Preliminary Data on Changes in Neural Tube Defect Prevalence Rates After Folic Acid Fortification in South America

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Several South American countries are fortifying wheat flour with folic acid. However, only Chile started in 2000 to add 2.2 mg/kg, providing 360 μg daily per capita, an acceptable dosage for preventing the occurrence of some neural tube defect (NTD) cases. ECLAMC (Spanish acronym for the Latin American Collaborative Study of Congenital Malformations) routinely monitoring birth defects in South America since 1976, surveyed the impact of this fortification. Data from 361,374 births occurred in 43 South American hospitals, distributed in five different countries, active throughout the 1999–2001 triennium, were selected from the ECLAMC network. Birth prevalence rates for three different congenital anomalies with similar expected prevalence rates, were surveyed by the Cumulative Sum Method (CUSUM) method. They were NTD, oral clefts (OC), and Down syndrome (DS). Expected values were derived from observations made in 1999, and CUSUM was applied to the consecutive series of 24 months covering years 2000 and 2001. Only one of three congenital anomaly types, NTDs, in only one of five sampled out countries, Chile, showed a significant decrease, of 31%, during the 2000–2001 biennium, corresponding to the birth of the periconceptionally fortified infants. The level of significance ($P < 0.001$) was reached in the 20th month after fortification started, corresponding to August 2001. This is the first observation of a significant decrease in the occurrence of NTD after folic acid food fortification in a population little influenced by confounders common in the developed world as pre-existing secular decreasing trends, and partially unregistered induced abortions.

KEY WORDS: folic acid; fortified flour; neural tube defects; anencephaly; spina bifida; birth defects monitoring

INTRODUCTION

Several South American countries are fortifying with folic acid, an important food staple, in their diet as wheat flour. However, only Chile, since the year 2000, is adding this micronutrient in large enough doses (2.2 mg/kg) to provide an estimated average of 360 μg daily per capita [Freire et al., 2000], which is similar to the recommended 400 μg [Recommendations, MMWR, 1992].

ECLAMC (Spanish acronym for the Latin American Collaborative Study of Congenital Malformations) [Castilla and Lopez-Camelo, 1990] is routinely monitoring birth defects since 1976. The permanent surveillance of birth defect prevalence rates, and risk factors

Participants of Latin American Collaborative Study of Congenital Malformations are listed at end of the paper under the heading "ECLAMC Participants."

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have tested hypothesis of teratogenicity in a highly polluted industrial area of Brazil [Monteleone-Neto and Castilla, 1994]; reported recent cases of embryopathy by thalidomide, indicated for the treatment of leprosy [Castilla et al., 1996], as well as by misoprostol misused to induce abortion [Orioli and Castilla, 2000]. Therefore, ECLAMC is a suitable epidemiology early alert system to identify changes in the prevalence rates of neural tube defects (NTD) and other birth defects after effective primary preventive measures, as folic acid fortification.

The impact of folic acid fortification on the prevalence rate of NTDs is being eagerly expected by many countries in order to establish similar fortification policies. This urgency prompted us to publish these preliminary results on the South American situation, even thought larger sample sizes will be available in the future, which will allow for more accurate statistical analyses.

MATERIALS AND METHODS

Design and Data Collection

In South America, the fortification of a food staple with appropriate dosage of folic acid for the prevention of congenital anomalies was only implemented in Chile. In this country, wheat flour fortification was regulated in 1999, implemented in January 1st 2000, and the fortified flour reached the bakeries, and therefore the population, 3–4 months later [Freire et al., 2000]. Thus, periconceptionally exposed babies might have started being born during the last quarter of year 2000 and first quarter of year 2001. Consequently, we surveyed the prevalence rates of three selected group of anomalies during the 1999–2001 triennium in ECLAMC hospital network in order to verify any possible decrease in their prevalence rates.

Data from 49 South American hospitals, active throughout the 1999–2001 triennium were selected from the ECLAMC maternity hospital network. These 49 selected hospitals were distributed in the following eight countries (number of hospitals in parenthesis): Argentina (20), Bolivia (3), Brazil (11), Chile (6), Ecuador (1), Paraguay (2), Uruguay (3), and Venezuela (3). Countries having less than 10,000 examined births in the year 1999 were excluded from further analysis, namely, Bolivia (7,251 births), Paraguay (3,503 births), and Ecuador (3,027 births). Examined births were defined as all liveborns plus stillborns weighting 500 g or more (about 23 weeks of gestation).

Data from the year 1999 were used as a baseline, from which the expected values of birth prevalence rate for the analyzed anomalies were derived. The observation period included 24 monthly intervals from January 2000 to December 2001.

Three congenital anomaly types having similar birth prevalence rates in South America, and for which prevention by folic acid supplementation was already proven or suggested were selected. These were: NTD, including anencephaly, open spina bifida, and cephalocele [Berry et al., 1999]; oral clefts (OC), including clefts of lip and/or palate [Tolarova and Harris, 1995]; and Down syndrome (DS), including clinically diagnosed cases, with and without cytogenetic confirmation [James et al., 1999; O'Leary et al., 2002]. Both isolated and associated or syndromic forms were included.

Statistical Analysis

The Cumulative Sum Method (CUSUM) method [Lucas, 1985], for monthly monitoring of the three selected groups of anomalies, in the years 2000–2001 was employed. The CUSUM parameters (reference value K, limit value H) were defined under the hypothesis that folic acid should reduce the prevalence rate of those anomalies in at least 20%. Average Run Length (ARL) of 500 ($\alpha = 0.002$ one tail) was used, under the null hypothesis of no reduction in the prevalence rate. The ARLs under the null hypothesis (ARL H0), the hypothesis of a reduction of at least 20% (ARL H1), and their respective CUSUM parameters for each anomaly in each country are shown in Table I. The number of

TABLE I. CUSUM Method: Parameter Setting by Anomaly Type and Country

Anomaly	Argentina	Brazil	Chile	Uruguay	Venezuela
Neural tube defects					
ARL-H0[a]	472.4	475.4	520.9	492.3	477.0
ARL-H1[b]	17.1	23.8	47.9	66.4	40.7
K[c]	8.0	6.5	2.2	1.3	2.6
H[d]	16.0	18.0	13.0	10.7	14.4
Cleft lip and/or palate					
ARL-H0[a]	532.2	512.6	471.0	501.4	499.6
ARL-H1[b]	21.7	36.1	63.4	72.6	68.7
K[c]	6.5	3.4	1.2	0.7	1.4
H[d]	17.0	16.8	10.0	9.6	11.4
DS					
ARL-H0[a]	499.2	481.6	630.9	487.4	495.0
ARL-H1[b]	20.5	38.6	52.2	152.1	62.5
K[c]	7.8	3.2	2.0	0.6	2.0
H[d]	17.8	14.6	11.0	8.6	15.0

[a]ARL-H0, Average Run Length under stability (null hypothesis).
[b]ARL-H1, Average Run Length to detect a reduction of at least 20%.
[c]K, reference value for the CUSUM.
[d]H, limit value for the CUSUM.

TABLE II. Number of Examined Births and Registered Cases of NTD, Cleft Lip and/or Palate (OC), and DS in 45 Hospitals of Five South American Countries, by Month (MO), in the 2000–2001 Period

	Argentina				Brazil				Chile				Uruguay				Venezuela			
MO	Births	NTD	OC	DS	Births	NTD	OC	DS	Births	NTD	OC	DS	Births	NTD	OC	DS	Births	NTD	OC	DS
01	5024	11	13	11	2510	6	1	6	1133	2	1	4	1042	1	0	2	1662	1	1	2
02	4752	7	3	16	2500	7	3	4	1022	4	0	2	1007	0	1	1	1413	2	1	4
03	4887	15	5	8	2668	6	2	5	1048	5	1	2	941	1	1	0	1518	3	1	3
04	4706	10	7	12	2478	7	6	4	1012	0	0	2	869	0	0	1	1514	0	2	3
05	4999	12	9	20	2611	7	3	3	1064	5	3	2	905	3	1	1	1558	3	2	5
06	4603	10	9	17	2394	4	2	4	1008	0	2	2	965	2	1	1	1489	2	1	2
07	4664	15	7	7	2424	9	1	3	1072	1	1	4	931	4	1	4	1504	2	2	2
08	4754	12	3	11	2377	10	1	6	1098	0	4	1	947	2	0	0	1698	2	1	2
09	4734	9	1	10	2454	12	7	5	1138	1	1	2	955	2	0	2	1857	0	0	1
10	4660	8	5	9	2402	12	4	9	1139	0	1	0	952	1	0	4	2059	2	1	3
11	4530	12	4	11	2304	11	3	4	1140	3	2	3	920	3	2	2	1785	5	4	1
12	4659	5	7	7	2437	7	0	7	1103	0	1	1	995	2	0	0	1721	0	3	3
13	4703	17	8	9	2476	4	2	3	1091	1	0	3	953	0	2	3	1478	5	1	0
14	4416	8	5	3	2250	7	1	4	1024	2	1	3	816	3	0	0	1242	3	3	1
15	4485	10	13	8	2381	12	5	7	1091	1	2	5	1002	3	2	3	1523	2	1	1
16	4164	9	9	13	2270	6	3	5	1043	3	2	4	294	1	0	1	1291	1	0	0
17	4269	14	2	11	2332	8	4	4	1101	4	3	1	909	4	0	0	1436	2	0	2
18	3803	14	5	8	2202	11	2	4	1066	1	0	2	840	0	0	2	1261	4	0	1
19	3789	9	4	7	2106	6	3	2	820	1	2	0	955	0	2	2	1505	3	4	4
20	3667	14	2	11	2222	3	4	2	865	1	1	1	937	2	4	1	1338	2	2	2
21	3548	8	3	9	1941	8	3	3	815	0	0	5	945	1	0	4	1595	0	1	3
22	3475	14	9	13	1993	9	3	2	884	0	0	1	874	1	2	1	1679	6	4	4
23	3092	22	10	6	1956	9	2	5	854	1	0	1	829	0	0	0	1787	4	3	2
24	2364	4	1	3	1725	4	4	4	808	1	1	2	924	2	0	2	1588	2	0	1
TOT	102747	269	144	240	55413	185	69	105	24439	37	29	53	21707	38	19	37	37501	56	38	52

TABLE III. Baseline Frequencies (Year 1999) for NTD, OC, and DS in 43 Hospitals of Five South American Countries

Country	Hospitals N	Births N	NTD N	Rate	OC N	Rate	DS N	Rate
Argentina	20	51123	113	22.10	91	17.80	109	21.32
Brazil	11	27767	87	31.33	45	16.21	43	15.49
Chile	6	10740	26	24.21	15	13.97	25	23.28
Uruguay	3	11145	18	16.15	10	8.97	9	8.08
Venezuela	3	18792	35	18.62	19	10.11	27	14.37

Rate, rate per 10,000 births.

monthly-observed cases for each anomaly was adjusted to the mean of monthly births as estimated for each country. Thus, the odds to erroneously state that the prevalence was reduced in 20% when that change was actually due to random chance is once in every 500 months, while, ARL $H1 = 40$ means we would, under the worse of the hypotheses, employ 40 months to detect such a decrease of 20% or greater.

RESULTS

The effective sample included 361,374 births occurred in the 1999–2001 period in 43 maternity hospitals from the ECLAMC net after exclusion of 40,430 births registered at six hospitals in Bolivia, Ecuador, and Paraguay.

The numbers of observed cases for the three selected anomaly types, and the number of examined births, by month and country of occurrence are shown in Table II.

Table III shows the baseline prevalence rates for the three selected anomaly types during 1999. Table IV shows the maximum values of CUSUM (S_i) and the month with a maximum CUSUM value (month) in the 24-month surveillance for the three selected anomalies in each country. Only NTD in Chile showed a significant ($S_i = 13.7 > H = 13$, $P < 0.001$) reduction of 31% detected on 20th month. The observed birth prevalence rate until that point was 16.60/10,000 (35 cases in 21,078 births), and the expected number of cases was 51.0 (observed/expected rate = 0.69; 95% CI: 0.48–0.97; Z test = −2.24, $P < 0.012$, one tail). Figure 1 displays the CUSUM values for NTD in each country sub-sample.

In Chile, and for an ARL-500, each of the three congenital anomaly types included as NTD showed decreases with CUSUM values near the limit of significance. At the end of the surveillance period (month 24) CUSUM values were $S_i = 8.04 < H = 9.2$ for anencephaly, $S_i = 8.17 < H = 9.8$ for spina bifida, and $S_i = 5.62 < H = 7.8$ for cephalocele (Fig. 2).

TABLE IV. Observed CUSUM's (S_i)[a] in NTD, OC, and DS by Country

	NTD		OC		DS	
Country	S_i[a]	Month[b]	S_i[a]	Month[b]	S_i[a]	Month[b]
Argentina	3.6	12	15.2	21	7.1	15
Brazil	5.3	6	13.8	14	2.6	22
Chile	13.7	20	3.6	23	4.0	20
Uruguay	3.9	23	2.2	10	0.6	2
Venezuela	10.1	12	4.6	18	7.7	18

[a]S_i, Max(0,K-Y_i + S_{i-1}), maximum observed cumulative sum during surveillance.
[b]Month, month in which the maximum cumulative sum was observed.

The actual number of cases for each of the three NTD types in the Chilean sub-sample are shown in Table V.

DISCUSSION

The South American scenario calls for food fortification rather than supplementation. (1) High infant mortality rates, ranging from 20 to 60 per thousand, make prevention of birth defects of low priority in public health, the only exception being Chile with a rate below 10/1,000. (2) Induced termination of pregnancy, being illegal in all 10 South American countries, precludes secondary prevention, leaving primary prevention strategies as the only available alternative. (3) The low proportion of intended pregnancies, estimated in less than 35% [Gadow et al., 1998], makes difficult any periconceptional intervention. (4) The low level of public awareness on the preventive effects of folic acid endangers compliance to multivitamin and folic acid supplementation [Castilla and Dutra, 1997; Castilla et al., 2000].

Secular trends for NTD and other major birth defects in the hospital based South American ECLAMC database are significantly increasing during the last 10 years [International, ICBD, 2001]. This is most probably due to increasing prenatal ultrasonographic diagnosis in countries where induced abortion is illegal, plus a consequent increasing rate of referral of those cases

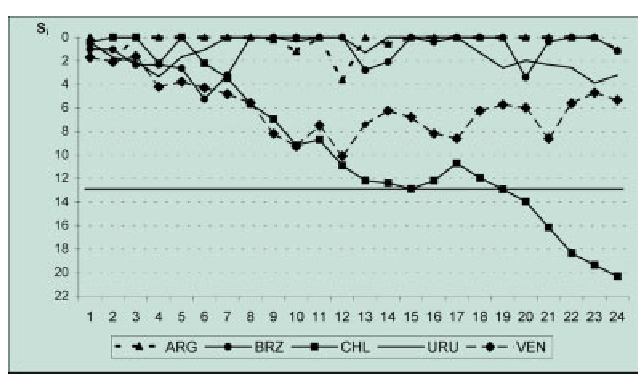

Fig. 1. Monthly surveillance by CUSUM method to detect a minimum decrease of 20% in the birth prevalence rate of neural tube defect in five South American countries. ARG, Argentina; BRZ, Brazil; CHL, Chile; URU, Uruguay; VEN, Venezuela; S_i, Max(0,K-Y_i + S_{i-1}); K, reference value; Y_i, the number of monthly observed cases for each anomaly adjusted to the mean of monthly births as estimated for each country; H, limit value.

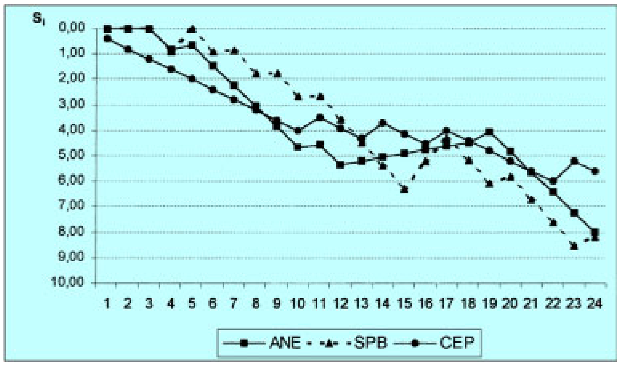

Fig. 2. Monthly surveillance by CUSUM method to detect a minimum decrease of 20% in the birth prevalence rate of three neural tube defect types in Chile. ANE, anencephaly; SPB, spina bifida aperta; CEP, cephalocele; S_i, Max$(0,K-Y_i+S_{i-1})$; K, reference value; Y_i, the number of monthly observed cases for each anomaly adjusted to the mean of monthly births as estimated for each country.

diagnosed in late pregnancy. This transfer of cases is more likely to occur from low to high complexity hospitals, the latter, more likely to participate in a research project as ECLAMC. Therefore, several factors in South America are working against the tested hypothesis of decrease by folic acid fortification in Chile.

With the available material, only one of three congenital anomaly types, NTD, in only one of five sampled out countries, Chile, showed a significant decrease during the 2000–2001 biennium, corresponding to the birth of the periconceptionally fortified infants.

These prompt results, being analyzed only 30 months after food fortification in Chile started, are obviously not free of flaws. The main pitfalls are: (1) a hospital-based system, affected by selective referral after prenatal diagnosis without the possibility of pregnancy interruption; (2) Small sample size, with insufficient numbers to analyze trends for specific types of NTDs; (3) The indirectness of the correlation, using intervention and outcomes at a given geographical unit (Chile) as a prove for cause-effect relationship, as in the "ecological fallacies."

Some strengths of these data can also be outlined, namely: (1) the highly comparable data among hospitals participating in the same project, following the same manual of operation, and periodically subjected to quality control and data validation [Castilla and Lopez-Camelo, 1990]; (2) the short time period of study (36 months), making unlikely the interplay of confounding effects from demographic changes as ethnicity, maternal age, or socio-economic status; (3) the promptness of this response from an active and experienced birth defects monitoring system; and (4) the clear cut results obtained with a data set that was not collected to test the hypothesis of NTD prevention by folic acid fortification.

TABLE V. Number of Examined Births and Number of Registered Neural Tube Defects (NTD) Cases by Anomaly Type and Year of Birth in Chile Sub-Sample

Year	1999	2000	2001
Births	10,740	12,977	11,462
Anencephaly	10	5	7
Spina bifida	11	15	6
Cephalocele	5	1	3
Total NTD	26	21	16

To our knowledge, this is the first observation of a significant decrease in the occurrence of NTD after folic acid food fortification in a population little influenced by confounders common in the developed world as pre-existing secular decreasing trends, and partially unregistered induced abortions. A decrease of at least 19% was reported as attributed to food fortification in the US [Honein et al., 2001]. The 31% reported here, even thought a minimal estimate also, will need further confirmation from more complete data sets, probably available in mid 2003.

ECLAMC Participants

The following doctors collected the information presented here in the ECLAMC network of 49 South American hospitals; Argentina: Graciela Ferreiro, Beatriz Minoli, Monica Rittler, Carlos Persini, Daniela Rottenberg, Viviana Cosentino, Mónica Jewtuszyk, Julio Quiroga, Mario Lerner, Martín Roubicek, Margarita Mussi, Susana Morales, Cristina Schneider, Mónica Ermini, Adriana Echegaray, Luisa Cárpena, Florentina Ponce, Carlos Negri, Mónica Menzio, Maria Cristina Mayer, Cesar Saleme, Carlos Quinteros, Juan Carlos Mereb; Bolivia: Saúl Rueda, Juan Manuel Jijena; Brazil: Aurea Gomes Nogueira, Rosana Canonaco, Marcus Moraes, Julio Cesar Leite, Pedro Armellini, Denise Cavalcanti, Marcos Aguiar, Valeria Fleck, Rui Locatelli Wolf, Mônica Pessoto, Eliana Ternes Pereira; Chile: Liliana Martínez, Emilia Toro Fuster, Nelson Rivera, Rodrigo Nazal, Aurora Canessa; Ecuador: Milagros Salto; Paraguay: José Luis Delgadillo, Marta Ascurra, Nidia Burró; Uruguay: Mariela Larrandaburu, Olga Ferro, Irene Rodríguez; Venezuela: Rosa Cedeño, Dania Guerra, Ana Brachó, Otto Sánchez.

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