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CHARACTERISTICS OF HUMAN MILK ANTIBODIES AND THEIR EFFECT IN RELATION TO THE EPIDEMIOLOGY OF BREASTFEEDING AND INFECTIONS IN A DEVELOPING COUNTRY

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INTRODUCTION

Most of us would believe that it is only in modern times that not all infants are exclusively breast-fed. However, even in ancient India and Europe[1] people were taught not to initiate breastfeeding at once, but to give other fluids and other materials, such as honey, that were likely to be contaminated. This is the custom still today in many traditional societies[2,3]. After breastfeeding has been started it is often incomplete ("partial breast-feeding"), but even then it may go on through the second year of life. There is information available that during previous centuries there was no breastfeeding at all in certain areas of Germany and Sweden. Infant mortality from diarrhea during the warm summer months in the early part of the 19th century was higher in such areas in northern Sweden than in adjacent regions where breastfeeding was the rule[4]. This presumably constitutes the first scientific evidence that breastfeeding can protect against infections.

Many studies claiming to demonstrate that breastfeeding protects against infection have been criticized for design and interference of confounding factors. However, other well controlled studies have been published which show protection from diarrheal disease[5,6,7,8,9], respiratory tract infections[8,9], and otitis media[11,12].

Many studies have demonstrated that most mothers in developing countries are breastfeeding, but little emphasis has been placed on determining the rate of exclusive breastfeeding, which is quite rare, with

partial breastfeeding dominating in many developing areas[2,13]. Feachem and Koblinsky[5] noted that there were striking differences in the diarrhea morbidity and mortality in relation to the type of feeding. Morbidity and mortality were lowest among those infants who were exclusively breast-fed, higher among those partially breast-fed, and highest among those not breast-fed at all.

BREASTFEEDING AND NEONATAL SEPSIS

The effect of breastfeeding on the occurrence of neonatal sepsis was recently studied in Lahore, Pakistan[14]. For each 42 consecutive cases of neonatal sepsis (age 3-28 days), 8 age-matched controls were sought from the same socio-economic circumstances, living in the same part of the city. We managed to locate between 4 and 8, or as a mean 6.4, such controls per case and thus the statistical analysis had to be made for an unmatched case-control study. Only one of the controls was exclusively breast-fed and the majority was partially breast-fed. However, many more cases than controls were not breast-fed. An odds ratio of 18 was found, strongly supporting the capacity even of partial breastfeeding to protect against neonatal sepsis in this poor community ($p<0.001$). Intense microbial exposure of infants in this community directly from birth onwards results in a gut flora with several potential pathogens[13]. These can be kept within the gut by partial breastfeeding once it starts 1-3 days after delivery[2].

Confounding factors may easily confuse the outcome of investigations of this kind but in the present study of neonatal sepsis the cases and controls were comparable as to age, sex, birth order, place and mode of delivery, hygiene of birth attendant, time between rupture of membranes and delivery, instruments used for delivery, and care of the cord, etc.[13].

THE MODE OF FEEDING IN A TYPICAL DEVELOPING AREA

In preliminary analyses of poor populations in Lahore, Pakistan, it became obvious that to properly describe the extent of breastfeeding it was necessary to take in regard a number of confounding factors[14]. Thus, the season of the year, the population group and the area of living were important. In a new prospective study, we have followed longitudinally 1,476 infants born into a population of 4,000 families, 1,000 from each of four groups living in different areas, a village outside Lahore, a mud hut area and the old city slum of Lahore, with an upper middle class group for comparison[16]. It was found that exclusive breastfeeding was rare, occurring initially only among 18% of the mothers in the village and 10% in the mud hut area after 1 month of lactation. Partial breastfeeding was predominant among the three poor groups whereas artificial feeding was most common in the upper middle class group. This was also the only group where commercial formulas were used to any major extent. Again, we noted the effect of the hot season. Mothers believe that their infants need extra fluid then. This can be seen as more children getting extra fluid during the months of April-September. In parallel, fewer get breast milk, presumably because they suck less, being less

thirsty given the extra fluid. These fluids may often be contaminated adding to the risk of gastrointestinal infections that are more common during the hot season.

Since exclusive breastfeeding was so rare, we decided to try to inform 300 mothers about the advantages of exclusive breastfeeding, showing, e.g., an educational puppet show on video in their home, together with oral information, using a flip chart, booklets and posters at altogether three visits during pregnancy[17]. The team from the project, together with the local traditional birth attendant who had also been specially trained, visited the mother regularly from delivery on for follow up.

The motivation was quite successful, e.g., increasing the rate of exclusive breastfeeding at 1 month after delivery about 40%, both in the village and in the urban slum, compared to the prospective longitudinal study where no special motivation was applied. Actually, the rate of partial breastfeeding also increased by about 40% at 1 month after delivery. The onset of breastfeeding was influenced as well, so that the percentage of neonates starting already at 24 hours of age had increased from 5 to 90% in the village and from about 15% to around 75% in the urban slum.

THE MODE OF FEEDING IN RELATION TO INFECTIONS IN THE LAHORE STUDY

In the longitudinal follow up of the 1,476 children in the four population groups, it was quite clear that breastfeeding protected against diarrheal disease[18]. This was obvious for all the population groups in the ages 1-23 months. Breastfeeding is here defined as partial or exclusive breastfeeding, the latter constituting a small group as mentioned above.

The degree of protection was evaluated by comparison of the prevalence of diarrhea among the breast-fed and the non-breast-fed. As can be seen in Fig. 1, the efficacy of protection was between 60-80% in the three poor groups and close to 40% in the control group among the youngest infants. Protection decreased after 9-12 months, remaining at 10-25% even at 24 months of age. The protection was seen year around both for the youngest (1-3 months) and the oldest age group tested (19-21 months) (Figs. 2 and 3). This is of special interest since during the warm months of April-September diarrhea is much more common and the infectious doses may also be higher. Still, breastfeeding, mainly partial breastfeeding, seems to protect just as well during the diarrhea season.

To try to determine if fluctuations unrelated to the termination of breastfeeding could have influenced the results, they were analyzed by comparing the rate of infections at monthly intervals 1, 2, and 3 months prior to and 1, 2, and 3 months after the termination of breastfeeding. Diarrhea became more prevalent after breastfeeding was stopped in all the population groups including the controls (Fig. 4), in the warm as well as the temperate season and in the different age groups. Such a change was not seen for upper and lower respiratory tract infections, but further analyses are required for these infections.

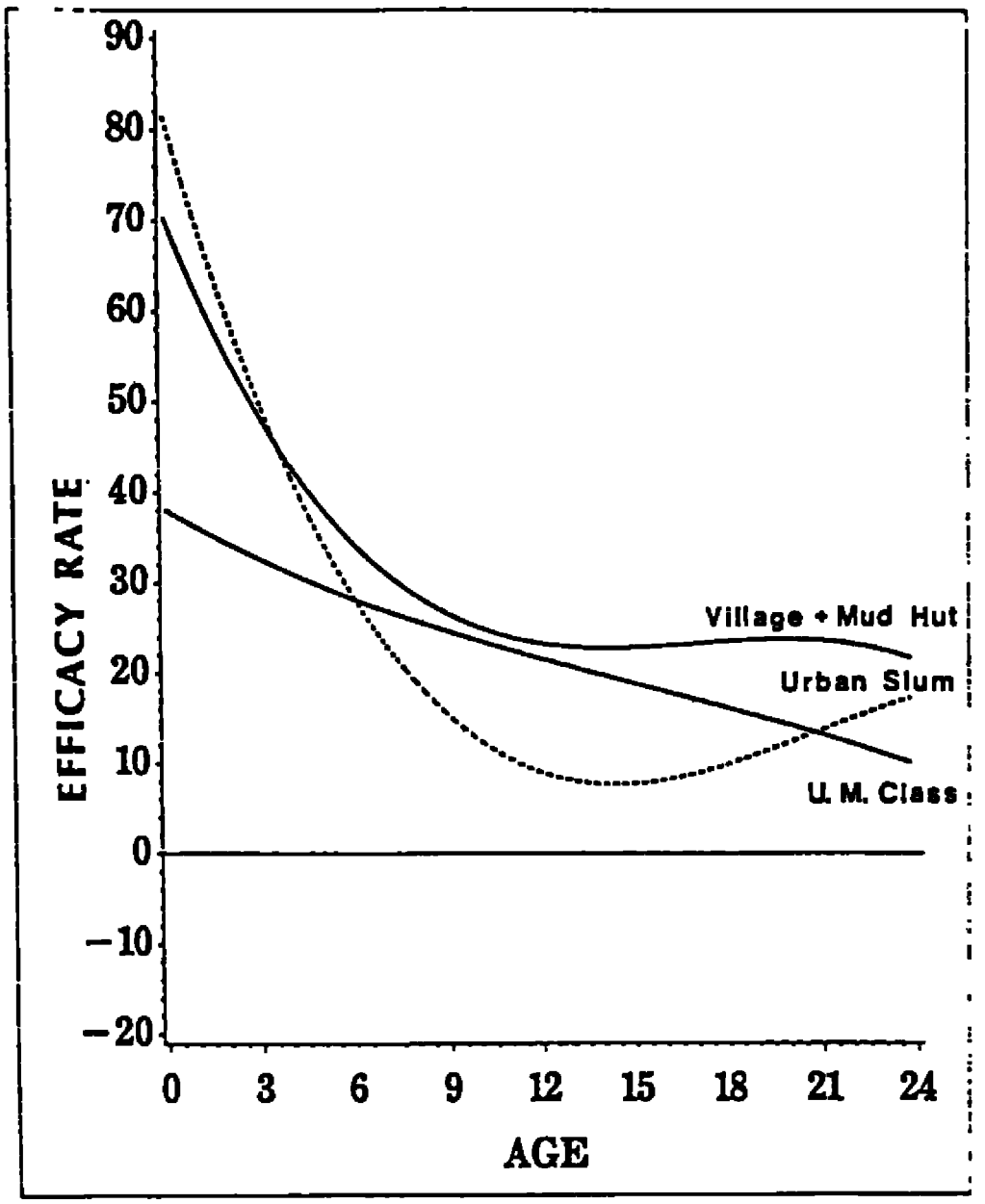

Figure 1. Efficacy of the protection of breastfeeding against diarrhea in three study areas. Efficacy, (E) is computed as the ratio between the incidence rate of diarrhea in the breast-fed infants (^{r}B) and in the non-breast-fed infants (^{r}C); $E = (1-{}^{r}B/{}^{r}C) \times 100$. The underlying observed values have been smoothed by a third degree polynomial function. Number of children for each area and age group is on the average 350 (range 23-935).

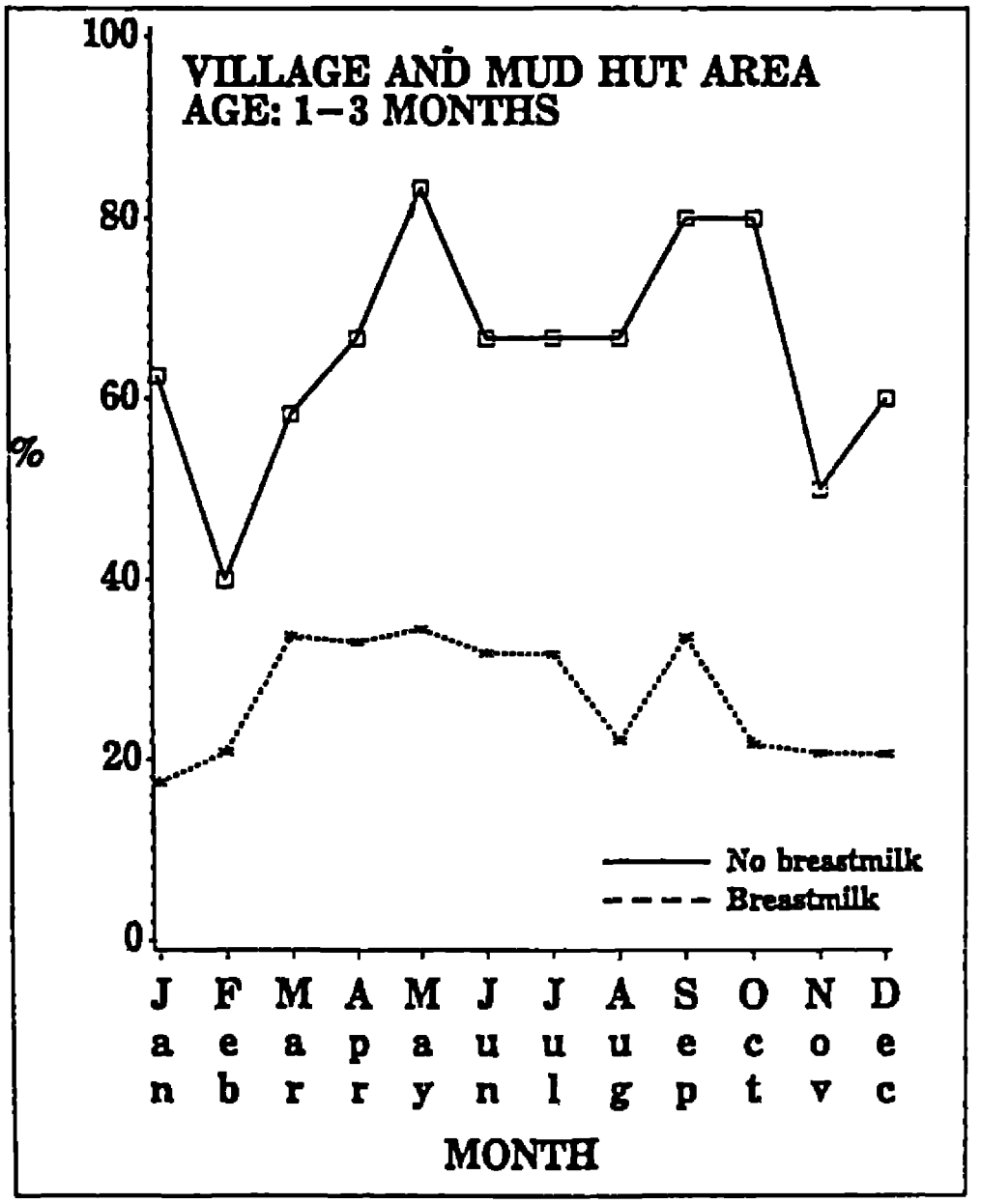

Figure 2. Incidence rate of diarrhea in the village and mud hut area analyzed per month of the year in the breast-fed and non-breast-fed infants, in the intervals 0-1, 1-2, and 2-3 months of age. About 40 observations/dot, range 6-195. April-June is the hot and dry season, July-September the hot and wet season.

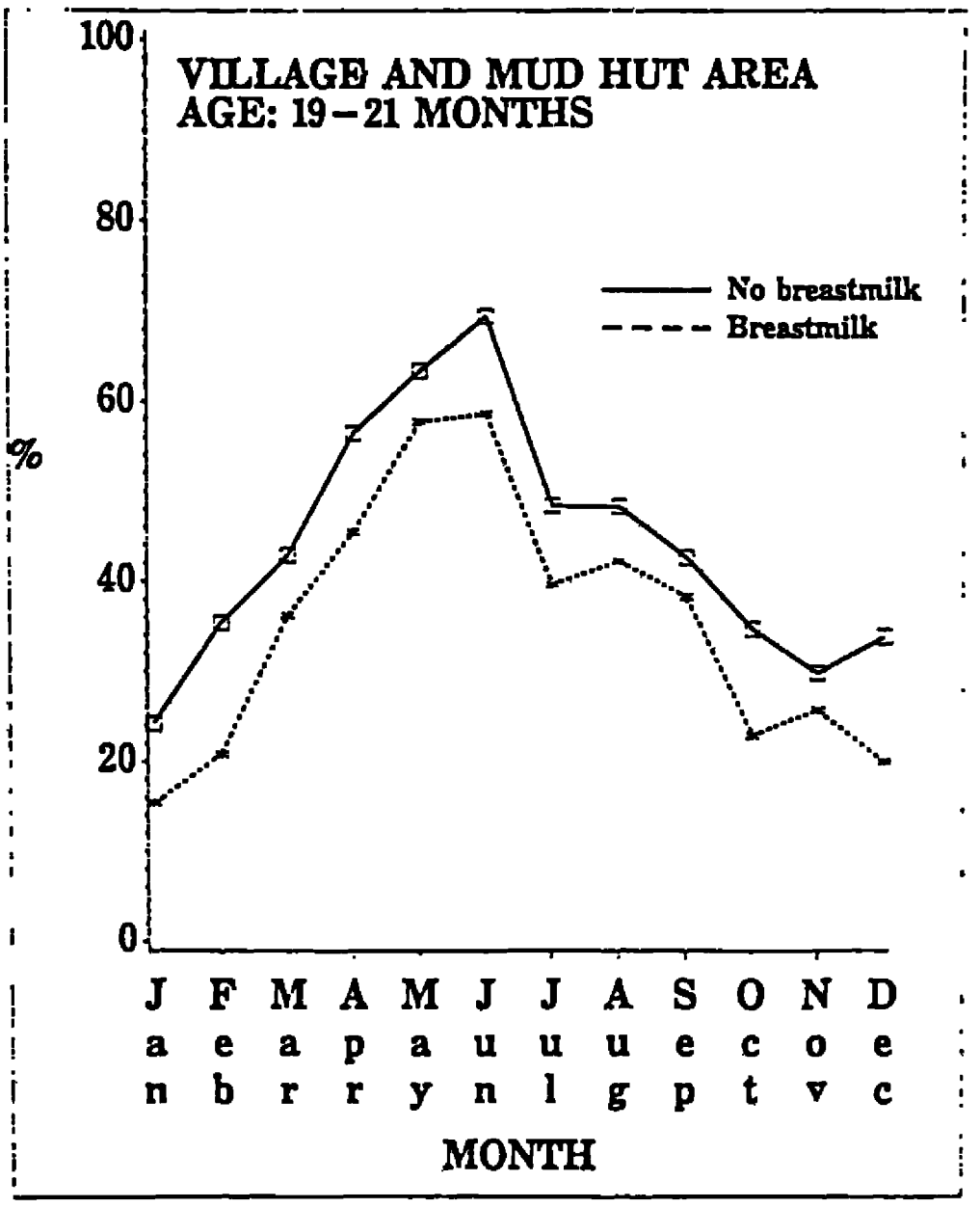

Figure 3. Same as Fig. 2 but for the age range 18-19, 19-20, and 20-21 months. Around 60-70 observations/dot, range 15-93.

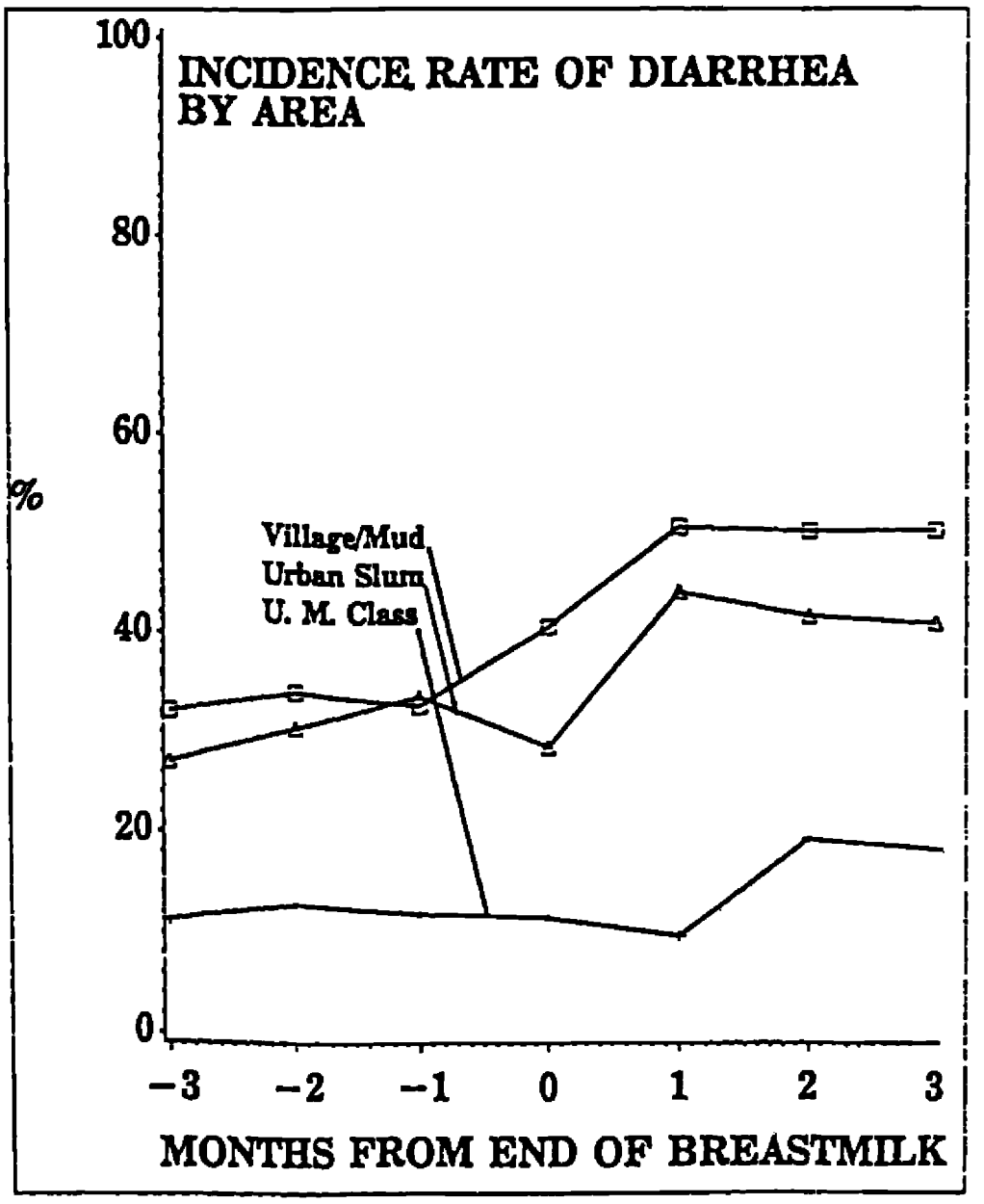

Figure 4. Diarrhea incidence rate by area of living, grouped monthly in relation to the time of termination of breastfeeding. Ages 1-24 months included. 300 observations/dot on the average, range 71-533.

Few studies of the protective role of human milk have been able to define the important host defense component among the many present in the milk. Glass *et al.*[19] demonstrated that protection against cholera in breast-fed infants is related to the milk secretory IgA (S-IgA) antibody titres against the *Vibrio cholerae* enterotoxin and lipopolysaccharide. More recently, Cruz *et al.*[9,20] found evidence for the role of milk S-IgA antibodies in the protection against ETEC in breast-fed infants.

These findings illustrate the importance of the predominant antibody in milk and all other exocrine secretions, S-IgA, which was actually first found in and isolated from human milk[21]. The appearance of milk S-IgA antibodies after intestinal antigenic exposure was an early example, both in experimental animals[22] and man[23] of the homing mechanism of Peyer's patch lymphocytes to peripheral sites within the mucosal immune system.

Some abberations have been noticed, however, in the expected titer increase of human milk S-IgA antibodies after intestinal exposure. We have seen that whereas parenteral vaccination may boost milk IgA antibodies[24], peroral vaccines may have the reverse effect[25,26]. At this conference similar findings in lactating mothers with intestinal infections have been presented by Cruz *et al.*, adding to his earlier observation that a perorally given food protein may decrease pre-exposure milk antibody titers[27]. These findings suggest that "oral tolerance" as seen in experimental animals may not be a consistent phenomenon in man, especially since decrease in milk S-IgA antibodies after oral exposure was usually accompanied by serum antibody increase.

We have tried to use avidities, or the relative affinity index, of human milk antibodies to gain more information about the milk S-IgA response. Avidities were determined by elution of the milk antibodies in an enzyme-linked immunosorbent assay (ELISA) by different molarities of KSCN, determining the molarity which eluted 50% of the antibodies[28]. It was noted that Swedish mothers had significantly higher relative affinity index of milk S-IgA antibodies to *E. coli* O antigen and diphtheria toxoid than Pakistani mothers[28]. The reason for this is not known, but one possible explanation could be poorer nutritional status of the Pakistani mothers. In a recent study, however, we followed the titres and avidities of S-IgA antibodies in the milk of undernourished Guatemalan mothers before and after a food supplementation of 440 kcal/day for 3 months. No increases in titres or avidities were seen, suggesting that the differences noted between the Swedish and Pakistani mothers might be due to causes other than the nutritional status.

Surprisingly, it was found that vaccination of lactating mothers in Pakistan with a whole cell cholera vaccine increased titres, but not avidities of the milk S-IgA antibodies against *V. cholerae* endotoxin[29]. This may suggest that the milk antibody avidities were already high, unable to increase any further on immunization. It might be a sign that many of the milk antibodies are the result of mature immune responses originating from memory cells migrating into the lactating mammary gland. Accordingly, this would also explain how the milk at one time can contain antibodies against so many bacterial species and serotypes, many more than the mother can have met recently.

Table 1. Antibody Levels and Avidities of Milk IgA Antibodies to *E. coli* 06 Antigen in Costa Rican and Swedish Mothers During Lactation. Antibody Levels were Expressed as % of a Reference and Avidity as the Molarity of KSCN Eluting 50% of Antibodies

	Mothers	Colostrum	3-12 Months milk
Antibody levels median (range)	Costa Rican n=17	80[a] (20-165)	27 (6-148)
	Swedish n=10	72[a] (27-133)	41 (20-161)
Avidities median (range)	Costa Rican n=17	1.75[b,c] (0.48-2.90)	1.3 (0.15-2.8)
	Swedish n=10	2.5[c] (1.1-3.05)	1.45 (0.9-1.95)

[a] Colostrum significantly higher ($p<0.001$) than 3-12 months milk

[b] Costa Rican mothers significantly lower than Swedish ($p<0.043$)

[c] Avidities significantly higher in colostrum than in mature milk, Costa Rican mothers $p<0.001$, Swedish mothers $p<0.0001$

High avidities were also found by Sennhauser *et al.*[30] for S-IgA antibodies to *E. coli* in milk from mothers of premature babies. Presumably, these antibodies are also not part of primary responses.

In a recent study, we compared the antibody levels and avidities of milk S-IgA antibodies to soybean protein in Japanese and Indian mothers[31]. The Japanese mothers eat much more soybean protein than the Indian mothers who were well nourished. Still, the Indian mothers had significantly higher titres ($p<0.01$) and lower avidities ($p<0.01$) of their milk IgA antibodies to the 7S soybean protein than the Japanese mothers. Obviously, we do not yet fully understand the development of avidity in mucosal immune response; further studies in experimental animals might be most helpful.

Recent analyses of milk samples from normally nourished Costa Rican women showed the same decrease from colostrum to mature milk in IgA antibody levels against *E. coli* 06 as Swedish mothers (Table 1) and as has been seen previously. However, the avidities against this single O antigen decreased significantly as well, both for the Swedish and the Costa Rican mothers. The avidities in the colostrum samples of the Costa Rican mothers were significantly lower than those of the Swedish mothers (Table 1). This is presumably not due to differences in nutritional condition. Neither are differences in microbial exposure or racial background obvious explanations.

BREASTFEEDING MAY ENHANCE VACCINE RESPONSES IN THE OFFSPRING

In a recent preliminary study, we compared the serum and secretory antibody responses to oral poliovirus and parenteral diphtheria and tetanus toxoids in breast-fed infants and infants on either of two isocaloric diets, one high (1.5 g/100 ml) and one low (1.1 g/100ml) in protein[31]. The S-IgA antibodies in saliva against the three vaccines were significantly higher in the breast-fed group than in the two formula-fed groups that did not differ and were therefore combined (Table 2). The fecal IgM antibodies to the tetanus and poliovirus vaccines were also significantly higher in the breast-fed infants, as were the S-IgA stool antibodies. The latter, but not the former, could have come from the mother's milk. The serum antibody responses in the breast-fed and the combined formula group did not differ significantly after the first two doses. However, 21-40 months later the breast-fed group had significantly higher IgG titres against diphtheria toxin and neutralizing antibodies against poliovirus, than the combined formula group (Table 2).

Table 2. Significantly Higher Antibody Level Increases in Breast- Compared to Formula-Fed Infants Against Oral Poliovirus and Parenteral Tetanus and Diphtheria Vaccines

Antibodies	Age (months)	After vaccine dose no	p-value
Salivary IgA			
against tetanus toxoid	4	2	<0.01
diphtheria toxoid	4	2	<0.01
poliovirus	4	2	<0.05
Fecal IgM			
against tetanus toxoid	3 and 4	1 and 2	<0.05 and <0.05
diphtheria toxoid	3 and 4	1 and 2	not sign.
poliovirus	3 and 4	1 and 2	<0.01 and <0.05
Serum IgG			
against tetanus toxoid	21-40	3	not sign.
diphtheria toxoid	21-40	3	<0.01
poliovirus	21-40	3	<0.001

Antibody determinations by ELISA

Recently, we have also compared the avidities of the serum antibodies after the vaccination. The avidities of the serum IgG antibodies to poliovirus increased significantly in all groups, but were not significantly higher in the breast-fed than in the two formula-fed groups (Table 3). The avidity of the serum IgG antibodies to diphtheria toxin also rose significantly in the breast-fed group and the low protein formula group. The antibody avidity 20-40 months after the 3rd dose was significantly higher in the breast-fed group

Table 3. Avidity Indexes of Serum IgG Antibodies after Vaccination in Relation to Mode of Feeding. Avidity Index Expressed as Molarity of KSCN Eluting 50% of the Antibodies

Mode of feeding	Before 1st dose	Before 2nd dose	2w After 2nd dose	20-40 Mo after 3rd dose
	Antigen: poliovirus type 1			
Breast-fed n=11	1.2[a] (0.2-2.9)[b]	0.5 (0.1-2.2)	0.4 (0.3-1.1)	2.0 (1.3-3.4) p<0.001
Low protein formula n=10	1.3 (1.1-2.4)	0.6 (0.3-1.2)	0.5 (0.3-0.8)	1.9 (0.9-3.0) p<0.001
High protein formula n=10	1.6 (1.0-3.2)	0.6 (0.2-0.8)	0.5 (0.3-0.7)	1.4 (1.2-2.9) p<0.001
	Antigen: diphtheria toxoid			
Breast-fed n=7	1.1 (0.4-2.6)	0.6 (0.2-1.9)	0.5 (0.2-1.4)	1.8[c] (1.4-3.2) p<0.005
Low protein formula n=9	0.8 (0.2-1.4)	0.5 (0.2-1.1)	0.5 (0.3-1.1)	1.5 (0.4-3.0) p<0.02
High protein formula n=5	1.4 (0.6-1.7)	0.9 (0.4-1.9)	0.4 (0.3-1.4)	0.9[c] (0.4-1.9) n.s.

[a] median
[b] range
[c] p<0.05 between breast-fed and high protein formula group

than in the high protein group (Table 3). However, the groups are small and this preliminary study must be confirmed by more extensive investigations.

The reason for this suggested enhancement of the vaccine responses in breast-fed compared to formula-fed infants is not clear. We propose that it may be due to the effects of anti-idiotypic antibodies present in human milk (Table 4A and B), just as we have proposed that maternal idiotypic and/or anti-idiotypic antibodies to poliovirus reaching the fetus via the placenta may

induce the S-IgA and IgM antibodies to poliovirus found in newborn saliva[33]. These antibodies are present in Swedish newborns, although wild or vaccine poliovirus strains are not normally present in Sweden due to vaccination of 99% of the population with inactivated poliovirus vaccine only. We have found such antibodies in the offsprings of mothers with IgA deficiency or hypogammaglobulinemia as well, thereby excluding the possibility that these secretory antibodies came from the mothers[34]. The immunoglobulin preparation given prophylactically to the hypogammaglobulinemic mothers contained anti-idiotypic as well as idiotypic antibodies to poliovirus[35],

Table 4A. Direct Binding of Presumed Anti-Idiotypes Against Poliovirus Type 1 in 4 Human Milk Samples to Solid Phase-Bound Monoclonal and Polyclonal Antibodies to Poliovirus Type 1

Maximal binding in OD, by ELISA

Milk sample no.	Monoclonal antibodies to poliovirus type 1			Pool of monoclonals nos I-IV	Polyclonal antibodies to polio-virus type 1
	no I	II	III		
1	0.8	0.7	0.7	0.8	1.6
2	0.7	0.8	0.7	0.6	0.9
3	0.5	0.3	0.4	0.2	0.5
4	0.5	0.4	0.4	0.2	0.9

Table 4B. Competitive Inhibition of Poliovirus Type 1 Antigen-Binding to Solid Phase-Bound Monoclonal and Polyclonal Anti-Polio Virus Type 1 with Presumed Anti-Idiotypes in 4 Human Milk Samples

Maximal inhibition in % by ELISA

Milk samples	Monoclonal anti-poliovirus type 1	Polyclonal calf anti-poliovirus type 1
1	100	39
2	100	40
3	100	32
4	100	63

presumably providing transplacental stimulus in the fetuses of the mothers with hypogammaglobulinemia. Both the idiotypic and anti-idiotypic antibodies were present in the cord blood of the newborns of the antibody deficient mothers[34].

In newborn mice, Stein and Söderström[36] showed that anti-idiotypes to an *E. coli* K antigen given to newborn mice directly or via the milk primed them to respond with protective immunity when vaccinated with the bacteria. At that age they would otherwise not respond to a capsular K polysaccharide. This observation is also in agreement with recent work from Ogra's group, showing a response with specific antibodies to respiratory syncytial virus in newborn mice after they had been given milk with virus antibodies from their mothers who had been immunized with idiotypes or anti-idiotypes[37].

If our assumption of the role in the fetus and neonate of idiotypes and/or anti-idiotypes is correct, it means that breastfeeding may not only protect by passive transfer of antibodies but even actively prime the immune response of the offspring.

THE ROLE OF BREASTFEEDING FOR INFANT MORTALITY AND BIRTHRATES

In countries with high infant mortality the main cause of death is infections. It is obvious that partial, or better, exclusive breastfeeding may help prevent or ameliorate many of these infections, reducing infant mortality. This result of breastfeeding is very important *per se*, but there may be even more important repercussions. It has been claimed, and the background data are rather convincing, that decreasing infant mortality is followed within a relatively short time span[38] by decreasing birth rate. In fact, it seems that family planning programs can enhance this connection once it exists, but they may not function well in the face of high infant mortality. To prevent the on-going population explosion it seems therefore mandatory to use all means to decrease infant mortality. In countries that have successfully done so recently, such as Costa Rica, a decrease in birth rates soon occurred.

The connection between infant mortality and birth rate is multifactorial, but one common and obviously major link is breastfeeding which when frequent and persistent, has a clear anti-conceptional effect[39].

Against this background it seems equally important to study the immunological capacities of human milk, to determine its favorable effects on the infant, and to promote exclusive breastfeeding, especially in developing countries.

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