 months or more) and of the borrowers of the larger loan (Tk 7,500.00) were significantly better-off compared to other categories. However, after controlling for background indicators, sex and age of the children, year of schooling of the mothers, the number of living children, age of the mothers, and the per capita monthly expenditure were the significant predictors of nutritional status of children.

Conclusion: Three years of involvement in the BRAC's development activities are probably too a little time to have a significant measurable effect on the children's nutritional status. However, the results of the study showed a numerical trend in the improvement of the nutritional status of children of the BRAC member households.

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Effect of Lathyrus Protein Concentrate on the Growth of Children

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Objective: Study the effect of a protein concentrate from *khesari dal* (Lathyrus peas) on the growth of malnourished children.

Methodology: A random sample of 30 second-degree malnourished children aged 1-3 year(s) was selected from the Kawran Bazar Beltola slum area. The children were divided into: (a) experimental group: those who received Lathyrus protein concentrate, and (b) control group: those who received a similar quantity of a cereal food, *suji*. Each child from both the groups was given fixed and measured quantity of food and vitamin at regular intervals for three months. The growth of each child was monitored by anthropometric measurement before and at regular intervals during the feeding period. Signs and symptoms of allergy, gastrointestinal disturbances, and clinical examinations of eyes, lips, tongues, etc. were recorded. A questionnaire was used for collecting information on the economic conditions, family background, and environment of the children. Data were analyzed by statistical method.

Results: The children of both the groups were suffering from second-degree malnutrition as analyzed by weight-for-age or weight-for-height. At the beginning of the experiment, the average weight of children in the experimental group was 6.82 kg and that of the control group was 7.37 kg. After 15 weeks of feeding, the average weight of children in the experimental group increased to 8.3 kg (mean weight gain 1.23 kg) and that of the control group to 8.05 kg (mean weight gain 0.97 kg). It means that weight gain was 26.8% more in the children taking Lathyrus protein concentrate feed. These children showed better resistance to diarrhoea and fever as revealed by the morbidity data.

Conclusion: The results of the study provide an encouraging indication of using the protein of a popular and vastly grown pea (*khesari dal*) as an excellent nutritional supplement for the undernourished children. Mass production of this easily cultivable legume and processing it to extract the concentrate to use it as a supplementary feed can help eliminate protein-energy malnutrition of a vast section of the population.

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Growth of the Newborns in Their Early Life (Two Weeks) According to Feeding Pattern in Rural Area of Bangladesh

Zeba Mahmud and Sadia A Chowdhury

Objective: Scrutinize the growth of the newborns for two weeks concurring to their feeding pattern.

Methodology: Over one hundred pregnant women, randomly selected and registered from 50 villages of Trishal thana of Mymensingh district, were prospectively followed up. The birth weight, length, head and chest circumference of their newborns were recorded within 24 hours of birth. Weight was collected daily, length, head and chest circumference were recorded weekly for two weeks following birth and their feeding pattern, and colostrum intake and prelacteal food were recorded daily using a structured questionnaire.

Results: The records show the mean weight at birth was 2.6±0.3 kg, length 46.4±2.7 cm, head circumference 32.9±1.4 cm, and chest circumference 314±21 mm with the female child, being less than those of the male in all aspects. Mean weight loss of 62 g occurred within the first 24 hours, but was recovered within 3 days. All the babies were given colostrum within 72 hours, 90% within the first 24 hours of birth, and this 90% lost less (60±15 g vs. 76±12 g) weight (RR=1.53). Prelacteal food, primarily sugar water and pond/tubewell water, had been given to 69% of the neonates, and those who were not given prelacteal food lost less weight (56± 9 g vs. 65±17 g) in the first 24 hours. Although all the mothers breastfed their infants, exclusive breast-feeding was virtually non-existent even in the first 14 days of the child's life with the evidence of feeding other liquids. There was no evidence of bottle-feeding or of intake of solid food in the sample. There was an increase in the growth indicators (weight, length, head, and chest circumference) of these children and interestingly more so for the female babies.

Conclusion: The mothers are not aware of exclusive breast-feeding as prelacteal feeding exists, and water is not considered a carrier of bacteria. Creation of intensive awareness and social mobilization for increasing breast-feeding are required.

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Clinical Nutrition

Serum Zinc and Vitamin A Status of Malnourished Children Fed a High-protein Diet during Recovery

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Objective: Evaluate the effect of a high-protein diet on growth, serum proteins, and micronutrient status of malnourished children.

Methodology: Sixty-nine children were randomized to receive either a high-protein (HP) or a standard protein (SP) diet for three weeks in the metabolic study ward, and then followed up at home for six months. Body weight and height were measured, and concentrations of serum proteins, retinol, and zinc were also determined on admission at 3 weeks, and at 6 months.

Results: The mean±SD height increment was 5.3±1.0 cm in the HP group compared to 4.2±1.1 cm in the SP group ($p < 0.001$). The mean increment of serum protein concentrations (7.5 g/L vs. 2.9 g/L) and retinol (45 µg/L vs. 20 µg/L) was significantly higher in the HP group compared to the SP group at 3 weeks, but not at 6 months. Inspite of higher dietary zinc intake in the HP group than in the SP group (5.7 mg/kg.d vs. 2.9 mg/kg.d), the serum zinc concentrations were not significantly different between the two groups.

Conclusion: Diet rich in protein, energy, and micronutrients fed during the recovery period enhances the growth of malnourished children, but does not have sustained effects on protein and micronutrient status.

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Standardized Management Reduces Mortality among Severely Malnourished Children with Diarrhoea

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Objective: Reduce mortality and cost of treating severely malnourished children with diarrhoea by following a standardized management protocol.

Methodology: The mortality rates and the cost of treatment were compared between severely malnourished children aged 0-5 years, who received standardized management (standardized group, SG) and conventional management (comparison group, CG). Key points of SG are the standardized use of rehydration fluids with emphasis on oral rehydration solutions (ORS), slower rehydration, appropriate feeding, routine micronutrient supplementation, antibiotics therapy, and proper management of complications. Conventional management included rehydration within 3-4 hours, giving antibiotics only if there were obvious features of infection, delay in feeding until rehydrated, and supplementing with micronutrients only when indicated. The SG included children admitted to one of the three clinical units from 1 January to 30 June 1997. Children admitted to the same clinical unit from 1 January to 30 June 1996 (before the protocol was implemented) constituted the CG. Odds ratios with 95% confidence intervals were calculated for outcome variables, like mortality rates.

Results: Admission characteristics of children in the SG (n=334) and CG (n=293) were comparable except that more SG children had oedema, acidosis, and *Vibrio cholerae* isolated from stools. Sixty percent of the children in the SG were successfully rehydrated with ORS rather than intravenous fluids compared to 29% in the CG (p=0.00001). The use of expensive antibiotics was less in the SG (p=0.0001). The SG children had fewer episodes of hypoglycaemia (15 vs. 26, p=0.02). Costs of laboratory tests, intravenous fluids, and antibiotics were significantly less in the SG. Thirty children (9%) died in the SG, whereas 49 (17%) died in the CG (p=0.003; OR 0.49, 95% CI 0.3-0.8).

Conclusion: Standardized management resulted in a 47% reduction in mortality among the severely malnourished children with diarrhoea, fewer episodes of hypoglycaemia, and lesser use of intravenous fluids. The use of expensive antibiotics as well as the cost of laboratory tests and intravenous fluids were also minimized. The use of a standardized approach should be considered in the care of severely malnourished ill children.

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Nutritional Rickets without Vitamin D Deficiency in the Chakaria Region of Bangladesh

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Objective: Explore the aetiology and characterize the clinical aspects of rickets in children in Chakaria.

Methodology: Fourteen rachitic children and 13 children identified by subjects' families as unaffected were evaluated clinically and investigated.

Results: The age of rachitic children (9 males and 5 females) varied from 36 to 98 months (mean 69 months); their parents reported them being symptomatic of rickets since an average of 24 months of age (range 0-48). Rachitic deformities included knock-knees (10), bowed legs (4), and sabre tibia (3). Ten of the 14 affected children had active rickets as determined by serum alkaline phosphatase activities > 350 U/L; 7 of the 12 children subjected to x-rays had radiographic evidence of active rickets. The rachitic children had a mean alkaline phosphatase activity of 492 U/L (range 198-834; controls with mean 206 and range 138-331; $p<0.0001$), a mean serum calcium level of 9.6 mg/dl (range 8.5-10.4, not significantly different from controls), a mean serum phosphorous level of 4.3 mg/dl (range 1.9-5.6; controls with mean of 5.2, range 4.3-5.8; $p<0.003$), a mean serum 25-OH-vitamin D level of 20 ng/mL (range 7-65 with two subjects less than 14; controls: mean 25, range 16-35; $p<0.008$), and a mean serum 1,25-(OH)2 vitamin D level of 131 pg/mL (range 78-190; control mean 73, range 22-144, and 9 of the 13 subjects above the upper limit of "normal" $p<0.0005$). Of the 10 children with active rickets, only two had low vitamin D levels (one with hypophosphataemia), and one had hypophosphataemia; calcium deficiency was the most likely aetiology of the rickets in at least seven of the affected children. Interestingly, three "unaffected" children had physical findings consistent with rickets (each with beaded ribs, one also with widened wrists, another also with knock-knees) without elevated alkaline phosphatase activities.

Conclusion: The results of the study demonstrate that active rickets in Chakaria is not usually associated with vitamin D deficiency, and that the clinical presentation of rickets in Bangladeshi children is similar to that of African children with calcium-deficiency rickets. Furthermore, the findings of rachitic deformities and elevated serum 1,25(OH)2 vitamin D levels among "unaffected" children suggest that subclinical calcium deficiency might be much more prevalent than previously suspected.

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Effect of Zinc and Vitamin A Supplementation in Undernourished Children with Persistent Diarrhoea in Bangladesh

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Objective: Evaluate and compare the efficacy of vitamin A and/or zinc supplementation on the clinical course of persistent diarrhoea in Bangladeshi children.

Methodology: A double-blind randomized, controlled trial was carried out with 96 moderately malnourished children (60%-75% of wt./age of 50th centile of NCHS standard) aged 6 months to 3 years with diarrhoea for more than 14 days. Children were randomly allocated in 4 groups. Each group (n=24) received either zinc (20 mg elemental zinc in two divided doses per day), or vitamin A (total 200,000 IU in twice daily doses over 7 days) or both (zinc plus vitamin A in a multivitamin syrup); and the control group received only multivitamins (except vitamin A) equal to that of the treatment groups for 7 days. Clinical data were collected by a physician, and stool output, consistency, and frequency were recorded by using metabolic balance for 7 days.

Results: The baseline characteristics of the 4 study groups were comparable. The total diarrhoeal stool output among the 4 groups over 7 days was significantly different (p<0.02). Cumulative stool weight reduced significantly (p<0.0001) from day 2 to day 7 in the children receiving zinc compared to the control group and also significantly reduced (p<0.02) from day 5 to day 7 in the children receiving vitamin A compared to the control group. Differences between zinc and vitamin A or zinc vs. zinc plus vitamin A were not significant (p=0.5, p=0.7 respectively). The cumulative stool frequency significantly reduced from day 4 to day 7 in the zinc group compared to the control group (p<0.001). No significant differences were found between any other pairs. The net gain in the body weight over the 7-day study period was significant only in the children receiving zinc compared to the control group (+100 g vs. -40 g, p<0.04). The percentage of children who had clinical recovery (passage of soft stool was taken as recovery) within 7 days was significantly greater in the zinc group (86%) compared to the control group (48%, p=0.007) or vitamin A (48%, p=0.007), but not with zinc plus vitamin A (68%, p=0.14).

Conclusion: The results indicate that zinc supplementation in persistent diarrhoea reduces stool output along with frequency, and promotes earlier recovery; and strongly suggest that zinc supplementation is a standard management for undernourished children with persistent diarrhoea.

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Zinc and Rehabilitation from Severe Protein-Energy Malnutrition: Higher Dosage Regime Associated with Increased Mortality

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Objective: Investigate the catch-up growth of children in severe protein-energy malnutrition, with particular reference to linear growth and the effect of zinc supplementation.

Methodology: One hundred forty-one children aged 6 months to 3 years were recruited after admission to a Nutritional Rehabilitation Unit in the Dhaka Shishu Hospital. They were randomized to receive either per oral 1.5 mg/kg for 15 days, 6 mg/kg for 15 days, or 6 mg/kg for 30 days of elemental zinc and followed up for 90 days.

Results: Anthropometric outcome variables included change in height-for-age (z-score), change in knee/heel length as measured by knemometer, change in weight-for-age and weight-for-height (z scores) and change in mid-upper arm circumference (MUAC), as well as change in skin-fold subscapular/triceps thickness. No anthropometric variable indicated significant improvement with increased zinc dosage, and in addition, mortality rates increased significantly in those exposed to high-dose zinc (6 mg/kg) as opposed to those exposed to low-dose zinc supplementation (1.5 mg/kg) with a Yates-corrected chi-square p value of 0.033 and a risk ratio of 4.55 (95% confidence limits: 1.09 <RR<18.8).

Conclusion: The study suggests that there is no extra benefit of higher-dose zinc supplementation. On the contrary, it seems to contribute to increased mortality in the severely malnourished children.

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Patterns of Infection in Severe Protein-Energy Malnutrition and their Effect on Recovery

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Objectives: Detect the incidence of infections and infestations among the severely malnourished children, and compare it with the age-matched control group admitted to the general wards for other reasons without any obvious signs of malnutrition.

Methodology: This prospective study was carried out on 50 severely malnourished children and 50 age-matched control with good nutritional status at the Nutrition Rehabilitation Unit of the Dhaka Shishu Hospital from August 1995 to January 1996.

Results: Of the 50 patients, 22 (44%), 15 (30%), and 13 (26%) were suffering from marasmic-kwashiorkor, marasmus, and kwashiorkor respectively. Most patients were aged less than one year and belonged to poor socioeconomic status. Thirty-two percent of the fathers and 58% of the mothers were illiterate; 54% of the families used *kancha* latrines, and only a few of the parents washed their hands with soap and water after defecation. Poverty, illiteracy, crowded unhygienic living conditions, poor personal hygiene, lack of safe drinking water, and poor sanitation were found to be the principal socioeconomic determinants of malnutrition and infection which proved that factors which predispose the host to malnutrition also predispose to infection, thus establishing the vicious circle of infection-malnutrition-infection. The severely malnourished children showed a very high incidence of infection (74% vs. 44%), particularly diarrhoea, respiratory tract infections, bacterial skin infection, and urinary tract infections. Besides infections, there were many complications of infection, e.g. anaemia, dyselectrolytaemia, which simultaneously needed equal attention, like that of controlling infections. The average duration of hospital stay of patients with infections was mean $\pm$ SD 14.6 ($\pm$8.11) days. Patients in whom no infection was detected were discharged after an average hospital stay of 6.57 ($\pm$3.13) days.

Conclusion: This showed a prolongation of hospital stay for the severely malnourished children complicated by different sorts of infections.

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Nutrition Policy

Inconsistencies in the Findings of the Bangladesh Child Nutrition Surveys

Radheshyam Bairagi1 and Riti Ibrahim Ahsan2

Objective: Investigate the reasons of inconsistencies in the findings in the Bangladesh child nutrition surveys.

Methodology: Although the regular personnel of the Bangladesh Bureau of Statistics (BBS) collected data on anthropometry and age in the previous surveys, 1995-96 data were collected by the locally recruited workers employed for this survey only. Data on 100 children included in the 1995-96 survey were collected also by the BBS personnel independently. A comparison of the data from the two sources formed the basis of this study.

Results: The average difference between the two sources (bias) was found to be about 1.8 months in age and 0.13 cm in mid-upper arm circumference (MUAC). The differences in weight and height were negligible. However, random error was found to be substantial data on height.

Conclusion: Bias in age was thought to be mainly responsible for the inconsistencies in malnutrition in different nutrition surveys in Bangladesh. There is a need to develop a tool for collecting data on age accurately, on the basis of a study in a demographic surveillance area where accurate data on age are available. It is also suggested that MUAC should be routinely collected in any nutrition survey until such a tool is fully developed. The importance of accuracy in w**eight and height data is emphasized.**

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Bangladesh National Plan of Action for Nutrition

MQ-K. Talukder

Objective: Discuss the principles of the Bangladesh Plan of Action for Nutrition. Bangladesh is one of the 159 countries which unanimously adopted a world declaration on nutrition at the International Conference on Nutrition (ICN) held in Rome in 1992. These nations pledged to prepare a National Plan of Action for Nutrition (NPAN) and to take appropriate actions for eliminating hunger and reducing all forms of malnutrition.

Methodology: The Ministry of Health and Family Welfare, Government of Bangladesh, constituted a National Working Committee and a subcommittee to prepare the NPAN. The Bangladesh National Nutrition Council was assigned to coordinate the task. Fifteen concerned ministries were involved in the preparation process. A series of meetings and workshops were held, and individual contributions were sought. The Food and Agricultural Organization (FAO) of the United Nations provided technical assistance and two national and international consultants.

Results: Five documents were prepared. The NPAN core document was developed on the basis of nine themes for nutrition action declared at the ICN. The NPAN contains a description of the existing nutrition situation in the country, defines the policy, objectives, and goals, outlines strategies, and provides the institutional framework for translating the plan into action. Various projects to be developed by the 15 ministries have been identified for implementation to combat malnutrition.

Conclusion: The NPAN has recently been adopted by the Government. Different programmes of the NPAN should help eradicate malnutrition by the year 2010

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National Food and Nutrition Policy: A Multisectoral Approach in Bangladesh

MA Mannan

Objective: Improve the nutritional status and the quality of life of the people, particularly the vulnerable groups.

Methodology: A draft national food and nutrition policy (FNP) was prepared by three experts taking into consideration the nutrition situation of the people of the country. It was presented in a workshop participated by policy-makers, sectoral stakeholders, and beneficiaries. The recommendations of the workshop were incorporated into the policy. It was finally modified based on the sectoral comments, and the policy dialogue initiated. The food and nutrition policy write-up was placed in the meeting of the Bangladesh National Nutrition Council (BNNC) and the Council of Ministers (Cabinet) for approval.

Results: The FNP, approved recently by both BNNC and the Cabinet, is intended to compliment the Government's other sectoral development policies. The strategy for effective implementation of the FNP is divided into four major sectors: (a) Food, agriculture, fisheries, livestock, and forestry for increased production, proper distribution, and food security; (b) Health, family welfare, and environment for primary health care, caring practices, disease control, sanitation, and hygiene; (c) Nutrition education and communication for the creation of awareness at different levels with formal and non-formal education; and (d) Community development and social welfare for poverty alleviation, income generation, and economic growth. To implement the various provisions of the FNP, a national plan of action for nutrition has been approved.

Conclusion: The FNP has the opportunity for its successful implementation since it has been developed through a multisectoral approach, and the stakeholders have the ownership. However, wide publicity at different levels, and strong coordination among the stakeholders during its implementation, monitoring and evaluation are required.

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Nutrition Initiative in Bangladesh: Is It a Blessing or Human Right?

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Objective: Review the National Food and Nutrition Policy (FNP) and the National Plan of Action for Nutrition (NPAN) to see if they would be able to meet the need of the country by the year 2010 through project like Bangladesh Integrated Nutrition Project (BINP).

Methodology: Secondary data were analyzed to review the nutrition initiatives taken in Bangladesh.

Results: Against all odds, the country has achieved some progress in various nutrition indicators. Stunting among children aged less than five years has reduced to 51.4% in 1995-1996 compared to 64.2% in 1992; coverage of vitamin A supplementation among children rose from 30% in 1980 to 84% in 1996; and consumption of iodized salt in the households increased to 67% in 1996 from 44% in 1995. All these marked major progresses in the selected indicators. Between 1993 and 1996, the average household consumption had increased at a rate of 8.2% annually. Despite these positive changes, malnutrition is still at an unacceptably high level. About 9 of the 10 children are malnourished in one way or the other; 70% of the pregnant women and children are anaemic; and death of about 650 children per day are attributed to malnutrition. Early childhood malnutrition, including anaemia and iodine deficiency disorder reduce learning ability and retard national efforts on education and development. The country, however, has made progress on several areas, viz. Cabinet's approval of the National Food and Nutrition Policy, finalization and approval of NPAN and launching the Bangladesh Integrated Nutrition Project (BINP). The FNP, NPAN, and BINP strive for achieving nutrition goals for Bangladesh. The FNP gives an overall policy, the NPAN provides a broad-based guideline of inter-sectoral nutrition plans, including activities under various ministries/sectors, and the BINP is the most active programme of the FNP and the NPAN.

Conclusion: Good nutritional status is the basic right of women and children. It is important that the issue of nutrition should be moved from blessing and welfare programme to the agenda of rights. Given the magnitude of the problems, immediate actions should be taken under the NPAN and FNP. The BINP activities should be expanded and enriched where necessary.

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Trends, Measures and Indicators of Food and Nutrition Security in Rural Bangladesh: Findings from the Nutritional Surveillance Project

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Objective: Describe the trends in nutrition and food security since 1990 with respect to market dependency, differences in production periods, lean and peak (seasonal) influences, and vulnerability.

Methodology: Data for this analysis came from the ongoing Nutritional Surveillance Project (NSP) of the Helen Keller International (HKI), Dhaka and the Institute of Public Health Nutrition (IPHN). Using a network of 19 partner organizations, the NSP monitors trends in nutritional status of children through bimonthly collection of data on nutrition, health, and socioeconomic and demographic variables from approximately 16,000 children and 14,000 households from 41 subdistricts throughout Bangladesh. 157,344 children were included in this analysis. Information on socioeconomic status, food consumption, and health status was collected using standardized questionnaire, and weights and heights were measured up to an accuracy of 100 g and 0.1 cm respectively.

Results: The study presents a comparative picture of the nutritional status of children in the 'vulnerable' group and 'non-vulnerable' group of households. The nutritional status of children (weight-for-age) improved in peak season. The gap between the two groups remained, and the magnitude of improvement was higher among the children from the non-vulnerable households. The nutritional status of children improved from Time 1 (T1) to T2 for both the groups with greater magnitude of improvement among the non-vulnerable households. Nutritional status deteriorated slightly from T2 to T3, with more deterioration among the vulnerable households. The nutritional status of children was significantly associated with lean season, vulnerability status, diarrhoea, gender and high rice-price period.

Conclusion: Children from the households that have land (non-vulnerable groups) benefited from the low rice-price period. These households consumed more of their own production and increased expenditure on other foods. Children from the vulnerable households also benefited in the low rice-price period. With relatively lower rice prices, these households purchased more rice from the market and had the opportunity to spend more on other foods. This increase in real income, due to the decline in rice prices, translates into the increased food availability. The increased food availability, evident by the increased per capita intake of rice and expenditure on other foods, is associated with declining levels of malnutrition.

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The Bangladesh Integrated Nutrition Project: The Secrets of Achievements

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Objective: Find out the association of information, education and communication (IEC) in relation to the outcome of intervention. The IEC activities have been identified as the heart of the community-based nutrition activities of the Bangladesh Integrated Nutrition Project (BINP), which was launched about two and a half years ago and is on the ground practically for little more than a year.

Methodology: This evaluation is based on both primary and secondary data collected from various sources and in various time-frames. The major sources of data were the monthly performance report, the fortnightly report prepared jointly by all stakeholders of the BINP, the monthly monitoring report prepared by the Ministry, IDA supervision Mission's report, IDA-UNICEF joint review Mission's report, the six-monthly progress report of the Project, field visits, interviews, and structured questionnaires. Data from all the primary and secondary sources were collected, analyzed and interpreted, but most data presented here are qualitative in nature.

Results: The knowledge, attitude and practice of supplementary feeding for the severely malnourished children were well known to all the mothers interviewed. The effect of supplementary feeding in reducing malnutrition was also known to them. It revealed that the role of IEC activities in reducing severe malnutrition was significant ($p<.001$). There was also a significant association between supplementary feeding of women and the outcome of pregnancy in terms of low birth weight and maternal mortality and knowledge. The IEC activities have changed the health and nutrition behaviour of the community with respect to growth monitoring and promotion, feeding of colostrum, infant feeding, caring practices of children and women, and nutritional status of the children aged less than two years in all the project thanas. All activities at the community were mutually supported and accentuated by the cooperation of both government (GO) and non-governmental organizations (NGO).

Conclusion: Effective IEC activities with the GO-NGO cooperation can result in positive nutrition behaviour, and improve the nutritional status of children and women of the community. Every activity to develop a nutrition vision in Bangladesh should have a strong IEC element and mutual support and cooperation of the local institutions, including NGO for longer sustainability.

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Women and Adolescent Nutrition

Association Between Anaemia and Socioeconomic Status among Non-pregnant Women in Rural Bangladesh

SM Ziauddin Hyder[1], Eva-Charlotte Ekström[1], AMR Chowdhury[2], and Lars-Åke Persson[2]

Objective: Determine the magnitude of social stratification of anaemia among the non-pregnant women in a rural area of Bangladesh. Current prevention and control programmes for anaemia have been limited in their effectiveness. A contributing factor for this limited effectiveness could be that there is a social stratification of anaemia among the women and that the programmes may not reach the strata of women with the highest prevalence of anaemia.

Methodology: The study was carried out in 12 villages of Fulbaria thana of Mymensingh district in March 1996. One hundred seventy-nine married healthy women aged 15-45 years were selected. Information on indicators of socioeconomic situation and haemoglobin concentration was collected through household visit. The socioeconomic indicators included household economic status, schooling of women, and land ownership. A socioeconomic score was developed where these three indicators were combined. Haemoglobin concentration was assessed on a fingerprick blood sample using a portable photometer and disposable cuvettes. Haemoglobin concentrations were categorized into normal (120+ g/L of haemoglobin), mild (100-<120 g/L), moderate (70-<100 g/L), and severe (<70 g/L).

Results: The overall prevalence of anaemia among the women was 73%. The prevalence of severe, moderate and mild anaemia was 1%, 21%, and 51% respectively. All the three indicators of socioeconomic situation were found to be associated with the prevalence of anaemia. Women without formal schooling had a prevalence of 78% compared to 68% for women with schooling (p<0.05). Women who lived in the households with less than 50 decimals of land had a prevalence of 79% compared to 63% if they had more land (p<0.05). Finally, women who perceived their economic situation as deficit had the prevalence of 83% compared to 68% among those who perceived it as non-deficit (p<0.05). The combined socioeconomic score was associated with anaemia in a stepwise manner, and the prevalence of anaemia increased with each additional negative socioeconomic indicator. Women exposed to all three negative aspects had a prevalence of 86% compared to 58% among those not exposed to any socioeconomic risk factors.

Conclusion: Anaemia is a highly prevalent health problem among women in rural Bangladesh. Although most women are affected, those from poor socioeconomic strata have the highest prevalence of anaemia. To improve the effectiveness of anaemia prevention and control programmes, it may prove to be of value to ensure that the programme coverage of women of the poorest socioeconomic strata is sufficiently high.

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Age at Menarche and Nutritional Status of Adolescent Girls in a Rural Area of Bangladesh

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Objective: Determine the age at menarche and its association with nutritional status of adolescent girls in a rural area of Bangladesh.

Methodology: A cross-sectional study was conducted in four villages of Rupganj thana of Narayanganj district. Data on 437 adolescent girls aged 10-17 years were collected during October-December 1996 using a pre-tested structured questionnaire, and the nutritional status was measured by weight, height, body mass index (BMI), mid-upper arm circumference (MUAC), and haemoglobin level.

Results: Of the 437 girls, 165 (37.8%) had commenced menarche. The mean age at menarche as determined by retrospective recall was 13 years (±0.89). The median age at menarche estimated by % menstruating girls in each age group was 13. The mean haemoglobin level was 9.5 g/dl; the prevalence of anaemia (Hb <11g/dl) was 90.2%; 42.6% of the girls had angular stomatitis, and 28.8% glossitis. Of the adolescents, 60.2% (n=263) were underweight (<5th centile, WHO-recommended reference) and 48.1% were stunted (<3rd centile, NCHS/WHO). The mean weight and BMI were significantly higher among the menstruating girls aged 13-15 years (p<0.01) than the non-menstruating girls. The mean height was found significantly higher among the menstruating girls aged 11-14 years (p<0.05).

Conclusion: The results of the study suggest that the mean age at menarche is 13 years in the rural area of Bangladesh studied. Adolescent girls aged 13-15 years who are heavier and taller than their age-peers are more likely to have commenced menstruation. Most adolescent girls were anaemic and undernourished. Hence, the adolescents require specific intervention to overcome their anaemia and improve their nutritional status for better reproductive health.

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Adolescent Nutrition in a Rural Community in Bangladesh

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Objective: Assess the nutritional status of adolescent boys and girls in a rural community of Bangladesh.

Methodology: Eight hundred three households, each containing at least one adolescent boy or girl, were selected consecutively from four purposively chosen villages in Rupganj thana of Narayanganj district. Initially, the guardians of the 1,483 healthy and unmarried adolescents aged 10-17 years (51% boys and 49% girls) were interviewed about family structure and socioeconomic status. Of these children, 906 (47% boys and 53% girls) from 597 households were weighed, had their height and mid-upper arm circumference (MUAC) measured and were clinically examined. Blood was then collected from 861 adolescents for haemoglobin estimation.

Results: The median monthly income per person in the 597 families was approximately Tk 554 (US$ 12). Of the household heads, 27% were labourers, 21% solvent farmers, 14 % ran small-sized businesses, and 6% unemployed. Although 86% of the 906 children were attending school, school attendance fell from 98% at 10 years of age to less than 63% at 17 years of age. Sixty-seven percent of these adolescents were thin (defined as BMI <5th centile of WHO-recommended reference). Seventy-five percent of the boys and 59% of the girls were thin. The prevalence of stunting (height-for-age <3rd centile) was 48% both for the girls and the boys. On clinical examination, angular stomatitis was found to be present in 46% of the adolescents; 27% had glossitis, 38% pallor, 11% dental caries, 3.2% an obviously enlarged thyroid, and 2.1% eye changes for vitamin A deficiency. According to INACG (International Nutritional Anaemia Consultative Group 1985) cut-off values for anaemia, 94% of the boys and 98% of the girls were anaemic.

Conclusion: Rural Bangladeshi adolescents suffer from high rates of malnutrition and almost universal anaemia, and boys are as malnourished and anaemic as girls.

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Knowledge, Attitude and Practice of Pregnant Women on Feeding Patterns in Bangladesh

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Objective: Study the beliefs and practices associated with diet during pregnancy to design appropriate educational strategies and to promote appropriate behavioural change.

Methodology: About 300 pregnant women of Muktagacha thana of Mymensingh district were selected for the study. A questionnaire was used for interviewing the pregnant women to obtain information regarding age at the time of pregnancy, parity, breast-feeding practices, antenatal care, taboos about food, household food habits, and food distribution. Twenty-four-hour dietary recall was used for obtaining information on daily dietary practices of the women.

Results: The mean age and parity of the women were 22±2 years and 3 respectively. The 24-hour food recall data reflected that there was very little variety in the diet, with rice being the primary component. Only 20% of the women had 3 or more serving of animal protein. Ninety-four percent reported having no yellow vegetables, 59% no fruit, and 65% no oil in their daily diets. Furthermore, 96% of the women were the last people to take food in the house. During pregnancy, 34% of the women were still breastfeeding their last child, even in their 3rd trimester. Of those who did not breastfeed, only 6% had stopped due to their pregnancy. Although only 3% of the women practised taboos about food, they avoided protein and calorie-rich foods, such as egg, hilsha, and mrigal fish. Conversely, 30% reported that the custom of consuming food, such as egg, milk, and green vegetables, was followed in their households. They mentioned a preference for dry cereals. An increased dietary intake during pregnancy, in terms of quantity, was reported by 65%, while 39% reported lower intakes.

Conclusion: To combat malnutrition, intersectoral programmes targeted toward poverty alleviation need to be undertaken. In addition, the whole family needs to be educated on the nutritional needs of pregnant women.

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Determinants of Haemoglobin Level during Pregnancy and Relationship with Pregnancy Outcome in Bangladeshi Urban Poor

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Objective: Investigate the determinants of haemoglobin (Hb) level during pregnancy and relationship with the pregnancy outcome in Bangladeshi urban poor.

Methodology: As part of a zinc supplementation trial, 559 women from Dhaka urban slums were enrolled between 12 and 16 weeks gestation. On enrollment, Hb and serum zinc levels were assessed. Anthropometric measurements (weight, height, and MUAC) were taken, and information was collected on reproductive history, socioeconomic status, and dietary intake, including the use of iron supplements. Women were prospectively followed up until delivery, and repeated Hb assays were performed at 7 months gestation.

Results: Mean Hb concentrations at enrollment and at 7 months gestation were 11.5±1.3 g/dl and 10.8±1.2 g/dl respectively (p<0.001) with 34.6% and 53.7% of the women classified as anaemic (Hb<11 g/dl) at baseline and at 7 months gestation respectively. Lower nutritional status (body mass index, MUAC, and serum zinc) and lower socioeconomic status were associated with lower Hb levels at baseline. The use of iron supplements was very low in this population: only 10 women (1.9%) reported to have taken iron tablets in the last 14 days, and no relationship between the use of iron tablets, and Hb levels was observed. The Hb levels at 4 months gestation were positively related to gestational length at birth as measured by LMP even after controlling for other possibly confounding variables. Birth weight, length at birth, infant chest-head and MUAC at birth were not related to the Hb levels at 4 months gestation. The Hb levels at 7 months gestation were not related to pregnancy outcome.

Conclusion: Anaemia in early pregnancy may be associated with a higher risk of prematurity based on LMP.

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Contraceptive Use and Maternal Weight among the Poor in Rural Bangladesh

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Objective: Examine if contraceptive method use is associated with maternal weight among the poor in rural Bangladesh, after controlling for the influence of demographic and socioeconomic confounding factors. Poor women in Bangladesh are believed to suffer from chronic malnutrition primarily because of low level of calorie as well as micronutrient consumptions. High fertility or repeated pregnancies and its associated resource-scarcity may also adversely affect maternal nutrition. Contraceptive use may prevent fertility-related maternal malnutrition. Moreover, the use of hormonal methods, like oral pills, may lead to weight gain which is recognized as a contraceptive side-effect in well-nourished populations. In Bangladesh, about 50% of the women use contraception, and over 20% of women use oral pills.

Methodology: The study includes 2,185 randomly selected married women of reproductive age from landless households in four rural thanas of Tangail and Mymensingh districts. Information on height, weight, perceived health, contraceptive use, and demographic and socioeconomic conditions was collected through a family-life survey conducted in 1993-1994. Body mass index (BMI) and perceived health were regressed on the type and use duration of contraceptive methods and control variables. Contraceptive methods were categorized as: pills, permanent methods, and condoms or traditional methods. Sixty-four injectables and 26 IUD cases were excluded from the analysis.

Results: The oral pill users had significantly higher BMI than the non-users. The BMI significantly increased with the duration of use of oral pills. Neither the use of permanent and other methods nor the durations of use of these methods were associated with BMI. The positive association between the use duration of oral pills and the BMI indicated that hormonal methods helped increase the poor women's weight. Women's perceived health was positively and significantly associated with the duration of pill use, but not with those of other methods meaning that weight gain was not a discomfort.

Conclusion: The prolonged use of oral pills seems to provide beneficial effect among the poor women. Further research is needed to better understand whether or not weight gain due to pill use provides nutritional benefit to poor women in Bangladesh.

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Impact of Lactational Performance on Calcium Metabolism through Bone Mass Density in Marginally Nourished Bangladeshi Women

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Objective: See the impact of breast-feeding behaviour/or pattern on bone mass density (BMD) in a group of Bangladeshi women.

Methodology: Four hundred Bangladeshi women aged 20-81 years were studied for their nutritional status, child birth events, breast-feeding behaviour, and effect of calcium metabolism ascertained by studying their BMD of distal end of radius and ulna during 1995-1996 in an urban postgraduate hospital in Dhaka. History of breast-feeding was ascertained, and reproductive performance records were taken. A bone densitometer –single-photon x-ray absorptiometry (SEXA) –was used for measuring the BMD.

Results: The mean age of the subjects was 41.9 ± 14.6 years, and the mean parity was 4.5 ± 2.9. The mean BMD of radius and ulna (g/cm2) was 0.42 ± 0.07. The results of the study showed that the BMD was negatively correlated with age of the subjects ($r=0.87$, $p<0.001$) and parity ($r=0.71$, $p<0.001$). The peak BMD was observed during the age of 25-39 years which declined after the age of 40 years. The BMD was negatively associated with the duration of total months of exclusive breast-feeding reported by the women ($r=0.42$, $p<0.00001$), the number of infants born ($r<0.38$, $p<0.00001$), the total number of months with lactational amenorrhoea ($r=0.25$, $p<0.01$), and the total months of partial breast-feeding of the infants born ($r=44$, $p<0.0001$). The mean BMD also reduced significantly with the increase in parity ($<p,0.001$), controlling for age, workload, body mass index, parity, and other lactational amenorrhoea (slope 6.8, $p<0.04$).

Conclusion: The results of the study suggest that the BMD of Bangladeshi women is negatively related with their prolonged breast-feeding pattern. The suggestion on policy implies that women's dietary adequacy in terms of the essential minerals is an important public health issue, and deserves attention to strengthen campaign for the protection and promotion of breast-feeding to improve child nutrition and survival.

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