Measuring effective coverage of measles immunization in low-resource settings: A method and application from the evaluation of Salud Mesoamérica 2015

Katherine Ellicott Colson

A thesis
submitted in partial fulfillment of the
requirements for the degree of

Master of Public Health

University of Washington
2014

Committee:
Bernardo Hernández-Prado
Ali Mokdad
Stephen Lim

Program Authorized to Offer Degree:
Master of Public Health
School of Public Health—Department of Global Health

University of Washington

Abstract

Measuring effective coverage of measles immunization in low-resource settings: A method and application from the baseline evaluation of Salud Mesoamérica 2015

Katherine Ellicott Colson

Chair of the Supervisory Committee:
Bernardo Hernández-Prado, Associate Professor
Department of Global Health

Background: Estimating vaccination coverage is challenging in resource-poor settings where accurate records are sparse. Household surveys, a key source of coverage information, typically capture data from child health cards and rely on maternal recall when cards are unavailable. Little is known about the accuracy of these sources in low- and middle-income countries. Moreover, such metrics provide estimates of crude coverage (vaccination), but do not capture the gap between crude and effective coverage (seroconversion). In this thesis, I harness data from the baseline evaluation of Salud Mesoamérica 2015 to compare survey-based estimates of measles immunization coverage to the seroprevalence of anti-measles antibodies in dried blood spots (DBS) collected from children in poor areas of Mexico and Nicaragua. This thesis addresses gaps in knowledge regarding the feasibility and validity of using DBS methods in low-resource settings, the accuracy of survey-based measures of vaccination coverage, and the prevalence of biological protection against measles (effective coverage) in the study areas.

Methods: Data for this study were collected as part of the baseline evaluation of Salud Mesoamérica 2015. Among the poorest 20% of the population in Mexico and Nicaragua, probability samples of 5,428 and 2,071 households (respectively) with resident women of reproductive age (15 to 49 years) or children under 5 years of age were selected for interviews. Surveys addressed maternal and child health topics such as antenatal and delivery care, immunization, child nutrition, and recent illness. During home-based interviews, consent was obtained to collect DBS, from children aged 12 to 23 months. ELISA methods for measuring the seroprevalence of measles immunoglobulin G (IgG) antibodies in dried blood spots were validated by comparing test results for matched serum and DBS samples. After establishing the validity of the method, I compare the levels and characteristics of measles vaccination coverage estimates based on maternal recall, health card documentation, and antibody presence. I assess how survey-based estimates of measles immunization coverage differ from serology-based estimates, and identify individual, maternal, household, community and health facility characteristics of children that have health card documentation of receiving measles immunization but who lack antibodies.

Findings: In both countries, survey-based sources of measles vaccination coverage were significantly higher (83% in Mexico and 85% in Nicaragua) than the seroprevalence of protective antibodies (68% in Mexico and 50% in Nicaragua). A large proportion of children in both settings (19% in Mexico and 43% in Nicaragua) had health card documentation of having received measles vaccine, but lacked antibodies. These discrepancies were geographically concentrated in several specific areas, particularly the North Atlantic Region of Nicaragua, suggesting that these differences are driven by variations in health service delivery rather than individual or household characteristics. In multivariate regression analysis, few factors aside from geography significantly increased the likelihood that a health card-positive child would lack antibodies. Clear and consistent geographic

patterns in both crude and effective coverage highlight that some surveyed areas are very high-performing while others are experiencing substantial challenges.

Conclusions: This study shows that using health cards and/or caregiver recall to estimate population protection against measles is unwise, because these sources overestimate effective coverage of measles immunization. Results from this study are of great importance as they point the areas and groups where protection against measles may have been wrongly assumed. These findings will enable the health authorities in Chiapas and Mexico to examine and address the reasons for the differences between crude and effective coverage.

Acknowledgements:

This study was funded by the Bill & Melinda Gates Foundation, the Spanish Agency for International Development Cooperation, and the Carlos Slim Health Institute, through the Inter-American Development Bank. I thank all the children and families who willingly participated in the study; local governments for the support extended to the study team; the ECOSUR and CIES-UNAN survey teams and supervisors for their contributions to data collection; Carlos Conde, María Olamendi-Portugal y Santa García-Cisneros at INSP for their contributions to dried blood spot laboratory analyses; Diego Ríos-Zertuche, Paola Zúñiga-Brenes, and Emma Iriarte at the Inter-American Development; Alan Potter at the Department of Laboratory Medicine at the University of Washington; and Marielle Gagnier, Erin Palmisano, Annie Haakenstad, and Brent Anderson at the Institute for Health Metrics and Evaluation. I would especially like to thank my committee members, Bernardo Hernandez, Ali Mokdad, and Stephen Lim for their generous time, guidance, and encouragement.

Table of Contents

Abstract 3

Acknowledgements: 6

Table of Contents 7

List of Tables 9

List of Figures 10

Chapter 1: Introduction 11

Chapter 2: Validation Study 14

Background 14

Methods 15

Sample collection 15

Laboratory procedures 17

Analysis 18

Results 20

Validation 20

Field-study 23

Discussion 28

Chapter 3: Applied Studies: Mexico and Nicaragua 32

Introduction 32

Methods 34

Study design and participants 34

Outcomes 36

Statistical Analyses 37

Results: Mexico 40

Results: Nicaragua.. 50

Chapter 4: Discussion and Conclusions 61

References 68

List of Tables

Table 1: Summary of study areas and sample 16

Table 2: DBS seropositivity sensitivity and specificity 22

Table 3: Summary of measles-specific IgG titers in DBS and DBS-matched serum 23

Table 4: Assay precision and reliability 23

Table 5: Characteristics of field study participants 25

Table 6: Household characteristics of the study sample in Chiapas 42

Table 7: Health facility characteristics of the study sample in Chiapas 43

Table 8: Relationship between crude and effective coverage of measles immunization in Chiapas ...45

Table 9: Crude and adjusted odds ratios of likelihood that card-positive children lack antibodies, Chiapas 49

Table 10: Household characteristics of the study sample in Nicaragua 52

Table 11: Health facility characteristics of the study sample in Nicaragua 53

Table 12: Relationship between crude and effective coverage of measles immunization in Nicaragua 55

Table 13: Crude and adjusted odds ratios of likelihood that card-positive children lack antibodies, Nicaragua 60

List of Figures

Figure 1: Linear relation between standardized OD values representing anti-measles IgG titers of matched DBS and serum samples 21

Figure 2: Distribution of DBS serum-equivalent values across microtiter plates 26

Figure 3: Distribution of 0 – 2.0 DBS serum-equivalent values across microtiter plates 26

Figure 4: Mean DBS serum-equivalent values by time between collection and processing 27

Figure 5: Mean DBS serum-equivalent values by altitude of sample collection 27

Figure 6: Mean DBS serum-equivalent values by hemoglobin concentration 28

Figure 7: Measles immunization coverage by source in Chiapas 45

Figure 8: Crude and effective coverage of measles immunization by municipality in Chiapas 47

Figure 9: Proportion of card-positive children lacking antibodies in Chiapas 48

Figure 10: Measles immunization coverage by source in Nicaragua 55

Figure 12: Crude and effective coverage of measles immunization by municipality in Nicaragua 57

Figure 13: Proportion of card-positive children lacking antibodies in Nicaragua 58

Chapter 1: Introduction

Estimating vaccination coverage is challenging in resource-poor settings where accurate records are sparse. Household surveys, a key source of coverage information, typically capture data from child health cards and rely on caregiver recall when cards are unavailable. Little is known about the accuracy of health cards and caregiver recall in low- and middle-income countries (LMIC), how accuracy may vary across demographics and settings in LMIC, or how crude coverage of vaccination compares to effective coverage (seroconversion) across populations and geographies. Errors may arise when caregiver recall is incorrect, health cards are lost, damaged or incomplete, errors are made during data entry, or confusion arises surround vaccination campaigns.[1] A recent review identified studies that compared vaccination information from health cards and caregiver recall to health provider sources (medical records, registries, direct observation) and found poor concordance, with median differences in coverage estimates ranging from -40 to +56 percentage points [2]. Furthermore, of the 45 studies meeting inclusion criteria, only 5 came from LMIC. Population-based surveys, particularly the USAID-funded Demographic and Health Surveys (DHS) and the UNICEF-funded Multiple Indicator Cluster Surveys (MICS), provide critical information on many public health indicators including vaccination, but are still reliant on health cards and caregiver recall.[1] There is a critical need to evaluate the accuracy of these sources, particularly in LMIC.

In addition to concerns about the accuracy of vaccination coverage estimates based on health cards and caregiver recall, such metrics provide estimates of crude coverage (vaccination), but do not capture the gap between crude and effective coverage (seroconversion). Effective coverage addresses the quality and health benefit of the service delivered, and only effective coverage captures biological protection to vaccine-preventable disease. In addition, immunity to vaccine-preventable diseases may wane in the years following vaccination,[3] and only serological studies can identify when this is the case, and if additional doses are needed. Measurement of effective coverage of immunization is extremely important for evaluating immunization programs and preventing outbreaks.

Because of the limitations of survey-based estimates of vaccination coverage and because of the value of effective coverage estimates, there is increasing interest in serological assays that measure immunity to vaccine-preventable diseases. Dried blood spots (DBS)—drops of blood collected from a finger stick and dried on filter paper—offer a promising, minimally invasive method for collecting blood samples for the measurement of biomarkers in low-resource settings. DBS are easy to transport and store, can be combined with other point-of-service tests such as hemoglobin measurement, do not require a centrifuge or immediate freezing, have less stringent regulations on shipment, and can be frozen for long periods of time.[4] Driven by recent advancements in methodology and sample collection in nonclinical arenas, a growing number of population surveys in developed and developing country settings are collecting DBS for the measurement of biomarkers including antibodies to infectious agents, HIV viral load and drug resistance, cholesterol, c-reactive protein, hormone levels, and metabolic and immune system indicators.[5–7]

In settings with a high burden vaccine-preventable illness, results from serosurveys can be used to track infections and herd immunity. In settings with low burden, serosurveys can be used to measure effective coverage of immunization, to assess the effectiveness of vaccination programs, and to evaluate the accuracy of other survey-based measures of vaccination coverage. For example, in Mexico and Nicaragua only three total measles cases have been confirmed in the past seven years, and therefore the vast majority of children with anti-measles antibodies will have acquired them through vaccination. Hence, the seroprevalence of protective antibodies to measles serves as a good indicator of vaccination program success.

In this thesis, I harness data from the baseline evaluation of Salud Mesoamérica 2015 to compare survey-based estimates of measles immunization coverage to the seroprevalence of anti-measles antibodies assessed using DBS methods, among children in poor areas of Mexico and Nicaragua. This thesis addresses gaps in knowledge regarding the feasibility and validity of using DBS methods in low-resource settings, the accuracy of survey-based measures of vaccination coverage, and the prevalence of biological protection against measles (effective coverage) in the study areas.

In Chapter 2, I describe the motivation, methods, and results of a study to validate a method for measuring the seroprevalence of measles immunoglobulin G (IgG) antibodies using dried blood spots collected in field surveys. After establishing the validity of the method, in Chapter 3, I compare the levels and characteristics of measles vaccination coverage estimates based on maternal recall, health card documentation, and antibody presence. In this section, I seek to answer the following questions:

1. Can the seroprevalence of measles antibodies be measured accurately using dried blood spot samples collected in remote field conditions?
2. How do survey-based estimates of measles immunization coverage differ from serology-based estimates?
3. What are the individual, maternal, household, community and health facility characteristics of children that have health card documentation of receiving measles immunization but who lack antibodies?

Finally, in Chapter 4, I interpret the findings from the applied studies, discuss the larger implications of the studies, and draw conclusions.

Chapter 2: Validation Study

Background

Measles is a highly infectious viral disease that causes more than 125,000 deaths around the world each year, most in children under five years of age.[8] Although measles was eliminated from the Americas in 2002,[9] the region remains vulnerable to imported cases and outbreaks, particularly in population clusters with low vaccination coverage. There are many poor, remote areas in Mesoamerica where little is known about population immunity to measles. Existing data is commonly derived from household surveys and public health records of immunization and disease, sources which are subject to bias and do not capture the gap between coverage (vaccination) and effective coverage (seroconversion).[10] This data can be significantly enhanced by measuring anti-measles antibody titers. Titer is typically determined by laboratory analysis of venipuncture blood serum, a standard practice in seroepidemiological monitoring. However, due to expense and logistical challenges, venipuncture blood is seldom collected in field surveys.[4,11,12] An alternative is to collect dried blood spots (DBS), drops of capillary blood obtained from finger or heel stick and dried on filter paper. DBS collection is an affordable, minimally invasive method of obtaining blood in non-clinical settings. DBS are simple to handle, and unlike conventional blood samples, do not require immediate centrifugation or freezing, are less biologically hazardous (resulting in non-stringent shipping regulations), and can be stored for long periods.[4] These advantages as well as ongoing improvements in assay methodology have led an increasing number of population surveys in both developed and developing countries to collect DBS. Biomarkers measured in DBS include HIV load and drug resistance, cholesterol, c-reactive protein, levels of hormones, and indicators of metabolic and immune system function, and antibodies to infectious agents.[5–7] There is, however, a need to develop additional methods of analyses; this led to this study's evaluation of a novel method of measuring anti-measles virus antibodies in DBS.

Previous DBS-based studies measuring measles immunity have evaluated methods for quantifying immunoglobulin M (IgM),[13–17] a biomarker of recent exposure to the virus, and immunoglobulin G (IgG),[14,17–23] a biomarker of past exposure to the virus or vaccine. IgG is most relevant for evaluating the effectiveness of immunization programs. The methods employed in the IgG-based studies included hemagglutination inhibition,[24–26] dot immunobinding,[21] RNA detection,[13–16,27,28] and enzyme immunoassays (EIA).[14,17,20,23] The EIA-related enzyme-linked immunosorbent assay (ELISA) is an attractive method given the commercial availability of a variety of kits and hence suitability for use in cross-study and cross-laboratory comparisons. In DBS, ELISA-based measurements of measles-specific IgG has been limited to studies using the Dade Behring Enzygnost ELISA,[18,22,29] there is therefore a need to evaluate additional ELISA kits to avoid dependence upon a single source. Further, since the DBS analyzed in one of these studies[22] was from venous blood, there is a need for additional evaluation of commonly-collected capillary DBS. I thus chose to validate an alternate commercial ELISA for measuring measles-specific IgG in capillary DBS, performing determinations of accuracy, precision, reliability, and feasibility, and comparing results from matched DBS and serum samples. The method was then applied to DBS collected during a large field study of one-year-old children targeted by immunization programs and living in low-resource settings in Mexico and Nicaragua.

Methods

Sample collection

DBS were collected by El Colegio de la Frontera Sur-Mexico (ECOSUR) and El Centro de Investigación y Estudios en Salud at the National Autonomous University of Nicaragua (CIES-

UNAN) as part of the baseline evaluation of Salud Mesoamérica 2015 (SM2015). SM2015 is a results-based financing initiative aimed at improving child and maternal health in the poorest populations in eight Mesoamerican countries, and uses the seroprevalence of measles antibodies as a primary indicator of health system performance. In designated municipalities in Nicaragua and in the Mexican state of Chiapas, localities (villages or towns) were selected with probability proportional to size. In selected localities, a complete census of housing and population was conducted to identify the most accurate and up-to-date sampling frame. Following the census, 30 households with resident women of reproductive age (15 to 49 years) or with children under 5 years of age were selected for interviews regarding maternal and child health topics such as antenatal and delivery care, immunization, child nutrition, and recent illness Surveys were conducted using computer-assisted personal interview (CAPI) programmed on DatStat Illume installed on Netbooks. The use of CAPI allowed instantaneous data transfer via a secure EMBED to the Institute for Health Metrics and Evaluation (IHME) where was continuously monitored for quality. During home-based interviews, consent was obtained to measure the height, weight, hemoglobin concentration, and to collect DBS, from children aged 12 to 23 months (nominally one year old); DBS were obtained from 71.9% (n=1134) and 86.2% (n=376) of the eligible children in Chiapas and Nicaragua, respectively. The final samples are summarized in Table 1.

Table 1: Summary of study areas and sample

	Mexico	**Nicaragua**
Designated SM2015 municipalities	56	23
Total SM2015 segments	8,163	1,455
Total segments in sample	181	90
Households censused	24,349	8,864
Households interviewed	5,428	2,071
Women interviewed	6,988	2,823
Children (aged 0 to 59) captured	6,499	2,236
DBS collected	1,134	454

Standard DBS collection procedures have been described elsewhere.[4] Briefly, the heel or finger was cleaned with alcohol and then punctured with a disposable lancet. Resulting blood drops were allowed to fall onto demarked circles on Whatman 903 filter paper. The DBS card was dried at room temperature for two hours and then inserted into a plastic bag with a desiccant, stored at 10-12°C in refrigeration for 15 to 20 days, and then shipped refrigerated to the National Institute of Public Health (INSP) Laboratory in Cuernavaca, Mexico.

For method validation analyses, venous blood samples and matched capillary DBS were collected from fifty children aged 8-22 months attending a pediatric hospital in Tuxtla Gutiérrez. Serum was isolated from the blood and stored frozen, and DBS were handled as above, by the same field workers, before shipment to INSP.

Permission to implement this research project was obtained from the Ministry of Health of Mexico. Ethical approval for this study was obtained from the institutional review boards of the University of Washington, the Mexico National Institute of Public Health (INSP), and the data collection agency El Colegio de la Frontera Sur-Mexico (ECOSUR). Participants in household surveys provided written informed consent. The study was done in compliance with national regulatory and ethics guidelines.

Laboratory procedures

INSP stored serum and DBS at -20°C until processed 0 to 12 months after collection. Samples were assayed for the presence of measles-specific IgG antibodies using the HUMAN Worldwide Diagnostics Measles IgG ELISA kit (Wiesbaden, Germany) consisting of buffers, reagents, and a 96-well microtiter plate pre-coated with measles antigen. A single blank substrate and duplicate positive and negative controls (calibrated against the International World Health Organization (WHO)

Preparation for Anti-Measles Serum) were assayed on each plate alongside study samples. The plates were processed through a series of incubation, wash, and reaction steps, ultimately resulting in a color change. The degree of color development of each well, which is directly proportional to the measles IgG antibody concentration in the specimen, was measured spectrophotometrically (Instrument Labsystems TEMS Multiskan MS, Helsinki, Finland). The optical density (OD) of the positive and negative controls was used to evaluate assay acceptability as well as to establish plate-specific positive and negative cutoff values. An assay was considered acceptable if the OD of the blank substrate was less than 0·150, the mean of the negative controls (MNC) was less than 0·250, the mean of the positive controls (MPC) was greater than 0·750, and the MPC to MNC ratio was greater than 5. Each assay conducted met these criteria.

In accordance with HUMAN's instructions, venous blood serum was diluted 1:100 with kit-supplied buffer. DBS samples were eluted based on the procedure described by Hogrefe et al.[30] To reconstitute DBS as hemolyzed liquid whole blood suitable for analysis, a 6mm diameter punch was eluted with 400µl phosphate buffered saline (PBS) for 14-18 hours at 6-8°C (500µl and 600µl PBS were found to reduce assay sensitivity). 100µl diluted serum or DBS eluate was pipetted into microtiter plate wells and the plate then processed as above. Equivocal results were retested and the second result was recorded as final. Technicians were blinded to the correspondence between DBS and serum samples.

Analysis

The blank substrate OD was subtracted from the OD of each well to correct for superfluous reactivity. Each well OD was then divided by the plate-specific cutoff value, MNC + (0.1*MPC), to standardize values across assays. A serum sample was considered measles-specific IgG positive if the standardized value was greater than 1.15, negative if less than 0.85, and equivocal if between these values.[31]

The efficacy of the DBS elution and assay procedures were assessed by comparing the results from analyses of the validation samples (Fig. 1). On the basis of previous work, data from these matched serum and DBS samples were expected to be linearly correlated.[4,32] The sensitivity, specificity, positive predictive value, and negative predictive value of the DBS assay were calculated using varying cutoff values and equivocal bandwidths established from the analyses of the serum samples (Table 2). Agreement of categorical (positive, negative, or equivocal) and quantitative results were determined using Cohen's kappa and Pearson's correlation coefficient, respectively (Table 3). The graphical association between DBS and serum values was also inspected for linearity.

The relationship between DBS and serum values was quantified using ordinary linear regression (OLS). Out-of-sample predictive performance was assessed by repeatedly withholding a random 20% of the data, fitting the linear model, and calculating the degree of agreement (kappa) between the serum and DBS categorical results. Models with the intercept suppressed and log-transformed independent and dependent variables were also evaluated. A linear model with intercept suppressed and untransformed inputs was selected based on superior in-sample (adjusted R^2) and out-of-sample predictive performance. This equation was used to convert the standardized values of the validation set DBS into serum-equivalent values, permitting direct comparisons between the DBS and serum. The equation was also used to convert the standardized values of the field-collected DBS, permitting calculation of measles-specific IgG seroprevalence.

Variations in the mean values of the SM2015 DBS versus the time between collection and analysis, child's altitude of residence, and hemoglobin concentration were examined. Multiple sensitivity tests were conducted to estimate the effect of various cutoffs and equivocal windows on overall seroprevalence.

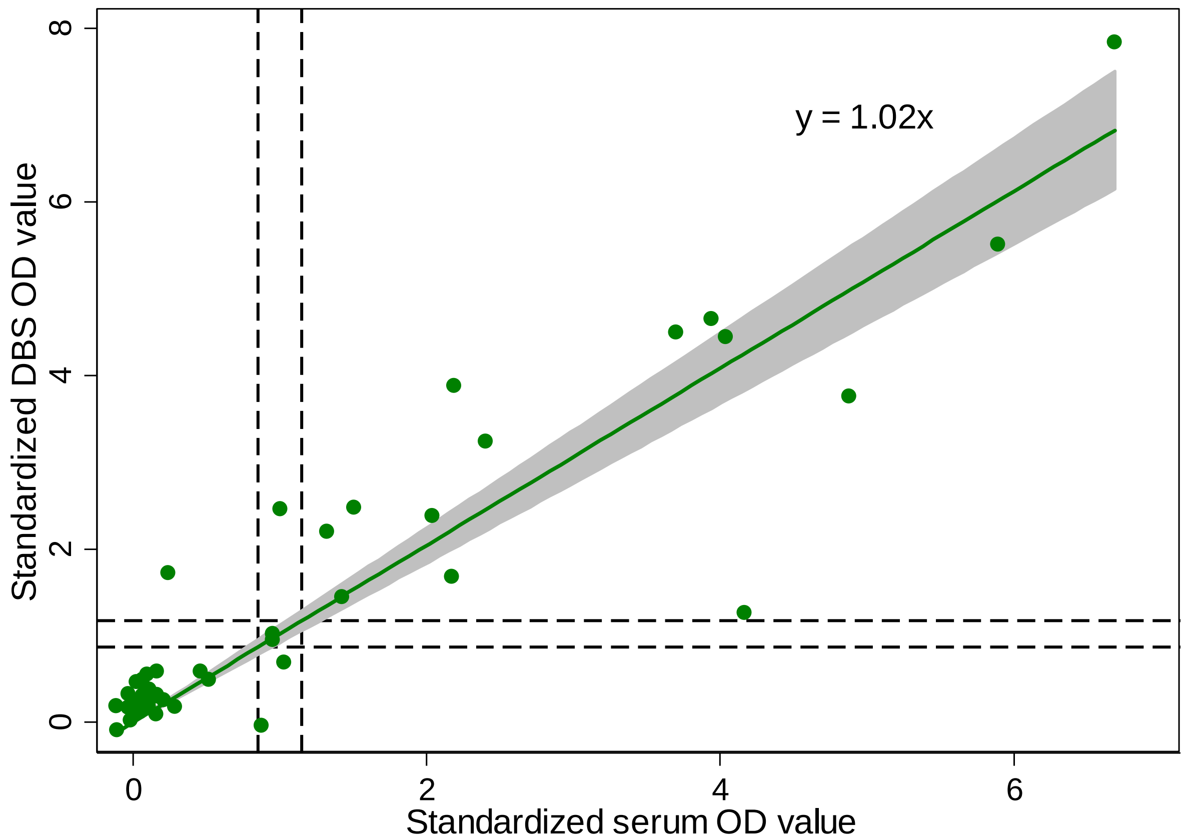
Analyses involved 23 assay runs, comprising 1439 single DBS, 149 duplicate DBS (i.e., result from first run equivocal), 23 duplicate positive and duplicate negative controls, and 18 replicates of a known positive DBS. Data from the repeatedly analyzed samples generated coefficients of variation (%CV) used to assess assay reliability and precision (Table 4). The lower limit of detection was defined as two standard deviations above the mean of the negative controls across all assays.[4]

Results

Validation

The donors of the validation set of samples (n=50) were 54% male with a mean age of 13.1 months (standard deviation: 4.5). No child had a reported history of measles infection, and 52% had a reported history of measles vaccination. Standardized values ranged from -0.11 to 6.69 for serum, and from -0.09 to 7.84 for DBS. Values from paired serum and DBS samples showed a high level of agreement and near direct equality (Fig. 1). Pearson's correlation coefficient was 0.92 (95% confidence interval (CI): 0.84 – 1.00). The intercept-suppressed conversion equation had an adjusted R^2 of 0.89 and a slope that was statistically indistinguishable from 1 (β=1.02, 95% CI: 0.92 – 1.12).

Figure 1: Linear relation between standardized OD values representing anti-measles IgG titers of matched DBS and serum samples



Dashed lines indicate the positive and negative cutoff values for measles-specific IgG seropositivity. The shaded area is the 95% confidence interval around predictions from the validation regression.

Maximal sensitivity and specificity were achieved with a cutoff between 1.00 and 1.20 with no equivocal window (Table 2). The width of the window had little effect except at very high or low cutoffs. For parsimony and consistency with HUMAN's instructions and the validation equation (Fig. 1), and because additional gains in sensitivity and specificity were negligible, a 1.02 cutoff and 0.15 equivocal window were selected. Using these parameters, the positive predictive value of the test was 87.5% and the negative predictive value was 93.8%.

Table 2: DBS seropositivity sensitivity and specificity

Cutoff (Standardized OD)	Equivocal window (+/- from cutoff)	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)
0.5	0	100	87.1	63.6	96.4
0.5	0.15	100	71	73.7	95.7
0.5	0.25	100	48.4	77.8	93.8
0.8	0	100	96.8	77.8	93.8
0.8	0.15	100	96.8	82.4	96.8
0.8	0.25	100	90.3	87.5	96.6
1	0	100	96.8	82.4	90.9
1	0.15	100	96.8	87.5	93.8
1	0.25	100	96.8	87.5	93.8
1.02	0	100	96.8	87.5	88.2
1.02	0.15	100	96.8	87.5	93.8
1.02	0.25	92.9	96.8	86.7	93.8
1.2	0	100	96.8	87.5	88.2
1.2	0.15	92.9	96.8	86.7	88.2
1.2	0.25	85.7	96.8	85.7	90.9
1.5	0	85.7	96.8	85.7	83.3
1.5	0.15	85.7	96.8	85.7	85.7
1.5	0.25	78.6	96.8	91.7	88.2

N = 50

There was a high level of agreement between results from analyses of the validation set DBS and DBS-matched serum (Table 3). The kappa was 0.85 (95% CI: 0.75 – 0.95) and the average misclassification from predictive simulations of the validation equation was 3.9% (not shown), indicating high concordance. Categorical results of measles-specific IgG in the serum samples were 62% negative, 10% equivocal, and 28% positive; the latter was significantly lower than the self-reported immunization coverage of 48%. This discrepancy between seroconversion and reported immunization may have been influenced by errors in self-report and/or from sample collection too soon after vaccination for development of a detectable IgG antibody titer.[33]

Table 3: Summary of measles-specific IgG titers in DBS and DBS-matched serum

DBS samples	Serum samples			
	IgG positive	IgG negative	IgG equivocal	*Total*
IgG positive	14	1	1	16
IgG negative	0	30	2	32
IgG equivocal	0	0	2	2
Total	14	31	5	50
Assay Parameters (95% CI)				
Kappa	0.85 (0.75, 0.95)			
% agreement	92.0 (84.5, 99.5)			
% sensitivity [a]	100 (99.7, 100)			
% specificity [a]	96.8 (91.9, 100)			

[a] Excluding equivocal results. 95% CI: 95% confidence interval.

Although the intra-assay %CV was low, the inter-assay %CVs were moderately high (Table 4). It is important to note that regardless of the elevated %CVs, sample categorization did not change with repeated measurements, and therefore the ELISA performed well within expectations for a semi-quantitative assay. Using the values of the standardized negative controls (mean 0.21 and standard deviation 0.14), the assay had a lower limit of detection of 0.47, which was well below the negative cutoff value of 0.85.

Table 4: Assay precision and reliability

Control type	Mean $\pm$ SD [a]	% CV	Sample size
Known positive DBS, inter-assay	3.50 $\pm$ 1.01	46.5%	18 runs
Kit positive control, inter-assay	1.90 + 0.47	24.8%	23 runs
Equivocal DBS, inter-assay	-	Mean: 30.7% Median: 25.8%	149 samples 2 runs per sample
Kit positive control, intra-assay	-	Mean: 8.4% Median: 3.0%	23 runs, 2 samples per run

[a] Units are OD values (non-standardized for kit-provided controls; standardized non-kit-provided controls)

Field-study

Table 5 shows the characteristics and DBS results for participants in the SM2015 study. The seroprotection of measles-specific IgG was 63.4% in Chiapas and 50.7% in Nicaragua. Although there was a large range of values across samples and assay runs, there was a clear distinction between the presence and absence of seroprotection (Fig. 2 and 3). The mean DBS serum-equivalent value is lower in Nicaragua, likely because immunization coverage is lower. In both countries, the sample represents poor, indigenous children aged 12 to 23 months living primarily in rural areas. Participants in Chiapas tended to live at higher elevations and to have lower rates of anemia than participants in Nicaragua. These two factors may have affected hematocrit (the proportion of whole blood that is erythrocytes rather than plasma) and thereby further increased the variation in the quantitative results. In the initial assays, there were 149 equivocal results (9.4%). After re-assaying these samples, 106 were clearly resolved and 43 remained indeterminate; there remains, however, a distinct divide between positive and negative samples (Fig. 2 and 3). Variations in the cutoff or the equivocal window (Table 2) had minimal effects (less than two percentage points) on the estimated prevalence of measles antibodies in the SM2015 population (not shown). The mean DBS serum-equivalent value did not statistically significantly differ nor systematically vary by time between sample collection and processing (Fig. 4), altitude of residence (Fig. 5), or hemoglobin concentration (Fig. 6).

Table 5: Characteristics of field study participants

Characteristic	Chiapas	Nicaragua
Sample size	1134	454
Age (months, mean $\pm$ SE)	17.5 $\pm$ 0.19	17.7 $\pm$ 0.23
Female (%)	50.3	50.0
Urban residence (%)	36.6	34.4
Indigenous [a] (%)	69.3	11.9
Altitude (meters, mean $\pm$ SE)	1184 + 21	632 + 17
Maternal education (% with high school education)	31.3	30.2
Household size (mean $\pm$ SE)	5.9 $\pm$ 0.07	6.0 $\pm$ 0.13
Anemia [b] (%)	32.7	47.1
Measles IgG positive (%)	63.4	50.7
Measles IgG negative (%)	34.6	44.9
Measles IgG indeterminate (%)	2.0	4.4
DBS serum-equivalent value (mean $\pm$ SE)	2.5 $\pm$ 0.07	1.4 $\pm$ 0.05

SD = standard deviation

[a] Child lives in household with indigenous language speakers

[b] Altitude-adjusted hemoglobin < 11.0 g/dl

Figure 2: Distribution of DBS serum-equivalent values across microtiter plates

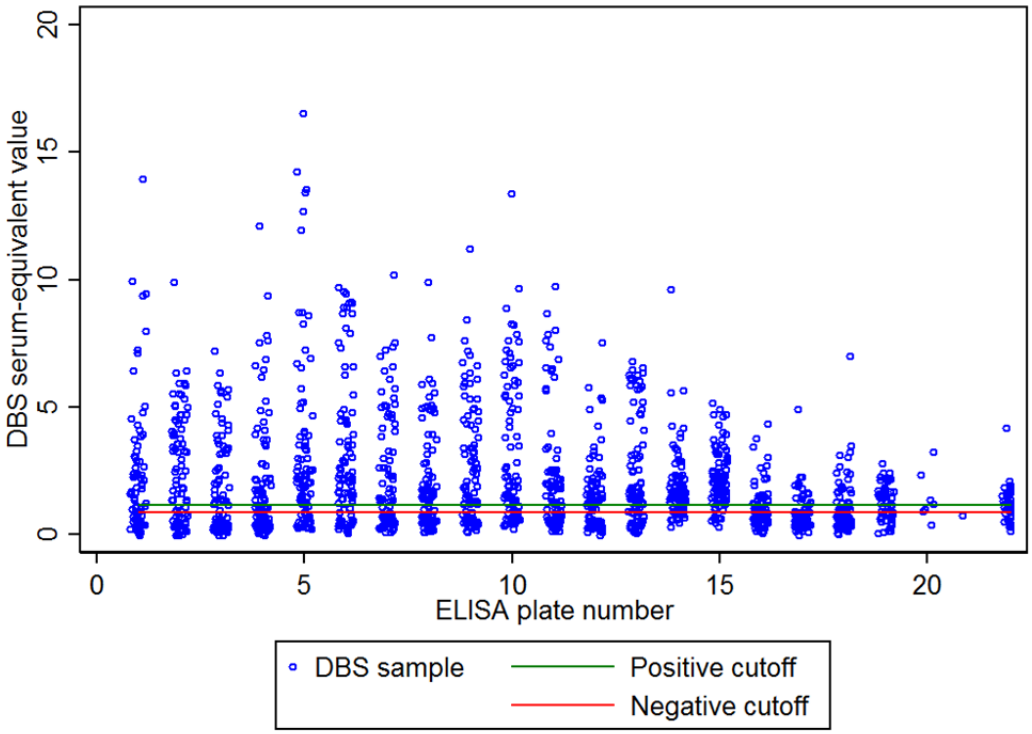


Figure 3: Distribution of 0 – 2.0 DBS serum-equivalent values across microtiter plates

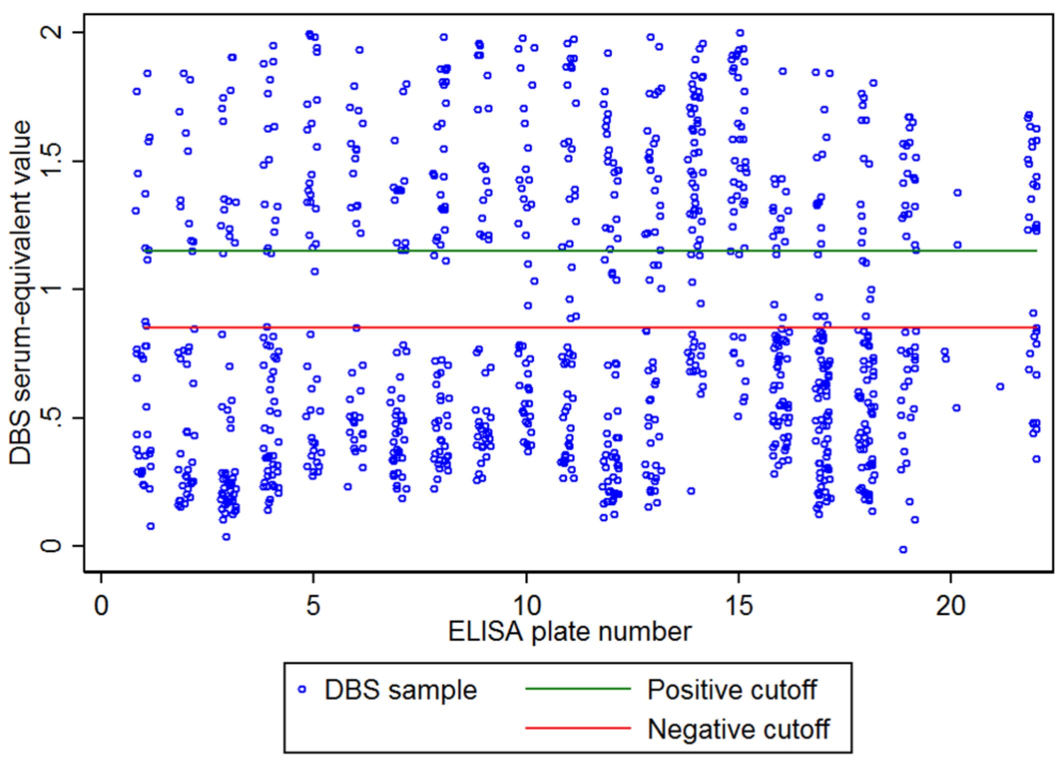
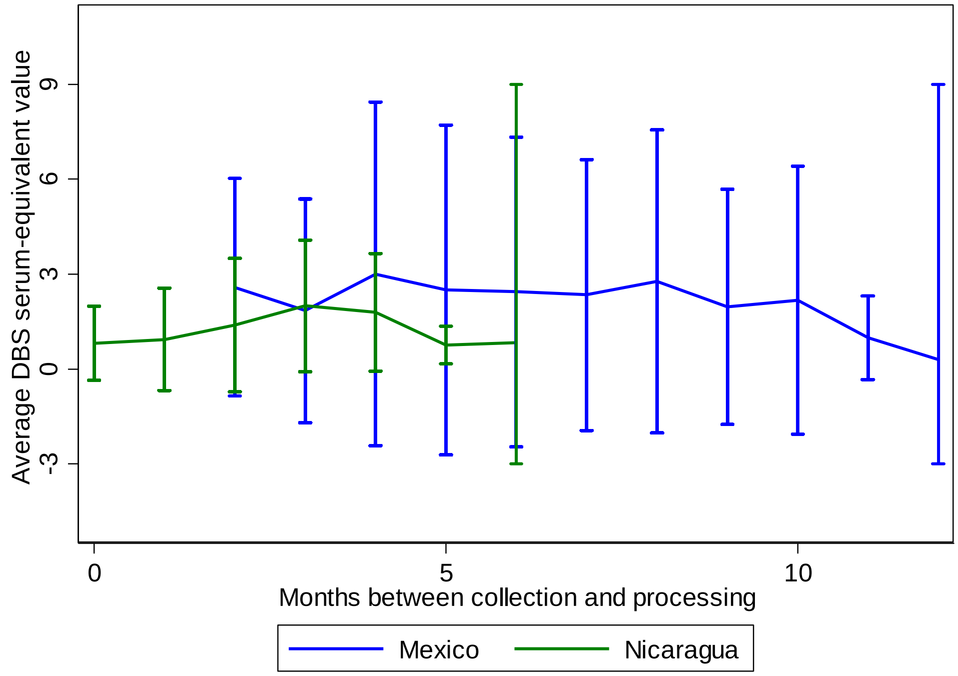
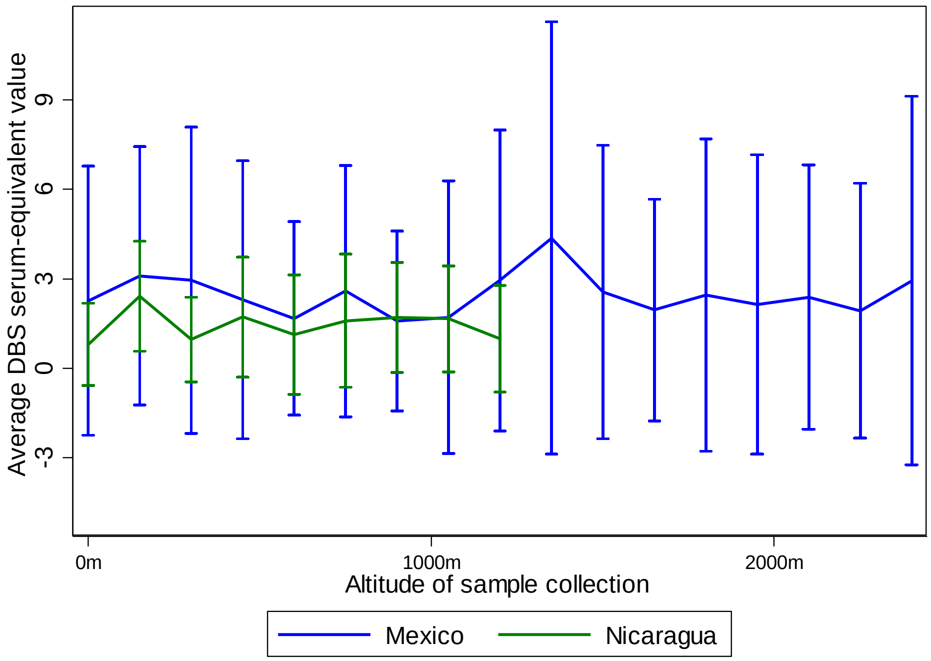


Figure 4: Mean DBS serum-equivalent values by time between collection and processing



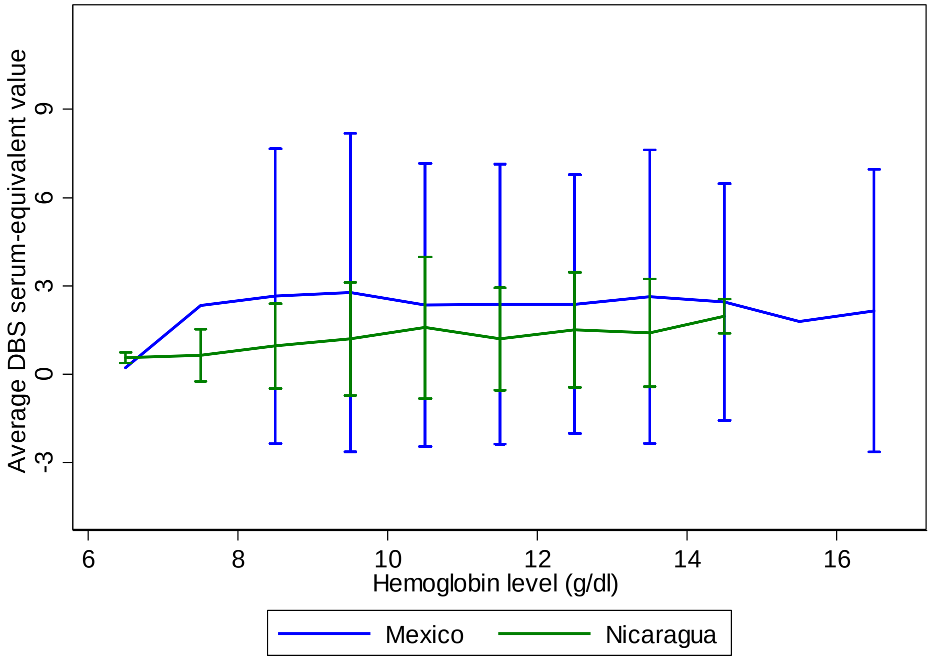
* Bars indicate 95% confidence interval

Figure 5: Mean DBS serum-equivalent values by altitude of sample collection



* Bars indicate 95% confidence interval

Figure 6: Mean DBS serum-equivalent values by hemoglobin concentration



* Bars indicate 95% confidence interval. Hemoglobin level is altitude-adjusted.

Discussion

This study establishes that the applied commercial ELISA test can be used to categorize individuals as having measles-specific IgG seroprotection through analyses of DBS. In support of this contention, I found that measles-specific IgG values in DBS and matched serum samples were strongly linearly related, highly correlated, and nearly directly equal. The capability of the HUMAN semi-quantitative ELISA to accurately categorize the level of measles-specific IgG in serum samples has been previously demonstrated (99-100% sensitivity and 91-100% specificity).[34] Here I similarly found that the HUMAN ELISA was suitable for evaluating DBS samples. Treating serum values as the standard, DBS results were shown to be 100% sensitive and 96.8% specific, and agreed in 46 of 50 (92%) cases. Thus the seroprotection categorization of the donors, which is of greatest clinical and policy relevance, from this study were highly consistent between serum and serum-matched DBS samples.

We demonstrated that it is feasible to conduct serosurveys using DBS samples collected in remote, low-resource settings. Similar to previous findings,[14–16,23,35] survey teams and participants found the DBS collection procedures to be feasible and acceptable. In the laboratory, use of a commercial ELISA was found to be practical and advantageous as all necessary supplies were provided, reducing time spent on validation and optimization. The regression equation generated from the validation study was applied to convert the measles-specific IgG values of the field study DBS samples into serum-equivalent measles-specific IgG values. Using this data, I observed a distinct division between protected and unprotected children. The results from the DBS were robust to the assay's lower limit of detection as well as to variations in cutoffs and equivocal windows. Re-assaying DBS with initially equivocal results enhanced the interpretability of the field findings.

This study had a few limitations. The results from DBS analyses using the HUMAN ELISA would be strengthened by validation against a second assay, particularly against the commonly used Enzygnost.[13,16,18,19,22,29] Although the high correlation I observed between serum and DBS indicates that the HUMAN ELISA was equally capable of recognizing measles-specific IgG in these sample types, additional research could substantiate that cellular material present in re-liquefied blood did not interfere with the detection of IgG in DBS. Serum collected and dried on separator cards[4] could help in that evaluation. While the HUMAN ELISA has been demonstrated to have minimal cross-reactivity with mononucleosis and to be unaffected by increasing anti-nuclear antibody titers,[34] and the use of negative controls adjusted for non-specific binding, the potential for cross-reactivity with other viruses or microbes cannot be fully discounted until further analyses are conducted.

Using a number of assumptions,[36,37] I expected 1.40 to 1.88μl serum to be present in a DBS sample well versus 1μl serum in a serum sample well. The observed 1.02 slope of the DBS versus serum comparison was then less than the expected 1.25 to 2.01, i.e., less DBS measles-specific IgG was recovered than anticipated. However, the high concordance between the results from DBS and DBS-matched serum suggests that the percentage of measles-specific IgG eluted from a DBS did not vary with concentration, and hence that any incomplete recovery did not affect the proficiency of the assay. The possible lack of full recovery may have stemmed from the use of potentially not fully optimized elution conditions; although these were similar to previous studies,[19] DBS elution has not yet been standardized. It is also possible that some loss of measles-specific IgG in DBS samples arose from sample degradation during collection, transportation, or storage. The lack of systematic variation in the mean values by duration of sample storage does, however, suggest that any sample degradation was minimal. In accord with this suggestion, previous studies have indicated that DBS can be frozen and stored for at least several months[14,20] and for as long as 2 years.[18,22] Additional research on sample stability in tropical low-resource settings where multiple cycles of freezing and thawing may occur would be beneficial.[4]

Immunization coverage in the Mesoamerican region is generally high, but this study highlights the importance of studying not only coverage, but also effective coverage (when assuming all seropositivity is due to vaccination, not disease exposure).[38] While studies of population measles serology in Mesoamerica have existed for more than 40 years,[39,40] and while the importance of serosurveys in tracking progress towards elimination is well established,[9,41] there has been little expansion or incorporation of serosurveys in routine monitoring. The procedures described herein can be incorporated into existing data collection protocols in resource-limited settings to monitor measles exposures, herd immunity, and effectiveness of immunization programs. These analyses provide a means of obtaining objective measures of health status and health system performance that overcome many challenges associated with using survey and administrative data alone. The use of DBS analyses will enhance future epidemiological studies and produce information that is particularly actionable by decision-makers in health.

Chapter 3: Applied Studies: Mexico and Nicaragua

Introduction

Measles is a highly infectious vaccine-preventable disease that causes more than 125,000 deaths around the world each year, most in children under five years of age.[8] Although endemic measles transmission was first interrupted in Mexico in 1997 and there have been no confirmed cases in Nicaragua since 1995, both countries remains vulnerable to imported cases and outbreaks, particularly in population clusters with low vaccination coverage.[42] Timely and accurate monitoring of vaccination coverage is critical for program planning and evaluation, resource allocation and prevention of future outbreaks. Indeed, in Mexico, lapses in coverage in poor, rural areas led to a devastating pandemic in 1989 and to reintroduction of endemic transmission in April 2000.[42] In both countries, existing data on vaccination coverage comes from national health surveys[43–48] which typically capture data from child health cards and rely on caregiver recall when cards are unavailable. These survey sources are subject to several sources of bias including sampling, misclassification, and measurement error.[10] Differences between survey-based estimates and other validating sources such as medical records range from -40 to +56 percentage points, a worrisome degree of inaccuracy. Administrative estimates are subject to error and bias from both numerator data (number of doses distributed) and denominator data (the number of persons who should have received the vaccine). Most importantly, these sources provide estimates of crude coverage (vaccination), but do not capture the gap between crude and effective coverage (seroconversion) [10].

There is growing interest in the use of seroepidemiological monitoring to monitor the effective coverage of immunizations including measles. [11,12,49,50] Dried blood spots (DBS), drops of capillary blood dried on filter paper, are an affordable, minimally invasive method of obtaining blood in non-clinical settings and are being used in an increasing number of population surveys in both developed and developing countries to measure biomarkers such as antibodies to infectious agents.[5,7] Two previous studies[18,29], as well as the study presented in Chapter 2, have validated methods for analyzing DBS for the presence of measles-specific immunoglobulin G (IgG) explicitly for this purpose. While these methods cannot distinguish between naturally occurring and vaccine-induced antibodies, in settings such as Mexico where measles incidence is low,[51] the vast majority of positive cases can be attributed to immunization. Hence, in low-measles settings, DBS are a valuable means of evaluating the accuracy of survey-based estimates of immunization coverage and the effectiveness of immunization programs.

To my knowledge, no previous studies have quantified the prevalence of protective antibodies against measles in a representative sample of children in poor regions of Nicaragua. In Mexico, several national health surveys[43,46] have collected DBS or blood samples, but to my knowledge, only one previous study[52] has quantified the prevalence of protective antibodies against measles in a representative sample of Mexican children. In this study, the proportion of seropositive samples from 1-4 year old children was 98.3%, and the authors concluded that vaccination coverage was adequate. While this result signals the success of the national immunization program, there is substantial evidence that national averages in health service delivery in Mexico mask significant subnational variation, with the poorest regions performing significantly worse than the wealthiest.[53–55] Indeed, the authors of the serological study suggested that 417,000 children are still susceptible, and it is likely that they are concentrated in poor, rural areas such as Chiapas.

In both countries, very little is known about the accuracy of crude immunization coverage estimates or the effectiveness of immunization programs in these areas. Moreover, little is known about why disparities between crude and effective coverage may exist, and in what populations these gaps may be concentrated. This lack of knowledge is particularly worrisome given that the communities most likely to experience outbreaks are also the populations for whom the least is known about population protection against measles.

To address this dearth of evidence, I aimed to quantify the difference between crude coverage and effective coverage of measles immunization among children aged 12 to 23 months in poor areas of Nicaragua and Chiapas, Mexico, and to identify individual, maternal, household, community and health facility characteristics that identify gaps between crude coverage and effective coverage. The study's findings provide new evidence to guide decision-making and to strengthen immunization programs for the underserved.

Methods

Study design and participants

Data for this study, including household and health facility surveys, were collected as part of the baseline evaluation of Salud Mesoamérica 2015 (SM2015). SM2015 is a results-based financing initiative aimed at improving child and maternal health in the poorest populations in eight Mesoamerican countries.

In designated SM2015 municipalities in the state of Chiapas in Mexico and the states of Jinotega, Matagalpa, Región Atlántico Norte (RAAN), Madriz, and Región Atlántico Sur (RAAS) in Nicaragua, localities were randomly selected for the evaluation with probability proportional to size using the most recent national census. A complete population census was conducted in selected localities and interviews were conducted with 30 randomly selected households with women of reproductive age (15-49 years) or children under 5 years old. Standardized multi-lingual household surveys were implemented using computer-assisted personal interview (CAPI) programmed on DatStat Illume installed on Netbooks. The use of CAPI allowed instantaneous data transfer via a secure EMBED to the Institute for Health Metrics and Evaluation (IHME) where was continuously monitored for quality.

The survey measured household characteristics and expenditure, access to health facilities, perceived barriers to care, exposure to social programs, women's educational and occupational backgrounds, current health status, recent illnesses and care received, pregnancy, reproduction, contraception, exposure to health interventions, antenatal, delivery and postnatal care, child nutrition, and immunization. Following home-based interviews with women and caregivers, professional nurses measured the height, weight and hemoglobin level of children less than 5 years old, and DBS samples from children aged 12 to 23 months. Methods employed for the collection and analysis of DBS have been described in detail elsewhere (reference methods paper). I determined that interviews with 4,734 and 2,464 households in Mexico and Nicaragua, respectively, were necessary to attain 80% power with alpha of 0.05 to detect targeted changes in key indicators for the initiative.

We identified health facilities operated by the Ministry of Health that serve populations in SM2015 municipalities using government-provided referral network data. In each country, ninety facilities were selected for interview, consisting of all facilities providing comprehensive and basic essential obstetric care services (31 in Mexico and 29 in Nicaragua) and a random sample of ambulatory facilities (with priority given to facilities nearest to surveyed households). In Mexico, facilities operated privately or by the Mexican Social Security Administration were not included because no SM2015 interventions occurred in these facilities. To assess facility policies, staffing and infrastructure, service provision and utilization, and supplies and functioning of key equipment and drugs, interviews with facility managers, an observation checklist, and administrative and medical records review were conducted. In particular, the survey recorded vaccination procedures and supplies, functioning of equipment used to store vaccines, temperature monitoring records for vaccine storage equipment, and temperature of all refrigerators at the time of the survey, obtained either from a thermometer outside the refrigerator, or from the monitoring charts for cases in which the thermometer was located inside the refrigerator.

Due to safety concerns in the department of Jinotega and RAAN in Nicaragua, data collection was halted before all selected localities and health facilities could be surveyed. Therefore, surveys were completed with 2,052 households (83% coverage) and 64 health facilities (71% coverage). To confirm that no bias was introduced, I compared socioeconomic, health services and demographic characteristics of surveyed and non-surveyed areas and found no differences between them.

Permission to implement this research project was obtained from the Ministries of Health of Mexico and Nicaragua. Ethical approval for this study was obtained from the institutional review boards of the University of Washington, the Mexico National Institute of Public Health (INSP), the data collection agency El Colegio de la Frontera Sur-Mexico (ECOSUR), and Nicaragua Center for Research and Health Studies (CIES) and the National Autonomous University of Nicaragua (UNAN). Participants in household and health facility surveys provided written informed consent. The study was done in compliance with national regulatory and ethics guidelines.

Outcomes

We defined crude coverage of measles immunization as the proportion of children who had at least one documented measles immunization on their health card. I defined crude coverage of complete immunization as the proportion of children immunization records for all nationally recommended vaccines for children 12-23 months old.[1] I captured self-reported immunization histories from caregiver recall for all children regardless of health card availability, so it was possible to compare recall with card documentation for the same children. I defined hypothetical maximum coverage as the proportion of children who either received measles immunization according to health card/caregiver recall, or who have not received measles immunization but whose health cards indicate that they received other immunizations at a time when they were eligible for measles immunization, and hence, represent missed opportunities for measles immunization. I defined effective coverage of measles immunization as the proportion of children with a positive DBS assay for measles-specific immunoglobulin G (IgG) antibodies. Assay methods employed in this study have been described and described in detail in Chapter 2. Although IgG is biomarker of both vaccination and natural exposure to measles virus, the latter is unlikely because there were no confirmed cases of measles in Mexico or Nicaragua in 2012 or 2013.[51] Although measles case reporting in these regions may be incomplete, natural exposure to measles is also unlikely because only 14 caregivers in Mexico and 4 in Nicaragua reported that their child had been diagnosed with measles at some point in their lives, and our survey teams saw no reports or cases of measles during data collection.

Statistical Analyses

We compared estimates of measles immunization coverage based on caregiver recall, child health cards, the combination of health card and recall, and seroprevalence of anti-measles antibodies. I examined concordance between sources for the same children, and calculated kappa,

[1] In Nicaragua: 1xBCG, 3xOPV, 3xPentavalent, 3xRotavirus, 3xPnemococcal conjugate, 1xMMR, and if older than 18 months, 1xDPT; in Mexico: 1xBCG, 3xPentavalent or if older than 18 months, 4xPentavalent, 3xHepB, 1xMMR, 3xPneumoccocal conjugate, and 2xRotavirus.

sensitivity, specificity, and positive and negative predictive values (PPV and NPV) of health card documentation in predicting serostatus.

Of particular interest are children with health card documentation of measles immunization who lack antibodies. To identify potential drivers of this discrepancy, I restricted the analysis to health card-positive children and used logistic regression to identify characteristics that increase the likelihood that a card-positive child will lack antibodies. I tested a wide variety of individual, maternal, household, community and health facility factors, and report crude and adjusted odds ratios.

In analyses of Mexico data, eighteen household characteristics and five health facility characteristics were included in the final regression models. These characteristics were the child age's (dichotomous: 18 months and older versus younger), child gender, whether the child had diarrhea in the two weeks preceding the survey, whether the child had a fever in the two weeks preceding the survey, childhood anemia, childhood underweight, maternal age (3 age groups: 15-19, 20-34, or 35-49), maternal age at first birth (dichotomous: before 20 years or after), maternal marital status (married, single, open union, or others), maternal literacy (full ability to read a written sentence in Spanish), maternal exposure to mass media (newspaper, television, or radio) in the week preceding the survey, whether the mother speaks an indigenous language, household asset score (proportion of assets inquired about that were owned by the household), travel time to usual health facility (in categories of 15 or 30 minutes), whether the household is a beneficiary of the social welfare program Oportunidades, whether the community is in an urban area, altitude of the segment (categories of 500 meters), the health jurisdiction of the segment, the number of doctors at the health facility attended for immunization, whether the health facility has sufficient electrical power to run all of its equipment at all hours, number of functional refrigerators owned by the facility,

facility has one or more broken cold storage units (refrigerators or cold boxes), and whether any refrigerator storing vaccines was out of the appropriate temperature range on the day of the survey (2-8°C). In Nicaragua, the set of covariates was used similar, except I also included availability of an improved water source at the household, maternal exposure to community health promoters (any face-to-face meetings in the last month), the Local Integrated Healthcare System geographic regions (SILAIS) of the locality, health facility has routine administrative meetings, and facility has doctor on staff, and I did not include household enrollment in Oportunidades, the number of doctors at the health facility attended for immunization or the number of functional refrigerators owned by the facility. Each of these factors was expected to have a potential relationship, direct or indirect, with the likelihood that a card-positive child would lack anti-measles antibodies.

We considered two methods for linking household and health facility data. First, by means of caregiver self-reported names and locations of the health facility usually attended for vaccinations, and second, by assigning facility characteristics of municipalities (Mexico) or SILAIS (Nicaragua) to the children living in that geographic unit. The first method is preferable, as the second likely involves some degree of contamination (households in one region attend facilities in another), which may weaken true associations between immunization and health facility characteristics in the data. For regression analyses, I linked as many children as possible via the first method, then linked the remaining children via the second method, and conducted regression analyses with the combined dataset.

In all analyses, I excluded 64 children (46 in Mexico and 18 in Nicaragua) whose health cards indicated that DBS collection occurred too soon (within 28 days) after vaccination for development of a detectable IgG antibody titer [33]. I retained 81 children (61 in Mexico and 20 in Nicaragua) with DBS samples whose health cards indicated previous measles vaccination, but no date was recorded, because among children with measles immunization dates recorded, the vast majority (94%) provided DBS more than 28 days after vaccination.

We generated survey weights prior to analysis and all coverage estimates, statistical tests and regressions took into account the household survey's multistage sampling design. Statistical analyses were conducted with Stata (version 13.1) and R (version 3.0.2). For all analyses, associations with a p value of less than 0.05 were regarded as significant.

We also conducted several sensitivity analyses. First, I tested the variability of the results when excluding children vaccinated between 7 and 35 days before DBS collection. Second, I specified regression model among card-positive and recall-positive children rather than card-positive children only. Third, I restricted the regression analysis to the subsample of children matching to health facilities by name.

Results: Mexico

Between July 25, 2012 and May 18, 2013 we surveyed 5,428 households with 6,988 women of reproductive and 6,465 children under five and collected DBS from 1134 children aged 12-23 months old. Table 6 shows the characteristics of DBS participants. The sample was 51% female with median age 17 months and high levels of recent illness and anemia. A majority of mothers were married or partnered homemakers in their twenties with primary education and an average of 3.3 children. Participants were mainly from poor, indigenous households in rural areas.

Characteristics of surveyed health facilities are presented in Table 7. A majority of facilities were ambulatory clinics. They tended to be heavily staffed but infrastructure issues such as lack of running water, electricity at all hours, insufficient electrical power, and broken equipment were not uncommon. While 89% offer vaccination, only 49% routine store vaccines. Importantly, only 23% had working, fueled emergency generators, 16% had at least one non-functional cold storage device (refrigerator, cold box), and among facilities storing vaccines, 20% had vaccine storage equipment that was observed to be outside the appropriate temperature range (2-8°C) on the day of the survey.

Table 6: Household characteristics of the study sample in Chiapas

	Univariate characteristic	Value (95% CI)
Child	Age <18 months	47.3 (44.2, 50.4)
	Female	51.0 (47.6, 54.3)
	Health: Excellent	11.4 (9.1, 14.2)
	Very good	15.2 (12.9, 17.8)
	Good	51.8 (48.4, 55.1)
	Moderate	19.3 (16.7, 22.1)
	Poor	2.3 (1.4, 3.7)
	Diarrhea last 2 weeks	17.6 (15.4, 20.0)
	Fever last 2 weeks	21.7 (18.8, 24.9)
	Anemic	33.5 (29.3, 38.1)
	Underweight	8.7 (6.7, 11.2)
	Wasted	2.0 (1.3, 3.3)
Maternal	Age (years): 15-19	11.5 (9.3, 14.1)
	20-24	28.3 (25.5, 31.2)
	25-29	27.3 (24.3, 30.6)
	30-34	17.5 (15.2, 20.0)
	35-39	10.4 (8.6, 12.6)
	40-44	4.2 (3.0, 5.8)
	45-49	0.8 (0.4, 1.6)
	Age at first birth <20 years	65.9 (62.3, 69.3)
	Parity (mean)	3.3 (3.1, 3.5)
	Marital status: married	35.1 (30.7, 39.7)
	single	1.7 (1.0, 2.8)
	open union	58.0 (53.3, 62.5)
	others	5.3 (4.0, 6.9)
	Literate	56.9 (52.5, 61.3)
	Education: none	15.2 (12.6, 18.3)
	literacy course	2.8 (1.6, 4.8)
	primary	51.9 (47.8, 56)
	secondary +	30.1 (26.3, 34.2)
	Occupation: paid employee	5.5 (3.9, 7.7)
	homemaker	93.3 (91, 95.1)
	other	1.2 (0.6, 2.2)
	Media exposure	70.4 (65.7, 74.7)
	Promotora meeting	19.1 (15.5, 23.4)
	In good health	61.0 (56.4, 65.4)
	Indigenous language	66.1 (58.5, 73)
Household	Father in home	92.1 (90.3, 93.7)
	Household size (mean)	5.9 (5.7, 6.2)
	Monthly expenditure: 0 - 499	10.5 (8.2, 13.4)
	500 - 999	18.3 (15.4, 21.7)
	1000 - 1999	33.0 (29.5, 36.7)
	2000 - 4999	32.9 (29.0, 36.9)
	5000 +	5.3 (3.8, 7.2)
	Asset score (mean)	0.2 (0.2, 0.2)
	Spanish interview	78.8 (72.8, 83.8)
	Indigenous language	69.4 (61.9, 75.9)
	Travel time (min) to HF: < 15	31.7 (26.4, 37.5)
	15 - 29	23.3 (19.9, 27.0)
	30 - 44	23.6 (19.8, 27.8)
	45 - 59	2.2 (1.3, 3.6)
	60 +	19.3 (14.1, 25.9)
	Seguro Popular	86.2 (83.5, 88.5)
	Oportunidades	64.7 (59.7, 69.4)
	Improved water source	87.6 (81.8, 91.8)
	Improved sanitation	64.1 (56.9, 70.7)
Community	% indigenous	67.0 (62.2, 71.7)
	Urban	34.5 (27.1, 42.8)
	Altitude: <500m	16.6 (11.1, 24.1)
	500m – 999m	25.9 (19.0, 34.2)
	1000m – 1499m	22.1 (15.0, 31.4)
	1500m – 1999m	12.4 (8.1, 18.7)
	2000m +	23.0 (16.6, 31.0)
		N = 1134

HF: Health facility. Values are percentages unless otherwise noted. 95% CI: 95% confidence interval. Estimates are survey weighted.

Table 7: Health facility characteristics of the study sample in Chiapas

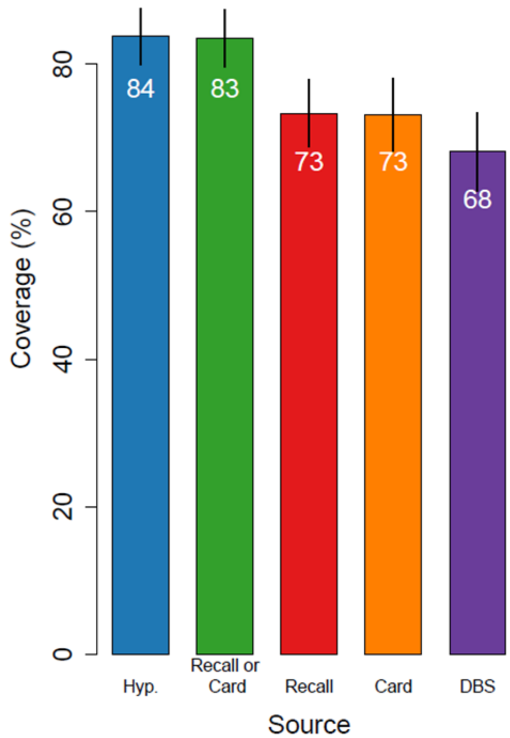
Univariate characteristic		Value (95% CI)	N
Roles and policies	Ambulatory facility	66.7 (55.9, 76.3)	90
	Seguro Popular accredited	57.8 (46.9, 68.1)	90
	Offers child services	91.1 (83.2, 96.1)	90
	Offers vaccination	88.9 (80.5, 94.5)	90
	Stores vaccines	48.8 (37.4, 60.2)	80
	Routine administrative meetings	80.0 (70.2, 87.7)	90
	Routine medical meetings	58.9 (48.0, 69.2)	90
Infrastructure	Running water	76.7 (66.6, 84.9)	90
	Internet access	21.1 (13.2, 31.0)	90
	Electricity	93.3 (86.1, 97.5)	90
	Sufficient electrical power for all equipment	73.8 (63.1, 82.8)	84
	Electricity at all hours	83.1 (72.9, 90.7)	77
	Electricity on all days	65.0 (53.5, 75.3)	80
	Generator	6.7 (2.5, 13.9)	90
	Working, fueled generator	11.9 (5.9, 20.8)	84
	National immunization scheme observed	84.0 (74.1, 91.2)	81
	Vaccination registry observed	87.7 (78.5, 93.9)	81
	1+ broken cold storage observed	15.6 (8.8, 24.7)	90
	1+ broken thermometer observed	23.9 (12.6, 38.8)	46
	Number of refrigerators (mean)	1.2 (0.1, 2.3)	38
	Number of cold boxes observed (mean)	1.8 (0.8, 2.7)	80
	Number of ice packs observed (mean)	1.7 (0.5, 2.8)	77
	Any refrigerator out of temperature range	20.0 (8.4, 36.9)	35
	Fridge temp recorded 2x/day in last 30 days	77.1 (59.9, 89.6)	35
Personnel	Has doctor	82.2 (72.7, 89.5)	90
	# of doctors (mean)	5.0 (-9.5, 19.4)	90
	# of nurses (mean)	7.1 (-30.9, 45.0)	90
	# of pediatricians (mean)	0.5 (-3.9, 4.9)	90
	# of auxiliary nurses (mean)	1.6 (-4.8, 8.0)	90
	# of lab technicians (mean)	1.1 (-7.5, 9.7)	90
	# of promotoras (mean)	1.5 (-4.4, 7.4)	90
	Total # of staff	20.0 (-45.1, 85.0)	90
Vaccine supply	Always receives number of vaccines ordered	51.3 (34.8, 67.6)	39
	Shortage of measles vaccine in last 6 months	5.6 (1.8, 12.5)	90
	Days between vaccine order and receipt	2.5 (-7.8, 12.9)	37
	During shortage: makes special order	43.6 (27.8, 60.4)	39
	borrows from other facility	28.2 (15.0, 44.9)	39
	does nothing	25.6 (13.0, 42.1)	39
	other	10.3 (2.9, 24.2)	39
	Vaccine orders placed at fixed times (vs as needed)	82.5 (67.2, 92.7)	40
	Anticipated shortage in last 3 months	52.6 (35.8, 69.0)	38
	MMR vaccine observed in stock	79.2 (65.9, 89.2)	53

Values are percentages unless otherwise noted. 95% CI: 95% confidence interval. Estimates are not survey weighted. MMR: measles, mumps and rubella.

Of 1262 eligible participants, 52% (n=654), 81% (n=1021) and 72% (n=1134) provided measles immunization information from recall, health card and DBS, respectively. There were considerable differences between coverage estimates depending on the source. Among 552 children with all three sources, survey-based estimates ranged from 73% (95% confidence interval (CI): 68 – 78%) for health cards to 83% (95% CI: 80 – 87%) for the combination of recall and health card (Figure 7). Effective coverage was substantially lower, at 68% (95% CI: 63 – 73%). Interestingly, coverage based on recall alone and health card alone were nearly identical, but when combining the two (the most commonly reported metric in developing countries) coverage was 10 percentage points higher. This finding indicates that a substantial proportion of children lacked health card documentation but receipt of immunization was recalled, or vice versa. Only 0.3% of children lacked measles immunization, but had documentation of a recent vaccination visit in which they could have received the vaccine. Hence, hypothetical maximum coverage was 84%.

Coverage classification according to health card versus DBS were in agreement for only 77% (95% CI: 74 – 79) of 895 children with both sources. Concordance, sensitivity, specificity, and positive and negative predictive values showed poor agreement (Table 8). Of particular concern, 19% of children with health card documentation of immunization did not have antibodies.

Figure 7: Measles immunization coverage by source in Chiapas



Hyp.: Hypothetical maximum coverage. Figure restricted to 552 children with all three sources, excluding children with DBS collection within 28 days of vaccination. Lines indicate 95% confidence intervals. Estimates are survey weighted.

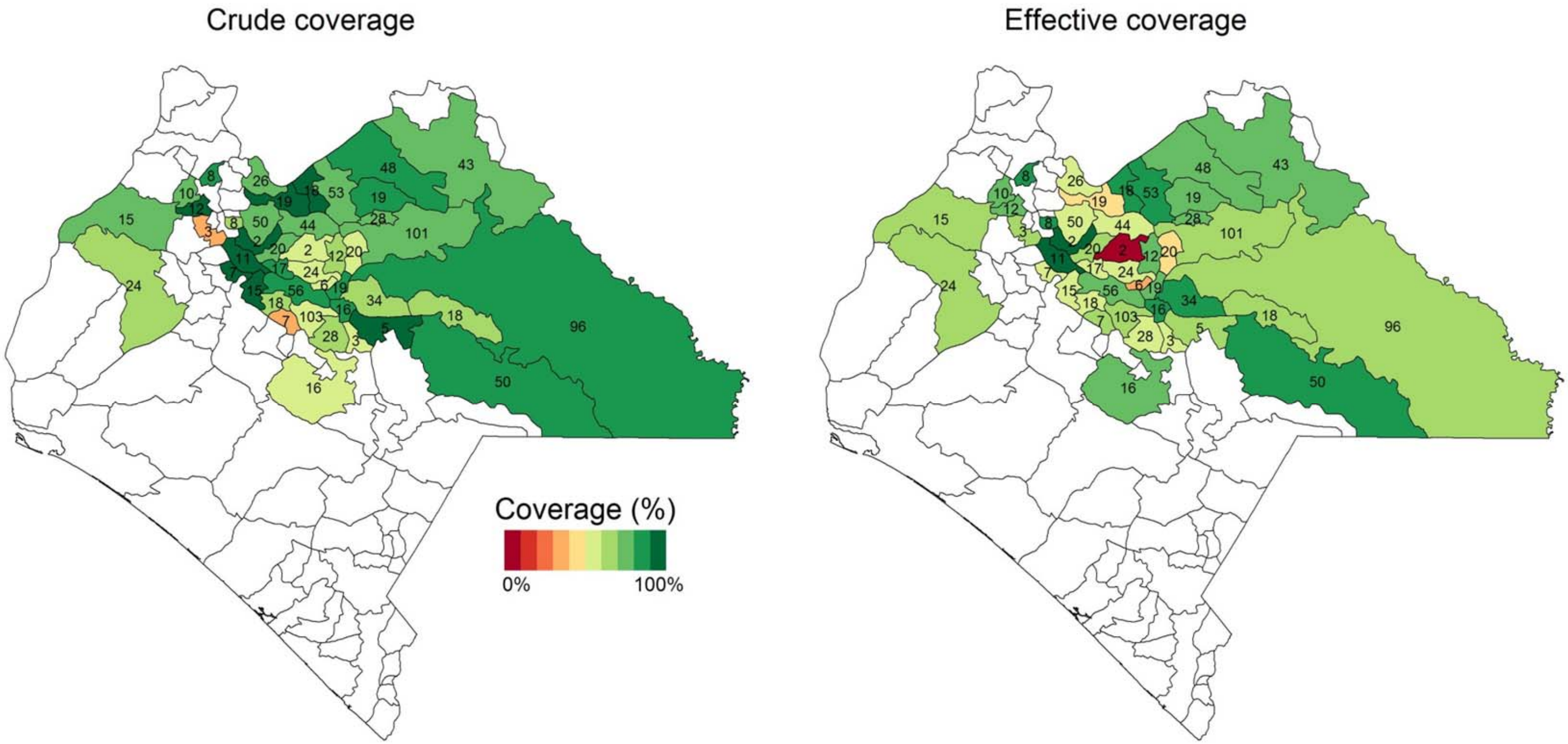
Table 8: Relationship between crude and effective coverage of measles immunization in Chiapas

Crude coverage*	**DBS effective coverage**		
	Yes	No	*Total*
Yes	59.8%	14.2%	74.0%
No	9.3%	16.7%	26.0%
Total	69.1%	30.9%	100%
Parameters (95% confidence interval)			
Sample size	895		
Kappa	0.43 (0.39, 0.46)		
% agreement	76.5 (73.7, 79.3)		
% sensitivity	86.6 (84.4, 88.8)		
% specificity	54.0 (50.8, 57.3)		
Positive predictive value	80.8 (78.2, 83.4)		
Negative predictive value	64.3 (61.2, 67.5)		

Table restricted to 895 children with both health card and DBS sources; excluding children with DBS collection within 28 days of vaccination. *Crude coverage: according to child health card. Estimates are survey weighted.

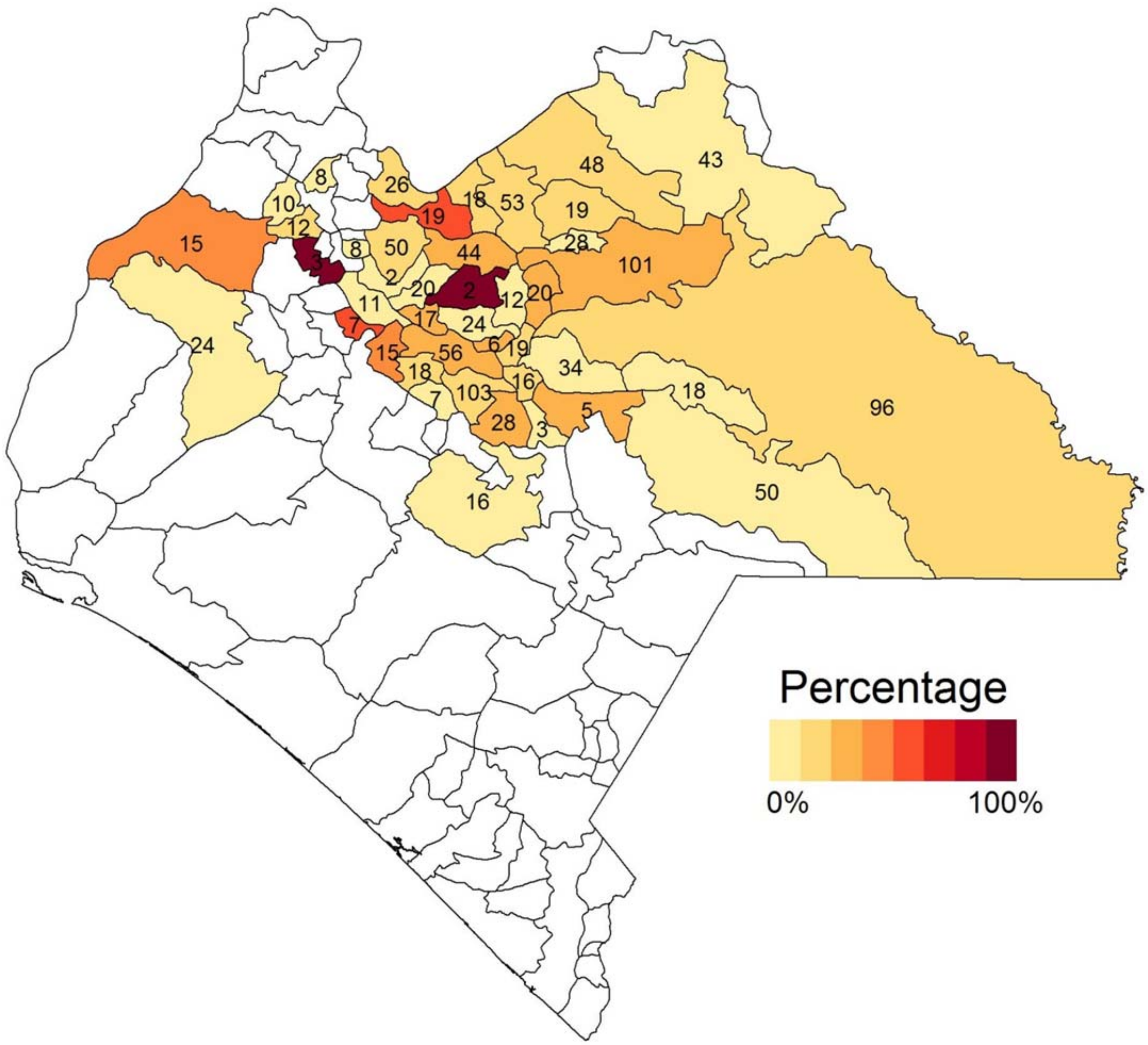
Comparisons of crude and effective coverage for each surveyed municipality are presented in Figure 8. Crude coverage was highest (100%) in Jitotol, Bochil, Chanal, Soyaló, Ixtapa, and Sabanilla, and lowest (33%) in San Lucas and Coapilla. At the health jurisdiction level, crude coverage was highest in jurisdiction XV (89%, 95% CI: 82 – 97%) and lowest in the jurisdiction of Valles Zoque (69%, 95% CI: 57 – 82%). Effective coverage was highest (100%) in Jitotol and Bochil, and lowest in Chalchihuitán (0%) and Mitontic (40%). Comparing jurisdictions, effective coverage was highest in jurisdiction XV (85%, 95% CI: 72 – 98%) and lowest in the jurisdiction of De Los Llanos (53%, 95% CI: 28 – 79%). In general, effective coverage followed geographic patterns similar to those of crude coverage, but overall levels were lower. Across municipalities, the proportion of card-positive children lacking antibodies ranged between 0% and 100% (Figure 9). At the level of the health jurisdiction, the highest proportion of card-positive lacking antibodies was in De Los Llanos (35%, 95% CI: 9 – 68%) and the lowest proportion was in Maya (9%, 95% CI: 0 – 28%). These proportions were statistically significantly greater than 0 in all surveyed jurisdictions (Valles Zoque, Mezcalapa, De Los Llanos, Altos Tsotsil Tseltal, De Los Bosques, Selva Lacandona, Maya, Tulijá Tseltal Chol, and Meseta Comiteca Tojolabal). Furthermore, there was substantial clustering of these discrepancies; half of such cases were concentrated in 8 of the 47 surveyed municipalities (Chamula, Chilón, Ocosingo, Simojobel, Tila, San Cristóbal de las Casas, Salto de Agua, Huitiupán). These clear and consistent geographic patterns highlight that some surveyed areas are very high-performing while others are experiencing substantial challenges. This pattern also reinforces the validity and accuracy of the findings—if there were errors in data collection or in the DBS ELISA procedure, we would likely observe a large gap or problem across all municipalities. Instead, I found that in many municipalities crude and effective coverage were identical, while in others, the gap was very large.

Figure 8: Crude and effective coverage of measles immunization by municipality in Chiapas



The number in each municipality indicates sample size. Estimates are survey weighted.

Figure 9: Proportion of card-positive children lacking antibodies in Chiapas



The number in each municipality indicates sample size. Estimates are survey weighted.

Among 783 card-positive children, I linked 197 children to 25 surveyed facilities by facility name and the remaining 586 by region. In regression analyses (Table 9), few factors increased the likelihood that a card-positive child would lack antibodies. Card-positive with mothers who were literate (could read aloud a written sentence presented to them) and single or in open unions (as opposed to married) were less likely to lack antibodies. Children from wealthier households were also less likely to have invalid vaccines. Of particular interest, and contrary to expectations, children from urban regions were far more likely to invalid vaccines that children from rural areas. These findings were consistent in all four sensitivity analyses, except in regression analysis restricted to the subsample of children matching to health facilities by name, in which no covariates were statistically significant.

Table 9: Crude and adjusted odds ratios of likelihood that card-positive children lack antibodies, Chiapas

Characteristic		Crude Odds Ratio (95% CI)	p	Adjusted Odds Ratio (95% CI)	p
Child	Age < 18 months	1.01 (0.65, 1.58)	0.9539	0.99 (0.60, 1.65)	0.9736
	Female	0.73 (0.50, 1.08)	0.1158	0.74 (0.50, 1.10)	0.1432
	Diarrhea in last 2 weeks	1.05 (0.59, 1.87)	0.8588	1.31 (0.72, 2.39)	0.3814
	Fever in last 2 weeks	0.87 (0.50, 1.52)	0.6267	0.83 (0.45, 1.53)	0.5562
	Anemic	1.12 (0.62, 2.02)	0.6989	1.01 (0.57, 1.78)	0.9793
	Underweight	0.82 (0.37, 1.81)	0.6256	0.90 (0.36, 2.24)	0.8138
Maternal	Age (years): 15-19 (ref)				
	20-34	1.51 (0.79, 2.86)	0.2115	1.54 (0.69, 3.46)	0.2938
	35-49	1.45 (0.78, 2.71)	0.2422	1.28 (0.70, 2.32)	0.4242
	Age at first birth <20 years	0.73 (0.45, 1.2)	0.2168	0.67 (0.38, 1.17)	0.1628
	Marital status: married (ref)				
	single	0.23 (0.03, 1.96)	0.1800	0.12 (0.02, 0.70)	0.0205 *
	open union	0.61 (0.36, 1.03)	0.0657	0.56 (0.31, 0.99)	0.0501
	others	1.19 (0.47, 2.96)	0.7147	1.08 (0.45, 2.61)	0.8675
	Literate	0.62 (0.36, 1.05)	0.0763	0.52 (0.29, 0.95)	0.0359 *
	Exposure to mass media	0.94 (0.58, 1.53)	0.8013	0.99 (0.56, 1.76)	0.9794
	Speaks indigenous language	0.82 (0.47, 1.43)	0.4892	1.04 (0.52, 2.09)	0.9143
Household	Asset score (mean)	0.07 (0.00, 1.31)	0.0770	0.04 (0.00, 0.68)	0.0288
	Time (min) to HF: < 15 (ref)				
	15 - 29	1.39 (0.73, 2.67)	0.3179	1.25 (0.63, 2.46)	0.5252
	30 - 44	1.47 (0.70, 3.06)	0.3102	1.33 (0.62, 2.83)	0.4634
	45 - 59	0.34 (0.04, 2.92)	0.3244	0.26 (0.04, 1.56)	0.1447
	60 +	0.71 (0.27, 1.90)	0.5012	0.51 (0.22, 1.19)	0.1236
	Oportunidades beneficiary	0.70 (0.40, 1.21)	0.1989	0.81 (0.46, 1.42)	0.4575

Community	Urban	2.29 (1.29, 4.08)	0.0055 *	2.24 (1.10, 4.60)	0.0295 *
	Altitude (meters): <500 (ref)				
	500 - 999	1.13 (0.49, 2.60)	0.7749	1.07 (0.51, 2.26)	0.8513
	1000 - 1499	1.06 (0.44, 2.56)	0.8962	0.91 (0.36, 2.27)	0.8343
	1500 - 1999	0.75 (0.22, 2.53)	0.6446	0.42 (0.07, 2.47)	0.3397
	2000 +	1.17 (0.51, 2.71)	0.7096	2.79 (0.43, 18.24)	0.2876
	Jur: Tulijá Tseltal Chol (ref)				
	Valles Zoque	0.7 (0.1, 4.87)	0.7221	0.99 (0.14, 6.74)	0.9908
	Mezcalapa	1.21 (0.32, 4.56)	0.7797	1.77 (0.38, 8.11)	0.4667
	De Los Llanos	0.00 (0.00, 0.00)	<0.0001 *	0.00 (0.00, 0.00)	<0.0001 *
	Altos Tsotsil Tseltal	0.56 (0.29, 1.08)	0.0863	0.44 (0.08, 2.37)	0.3400
	De Los Bosques	1.16 (0.49, 2.76)	0.7376	1.41 (0.49, 4.07)	0.5240
	Norte	0.54 (0.07, 4.29)	0.5621	0.49 (0.08, 3.05)	0.4501
	Selva Lacandona	0.70 (0.20, 2.37)	0.5634	0.65 (0.15, 2.86)	0.5739
	Maya	0.26 (0.07, 0.89)	0.0344	0.26 (0.06, 1.17)	0.0821
	Meseta Comiteca Tojolabal	0.54 (0.22, 1.35)	0.1918	0.94 (0.23, 3.80)	0.9315
Health facility	# of doctors	1.02 (0.95, 1.09)	0.5897	1.03 (0.94, 1.14)	0.5043
	Sufficient electrical power	0.78 (0.21, 2.98)	0.7224	0.33 (0.06, 1.71)	0.1905
	# of refrigerators	1.11 (0.48, 2.53)	0.8124	0.80 (0.21, 3.10)	0.7515
	1+ broken cold storage observed	1.34 (0.67, 2.68)	0.4100	1.20 (0.46, 3.12)	0.7046
	Any refrigerator out of temperature range	1.22 (0.49, 3.02)	0.6757	1.99 (0.67, 5.93)	0.2216

N = 541 observations. Ref: reference category. Jur: Health jurisdiction. 95% CI: 95% confidence interval. * p<0.05. Adjusted odds ratios correspond to coefficients from a multivariate survey-weighted logistic regression including all listed covariates.

Results: Nicaragua

Between March 1, 2013 and September 3, 2013 we surveyed 2,052 households with 1,713 women of reproductive and 1,403 children under five and collected DBS from 454 children aged 12-23 months old. Table 10 shows the characteristics of DBS participants. The sample was 51% female with median age 18 months and high levels of recent illness (18%) and anemia (47%). A majority of mothers were married or partnered homemakers in their twenties with primary education and an average of 2.6 children. Participants were mainly from poor, indigenous households in rural areas.

Characteristics of surveyed health facilities are presented in Table 11. A majority of facilities were ambulatory clinics. Most (86%) were ambulatory clinics and less than half staffed a doctor. A notable proportion lacked basic infrastructure such as running water (25%) and electricity (5%). Most facilities (84%) routinely store vaccines, but some report challenges in vaccine supply. For example, 28% of facilities reported not always receiving the number of vaccines ordered and 8% of facilities did not have combined measles, mumps, and rubella (MMR) vaccine in stock on the day of the survey. Challenges in cold chain management were also present: Only 57% of facilities displayed temperature monitoring charts for every functional refrigerator and even fewer (29%) had recorded temperatures twice daily on monitoring charts during the last 30 days. Of particular concern, 71% of facilities had at least one broken refrigerator or cold box and 7% had at least one vaccine storage unit outside of the appropriate temperature range (2-8°C) on the day of the survey.

Table 10: Household characteristics of the study sample in Nicaragua

	Univariate characteristic	Value (95% CI)
Child	Age < 18 months	34.0 (28.0, 40.6)
	Female	51.3 (44.8, 57.9)
	Child health: Excellent	16.9 (12.1, 22.9)
	Very good	24.3 (19.7, 29.6)
	Good	29.6 (24.3, 35.5)
	Moderate	26.2 (20.7, 32.4)
	Poor	3.1 (1.5, 6.0)
	Recent diarrhea	18.8 (14.0, 24.9)
	Recent fever	18.1 (13.9, 23.3)
	Anemic	46.7 (40.9, 52.5)
	Underweight	1.9 (0.6, 5.6)
	Wasted	0.2 (0, 1.7)
	Stunted	11.1 (7.4, 16.5)
Maternal	Age (years): 15-19	14.4 (10.6, 19.3)
	20-24	31.4 (26.3, 36.9)
	25-29	24.7 (20.4, 29.5)
	30-34	14.3 (9.9, 20.1)
	35-39	10.2 (7.1, 14.5)
	40-44	4.4 (2.5, 7.7)
	45-49	0.6 (0.1, 2.3)
	Age at first birth <20 years	67.6 (61.7, 73.1)
	Parity (mean)	2.6 (2.3, 2.8)
	Marital status: single	31.2 (25.5, 37.6)
	married	18.0 (13.0, 24.5)
	open union	45.3 (39.9, 50.8)
	others	5.4 (3.3, 8.7)
	Literate	71.8 (65.7, 77.2)
	Education: none	11.4 (7.7, 16.6)
	primary	48.8 (41.7, 55.9)
	secondary	28.2 (23.4, 33.5)
	university +	11.6 (6.8, 19.2)
	Occupation: paid employee	12.5 (8.6, 17.9)
	homemaker	84.5 (78.9, 88.8)
	other	3.0 (1.3, 6.5)
	Media exposure	88.5 (83.0, 92.4)
	Promotora meeting	1.4 (0.6, 3.5)
	In good health	64.7 (59.2, 69.8)
	Indigenous	12.1 (5.5, 24.4)
Household	Father lives in home	72.9 (65.9, 78.9)
	Household size (mean)	5.9 (5.5, 6.2)
	Exp.: 0 - 499	2.8 (1.2, 6.7)
	500 - 999	5.7 (3.5, 9.0)
	1000 - 1999	22.0 (16.7, 28.4)
	2000 - 3499	26.4 (21.9, 31.4)
	3500 - 5499	20.3 (15.0, 26.9)
	5500 - 8999	14.1 (10.1, 19.3)
	9000+	8.7 (5.5, 13.5)
	Asset score (mean)	24.9 (23.4, 26.3)
	Interview in Spanish	97.9 (93.8, 99.3)
	Indigenous	13.2 (6.1, 26.2)
	Time to HF:< 15 min	30.8 (22.8, 40.2)
	15 – 29	21.7 (16.7, 27.6)
	30 – 44	18.3 (13.5, 24.3)
	45 – 59	1.4 (0.4, 4.9)
	60 +	27.8 (20.5, 36.4)
	Improved water	82.6 (77.2, 86.9)
	Improved sanitation	14.7 (9.7, 21.6)
Community	Urban	34.9 (22.5, 49.8)
	Altitude: <500m	37.0 (24.1, 52.0)
	500 – 999m	46.7 (33.3, 60.5)
	1000m +	16.4 (9.2, 27.5)
	SILAIS: Jinotega	37.8 (25.7, 51.6)
	Matagalpa	28.9 (18.1, 42.8)
	RAAN	21.5 (11.1, 37.5)
	Madriz	11.8 (6.2, 21.3)

N = 454. Values are percentages unless otherwise noted. HF: Health facility. Exp: monthly household expenditure in Córdobas. Min: minutes. m: meters. RAAN: Región Atlántico Norte. 95% CI: 95% confidence interval. Estimates are survey weighted.

Table 11: Health facility characteristics of the study sample in Nicaragua

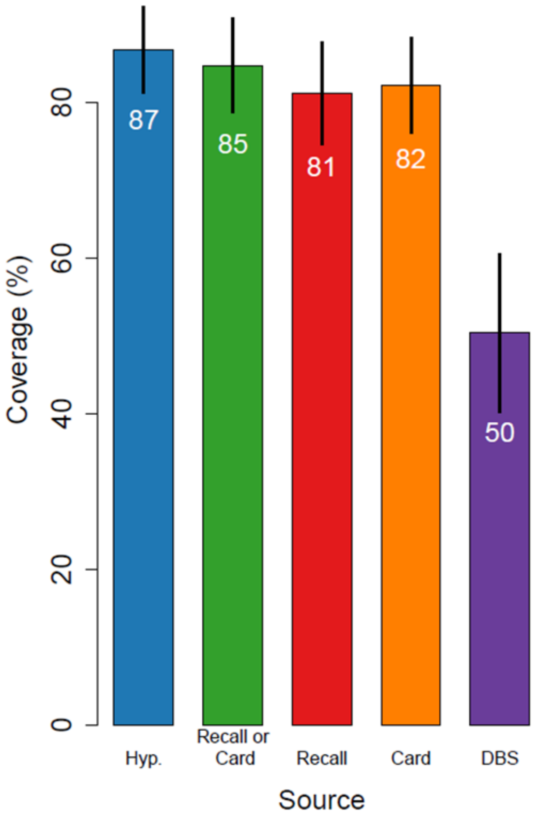
	Univariate characteristic	Value (95% CI)	N
Roles and policies	Ambulatory facility	85.9 (75, 93.4)	64
	Secretaría de Salud accredited	92.2 (82.7, 97.4)	64
	Offers child services	100 (94.4, 100)	64
	Offers vaccination	95.3 (86.9, 99.0)	64
	Stores vaccines	78.1 (66.0, 87.5)	64
	Routine administrative meetings	81.2 (69.5, 89.9)	64
	Routine medical meetings	73.4 (60.9, 83.7)	64
Infrastructure	Running water	75.0 (62.6, 85.0)	64
	Internet access	10.9 (4.5, 21.2)	64
	Electricity	92.2 (82.7, 97.4)	64
	Sufficient electrical power for all equipment	76.6 (64.3, 86.2)	64
	Electricity at all hours	72.6 (59.8, 83.1)	62
	Electricity on all days	54.2 (40.8, 67.3)	59
	Generator	7.8 (2.6, 17.3)	64
	Working, fueled generator	20.3 (11.0, 32.8)	59
	National immunization scheme observed	68.4 (54.8, 80.1)	57
	Vaccination registry observed	87.9 (76.7, 95.0)	58
	1+ broken cold storage observed	43.9 (30.7, 57.6)	57
	1+ broken thermometer observed	6.5 (0.8, 21.4)	31
	Number of refrigerators (mean)	1.3 (-0.6, 3.3)	50
	Number of ice packs observed (mean)	1.8 (0.7, 2.8)	60
	Any refrigerator out of temperature range	7.0 (1.5, 19.1)	43
	Fridge temp recorded 2x/day in last 30 days	40.4 (27.0, 54.9)	52
Personnel	Has doctor	48.4 (35.8, 61.3)	64
	# of doctors (mean)	2.0 (-8.0, 12.0)	64
	# of nurses (mean)	5.3 (-22.3, 32.9)	64
	# of pediatricians (mean)	0.5 (-2.1, 3.0)	64
	# of auxiliary nurses (mean)	4.2 (-17.8, 26.2)	64
	# of lab technicians (mean)	0.8 (-4.0, 5.6)	64
	# of promotoras (mean)	0.4 (-3.5, 4.2)	64
	Total # of staff	13.4 (-48.1, 74.8)	64
Vaccine supply	Always receives number of vaccines ordered	76.0 (61.8, 86.9)	50
	Shortage of measles vaccine in last 6 months	0.0 (0.0, 26.5)	12
	Days between vaccine order and receipt	1.4 (-1.0, 3.8)	48
	During shortage: makes special order	80.0 (66.3, 90.0)	50
	borrows from other facility	10.0 (3.3, 21.8)	50
	does nothing	6.0 (1.3, 16.5)	50
	other	6.0 (1.3, 16.5)	50
	Vaccine orders placed at fixed times (vs as needed)	69.4 (54.6, 81.7)	49
	Anticipated shortage in last 3 months	32.0 (19.5, 46.7)	50
	MMR vaccine observed in stock	91.3 (79.2, 97.6)	46

Values are percentages unless otherwise noted. 95% CI: 95% confidence interval. Estimates are not survey weighted.

Of 475 eligible participants, 85% (n=406), 80% (n=382) and 96% (n=458) provided measles immunization information from recall, health card and DBS, respectively. Among 299 children with all three sources, coverage according to health cards was 82% (95% confidence interval: 76 – 88%), coverage according to caregiver recall was 81% (95% CI: 75 – 88%), and coverage according to the combination of health cards and recall (the most commonly reported metric in developing countries) was 85% (95% CI: 79 – 91%) (Figure 10), but these differences were not statistically significant. Crude coverage of complete vaccination was only 53% (55 – 62%). Worrisomely, effective coverage was substantially lower, at only 50% (95% CI: 40 – 61%). Only 2.3% of children lacked measles immunization, but had documentation of a recent vaccination visit in which they could have received the vaccine. Hence, hypothetical maximum coverage was 87%.

Coverage classification according to health card versus DBS were in agreement for only 61% (95% CI: 55 – 66) of 329 children with both sources. Concordance, sensitivity, specificity, and positive and negative predictive values showed poor agreement (Table 12). Of particular concern, 43% of children with health card documentation of immunization did not have antibodies.

Figure 10: Measles immunization coverage by source in Nicaragua



Hyp.: Hypothetical maximum coverage. Figure restricted to 299 children with all three sources, excluding children with DBS collection within 28 days of vaccination. Lines indicate 95% confidence intervals. Estimates are survey weighted.

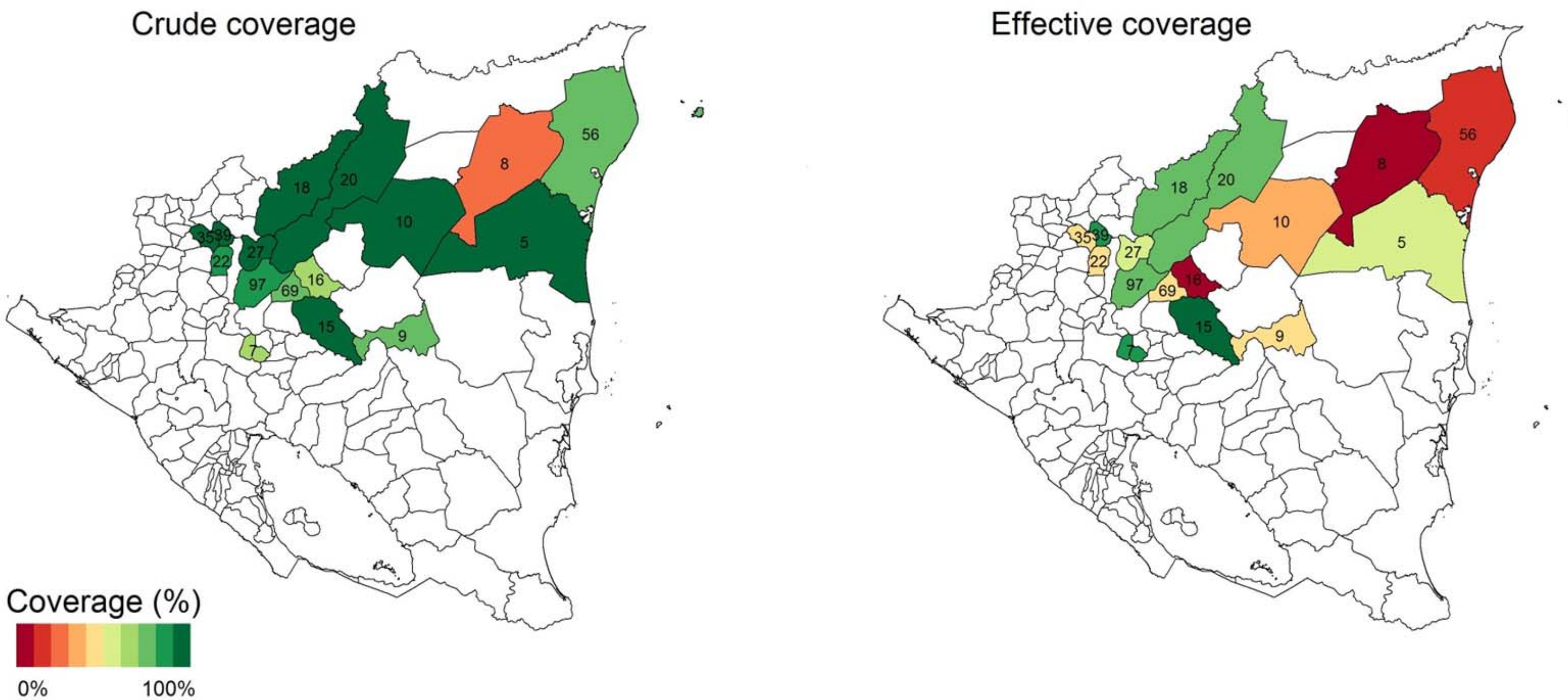
Table 12: Relationship between crude and effective coverage of measles immunization in Nicaragua

Crude coverage*	**DBS effective coverage**		
	Yes	No	*Total*
Yes	46.7%	35.4%	82.2%
No	3.7%	14.2%	17.8%
Total	50.7%	49.6%	100%
Parameters (95% confidence interval)			
Sample size	299		
Kappa	0.21 (0.10, 0.33)		
% agreement	60.9 (55.4, 66.4)		
% sensitivity	92.8 (88.8, 96.7)		
% specificity	28.6 (20.8, 36.3)		
Positive predictive value	56.9 (50.8, 63.0)		
Negative predictive value	79.5 (68.0, 91.1)		

Figure restricted to 329 children with both health card and DBS sources; excluding children with DBS collection within 28 days of vaccination. *Crude coverage: according to child health card. Estimates are survey weighted.

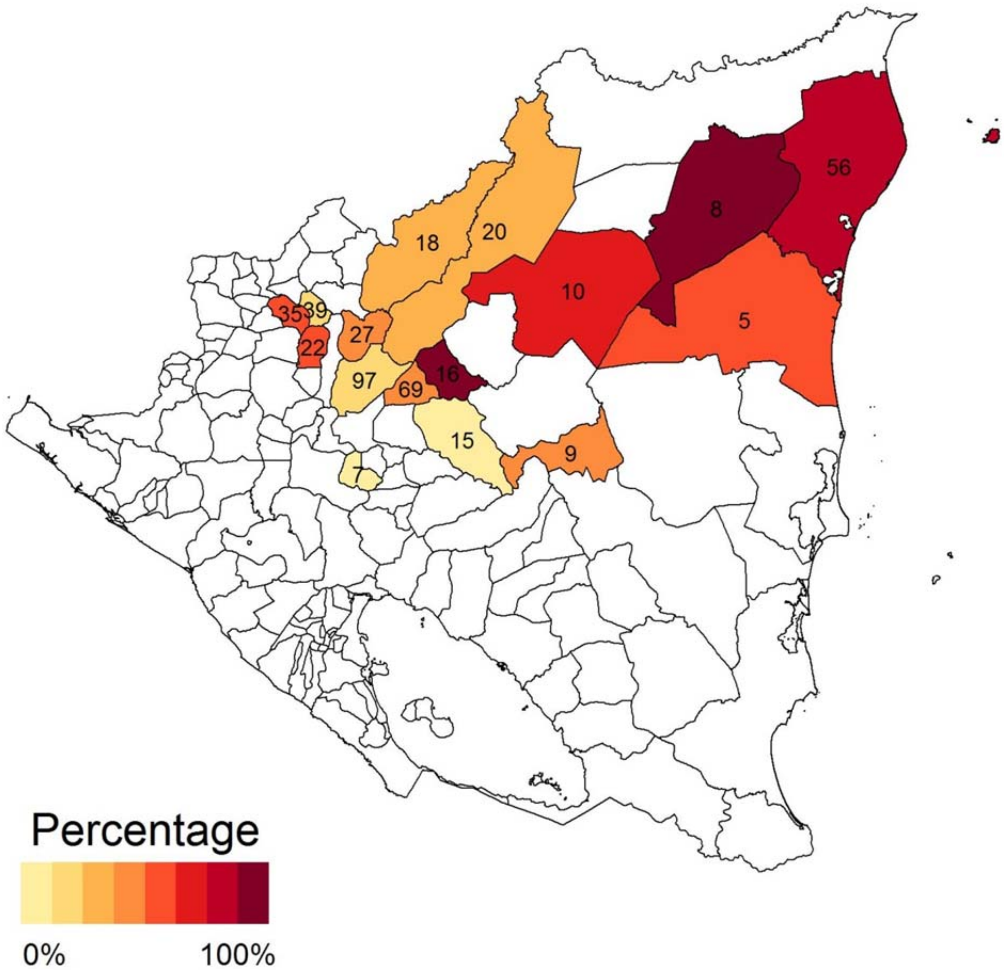
Comparisons of crude and effective coverage for each surveyed municipality are presented in Figure 12. Crude coverage was highest (100%) in Siuna and Prinzapolka, and lowest in Rosita (20%) and Rancho Grande (61%). Effective coverage was highest in Matiguás (92%) and Terrabona (83%), and lowest in Rosita (0%) and Rancho Grande (7%). In general, effective coverage followed geographic patterns similar to those of crude coverage, but overall levels were lower. Across municipalities, the proportion of card-positive children lacking antibodies ranged between 0% and 100% (Figure 13). In all municipalities except Terrabona, these proportions were statistically significantly greater than 0. Furthermore, there was substantial clustering of these discrepancies: more than half of such cases were concentrated in 4 of the 16 surveyed municipalities (Jinotega, Tuma-La Dalia, San Juan Río Coco and Puerto Cabezas). Moreover, the proportion of health card-positive children lacking antibodies was statistically significantly higher in RAAN than in other departments. These clear and consistent geographic patterns highlight that some surveyed areas are very high-performing while others are experiencing substantial challenges. This pattern also reinforces the validity and accuracy of the findings—if there were errors in data collection or in the DBS ELISA procedure, we would likely observe a large gap or problem across all municipalities. Instead, I found that in many municipalities crude and effective coverage were identical, while in others, the gap was very large.

Figure 11: Crude and effective coverage of measles immunization by municipality in Nicaragua



The number in each municipality indicates sample size. Estimates are survey weighted.

Figure 12: Proportion of card-positive children lacking antibodies in Nicaragua



The number in each municipality indicates sample size. Estimates are survey weighted.

Among 301 card-positive children, I linked 90 children to 20 surveyed facilities by facility name and the remaining 211 by SILAIS. In regression analyses (Table 13), few factors increased the likelihood that a card-positive child would lack antibodies. In adjusted analyses, card-positive children were more likely to lack antibodies if they were poorer, male, underweight, or living in rural areas, or if they had mothers who are moderately educated, single, or exposed to mass media (newspaper, radio, television) on a weekly basis. Interestingly, children attending facilities that report having routine administrative meetings, and indication of high-quality management, were less likely to lack antibodies. The strongest predictor was residence in RAAN. Factors that were not statistically significant predictors include: child age, child health status, child recent illness, anemia, stunting, wasting, maternal age, maternal parity, maternal age at first birth, maternal education, maternal literacy, maternal exposure to community health promoters, maternal health status, indigenous ethnicity, household expenditure, household asset score, travel time to usual health facility, household availability of improved water source, household availability of improved sanitation, or altitude of residence. Health facility factors that were not statistically significant predictors include: facility level, receipt of referrals, personnel, electricity, frequency of blackouts, water supply, internet access, generator ownership and functioning, facility roles and policies, vaccine procedures and policies, vaccine supplies, and availability and functioning of cold chain infrastructure. These findings were consistent in all sensitivity analyses.

Table 13: Crude and adjusted odds ratios of likelihood that card-positive children lack antibodies, Nicaragua

Characteristic		Crude odds ratio (95% CI)	p	Adjusted odds ratio (95% CI)	p
Child	Age < 18 months	0.59 (0.31, 1.13)	0.1160	0.72 (0.31, 1.69)	0.4589
	Female	0.66 (0.34, 1.28)	0.2223	0.40 (0.16, 0.96)	0.0486 *
	Diarrhea in last 2 weeks	1.26 (0.55, 2.90)	0.5856	0.69 (0.25, 1.90)	0.4754
	Fever in last 2 weeks	1.22 (0.52, 2.85)	0.6561	1.83 (0.68, 4.94)	0.2394
	Anemic	1.36 (0.85, 2.20)	0.2080	1.45 (0.75, 2.79)	0.2745
	Underweight	5.50 (0.56, 54.00)	0.1484	8.80 (1.64, 47.27)	0.0167 *
Maternal	Age (years): 15-19 (ref)				
	20-34	0.66 (0.32, 1.39)	0.2823	0.54 (0.18, 1.61)	0.2813
	35-49	1.33 (0.65, 2.74)	0.4346	2.25 (0.92, 5.54)	0.0863
	Age at first birth <20 years	0.94 (0.46, 1.90)	0.8607	0.81 (0.33, 2.00)	0.6554
	Marital status: married (ref)				
	single	1.54 (0.53, 4.45)	0.4258	3.51 (1.07, 11.50)	0.0469 *
	open union	1.43 (0.71, 2.89)	0.3186	1.66 (0.62, 4.43)	0.3183
	others	2.21 (0.57, 8.58)	0.2575	3.67 (0.54, 25.22)	0.1956
	Literate	0.83 (0.42, 1.65)	0.5925	1.66 (0.73, 3.77)	0.2363
	Promotora exposure	1.06 (0.15, 7.34)	0.9494	3.38 (0.44, 25.88)	0.2496
	Exposure to mass media	0.36 (0.12, 1.12)	0.0828	0.26 (0.08, 0.85)	0.0337 *
	Indigenous	4.09 (1.11, 15.06)	0.0381 *	1.69 (0.39, 7.33)	0.4890
Household	Asset score (mean)	0.02 (0.00, 0.29)	0.0052 *	0.16 (0.01, 2.30)	0.1878
	Time (min) to HF: < 30 (ref)				
	30 - 59	1.33 (0.59, 3.01)	0.4972	0.86 (0.34, 2.15)	0.7459
	60 +	0.61 (0.27, 1.38)	0.2406	0.49 (0.22, 1.10)	0.0932
Community	Improved water	0.45 (0.22, 0.91)	0.0314 *	0.47 (0.17, 1.29)	0.1516
	Urban	0.60 (0.20, 1.81)	0.3715	0.19 (0.05, 0.71)	0.0196 *
	Altitude (meters): <500 (ref)				
	500 - 999	0.68 (0.25, 1.86)	0.4555	2.05 (0.45, 9.32)	0.3594
	1000 +	0.42 (0.13, 1.40)	0.1641	1.23 (0.18, 8.22)	0.8338
	SILAIS: Jinotega (ref)				
	Matagalpa	1.48 (0.58, 3.80)	0.4161	0.62 (0.12, 3.25)	0.5777
	RAAN	6.87 (2.11, 22.40)	0.0022 *	24.15 (1.99, 292.77)	0.0180 *
	Madriz	1.14 (0.37, 3.49)	0.8149	3.64 (0.44, 30.28)	0.2415
Health facility	Routine administrative meetings	0.10 (0.00, 2.40)	0.1596	0.01 (0.00, 0.38)	0.0195 *
	Doctor on staff	1.30 (0.23, 7.30)	0.7704	0.08 (0.00, 16.19)	0.3601
	Sufficient electrical power	1.76 (0.45, 6.82)	0.4180	1.08 (0.09, 12.83)	0.9543
	# of refrigerators	1.22 (0.72, 2.07)	0.4696	3.20 (0.95, 10.76)	0.0703
	1+ broken cold storage	0.26 (0.06, 1.22)	0.0933	6.71 (0.33, 135.45)	0.2238
	Any refrigerator not 2-8°C	7.09 (0.06, 785.4)	0.4177	0.19 (0.01, 4.45)	0.3116

N = 237 observations. Ref: reference category. 95% CI: 95% confidence interval. * p<0.05. Adjusted odds ratios correspond to the coefficients from the multivariate survey-weighted logistic regression including all listed covariates.

Chapter 4: Discussion and Conclusions

To my knowledge this is the first study to show effective coverage of measles vaccination in Mexico or Nicaragua compared to card coverage. The data show that the use of cards and/or recall is misleading and masks variations in coverage. These results are of great importance as they point the areas and groups where protection against measles may have been wrongly assumed. These findings will enable the health authorities in Chiapas and Mexico to examine and address the reasons for the differences between crude and effective coverage. Indeed, concerns about this gap in Mexico have been raised previously[42], and this study allows us to test these concerns.

Our estimates of measles vaccination coverage according to card and/or recall were slightly higher than those previously reported for SM2015 municipalities. In the 2011-2012 Mexico National Health and Nutrition Survey (ENSANUT),[48] measles vaccination coverage according to card and recall among children aged 12-23 months living in SM2015 municipalities was 79% (95% CI: 75 – 83%). Likewise, in the 2011-2012 DHS ENDESA, measles vaccination coverage according to card and recall among children aged 12-23 months living in SM2015 municipalities was 75.5% (95% CI: 64.4 – 86.6%). This similarity further confirms the validity of the findings.

Of note, in Nicaragua the lowest coverage levels (according to recall and card) were reported in RAAN in both the SM2015 survey (73.9%) and the 2011-2012 DHS ENDESA (65.6%). Indeed, I found the highest proportion of card-positive children lacking antibodies (73.2%) in RAAN. This region is very remote area and requires several hours of travel by land or river. In fact, it was necessary to purchase electricity generators for SM2015 surveyors to power netbooks for data collection while in the field.

Our findings revealed that in poor areas of Mexico, one in five children with health card documentation of receipt of measles immunization lacked antibodies, and in poor areas of Nicaragua, nearly half of such children lacked antibodies. Unfortunately, survey-based estimates of measles immunization coverage are weak predictors of population immunity. Thus, current national and subnational estimates of immunization coverage based on the combination of maternal recall and children's health cards may be over-estimating actual protection against vaccine-preventable diseases. This finding is alarming and requires immediate attention. Moreover, this study showed that the crude coverage of complete immunization (including BCG, hepatitis B, pentavalent, rotavirus, pneumococcal, and MMR vaccine) among the study population was only 33% in Mexico and only 53% in Nicaragua. Hence, complete effective coverage can be expected to be even lower if the same patterns observed for measles are present in other immunization.

We did not observe any missed opportunities for measles vaccination in the region. Children ages 12-23 months attended health facilities for immunization an average of 7 times in Mexico and 5 times in Nicaragua. This finding is consistent with the national vaccination schedules for both countries which recommend five visits by 12 months of age (at birth, 2, 4, 6, and 12 months). Thus, children in these settings are attending health facilities a sufficient number of times to be fully immunized, but many do not have protective antibodies against measles.

There are many possible explanations for the observed discrepancies. First, measles vaccine efficacy is imperfect, even under ideal conditions. Previous trials of measles immunization for 12 month old children in the Latin American region have observed efficacy between 74% and 100%, but most were above 93%.[56,57] Poor nutrition and recent illness were common among study participants and may also contribute to lower vaccine efficacy.[57] However, there was no indication that gaps were due to these factors. Second, there may be errors in health card documentation in which some marked vaccines may not have actually been delivered. In Mexico, benefits from the conditional cash transfer program Oportunidades are only provided if children are fully immunized, a stipulation which might motivate families or providers to mark vaccines on health cards even when they have not been administered. On the other hand, there may be cases in which vaccines are administered but not documented on health cards, a scenario that is particularly likely during national health weeks. Third, interruptions in the cold chain during vaccine transport, storage or delivery may have rendered vaccines ineffective. Fourth, there may be limitations in sensitivity of the ELISA or DBS elution methodology employed to test for measles-specific IgG. However, in the validation study presented in Chapter 2, these methods were 100% sensitivity. Finally, it is possible that some vaccines were prepared or administered incorrectly.

The fact that discrepant cases were clustered in a small number of municipalities suggests that the cause for these discrepancies is driven by differences in health service delivery rather than individual or household characteristics. The observation of both very high and very low coverage levels within the same states indicates that system-level factors are driving the gap between coverage and effective coverage. This variation also confirms that the DBS test was both sensitive and accurate; if there were errors in data collection or in DBS analyses, there would be a large gap or problem across all municipalities. Instead, I found that in many municipalities crude and effective coverage were identical, while in others, the gap was very large. Our finding of clear and consistent geographic patterns highlights that some surveyed areas are very high-performing while others are experiencing substantial challenges.

The survey data included information that allowed me to investigate some of the drivers of this finding. Although it is possible that errors in health card documentation of vaccination may play a role, there were no indications of such practice. The data suggest that issues in the cold chain are the main driver of the findings. Unfortunately, samples of the vaccines from health facilities were not collected to examine whether measles vaccines maybe invalid or attenuated.

In Mexico, I found that maternal literacy and greater household wealth decreased the likelihood that a card-positive child would lack antibodies. That is, child health card documentation is more accurate for children from wealthier households with literate mothers. It is possible that more affluent and educated mother check the vaccination card of their children to ensure that a vaccine was given when marked. However, it is more plausible that the parents take their children to better run facilities and they avoid those with potential problems such as lack of electricity. We also found that card-positive children from urban areas were more likely to lack antibodies than their counterparts living in rural areas. This finding contrasted with the expectation that rural areas would be more likely to experience cold chain interruptions, and therefore children from these areas would be more likely to lack antibodies. One possible explanation is that rural facilities with weak electrical systems often do not store vaccines. Indeed, SM2015 interview teams frequently observed electricity challenges in both urban and rural areas, but only 49% of the surveyed facilities routinely store vaccines, and the majority of these are in urban areas. Another possible explanation is that differences in management between and rural facilities may affect vaccination practices and vaccine integrity. Moreover, it is possible that in poor communities families move to urban areas seeking employment. It is possible that such families live in poor areas of urban settings where the health facilities delivering services are overwhelmed by increased demand.

In Nicaragua, I found that several individual, maternal, community and health facility characteristics decreased the likelihood that a card-positive child would lack antibodies. Child health card documentation was more accurate for healthy weight female children living in urban areas or Jinotega whose mothers are married and have weekly exposure to mass media, and who attend health facilities that have routine administrative meetings. It is possible that families living in more affluent areas attend facilities that are better managed with better trained staff and therefore have better vaccination practices, vaccine integrity, and health card marking practices. It is also possible that few health facility characteristics were significant predictors because some children may receive vaccines through mobile units or vaccination campaigns.

We estimated that the prevalence of protective antibodies against measles was only 68% in Mexico and 50% in Nicaragua which raises the question of why no apparent outbreaks have occurred. One explanation is that the level of immunization coverage required for herd immunity in the surveyed populations is unknown. Given the unique, rural setting in which the participants live, the level of immunity may be sufficient to prevent a sustained outbreak. During data collection, field teams did not observe or hear of any measles outbreaks. Alternatively, in the absence of active surveillance in the region, it is also possible that some measles cases have occurred. While there were no confirmed cases of measles in either country in 2012 or 2013, between January 1st 2014 and May 5th 2014 there were 1021 suspected and 2 confirmed cases in Mexico and 41 suspected cases in Nicaragua.[51,58] In addition, in Mexico the ENSANUT 2012 showed recent declines in vaccination coverage,[48] which might explain this recurrence. Furthermore, if measles is present in these settings, it is possible that some surveyed children may have antibodies from natural exposure rather than immunization, implying that effective coverage of immunization may be even lower than estimated.

Our study is subject to several limitations. First, immunization data was collected from a relatively small sample of children which limited statistical power to detect significant patterns across demographic, geographic, and health facility factors. Second, the focus of this study was exclusively on protective antibodies against measles as a biomarker for measles immunization. Indeed, this analysis would have been strengthened by the quantification of antibodies against other vaccine-preventable diseases. Vaccines vary in efficacy, sensitivity to heat, and number of doses required to induce immunity, so the observed patterns for measles may not apply to other immunizations. Third, my ability to match participants to health facilities was limited. Only 25% of card-positive children in Mexico and 30% of such children in Nicaragua could be linked by name to interviewed health facilities. While the remaining participants could be linked by geographic region, households rarely reported attending the health facility closest to their home. As a result, the effects of health facility characteristics on antibody status may be diluted. However, the findings of this study were unchanged when restricting only to those households linked to health facilities by name, or only those households linked by region. Fourth, no interviews were conducted with private facilities or facilities operated by the Mexican Social Security Institute. The proportion of surveyed participants that attend these facilities is unknown, and attendance at these facilities may explain why no health facility characteristics were significant in the presented analyses. In addition, there was a moderate degree of non-response during the census, household survey and DBS collection. Our survey weights included some post-stratification which helps address this issue, but it is possible that non-response may have biased the results. Finally, SM2015 data is based on self-reported information from a household survey and may be subject to self-reporting bias.

On the other hand, this study has several strengths. First, the surveys had large sample sizes. For example, the most recent national health and nutrition surveys[46,48] collected immunization data from 723 and 223 children aged 12-23 months in SM2015 areas in Mexico and Nicaragua, respectively, compared to 1134 and 454 in this study. Second, study procedures incorporated electronic data capture and standardized methodology to ensure high data quality. Third, a new census was conducted in each selected primary sampling unit to ensure accurate denominator data for sampling, rather than using the most recent national population census. Indeed, when comparing the total population in each segment to existing census data, there were large differences.

Our findings are crucial to immunization activities in Mexico and Nicaragua. The use of DBS to estimate effective coverage instead of coverage based on cards and/or recall is the right approach for ensuring the health and wellbeing of children in these regions. This study's findings showed that such approach should be adopted and used in future immunization work. In fact, the Government of Mexico has adopted this approach based on findings form this study. Clearly knowledge of this issue is the first step in solving it. I was able to raise some potential issues that could explain the findings, which call for aggressive monitoring of the vaccine cold chain. Moreover, they call for random inspection of vaccine integrity. These steps will help ensure that high-quality immunizations are used to achieve high effective coverage of measles immunization in Mexico and Nicaragua.

References

1 Cutts FT, Izurieta HS, Rhoda DA. Measuring Coverage in MNCH: Design, Implementation, and Interpretation Challenges Associated with Tracking Vaccination Coverage Using Household Surveys. *PLoS Med* 2013; **10**: e1001404.

2 Miles M, Ryman TK, Dietz V, Zell E, Luman ET. Validity of vaccination cards and parental recall to estimate vaccination coverage: A systematic review of the literature. *Vaccine* 2013; **31**: 1560–8.

3 Davidkin I, Jokinen S, Broman M, Leinikki P, Peltola H. Persistence of Measles, Mumps, and Rubella Antibodies in an MMR-Vaccinated Cohort: A 20-Year Follow-up. *J Infect Dis* 2008; **197**: 950–6.

4 McDade TW. Development and validation of assay protocols for use with dried blood spot samples. *Am J Hum Biol Off J Hum Biol Counc* 2014; **26**: 1–9.

5 McDade TW, Williams S, Snodgrass JJ. What a drop can do: dried blood spots as a minimally invasive method for integrating biomarkers into population-based research. *Demography* 2007; **44**: 899–925.

6 Johannessen A. Dried blood spots in HIV monitoring: applications in resource-limited settings. *Bioanalysis* 2010; **2**: 1893–908.

7 National Research Council (US) Committee on Advances in Collecting and Utilizing Biological Indicators and Genetic Information in Social Science Surveys. Biosocial Surveys. Washington (DC), National Academies Press (US), 2008http://www.ncbi.nlm.nih.gov/books/NBK62436/ (accessed 12 Mar2014).

8 Lozano R, Naghavi M, Foreman K, *et al.* Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: a systematic analysis for the Global Burden of Disease Study 2010. *The Lancet* 2012; **380**: 2095–128.

9 Castillo-Solorzano C C, Matus CR, Flannery B, Marsigli C, Tambini G, Andrus JK. The Americas: paving the road toward global measles eradication. *J Infect Dis* 2011; **204 Suppl 1**: S270–278.

10 Cutts FT, Izurieta HS, Rhoda DA. Measuring coverage in MNCH: design, implementation, and interpretation challenges associated with tracking vaccination coverage using household surveys. *PLoS Med* 2013; **10**: e1001404.

11 Parker SP, Cubitt WD. The use of the dried blood spot sample in epidemiological studies. *J Clin Pathol* 1999; **52**: 633–9.

12 Snijdewind IJM, van Kampen JJA, Fraaij PLA, van der Ende ME, Osterhaus ADME, Gruters RA. Current and future applications of dried blood spots in viral disease management. *Antiviral Res* 2012; **93**: 309–21.

13 Riddell MA, Leydon JA, Catton MG, Kelly HA. Detection of measles virus-specific immunoglobulin M in dried venous blood samples by using a commercial enzyme immunoassay. *J Clin Microbiol* 2002; **40**: 5–9.

14 De Swart RL, Nur Y, Abdallah A, *et al.* Combination of reverse transcriptase PCR analysis and immunoglobulin M detection on filter paper blood samples allows diagnostic and epidemiological studies of measles. *J Clin Microbiol* 2001; **39**: 270–3.

15 El Mubarak HS, Yüksel S, Mustafa OM, Ibrahim SA, Osterhaus ADME, de Swart RL. Surveillance of measles in the Sudan using filter paper blood samples. *J Med Virol* 2004; **73**: 624–30.

16 Uzicanin A, Lubega I, Nanuynja M, *et al.* Dried blood spots on filter paper as an alternative specimen for measles diagnostics: detection of measles immunoglobulin M antibody by a commercial enzyme immunoassay. *J Infect Dis* 2011; **204 Suppl 1**: S564–569.

17 Helfand RF, Keyserling HL, Williams I, *et al.* Comparative detection of measles and rubella IgM and IgG derived from filter paper blood and serum samples. *J Med Virol* 2001; **65**: 751–7.

18 Samoĭlovich EO, Svirichevskaia EI, Ermolaev MA. [Comparative assessment of determination of measles virus IgG antibodies in blood serum and dried whole blood]. *Vopr Virusol* 2008; **53**: 46–8.

19 Mercader S, Featherstone D, Bellini WJ. Comparison of available methods to elute serum from dried blood spot samples for measles serology. *J Virol Methods* 2006; **137**: 140–9.

20 Condorelli F, Scalia G, Stivala A, *et al.* Detection of immunoglobulin G to measles virus, rubella virus, and mumps virus in serum samples and in microquantities of whole blood dried on filter paper. *J Virol Methods* 1994; **49**: 25–36.

21 Condorelli F, Ziegler T. Dot immunobinding assay for simultaneous detection of specific immunoglobulin G antibodies to measles virus, mumps virus, and rubella virus. *J Clin Microbiol* 1993; **31**: 717–9.

22 Riddell MA, Byrnes GB, Leydon JA, Kelly HA. Dried venous blood samples for the detection and quantification of measles IgG using a commercial enzyme immunoassay. *Bull World Health Organ* 2003; **81**: 701–7.

23 IBRAHIM SA, ABDALLAH A, SALEH EA, OSTERHAUS ADME, DE SWART RL. Measles virus-specific antibody levels in Sudanese infants: a prospective study using filter-paper blood samples. *Epidemiol Infect* 2006; **134**: 79–85.

24 Nakano JH, Miller DL, Foster SO, Brink EW. Microtiter determination of measles hemagglutination inhibition antibody with filter papers. *J Clin Microbiol* 1983; **17**: 860–3.

25 Wassilak SG, Bernier RH, Herrmann KL, Orenstein WA, Bart KJ, Amler R. Measles seroconfirmation using dried capillary blood specimens in filter paper. *Pediatr Infect Dis* 1984; **3**: 117–21.

26 Rawat D, Chakravarti A, Yadav S. Study of measles antibody in children. *Indian Pediatr* 2001; **38**: 1286–9.

27 Katz RS, Premenko-Lanier M, McChesney MB, Rota PA, Bellini WJ. Detection of measles virus RNA in whole blood stored on filter paper. *J Med Virol* 2002; **67**: 596–602.

28 Mosquera M del M, Echevarría JE, Puente S, Lahulla F, de Ory F. Use of whole blood dried on filter paper for detection and genotyping of measles virus. *J Virol Methods* 2004; **117**: 97–9.

29 Novello F, Ridolfi B, Fiore L, *et al.* Comparison of capillary blood versus venous blood samples in the assessment of immunity to measles. *J Virol Methods* 1996; **61**: 73–7.

30 Hogrefe WR, Ernst C, Su X. Efficiency of Reconstitution of Immunoglobulin G from Blood Specimens Dried on Filter Paper and Utility in Herpes Simplex Virus Type-Specific Serology Screening. *Clin Diagn Lab Immunol* 2002; **9**: 1338–42.

31 HUMAN Diagnostics Worldwide. Measles IgG ELISA Test for the Detection of IgG Antibodies to Measles Virus in Human Serum: Complete Test Kit. Wiesbaden, Germany, HUMAN, 2011.

32 Stöckl D, Dewitte K, Thienpont LM. Validity of linear regression in method comparison studies: is it limited by the statistical model or the quality of the analytical input data? *Clin Chem* 1998; **44**: 2340–6.

33 Helfand RF, Kebede S, Gary HE Jr, Beyene H, Bellini WJ. Timing of development of measles-specific immunoglobulin M and G after primary measles vaccination. *Clin Diagn Lab Immunol* 1999; **6**: 178–80.

34 HUMAN Diagnostics Worldwide. Design verification: Measles IgG. 2008.http://www.human.de/data/gb/vr/el-measg.pdf (accessed 1 Apr2014).

35 Shell-Duncan B, McDade T. Use of combined measures from capillary blood to assess iron deficiency in rural Kenyan children. *J Nutr* 2004; **134**: 384–7.

36 León-Velarde F, Gamboa A, Chuquiza JA, Esteba WA, Rivera-Chira M, Monge CC. Hematological parameters in high altitude residents living at 4,355, 4,660, and 5,500 meters above sea level. *High Alt Med Biol* 2000; **1**: 97–104.

37 Recommendations to Prevent and Control Iron Deficiency in the United States. *Morb Mortal Wkly Rep* 1998; **47**: 1–36.

38 Santos JI. Vaccination in Mexico in the context of the 'vaccine decades': achievements and challenges. *Gac Médica México* 2014; **150**: 180–8.

39 Golubjatnikov R, Elsea WR, Leppla L. Measles and rubella hemagglutination-inhibition antibody patterns in Mexican and Paraguayan children. *Am J Trop Med Hyg* 1971; **20**: 958–63.

40 Golubjatnikov R, Leppla L, Filloy L. [Hemagglutination inhibiting antibodies to measles, rubella, and parotitis. Study of their prevalence in a community of the Mexican plateau]. *Salud Pública México* 1970; **12**: 603–9.

41 Castillo-Solorzano C, Marsigli C, Danovaro-Holliday MC, Ruiz-Matus C, Tambini G, Andrus JK. Measles and rubella elimination initiatives in the Americas: lessons learned and best practices. *J Infect Dis* 2011; **204 Suppl 1**: S279–283.

42 Santos JI, Nakamura MA, Godoy MV, Kuri P, Lucas CA, Conyer RT. Measles in Mexico, 1941-2001: interruption of endemic transmission and lessons learned. *J Infect Dis* 2004; **189 Suppl 1**: S243–250.

43 National Institute of Statistics and Censuses (Nicaragua), Ministry of Health (Nicaragua), Macro International, Inc. Nicaragua Demographic and Health Survey 2001. Calverton, United States, Macro International, Inc.

44 Ministry of Health (Nicaragua), National Institute for Development Information (Nicaragua). Living Standards Measurement Survey 2005. Managua, Nicaragua, National Institute for Development Information (Nicaragua).

45 Division of Reproductive Health-Centers for Disease Control and Prevention (CDC), National Institute for Development Information (Nicaragua). Nicaragua Reproductive Health Survey 2006-2007. Managua, Nicaragua, National Institute for Development Information (Nicaragua).

46 Ministry of Health (Nicaragua), National Institute for Development Information (Nicaragua). Nicaragua National Demographic and Health Survey 2011-2012. Managua, Nicaragua, National Institute for Development Information (Nicaragua).

47 National Institute for Public Health (Mexico). Mexico National Survey of Health and Nutrition 2005-2006. Cuernavaca, Mexico, National Institute for Public Health (Mexico).

48 National Institute of Public Health (Mexico). Mexico National Survey of Health and Nutrition 2011-2012. Cuernavaca, Mexico, National Institute of Public Health (Mexico).

49 Pomelova VG, Osin NS. [Prospects of the integration of dry blood spot technology with human health and environmental population studies]. *Vestn Ross Akad Meditsinskikh Nauk Ross Akad Meditsinskikh Nauk* 2007; : 10–6.

50 Spooner N. A glowing future for dried blood spot sampling. *Bioanalysis* 2010; **2**: 1343–4.

51 WHO vaccine-preventable disesases: monitoring system 2014 global summary. Measles reported cases. , World Health Organization, 2014http://apps.who.int/immunization_monitoring/globalsummary/timeseries/tsincidencemeasles.html (accessed 29 May2014).

52 Ruiz-Gómez J, Valdespino JL, Olaiz-Fernández G, Arias-Toledo E, Sepúlveda J. Encuesta serológica nacional del sarampiónen niños: evidencias para su eliminación. (Spanish). *Measles Serol Surv Mex Child Evid Its Elimin Mex Engl* 2007; **49**: S370–S376.

53 Gakidou E, Lozano R, González-Pier E, *et al.* Assessing the effect of the 2001-06 Mexican health reform: an interim report card. *Lancet* 2006; **368**: 1920–35.

54 Mexican Ministry of Health. Effective coverage of the health system in Mexico 2000-2003. Federal District, Mexico, Mexican Ministry of Health, 2006.

55 Lozano R, Soliz P, Gakidou E, *et al.* Benchmarking of performance of Mexican states with effective coverage. *The Lancet* 11; **368**: 1729–41.

56 WHO Department of Immunization, Vaccines and Biologicals. The Immunological Basis for Immunization Series: module 7: measles- Update 2009. Geneva, Switzerland, World Health Organization, 2009http://whqlibdoc.who.int/publications/2009/9789241597555_eng.pdf (accessed 10 Jun2014).

57 Collaborative Study by the Ministries of Health of Brazil, Chile, Costa Rica, Ecuador, and the Pan American Health Organization. Seroconversion rates and measles antibody titers induced by measles vaccine in Latin American Children 6-12 Months of Age. *Bull Pan Am Health Organ* 1982; **16**.

58 Reported measles cases and incidence rates by WHO Member States 2013, 2014 as of 05 May 2014. http://www.who.int/immunization/monitoring_surveillance/burden/vpd/surveillance_type/active/measlesreportedcasesbycountry.pdf?ua=1.