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EPIDEMIOLOGY

Titres of class-specific antibodies against *Shigella* and *Salmonella* lipopolysaccharide antigens in colostrum and breast milk of Costa Rican, Swedish and Vietnamese mothers

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Summary

Enzyme immunoassays (EIA) were used to estimate titres of class-specific antibodies against purified and chemically defined phenol-water-extracted lipopolysaccharide (LPS) antigens of *Salmonella* serogroup B (BO), *Shigella dysenteriae* type 1, *Plesiomonas shigelloides* (the same O-antigen as *Shigella sonnei*) and *Shigella flexneri* Y. Titres in colostrum and breast milk of Swedish, Vietnamese and Costa Rican mothers from various socioeconomic conditions were compared. The antibodies were mainly of the IgA isotype. IgM antibodies were also present, but only very low concentrations of IgG were found. In Costa Rican mothers, the IgA antibody titres were significantly higher ($P < 0.05$) in women of low and middle socioeconomical conditions than were those in mothers of high socioeconomical level. The low titres in the last group were comparable to those found in Swedish mothers. The IgA antibody titres found in Vietnamese mothers were similar to those of Costa Rican mothers from the low and middle socioeconomic conditions, being highest against *S. flexneri* Y LPS. The IgM antibody titres were also highest in Vietnamese mothers, immediately followed by the Costa Rican mothers of low socioeconomic conditions. The low IgM titres in the Costa Rican women of high socioeconomic level were comparable to those seen in Swedish mothers. The results suggest that, in Costa Rica and Vietnam, *S. flexneri* is the most prevalent *Shigella* sp. causing infection and that *Salmonella* serogroup B infections are rare in all three countries. The results also show that the antibody repertoire in colostrum and breast milk varies. Furthermore, in addition to the prevalence of a specific micro-organism in a determinded geographical area, such differences may be associated mainly with exposure to certain pathogens in particular socioeconomic conditions.

Introduction

Specific antibodies, mainly of the sIgA isotype, against various microbial agents have been described in human colostrum and breast milk.[1–8] Their presence in this, as in other secretions, may be explained by the migration and

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homing[9–11] of lymphocytes, previously committed in the gut lymphoid tissues, into the mammary gland, which is considered as an immunological organ of the secretory common mucosal immune system.[10] It is likely that such antibodies are important in the protection of the breast-fed baby.[3,12–15]

Studies in various countries have demonstrated that the antibody content of colostrum and milk, related to enteric microbial agents, reflects the gut microbial flora prevalent in the country in question.[1,4,5,7,8] It might be expected that the colostrum and breast milk of women living in less-developed countries, mainly in tropical or subtropical regions, probably have a greater variety of antibodies than of women from industrialised nations in temperate climates. Within climatic zones, many differences reflecting the socio-economical conditions are observed. Differences in exposure to pathogenic micro-organisms, obviously associated with features such as personal hygiene, habitat, and environment, may also be reflected. Women living in overcrowded slums or other places with poor sanitation and inadequate environmental conditions, may well have both a broader repertoire and higher titres of milk antibodies than women in better socioeconomic conditions.

The aim of the present study was to compare the titres of class-specific antibodies against certain defined *Shigella* and *Salmonella* lipopolysaccharide (LPS) antigens, in the colostrum and milk of women from different geographical regions, with those of mothers in same country but living under different socioeconomic conditions.

Materials and methods

Study populations

Costa Rican mothers

From February to September 1989, healthy Costa Rican mothers living in the metropolitan area of San José, were recruited at delivery from low, middle and high socioeconomic conditions, as defined by the total income of the household and other specific features mentioned below. All mothers were healthy and had not suffered from any disease during pregnancy; nor were they considered as high-risk patients. Mothers who had cesarean section or who were delivered of premature children were not included; neither were those who for any reason were not representative of the specific group. Mothers were informed about the purpose of the study and written consent was obtained. Forms including socioeconomic and clinical information were completed for each mother.

Low socioeconomic (Low) group. The 34 mothers included in this group were recruited at the Section of Gynecology-Obstetrics and Neonatology of the San Juan de Dios Hospital (SJDH), San José, Costa Rica. They had a very low family income (less than 20000 colones per month; 1 dollar = 85 colones), which correlated with poor living conditions in overcrowded slums or at marginal areas of the capital (confirmed by personal observations). Prenatal care at Centres of the Ministry of Health and lack of cover by the Social Security System were considered as key indicators for selection of this group. Information was obtained directly from the hospital register, personal observation of the living conditions and from the mothers.

Middle socioeconomic (Middle) group. The 37 mothers in this group were also selected at the SJDH. The middle sector in Costa Rica is not a homogenous group. For the purpose of the present study, however, women considered as belonging to the middle socioeconomic stratum were those who had intermediate incomes (30000–70000 colones per month) and living conditions, as compared to those of the low or high socioeconomic groups. As specific criteria for selection, private prenatal care in most cases or, alternatively both private and Social Security care were considered. All of these mothers attended a Social Security system hospital (SJDH) for delivery.

High socioeconomic (High) group. The 23 mothers of the high socioeconomic stratum were recruited at two private clinics in San José, The Clínica Bíblica and The Clínica Santa Rita. Both are considered to be the most expensive clinics in Costa Rica. All women in this group had high household incomes (more than 150000 colones per month) and excellent living conditions as compared to the previous groups.

Vietnamese mothers

This group consisted of 39 mothers from Hanoi, recruited during 1988, at the Ngo Quyen Delivery Station and in the Trang Thi Hospital. Only mothers who had delivered full-term children were included. The women in this population lived under poor socioeconomic conditions and were undernourished as compared to those of the other groups.

Swedish mothers

Group 1. This consisted of 17 healthy Swedish mothers, recruited from December 1989 to February 1990, at the Gynecology Department of The Huddinge Hospital in Stockholm. Colostrum was studied from this group.

Group 2. This consisted of 26 Swedish mothers from a follow-up study in the Department of Medical Nutrition, Huddinge Hospital, during 1984–1985. Immature and mature breast milk was analysed in this group. All women belonged to the middle-income class and were living under good socioeconomic conditions.

Recruitment, collection and processing of colostrum and milk

The Costa Rican, the Swedish (group 1) and the Vietnamese mothers were recruited at delivery. By use of electric breast pumps at the hospital or manual breast pumps at home, specimens of colostrum and breast milk were obtained on days 1, 2, 3, 4, 7 and 30; 1 to 2: and 1 to 5, 30, 90 and 180, *post partum*, respectively. Breast milk from Swedish mothers of group 2 was collected on days 14 and 30 after delivery. Specimens were centrifuged at 10000 *g* for 10 min at 4 °C. The clear middle part of each was transferred to a plastic vial and frozen until used. The Costa Rican specimens (kept at −70 °C) and the Vietnamese specimens (kept at −30 °C) were transported in dry-ice to Sweden and kept there at −20 °C until analysed. The Swedish specimens of group 1 were processed as already described and kept frozen at −20 °C until assayed. The Swedish collection from group 2 had been centrifuged previously and kept frozen at −20 °C.

Enzyme immunoassays (EIA)

The highly purified and chemically defined phenol-water-extracted lipopolysaccharide (LPS) antigens used in this study have been previously described: *Shigella flexneri* type Y LPS,[16,17] *Plesiomonas shigelloides* LPS (same O-antigen as *Shigella sonnei*),[18] *Shigella dysenteriae* serotype 1,[19] and *Salmonella* serogroup B (BO).[20] The EIA was performed according to Engvall and Perlmann,[21] as modified by Karlsson *et al.*[22] Briefly, the wells of plastic plates (Nunc, Immunoplates, Copenhagen, Denmark) were coated with 100 μl of LPS antigen: 5 μg ml^{-1} for *S. flexneri* type Y, and 10 μg ml^{-1} for *Salmonella* BO, *S. dysenteriae* type 1 and *P. shigelloides*, in 0·05 M carbonate buffer, pH 9·6, at room temperature for 18 h. For colostrum and milk, titrations were performed from 10^{-1} to 10^{-5} dilutions in order to estimate the end-point titre and to determine the optimal sample dilution to be used. One sample with an intermediate titre was used as an internal control for the immunoassays. Alkaline phosphatase-conjugated antibodies (Dakopatts, Copenhagen, Denmark) used were: rabbit anti-human IgA (α chains), 1 in 2000, IgM, 1 in 2000 and IgG, 1 in 4000.

The EIA titres were expressed as relative titres defined as the optical density (OD) after 100 min times the dilution (= 1000). High IgA titres in the individual samples were defined as relative titres over the cut-off value (mean value + 2 S.DS) for the specific antigen in the Swedish group.

Total immunoglobulin determinations in Costa Rican samples

Total colostrum and milk IgA, IgM and IgG concentrations were determined by means of nephelometry. The Behring Nephelometer Analyser (BNA) was used for this purpose.[23]

Statistical methods

Analysis of variance on the overall ranks, general linear model, with simultaneous comparisons made by Tukey's Studentised Range Test was used to determine differences between the Costa Rican groups. The non-parametric Kolmogorov–Smirnov Test was used for individual comparisons between populations. The χ^2 test (1 df) was used for comparing the independent samples.

Results

Costa Rican populations

Basic information about the three groups of mothers is summarised in Table I. Mothers in each group were distributed in similar age ranges from 17 to 43 years of age. Higher parity, defined as the number of children born alive, was found in the low socioeconomic group of mothers, while the high socioeconomic had the lowest. The women in the low group had a lower level of education than women in the middle and, particularly the high socioeconomic group.

Table I *Characteristics of the three groups of Costa Rican mothers according to the socioeconomic groups*

Characteristic	Socioeconomic groups		
	Low	Middle	High
Number of mothers	34	37	23
Age (years)	25·5* (17–43)†	26·2 (17–35)	29·9 (22–42)
Parity	3·2 (1–10)	2·2 (1–7)	2·1 (1–5)
Education (years)	6·1 (0–11)	12·0 (6–17)	14·6 (9–18)
Gestational age (weeks)	39·5 (37–42)	39·6 (37–42)	38·9 (37–40)
Weight at birth (g) of the newborn	3119 (2520–4300)	3219 (2225–3800)	3073 (2300–3860)

* Mean.
† Ranges.

Table II *Total IgA concentrations (g/l) in breast milk of Costa Rican mothers from different socioeconomic groups*

Day after delivery	Socioeconomic groups		
	Low	Middle	High
1	18·4 (21·3)* (1·3–65·9)† $n = 20$	18·0 (16·0) (1·4–53·1) $n = 27$	21·7 (24·5) (2·7–71·5) $n = 10$
2	6·5 (8·0) (0·6–32·2) $n = 29$	4·9 (6·3) (0·7–25·7) $n = 30$	6·6 (13·7) (0·7–63·0) $n = 20$
3	2·3 (1·7) (0·5–7·9) $n = 34$	2·2 (2·0) (0·1–9·1) $n = 36$	1·8 (0·9) (0·3–3·9) $n = 20$
4	1·5 (1·0) (0·1–5·0) $n = 33$	1·5 (1·5) (0·1–7·1) $n = 32$	1·1 (0·6) (0·3–2·6) $n = 22$
7	1·3 (1·0) (0·5–4·7) $n = 34$	1·1 (0·8) (0·4–4·7) $n = 28$	1·0 (0·8) (0·3–4·1) $n = 23$
30	2·1 (3·9) (0·3–21·1) $n = 33$	1·4 (3·0) (0·1–14·5) $n = 35$	0·9 (1·7) (0·3–8·0) $n = 21$

* Mean (S.D.).
† Ranges.

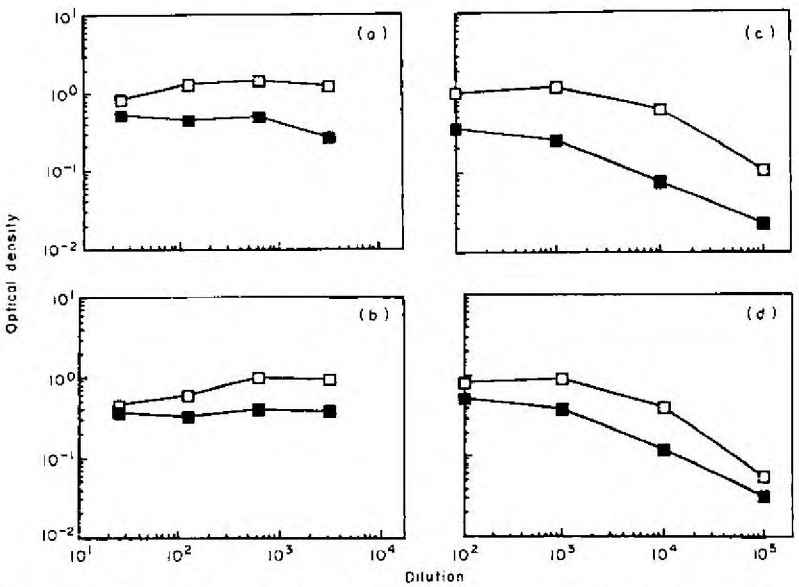

Fig. 1. Titration of IgA (□) and IgM (■) antibodies against *Shigella flexneri* Y LPS in two samples of colostrum (2–1 and 12–1), expressed as optical density (λ = 405 nm, 100 min). (a) and (b) show the titrations from $2{\cdot}5 \times 10^{-1}$ to $3{\cdot}2 \times 10^{-3}$ dilutions for samples 2–1 and 12–1 respectively. (c) and (d) show the end-point titrations from 10^{-2} to 10^{-5} dilutions for samples 2–1 and 12–1 respectively.

Total immunoglobulin concentrations in Costa Rican samples

Total IgA concentrations in colostrum and milk during the first month post partum

The highest IgA concentrations were found in initial colostrum (Table II). The mean values decreased from 18·4 (S.D. = 21·3), 18·0 (16·0) and 21·7 (24·5) g/l found at day 1 to 6·5, 4·9 and 6·6 g/l at day 2, in the low, middle and high socieconomic groups, respectively. This is approximately a three-fold decrease from day 1 to day 2. A similar decrease was observed between days 2 and 3, where the total IgA concentration of colostrum decreased from 6·5 in the low, 4·9 in the middle and 6·6 g/l in the high socioeconomic groups to 2·3, 2·2 and 1·8 g/l respectively. From day 4 to day 30 the values remained constant in the three groups at around 1·1–2·0 g/l. No differences were found in respect of total IgA concentrations in mothers of the low, middle and high socioeconomic groups.

Total IgM concentrations in colostrum and milk during the first month post partum

Day 1 IgM concentrations were 4·3 (7·3) 2·0 (1·9), 1·9 (2·4) g/l in the low, middle and high socioeconomic groups, decreasing on day 2 to 1·0 (1·9), 0·5 (0·7), and 0·9 (1·6) g/l, respectively. Thereafter, only IgM values less than 0·3 g/l were found.

Total IgG concentration in colostrum and milk during the first month post partum

The IgG concentrations in colostrum and milk were very low, below 1·9 g/l, on day 1 in all groups.

Specific antibodies against LPS antigens

Colostrum and milk samples were first tested by EIA and use of serial dilutions in order to determine a suitable dilution for examination of all samples. The results of the titrations are shown in Fig. 1. Samples diluted 1 in 25 gave low optical density values. The absorbance increased, or a plateau was observed, with higher dilutions; then titres started to decrease [Fig. 1(a) and (b)]. End-point titration curves from 10^{-2} to 10^{-5} are shown in Fig. 1(c) and (d). Since a 'pro-zone' phenomenon, particularly in the IgA antibody curve, was observed at low dilutions of colostrum or milk, 10^{-3} was chosen as an optimal dilution for the samples.

Costa Rican mothers

IgA-specific antibody titres in colostrum and milk during the first month post partum

The highest relative IgA antibody mean titres against the four antigens were observed in colostrum 24–48 h after delivery [Fig. 2(a)]. The IgA mean titre of 830 (S.D. = 380) to *Salmonella* B (BO), in the low socioeconomic group, found at day 1, showed a four-fold decrease to 210 (210) at day 4. From day 4 to day 30 the values remained at similar levels near to a relative-titre of 200. During the first 4 days, a similar pattern was observed in the middle and high socioeconomic groups where the mean titres decreased four-fold from 790 (550) to 130 (140) and from 510 (290) to 110 (80), respectively. From day 4 to day 30 they continued to fall almost two-fold from 230 (210) to 110 (130) in milk samples from the middle socioeconomic group, and from 110 (80) to 80 (130) in the high socioeconomic group. This accounts for a seven-fold decrease as compared to day 1 *post partum*.

IgA antibodies to *S. dysenteriae* type 1 followed the same pattern, decreasing four-fold from a relative mean titre of 900 (510) in the milk samples from the low, 840 (550) in those from the middle and 460 (260) in those from the high socioeconomic groups at day 1 to 250 (220), 210 (180), and 110 (80), respectively, in samples collected at day 4. The titres stayed constant in the low socioeconomic group from day 7 to day 30, whereas the mean titres of the middle and high socioeconomic groups continued to decrease six- and five-fold, respectively, to 140 (180) and 100 (140) at day 30 *post partum*.

Shigella sonnei IgA antibody titres decreased from 870 (490) in the low, 780 (510) in the middle and 330 (180) in the high socioeconomic groups to 260 (460), 140 (200) and 70 (90), representing a four-, six- and five-fold decrease from day 1 to day 30, respectively.

Antibodies to *S. flexneri* Y showed a similar decrease from a mean relative-titre of 1290 (630) in the low, 1140 (610) in the middle, and 530 (340) in the high socioeconomic groups to 340 (280), 340 (260), and 150 (160) at day 4 and then to 330 (370), 220 (260) and 120 (130) at day 30, respectively,

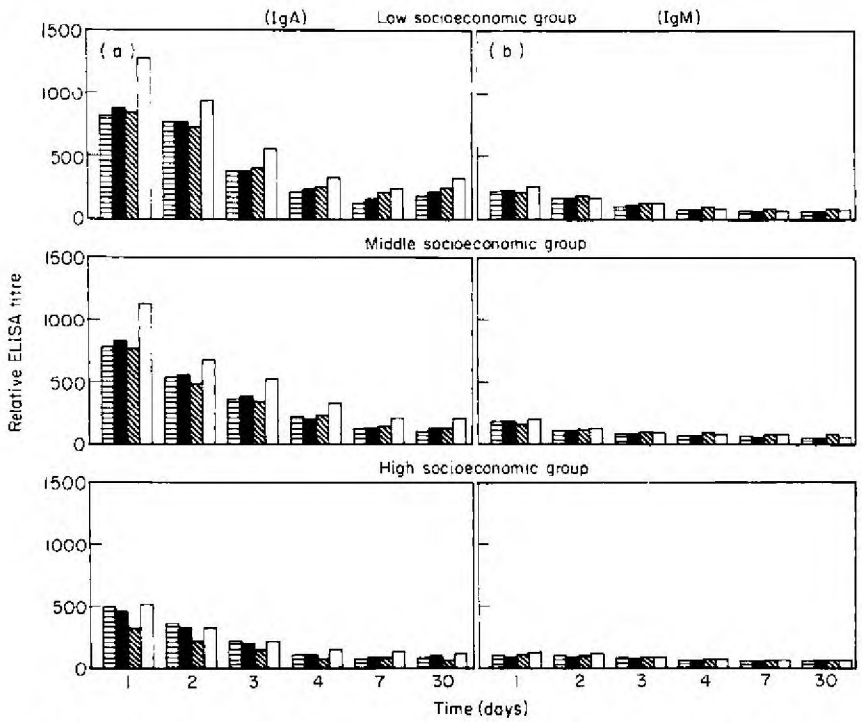

Fig. 2. Relative titres of IgA (a) and IgM (b) antibodies against the four lipopolysaccharide (LPS) antigens and found in Costa Rican mothers of different socioeconomic groups, during the first month after delivery. Bars correspond to: *Salmonella* BO (▤), *Shigella dysenteriae* type 1 (■), *P. shigelloides* (the same O-antigens as *S. sonnei*) (▧) and *S. flexneri* Y (□).

accounting for a total of four-, five- and four-fold decreases during the first month *post partum* for the low, middle and high socioeconomic groups, respectively.

The sIgA relative titres to *Salmonella* serogroup B (BO), *S. dysenteriae* type 1, *S. sonnei* and *S. flexneri* Y LPSs were significantly higher ($P < 0{\cdot}05$) in the samples from mothers of the lower socioeconomic groups compared to those of the higher groups. This difference persisted during the first month after delivery, despite the sharp decrease in titre observed at day 4 *post partum* [Fig. 2(a)]. Samples from mothers of the middle socioeconomic group showed titres which were intermediate between those of the low and high groups. There was not statistical significance in the antibody titres of mothers from the low and middle socioeconomic groups ($P > 0{\cdot}05$), except for the *S. dysenteriae* LPS ($P < 0{\cdot}05$). The antibody titres were significantly higher in mothers of the middle socioeconomic group than in mothers of the high socioeconomic group ($P < 0{\cdot}05$).

IgM-specific antibody titres in colostrum and milk during the first month post partum

IgM antibody against the LPSs studied was also present in colostrum and milk [Fig. 2(b)]. The highest IgM antibody titres were found in the early colostrum. Antibodies against *Salmonella* B (BO) decreased three-fold in the

Table III *Titres of IgA and IgM antibodies against four lipopolysaccharide antigens and found in breast milk from Swedish women*

Day after delivery	*n*	Antigens: *Salmonella* spp. mean OD (S.D.)†	*Shigella dysenteriae* mean OD (S.D.)	*S. sonnei* mean OD (S.D.)	*S. flexneri* mean OD (S.D.)
			IgA		
1	17	490 (310)	490 (370)	540 (340)	630 (400)
14	26	90 (50)	100 (70)	120 (70)	130 (60)
30	26	60 (30)	80 (50)	100 (60)	110 (40)
			IgM		
1	17	120 (70)	160 (90)	170 (100)	220 (160)
14	26	30 (20)	30 (40)	40 (30)	30 (20)
30	26	20 (10)	20 (20)	30 (20)	20 (10)

* OD, Optical density.
† S.D., Standard deviation.

low socioeconomic group from 230 (S.D. = 180) at day 1 to 70 (130) at day 4. In the middle and high socioeconomic groups a decrease was also observed from relative titres of 180 (200) to 70 (40) and from 110 (70) to 70 (40) during the first 4 days *post partum*. After that, only very low titres (of about 50) were found in samples from all groups. Antibodies to *S. dysenteriae* type 1 showed the same decline from 220 (180) to 60 (30), 180 (150) to 50 and 100 to 70 in the low, middle and high socioeconomic groups respectively. Specific IgM antibodies to *S. sonnei* also had a two-fold decline from 220 in the low, 170 in the middle and 120 in the high socioeconomic groups to 80, 80 and 70, respectively, at day 30 *post partum*. The IgM antibodies to *S. flexneri* Y in the low socioeconomic group decreased three-fold from a relative titre of 260 (190) at day 1 to 90 (40) at day 4 and then to 70 (70) at day 30 *post partum*. They likewise declined from 210 (140) to 90 (50) and to 60 (30) (three-fold) in the middle socioeconomic group. In the high socioeconomical group the mean relative titres dropped from 140 (90) to 70 (40) at day 4 and to 60 (40) at day 30 (two-fold decrease).

The IgM antibody titres showed the same pattern as IgA, having a tendency to be highest in the low socioeconomic group during the early days after delivery and decreasing markedly at day 4 *post partum*. Thereafter, the titres remained at a low constant level during the first month *post partum*. The IgM antibody titres in the middle socioeconomic group were similar to those of the low socioeconomic group during the first 2 days after delivery. Thereafter, the relative titres seen in this group became low and similar to those observed for the high socioeconomic group.

Table IV *Titres of IgA and IgM antibodies against four lipopolysaccharide antigens and found in breast milk from Vietnamese women*

Day after delivery	*n*	Antigens: *Salmonella* spp. mean OD (S.D.)†	*Shigella dysenteriae* mean OD (S.D.)	*S. sonnei* mean OD (S.D.)	*S. flexneri* mean OD (S.D.)
			IgA		
1–2	15	670 (240)	850 (400)	780 (450)	1520 (610)
3–5	23	440 (220)	620 (420)	500 (300)	890 (570)
30	36	160 (10)	210 (210)	140 (90)	480 (510)
90	30	130 (80)	170 (200)	130 (90)	450 (460)
180	17	160 (150)	190 (150)	180 (400)	460 (400)
			IgM		
1–2	15	370 (250)	350 (170)	350 (170)	460 (210)
3–5	23	200 (90)	220 (160)	220 (90)	260 (170)
30	36	110 (60)	110 (40)	110 (40)	110 (40)
90	30	110 (50)	110 (50)	120 (50)	120 (50)
180	17	100 (50)	100 (50)	120 (70)	110 (70)

* OD, Optical density.
† S.D., Standard deviation.

Swedish mothers

IgA antibody titres in the Swedish mothers

IgA antibodies to *Salmonella* BO (Table III) had a relative mean titre of 490 in colostrum from group 1 of Swedish mothers. Those relating to *S. dysenteriae* type 1 and *S. sonnei* were 490 and 540, respectively, at days 1–2 after delivery. Antibodies to *S. flexneri* Y had titres of 630 in the same group. In the breast milk of Swedish mothers of group 2, only relatively low titres against the four LPSs, of about 130–90, were found at day 14 after delivery and 110–60 at day 30 *post partum* (Table III).

IgM antibody titres in the Swedish mothers

Relatively low titres of 120, 160, 170 and 220 relating to *Salmonella* BO, *S. dysenteriae*, *S. sonnei* and *S. flexneri*, respectively, were found in the colostrum of the Swedish mothers of group 1 and only very low values, less than those of the background for the EIA, of about 30–40 were observed in the breast milk of mothers in group 2 (Table III).

Vietnamese mothers

IgA antibody titres in the Vietnamese mothers

The highest antibody titres were also found here in the early colostrum, decreasing markedly to relatively low values at 30 days after delivery (Table IV). The IgA antibody titres relating to the *S. flexneri* Y LPS decreased from a relative mean titre of 1520, observed at day 1, to 480 at day 30 *post partum*.

Similarly, *S. dysenteriae* and *S. sonnei* antibody titres declined from 850 and 780 at day 1 to 210 and 140, respectively, at day 30. Antibodies to *Salmonella* BO were also found to decrease from 670 at delivery to 160 a month later. Thereafter, only relatively low antibody titres were found up to day 180, except for those relating to *S. flexneri* Y, which remained at around 450 at days 30–180 *post partum*.

IgM antibody titres in the Vietnamese population

Mean relative antibody titres of 350–370 in respect of *Salmonella* BO, *S. dysenteriae*, *S. sonnei* and of 460 in respect of *S. flexneri* LPSs were found at days 1–2 after delivery (Table IV). Thereafter, the titres decreased to 110 against the four LPSs at day 30 and remained constant up to day 180.

Comparison of the relative mean IgA antibody titres in Costa Rican, Vietnamese and Swedish mothers

The highest relative IgA antibody titres in respect of *Salmonella* BO LPS were found in the low and middle socioeconomic groups of Costa Rican mothers. Similar relative mean titres were observed in the Vietnamese mothers [Fig. 3(a)]. The titres of the high socioeconomic group of Costa Rican and Swedish mothers (group 1) were similar and lower than those of the former three groups. Differences in the antibody titres in respect of the *Salmonella* LPS in the low socioeconomic Costa Rican groups and in Swedish mothers were observed but were not statistically significant ($P > 0{\cdot}05$). The difference between Vietnamese and Swedish mothers was also not significant ($P > 0{\cdot}05$). The antibody titres at days 3–5 were similar in the low and middle socioeconomic groups of Costa Rican and Vietnamese mothers, whereas in the high socioeconomic group of Costa Rica the values were significantly lower ($P < 0{\cdot}05$). In the Swedish group, samples of colostrum were not collected at that time after delivery. From days 7 to 30, only relatively low antibody titres were found but differences were still observed.

The relative antibody titres relating to *S. dysenteriae* and *S. sonnei* at day 1 *post partum* showed a similar pattern, being around 800 in the Costa Rican low and middle socioeconomic groups as they were in the Vientamese group [Fig. 3(a)]. Those titres of antibodies against these two LPS antigens in the Costa Rican mothers of the high socioeconomic group, as in the Swedish group of mothers, were significantly lower ($P < 0{\cdot}05$). At days 3–5 after delivery, the Vietnamese mothers showed a tendency to have higher antibody titres than the other groups, mainly in respect of the *S. dysenteriae* LPS.

The IgA antibody titres observed on day 1 in respect of the *S. flexneri* Y LPS were higher ($P < 0{\cdot}05$) in the Vietnamese group than in the other groups, except for the low socioeconomic group of Costa Rican mothers [Fig. 3(a)]. This difference persisted during the first month after delivery.

Comparison of the IgM antibody titres in Costa Rican, Vietnamese and Swedish mothers

Low IgM antibody titres in respect of the four LPSs were also found [Fig. 3(b)]. The highest titres were found at day 1 after delivery, showing a tendency to be higher in the Vietnamese mothers than in the other groups. The

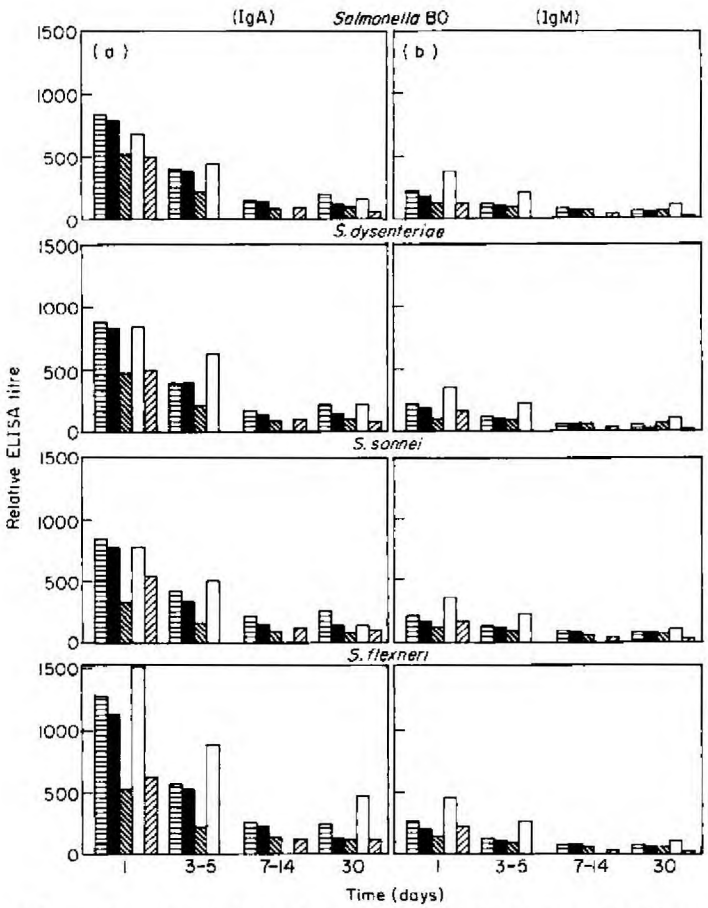

Fig. 3. Comparison of the relative titres of IgA (a) and IgM (b) antibodies against the four LPS antigens as determined by ELISA and found in colostrum and breast milk of Costa Rican mothers from the low (▤), middle (■) and high (▧) socioeconomic groups, Vietnamese mothers (□) and Swedish mothers (▨) during the first month after delivery.

IgM titres found in the high socioeconomic group of Costa Rican mothers and in the Swedish women were the lowest. The highest IgM antibody titres were found in respect of *S. flexneri* in all groups and, in particular, in the colostrum and milk of the Vietnamese mothers. The differences were statistically significant ($P < 0{\cdot}05$) with respect to the Swedish mothers and to the high socioeconomic group of Costa Rican mothers.

IgG antibody titres

The IgG antibody titres were very low (< 50) in all groups.

Samples with high IgA titres in the Costa Rican, Swedish and Vietnamese mothers

The numbers of colostrum samples with high IgA titres (cut-off + 2 S.D. of the corresponding Swedish value) in the various populations are shown in Table V. Of the Costa Rican colostrum samples of the low socioeconomic group,

Table V *Number of colostrum samples (days 1–2) with high IgA antibody titres* in Costa Rican, Swedish and Vietnamese mothers*

Group	LPS			
	Salmonella spp.	*Shigella dysenteriae*	*S. sonnei*	*S. flexneri*
Costa Rica				
Low	10/35† (29%)	9/35 (26%)	8/35 (23%)	15/35 (43%)
Middle	7/37 (19%)	7/37 (19%)	6/37 (16%)	11/37 (30%)
High	0/22	0/22	0/22	1/22 (5%)
Vietnam	1/15 (7%)	3/15 (20%)	3/15 (20%)	8/15 (53%)
Sweden	0/17	1/17 (6%)	1/17 (6%)	0/17

* Above the cut-off OD value (mean value + 2 S.D. for the specific antigen in Swedish group 1). Cut-off value for *Salmonella* BO = 1110, *S. dysenteriae* = 1230, *S. sonnei* = 1220 and *S. flexneri* = 1430.

† Number of samples with high titre/total number of samples.

29% had titres above the cut-off value in respect of the *Salmonella* BO, whereas only 7% of the Vietnamese samples were high. In the Costa Rican samples from the low socioeconomic group, 26% had high IgA titres in respect of *S. dysenteraie*, being similar to 20% found in the Vietnamese group. Samples with high titres were 23% and 20%, respectively, in relation to *S. sonnei* in the two groups. In respect of *S. flexneri*, 53% of the Vietnamese samples and 43% of those in the low socioeconomic Costa Rican group had titres above the cut-off value. In the high socioeconomic Costa Rican group, as in the Swedish group, low titres were found. The differences between the values of the Costa Rican low and middle socioeconomic groups and those of the Vietnamese group were not significant ($P > 0{\cdot}05$) according to the χ^2 test.

Discussion

Studies of specific antibodies in breast milk are influenced by several factors such as timing of the collection of samples. We observed that the titres decreased rapidly during the first 4 days *post partum* (Tables II, IV; Fig. 2), which suggests that comparisons should be based on analysis of colostrum. Adequate titration and the selection of a specific sample dilution avoid possible 'pro-zone' phenomena, which we observed in the EIA when low dilutions of the sample were assayed [Fig. 1(a) and (b)]. Thus, in this study much higher relative titres were recorded compared to those of earlier investigations using no, or only few dilution steps.[4,5,7] Furthermore, the use of chemically defined microbial antigens permits the determination of a specific antibody response directed against epitopes on the O-chain of each LPS.[16]

As expected, the antibody content of colostrum and milk from the Costa

Rican, Swedish and Vietnamese mothers was mainly of the IgA isotype. Furthermore, the dimeric or polymeric secretory component-bound IgA[15] represents more than 90% of the total IgA in colostrum and breast milk.[10,24] Specific IgM antibodies were also found. They may reflect recent infections since their concentrations were higher ($P < 0.05$) in more exposed populations, particularly those against *S. flexneri* in the Vietnamese group (Table IV). Only very low IgG titres of antibody against the LPSs assayed were found (data not shown).

The results of the present study provide support to already available data, indicating that the repertoire of antibodies to microbial agents reflects the antigenic composition of the gut flora prevalent in a specific geographical area[1,5,7,8] and, therefore, the secretory immunity of the mother, particularly at the intestinal level. Moreover, we demonstrated that differences, which correlated to the socioeconomic conditions, probably reflect the degree of exposure to the pathogenic micro-organisms. As a logical conclusion, features such as poor hygienic and environmental conditions, prevalent mainly in marginal sectors of the society, facilitate contact with pathogenic bacteria. Obviously, women living in conditions of good sanitation and hygiene are less exposed to enteric pathogens. For less privileged mothers, frequent exposure to these micro-organisms leads to both primary antibody responses and natural enhancement of the secretory immunity, including antibodies in breast milk. Thus, the antibodies in breast milk appear to be a good indicator of the immune response to gut-associated enteropathogenic agents.

The results observed in samples from Costa Rican mothers of the middle socioeconomic group are probably related to the heterogeneity of this group, which in Costa Rica is not easily defined. Because of the location of the San Juan de Dios Hospital, it may be that the section of the population attending that medical centre belongs mainly to the low–middle or middle–middle groups. Even so, we observed significant differences ($P < 0.05$) in the *S. dysenteriae* type 1 antibody titres of colostrum and milk between the low and middle groups. This pathogen has been associated mainly with very poor hygienic conditions.

Several authors have shown that severe malnutrition in mothers from very poor traditional societies influences not only the volume[25] but also the quality of antibody in their milk. Furthermore, diminished antibody avidity[26] has been demonstrated in breast milk of malnourished mothers. Another study concluded, however, that there was no difference between privileged and underprivileged and malnourished Guatemalan women in their ability to respond with a wide range of antibodies against a pool of somatic antigens from eight indigenous serotypes of *Escherichia coli*.[4] In the same groups of mothers, however, the specific IgA titres of antibody to *Salmonella* and *Shigella* LPS antigens appeared to be higher in the urban privileged group.[5] Therefore, severe malnutrition may in fact interfere with conclusions regarding the relationship between the degree of exposure to enteric pathogens and the repertoire of antibodies in colostrum and milk. Nevertheless, the improving health plus specific nutritional programmes in Costa Rica during recent years[27] permits us to assume that the nutritional status was equal in all mothers. The fact that there was no difference in the total content of IgA, IgM

or IgG in the milk of Costa Rican mothers from the different socioeconomical conditions supports this conclusion (Table II). It confirms not only that the nutritional status was similar but also that the observed differences in the antibody titres in the various socioeconomic groups actually correspond to the differing proportions of the total immunoglobulin content of their breast milk. The differences observed in the kinetics after delivery are mainly attributed to a rapid decrease not only of the antibody content and total immunoglobulin concentration during the first 2 days after delivery, but also to the increasing volume of milk during the following week, which reaches a more stable volume and concentration after the second week *post partum*.

The titres of antibody against *S. flexneri* LPS were the highest (Figs 2 and 3). This suggests that in Costa Rica and Vietnam, *S. flexneri* may be more prevalent than the other shigellae (Table IV; Fig. 3). Despite the indicators of good health generally in Costa Rica, there are various vulnerable groups.[27] Of these, people living in slums still play a relatively important role in the morbidity of infectious diseases, including diarrhoea. As far as we know, shigellosis is not a major cause of diarrhoea in Costa Rica, but the high titres of *S. flexneri* antibody found in colostrum and milk of Costa Rican mothers suggest that *S. flexneri* may be a prevalent pathogen among people of the low- and middle-income classes. It should be kept in mind, however, that shigellosis may be a mild disease not requiring medical attention, thus being unrecorded in epidemiological surveys. In fact, recent studies of shigellosis suggest that more than 90% of affected persons have mild diarrhoea only.[28]

Comparison of the Costa Rican women of the low socioeconomic group with the Vietnamese women showed that the Vietnamese (who lived under poor hygienic and sanitary conditions) had similar titres of antibodies against the LPSs assayed. The titres of antibody against *S. flexneri* Y were higher in the Vietnamese group, a fact which correlates with the already determined high prevalence of shigella infections in Vietnam.[17] Our results show that the Vietnamese mothers had a good repertoire of antibodies that reflected the high prevalence of *Shigella* spp. and also that their state of nutrition may have led to adequate antibody production. As mentioned above, several authors have suggested that malnutrition is associated with differences not only in the quantity[24–26] but also in the avidity of milk antibodies. We have not specifically tested avidity but the EIA we used favours the measurement of high-avidity antibodies.

The low antibody titres against enteric pathogens in colostrum and milk from Swedish mothers were not surprising, because of the low prevalence of *Shigella* spp. and *Salmonella* spp. in this country. The similar low titres in the Costa Rican mothers of the upper class, who have good living conditions, lead us to conclude that they are little exposed to these enteropathogens despite their prevalence in Costa Rica.

The low individual IgA titres against the *Salmonella* BO LPS in Vietnamese breast milk (Table V) indicates that *Salmonella* spp., at least of serogroup BO, are not very prevalent in Vietnam.

The results of the present study give basic information on the content of antibodies against LPS antigens related to common enteropathogens of breast milk of mothers from different geographical areas and mothers of different

socioeconomic conditions in the same country. The fact that mothers in various countries and of different nutritional conditions have a good antibody repertoire in colostrum and milk indicates that policies to implement oral vaccination of mothers, promote breast feeding, environmental sanitation and improvement in health and social development may help mothers to protect their babies against microbial diseases.

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