July - December, 2009

Volume 20 No. 2

Special Anniversary Issue

Center for Studies of Sensory Impairment, Aging, and Metabolism (CeSSIAM)

BULLETIN OF RESEARCH ABSTRACTS

Editorial Board

- Dr. Fernando Beltranena
- Dr. Carlos Castellanos
- Dr. Gustavo Hernández Polanco
- Ms. Rosa Amalia de Vásquez

Editors

- Dr. Marieke Vossenaar
- Dr. Mónica Orozco
- Dr. Noel W. Solomons

Postal Address:
CeSSIAM in Guatemala
P.O. Box 02-5339
Section 3136/GUATEMALA
Miami, FL 33102-5339, USA

Visiting Address:
CeSSIAM
17 avenida 16-89 (interior)
zona 11 Anillo Periférico
Guatemala City, 01011
Guatemala

Email:
cessiam@guate.net.gt
Tel/Fax: ++(502) 24733942

Inside this issue:

Editorial

TWENTY-FIVE YEARS OF CeSSIAM: "OPERATION OPENNESS AND OPPORTUNISM"

It seems like only yesterday that CeSSIAM was celebrating its 20th Anniversary with plans for an academic week in July 2005 in Guatemala. Economic times make our 2010 plans more modest and appropriate to the financial circumstances of the day. But it is just and necessary that we recognize in many, small ways, the legacy of our institution. This begins with the founding co-directors of CeSSIAM, Gustavo Hernandez-Polanco, Fernando Beltranena, and the late Oscar Pineda. These were professionals who shared a vision of modern academic activities, separated from any specific set of traditions and informed by the new questions in health and by a global international sounding board. With that group must be placed our patron spirit, the late Doña Elisa Molina de Stahl, founding President of the National Committee for the Blind and Deaf and builder of the Eye and Ear Hospital, which bears the name of her uncle, Dr. Rodolfo Robles V. It was that institution that provided us a roof over our head and legitimacy in society during the first 15 years. She knew the meaning of investment in the future.

The original band of young physicians and medical students, who first climbed the stairs of the Robles Hospital, Francisco Rosales, Carolinas Barillas-Mury, and Carolina Vettorazzi on July 1, 1985 were the spark that lit the wick. Each has since distinguished themselves in biomedical research. The original constellation of the centers divisions were: Sensory Impairment; Vitamin A; Gerontology; and Child Health. We are indebted to the respective leadership given by the cadre of your physicians: Carlos Grazioso, Jesus Bulux, Manolo Mazariegos, and Susana Molina. The staffing of the "divisions" came primarily from local thesis students and international exchange students. Germany, the Netherlands, Canada and the USA were the major sources of international students through the early years and still after 25 years. We have also had creative research collaborators from the same four nations, as well as from Central America.

We must recognize with special enabling efforts on the financial front made by Alan Kligerman, the AkPharma Inc, Nevin Scrimshaw and the Nevin Scrimshaw International Nutrition Foundation. The continuous donations from AkPharma and the institutional back-up provided by the Foundation combined to solve complex problems of financing from international agencies, to major industries to the National Institutes of Health. The 20th Anniversary celebration in Guatemala might have been the turning point, as Klaus Schűmann has joined us on the financial side, as well, with the initial funding by his mother, Frau Hildegard Grunow, of a Leadership Learning Center to house the visiting students and post-doctoral fellows on our campus. With the untimely passing of our benefactor, Prof. Schűmann created the Hildegard Grunow Foundation in Munich as a memorial to his mother's generosity. It has quickly become the *opening* through which the seed money investments for novel departures in contemporary research began to flow.

Volume 20 No. 2

If the 25 years of CeSSIAM could be considered a mission, we could call it: "Operation Openness and Opportunism." Our formula for progress and progression has been to open our eyes and our senses to the relevant scientific question of the moment, and to open up our hospitality to exchange-students and collaborative arrangements with international academic colleagues. We have not hesitated to open up our communication in posters and platform sessions at scientific meetings and in the pages of scientific journals. Perhaps the most important aspect of the openness policy is that of maintaining an open ***mind*** to emerging concepts and innovative technology. From the most simple and practical level of a color-strip dipstick to detect the presence of *Giardia intestinalis* in feces, to the most sophisticated single nucleotide polymorphism (SNP) technology in characterizing ethnic genetic variance, our recent years have embraced opportunities that serendipitously presented themselves in our path.

With 25 years under our belt, the openness ethos is still strengthening as a guiding principle of the Center, and the opportunities have not stopped appearing. As we look forward to the first year of the next pentad of our history, the openness principle points toward the pursuit of a collaboration to complement the non-invasive, bloodless measure of hemoglobin with a companion method for detecting iron depletion based on application of a probe to the skin. The daily consumption of a common bioactive lipid in breast milk (gangliosides) may suppress intestinal colonization with the pathogenic protozoa, *G. intestinalis*. The innovative Positive Deviance approach to health and wellness guidance, developed and advanced in recent CeSSIAM studies, is poised to guide the search for ways to overcome the barrier to full adherence to the WHO recommendation for 6 months of exclusive breast feeding and to address the complex causality of short stature, the leading nutritional problem of Guatemala. Our successful collection of DNA material in cells in saliva for genetic screening may soon be joined by with transcriptional analyses of messenger RNA, harvested from buccal cells desquamated into saliva. These are the ideas on the drawing board opening the pathway into the next round of research for the Center. Despite our quarter century of age, our ideas and plans still point firmly to tomorrow.

"Perhaps the most important aspect of the openness policy is that of maintaining an open mind to emerging concepts of innovative technology"

Noel W. Solomons MD

Fecal production of reactive oxygen species (ROS) in response to supplementation with three types of iron supplements in healthy male adults

Mónica Orozco, Noel W Solomons, and Klaus Schümann

Safety and efficacy are important criteria to consider when it comes to selecting iron supplements to treat iron deficiency and iron deficiency anemia (IDA). Research assessing the efficacy of several forms of iron supplements is abundant; but only recently, the ability of therapeutic iron supplements to catalyze the production of free radicals has drawn much attention due to the safety concerns this poses. Previous experiences have demonstrated that iron supplementation can deplete the antioxidant capacity state of human feces (Orozco, et al, 2010). Alterations on this state may severely harm the intestinal tract since the increased production of free radicals has been positively associated with a higher risk of colon cancer (Lund, 1998). Several iron supplements have demonstrated to be efficient in treating IDA, but little is known about their capacity to generate free radicals in the intestinal lumen.

In this study we compared the ability of three iron supplements -- ferrous sulfate ($FeSO_4$), sodium iron EDTA (NaFeEDTA), and iron polymaltose (IPM) -- to produce free radicals in fecal samples from apparently healthy male adults. Ten men aged 18 – 56 took the three supplements for 6 days each, in a randomized fashion. Each supplementation period was followed by a washout period. A baseline period of three days was also included in the experimental design. Subjects took daily iron doses of 100 mg of each of the iron treatments and provided 3 fecal samples at the end of each supplementation and washout stage. The buffering capacity of fecal material to quench free-radical generation, an indirect measure of *in situ* luminal oxidation, was assessed with an HPLC-based method adapted from Owen et al (2000), with some modifications. To determine if there was an association between the amount of residual iron and ROS production in the feces, we also assessed non-heme iron with a commercial spectrophotometrically-based assay, the Feren-B-Method kit (Bioanalytic, Umrich, Freiburg, Germany). Baseline and final C-Reactive Protein (CRP) concentrations were analyze at the clinical laboratory of Sanatorio Nuestra Señora del Pilar in Guatemala City. A cut-off point of < 5 mg/L was used as a criterion for a normal state of systemic inflammation.

Results showed that all 10 subjects had CRP values below the threshold criterion of 5 mg/dL before the study. After 3 rounds of iron no differences among the ROS responses to any of the three compounds were obseved. **Figure 1A** shows identical concentrations of residual fecal non-heme iron with each of the three iron compounds supplemented at a daily dose of 100 mg Fe each. These values were significantly greater than non-supplementation specimens by MANOVA ($p<0.001$). On **Figure 1B**, it can be observed that there were no differences among the ROS responses to any of the three compounds.

In conclusion, daily consumption of 100 mg of elemental iron from each of three oral iron compounds produced identical concentrations of residual iron into the stool and identical degrees of attenuation of total fecal antioxidant capacity as indicated by *in situ* generation of ROS.

References:

Orozco MN, Solomons NW, Schümann K, Friel JK, and Mendizábal de Montenegro AL. 2010. J Nutr. In press.

Lund EK, Wharf SG, Fairweather-Tait S and Johnson IT. 1998. Increases in the concentrations of available iron in response to dietary iron supplementation are associated with changes in crypt cell proliferation in rat large intestine. J Nutr. 128:175-179.

Owen, RW, Spiegelhalder B and Bartsch H. 2000. Generation of oxygen reactive species by faecal matrix. Gut. 46:225-232.

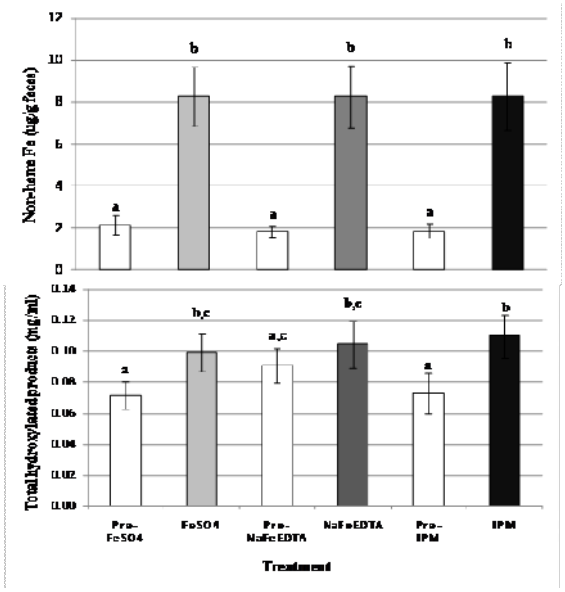


Figure 1. A) Fecal concentration of non-heme Fe for pre-supplementation periods during which no oral Fe was ingested (open bars), and during the respective periods of oral supplementation with each Fe compound (shaded bars). B) Fecal in situ ROS responses for pre-supplementation and during the respective periods of supplementation with each iron compound (shaded bars). Bars not sharing common superscript letters were significantly different ($p<0.05$) by MANOVA.

Mónica Orozco is a post-doctoral fellow at CeSSIAM

Noel W. Solomons is the Scientific Director of CeSSIAM

Klaus Schümann is the Director of the Research Center for Nutrition and Food Sciences, Technische Universität München in Germany.

Calibration of digital readings (Masimo RAD-87) with whole blood for the field diagnosis of anemia

Caitlin R. Crowley, Gabriela Montenegro-Bethancourt, Claudia Arriaga, Noel W. Solomons, and Klaus Schümann

Blood hemoglobin concentrations, is a method used for diagnostic anemia. We designed a study to evaluate the validity and reliability of a new, non-invasive device to be used for measuring hemoglobin (Hb) values. By using the RAD-87 [Masimo Corporation, Irvine, CA], we analyzed the readings taken at times: 5, and 10 min, and compare them to the values from conventional blood draw method.

We had a sample of 80 subjects evaluated using both methods. Adult males (n=40) from San Francisco El Alto Totonicapan (2600 m.a.s.l), and, pregnant women (n=40) from Retalhuleu (240 m.a.s.l) were recruited to attain maximum range of Hb values. To assess reproducibility, ten control subjects were tested repeatedly over a period of ten days using the non-invasive device. Eight additional subjects were four times over two-hour intervals within a 24-h period to determine within day variability. Descriptive statistics and regressions, including the Pearson product moment and Spearman Rank Order Correlation Coefficients, were calculated using SPSS version 17. World Health Organization thresholds for anemia were used for diagnostic discrimination of screening (digital readings) versus reference standard (whole blood) to generate sensitivity, specificity, and positive and negative predictive values.

Overall hemoglobin values with conventional whole blood samples ranged from 9.1 g/dL to 19.5 g/dL. Mean value 13.5 ± 2.6 g/dL and median 13.3 g/dL

Comparison of whole blood Hb concentrations with the non-invasive device: Overall, digital readings showed a direct, positive correlation with Hb values determined by the invasive method (**Figure 1**). Measurements taken at 10 min had a slightly stronger Pearson correlation ($r = 0.81$) than those taken at 5 min ($r = 0.75$). Readings taken by the non-invasive device tended to be slightly lower than the conventional blood draw method. Hemoglobin values ranged from 8.9 g/dL to 15.7 and 15.9 g/dL at 10 and 5 min, respectively, with a median value of 11.9 g/dL. Therefore, mid- and low-range values were captured fairly accurately, but greater limitation was seen at the upper limit. MANOVA and LSD post hoc analysis showed that whole blood values were significantly different from both digital readings ($p<0.001$), though no difference was found between 10- and 5- min intervals ($p=0.966$).

The non-invasive device was moderately sensitive for classifying anemia at higher cut-off values. Sensitivity was highest for both 10 and 5 min at a cut-off value of 13.0 g/dL (97% within the total population, 100% among the female population). Specificity, however, was greatest at the lowest cut-off values for determining anemia. When used to assess plethoric values among the male subgroup and the entire population, the digital device failed in sensitivity, but was 100% specific. Individual sensitivity, specificity, and positive and negative predictive values for each cut-off is shown in **Table 1**.

The urgency of applying a system of screening and differentiated administration of iron, which would be facilitated by an effective non-invasive device, emerged after the publication of the study which took place in Pemba, Tanzania [1]. While iron-supplementation helped children with iron-deficiency anemia to cope with sequels of anemia tropica, the number of hospital admissions and deaths increased among iron-replete children. Thus, a device such as the Rad-87 holds promise for serving as a field-friendly screening apparatus. Improved sensitivity and specificity in the anemic range and a shorter determination period, however, are desirable.

Acknowledgements:

We are very thankful for the collaboration of the personnel of the local health Centers: Centros de Salud de: San Francisco El Alto, El Asintal and Retalhuleu. We appreciate the assistance from Gloria Hidalgo, Juergen Kotzerke; Dr. Michael Schietzel; and the Hildegard Grunow Foundation for funding.

References:

Sazawal S, Black RE, Ramsan M, Chwaya HM, Stoltzfus RJ, Dutta A, Dhingra U, Kabole I, Deb S, Othman MK, Kabole FM. Effects of routine prophylactic supplementation with iron and folic acid on admission to hospital and mortality in preschool children in a high malaria transmission setting: community -based, randomised, placebo-controlled trial. Lancet 2006;367:133-43.

Caitlin R. Crowley is a research fellow at CeSSIAM

Gabriela Montenegro-Bethancourt, is a research nutritionist at CeSSIAM

Claudia Arriaga, is a research fellow at CeSSIAM

Noel W. Solomons, is the Scientific Director at CeSSIAM

Klaus Schümman, is the Director of the Research Center for Nutrition and Food Sciences, Technische Universität München in Germany.

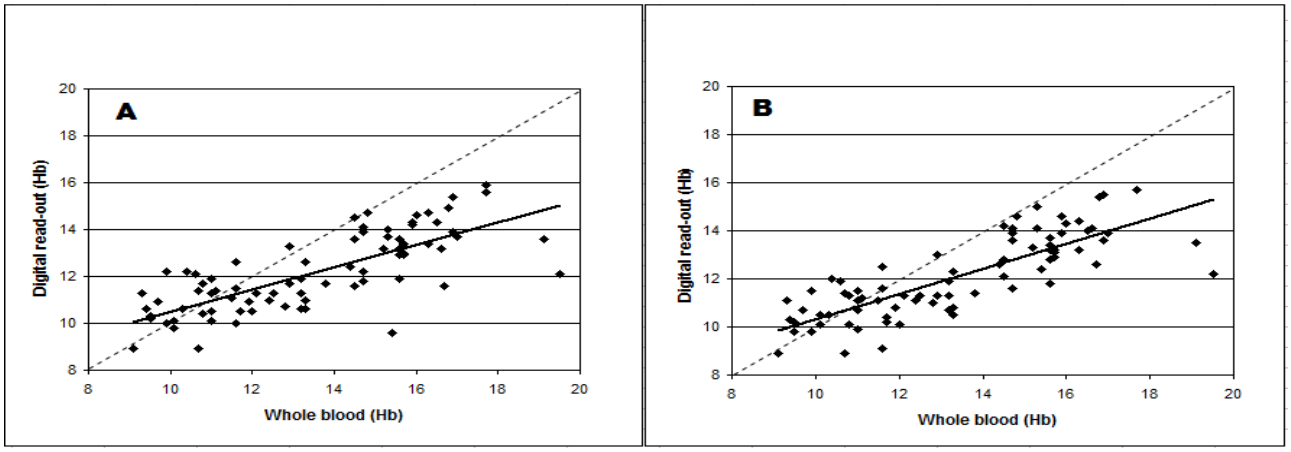


Figure 1. Scattergrams of paired values for hemoglobin in g/dL in the 80 selected volunteers in the sample. The horizontal axis represents the whole blood Hb values. The vertical axis represents the digital reading from the non-invasive device. The discontinuous diagonal line represents the 45º line of identity. The dark solid line represents the least-squares regression line. Panel A displays the 10-min digital reading. The Pearson correlation coefficient is r = 0.81, p <0.001. Panel B displays the 5-min digital reading. The Pearson correlation coefficient is r = 0.75, p <0.001.

cut-offs (g/dL)	Digital (10-min)								Digital (5-min)							
	n=80				n=40				n=80				n=40			
	SEN	SPE	PPV	NPV	SEN	SPE	PPV	NPV	SEN	SPE	PPV	NPV	SEN	SPE	PPV	NPV
<11.0	65	84	52	90	65	57	52	68	65	83	50	90	67	57	52	68
<11.5	82	72	53	91	82	22	56	50	77	74	53	90	77	28	57	50
<12.0	93	73	66	95	93	9	73	33	86	67	63	85	86	9	76	14
<13.0	97	61	67	96	100	0	83	nc	97	59	66	96	100	0	83	nc
>16.0	0	100	nc	84	0	100	nc	68	0	100	nc	84	0	100	nc	68
>17.0	0	100	nc	95	0	100	nc	90	0	100	nc	nc	0	100	nc	90

Table 1. Sensitivity, Specificity and Predictive Accuracy of Digital Readings of Hemoglobin Concentration at Different Diagnostic Cut-off Criteria for Anemia Classification. SEN = sensitivity | SPE = specificity | PPV = positive predictive value | NPV = negative predictive value For cut-offs <13 g/dL the female subpopulation (n=40) is used, corresponds to the first 4 data rows;For cut-offs >16 g/dL the male subpopulation (n=40) is used, corresponds to the last 2 data rows.

Giardia prevalence estimates in Quetzaltenango children in day-care centers using two diagnostic methods for coproantigens: Enzyme-Linked Immunosorbent Assay (ELISA) and color indicator strips (DIPSTICKS)

Gabriela Montenegro-Bethancourt, María José Ríos, and Gloria Hidalgo

Giardia intestinalis, is responsible for more than 10% of diarrhea cases around the world (WHO) [Kosek et al, 2003). Traditional diagnosis for *giardiasis* consists of microscopic examination of fecal samples. This method has low sensitivity, when a single fecal sample is examined, but when 3 separated stool samples are examined 85-90% of cases are detected. This is however, not practicable, time consuming and requires skilled technicians. Immunoassays are more sensitive and specific in Giardia detection. Highly sensitive and specific enzyme-linked immunosorbent assays (ELISA) that detect an antigen of *Giardia* in stool samples can also be used for this purpose. However, the high costs and the need of specialized equipments limits the use of ELISA kits, and other methods. With the idea of practicability, commercial kits have been developed to enhance the rapid detection of *Giardia*. One of this is the RIDA® Quick Giardia, R-Biopharm, with higher sensitivity to detect infections in stool specimens even during the absence of cyst passage or visible signs of trophozoites (García et al, 2000; Youn et al, 2009). This test detects cell wall proteins of Giardia cysts and trophozoites using monoclonal antibodies.

The objective of this study was to evaluate the RIDA® Quick Giardia test as a rapid method for diagnosis of Giardiasis in children, and ELISA coproantigen test (Prospect® *Giardia* Microplate Assay) as the gold standard.

Fecal samples from 172 children (93 girls and 79 boys), aged 18-72 m, from 2 urban and 6 rural day care centers were collected. Samples were collected by parents in a special fecal collection container and frozen at -20 ºC until analyzed. Stool samples were analyzed with the ProSpec T® Giardia Microplate Assay (Alexon Trend Minnesota, USA) ELISA method in a single-assay. Samples that tested positive with the ELISA method were then analyzed with the RIDA®-Quick Giardia dipsticks (R-Biopharm AG, Darmstadt, Germany). For the ELISA tests, a cut-off point was calculated to determine positive and negative cases. Only ELISA positive samples were used for RIDA® analysis. Inmunocromatographic results were expressed on a visual scale as positive, if 2 lines showed (control and sample), and, negative when only one line appeared (control).

Diagnosis of giardiasis by ELISA for copro-Ag detection was positive in 51 cases (29.7%). Prevalence by area was 25.5% in the urban and 31.2% in the rural population. Of the 51 cases that testes positive with the ELISA, 26 (51.0%) teste positive with the RIDA® test. Except in one case, it was noticed that values with absorbance > 2.099 with ELISA tested positive with RIDA®. Overall, 26 samples were positive with both methods.

We conclude that when available, ELISA can be a simple, rapid, and sensitive method for the detection of *G.intestinalis*, even in a single fecal analysis. Prevalence rates with ELISA where proportionally higher (2 folds) compared to the other method. Similar studies comparing those two methods, attribute differences based on the fact that ELISA test is able to detect a *Giardia* antigen (GSA65) that is a glycoprotein common to all the isolates of *Giardia* spp., whereas only cell wall proteins of Giardia cysts and trophozoites are detectable by the immnunochromatographic kit. However, due to its simplicity, RIDA Quick Giardia Test System ®, can be a valuable tool for initial giardiasis screening, especially at field work where no other methods are available. Low concentrations of parasitic cysts in the samples can reduce the sensitivity of the RIDA®. Cost and availability of the commercial kits, might limit its use in routine testing in developing countries. Potentially, these methods should be seen as supplementary/complementary methods to the traditional microscopy method (with multiple samples), in unprocessed stool samples.

References:

Kosek M, Bern C, Guerrant RL. The global burden of diarrhoeal disease, as estimated from studies published between 1992 and 2000. Bull World Health Organ. 2003;81(3):197-204.

Garcia, L.S,R y Shimizu and C.N. Bernard. 2000. Detection of *Giardia lamblia, Entamoeba histolytical/Entamoeba dispar,* and *Cryptosporidium parvum* antigens in human fecal specimens using the Triage parasite panes enzyme immunoassay. J Clin Microbiol. 2000; 38:3337-40

Youn S, Kabir M, Haque R, Petri WA Jr. Evaluation of a screening test for detection of giardia and crystoporidium parasites. J Clin Microbiol; 2009; 47:451-2

Gabriela Montenegro Bethancourt is a Research Nutritionist at CeSSIAM

María José Ríos, is a Physician at CeSSIAM

Gloria Hidalgo is Chemical Biologist, Head of the Clinical Laboratory, Hospital La Democracia, Quetzaltenango

Introduction of first foods to Guatemalan infants

María José Soto-Méndez, Raquel Campos, Liza Hernández, and Noel W Solomons

The WHO recommends exclusive breastfeeding until 6 mo and adequate complementary feeding (CF) thereafter. The choice of CF, and associated nutrient intake, is related to local traditions and the economic status of the caretaker. As part of a larger study to determine the contribution of nutrients from CF, we examined the timing and choice of the first foods introduced to infants in Guatemala.

Sixty-four infants from Santo Domingo Xenacoj, a rural area of Guatemala, and 64 infants from Guatemala City, an urban area, participated in the study. Infants were aged 6 to 11 mo upon recruitment. As part of an extensive interview with the mothers, the specific questions utilized for our aim were the age of introduction of the first food, the first foods the mother or caretaker gave to the children and the person who advised it.

In the rural area the first food item had been offered to the infant between the 3rd to the 9th mo of age (median, 5th month). Rural mothers often mentioned a number of different items in the category of "first food" when presented with the question. The most commonly named item, among the 30 different items mentioned, was white bread roll (**Figure 1**). The three persons who were most influential in suggesting the timing and the nature of the first food were ranked as: maternal grandmother (38%), sister (23%), and the mother herself (22%).

Infants living in the urban area had received their first food, anywhere between the 2nd to the 9th mo, (median, 5th mo). Urban women named only one food or beverage item in response to the query on the first food offered to the infant, namely commercial baby food (**figure 2**). When asked who had made the determinant suggestion as to what would be the first food given to the child and when, 45% listed the maternal grandmother, and 25% listed themselves.

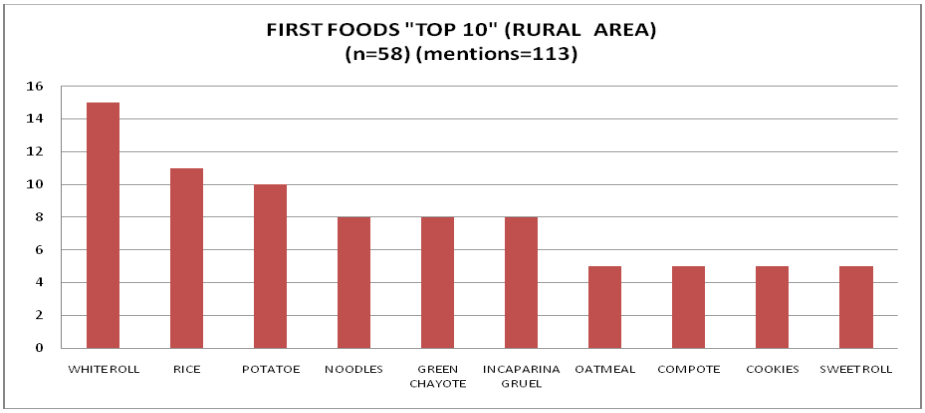
In conclusion, we can confirm that complementary foods were not introduced in accordance to the WHO recommendations in terms of timing or choice of food items given. Interestingly, commercial or industrialized foods were often the first foods to be introduced in both areas examined.

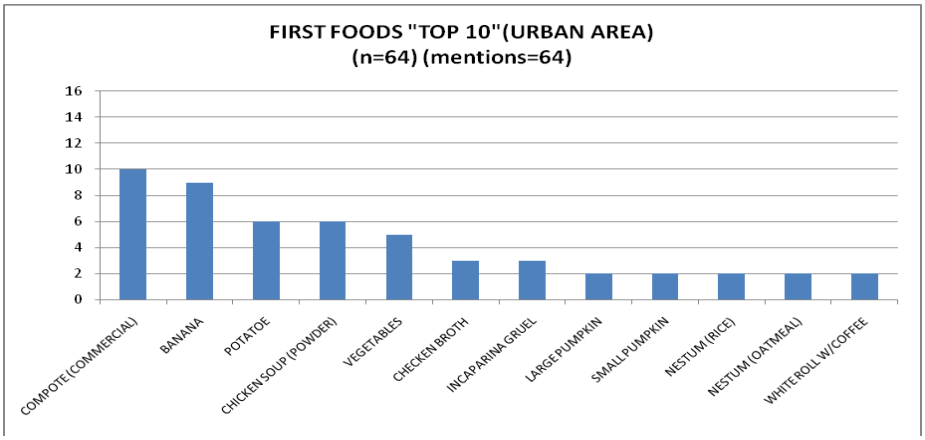
Reference:

Food And Agriculture Organization of the United Nations, World Health Organization & United Nations University (2001). Report of a Joint FAO/WHO/UNU Expert Consultation. Rome: FAO/WHO/UNU.

María José Soto , Raquel Campos and Liza Hernández are Research Nutritionists at CeSSIAM

Noel W Solomons is the Scientific Director of CeSSIAM





Figures 1 and 2: Ten food items most commonly mentioned as "first introduced foods" by caretakers in the rural and urban areas, respectively

This document was created with Win2PDF available at http://www.win2pdf.com.
The unregistered version of Win2PDF is for evaluation or non-commercial use only.
This page will not be added after purchasing Win2PDF.