OPEN ACCESS

Microbiological & Immunological Communications

DOI: doi.org/10.55627/mic.003.01.0513

Research Article

Sero-surveillance of Measles Virus Seropositivity Amongst Vaccinated Children of Rural Areas of Sindh

Zeb Hussain[1]*, Ambreen Fatima[2], Muhammad Sameer Qureshi[1], Asif Iqbal Khan[1], Shaheen Sharafat[2]

[1]Department of Clinical Laboratory Sciences, Dow Institute of Medical Technology, Dow University of Health Sciences, Karachi, Pakistan
[2]Department of Pathology, Dow International Medical College, Dow University of Health Sciences, Karachi, Pakistan
***Correspondence**: Zeb.hussain@duhs.edu.pk

Abstract

Measles remains a serious public health concern worldwide, and outbreaks persist despite large vaccination campaigns, particularly in low-income areas. This study seeks to contribute to the existing body of information by analyzing antibody levels in vaccinated newborns, determining the durability of protection, and identifying potential vaccination gaps. Understanding the measles seroprevalence in this specific area is critical in order to optimize vaccination strategies and ensure the continuous protection of vulnerable people in rural Sindh, Pakistan. Serum samples were collected from 173 children using standard sample collection protocol. Measles-specific IgG antibodies were quantified using enzyme-linked immune sorbent assay (ELISA) at the Department of Microbiology Dow Diagnostic Research and Reference Laboratory (DDRRL). The study recruited children with an average age of 41.3 months, evenly split between males (n=88) and females (n=81), all vaccinated as per their parents' records. The measles IgG antibodies seropositivity ratio in children among the vaccinated population was 53 (30.6%), and seronegative 120 (69.4%) out of 173. The study revealed alarming rates of measles susceptibility among vaccinated children in Pakistan, despite analyzing various factors, including demographics and socio-economic status, no significant associations with measles susceptibility were found. These findings emphasize the need for improved vaccination strategies to address deficiencies in immunity levels and mitigate the risk of measles outbreaks in the region.

Keywords: ELISA, Immunoassay, EMRO, Pakistan, Sindh, Karachi

1. Introduction

Measles is a continuously progressing disease in various parts of the world affecting thirty million people every year. In 2000, the global annual measles incidence stood at 145 cases per million people. However, thanks to the swift implementation of immunization programs across the globe, this incidence saw a remarkable 83% reduction, reaching 25 cases per million people(Dabbagh et al. 2018). despite of outstanding efforts of the Expanded Program on Immunization (EPI) and various Non-government Organizations measles eradication seems an issue of global concern. From 2001 to 2014, the epidemiological status of the disease has remained epidemic in Pakistan(Greenwood 2014). For Pakistan, Measles disease is still a high-priority public health problem(Sheikh et al. 2011).

In Pakistan, the recorded instances of measles virus were 1,978 confirmed cases in November 2019(Muzzamil et al. 2023). This figure escalated to 8,500 in 2020, followed by a rapid surge to

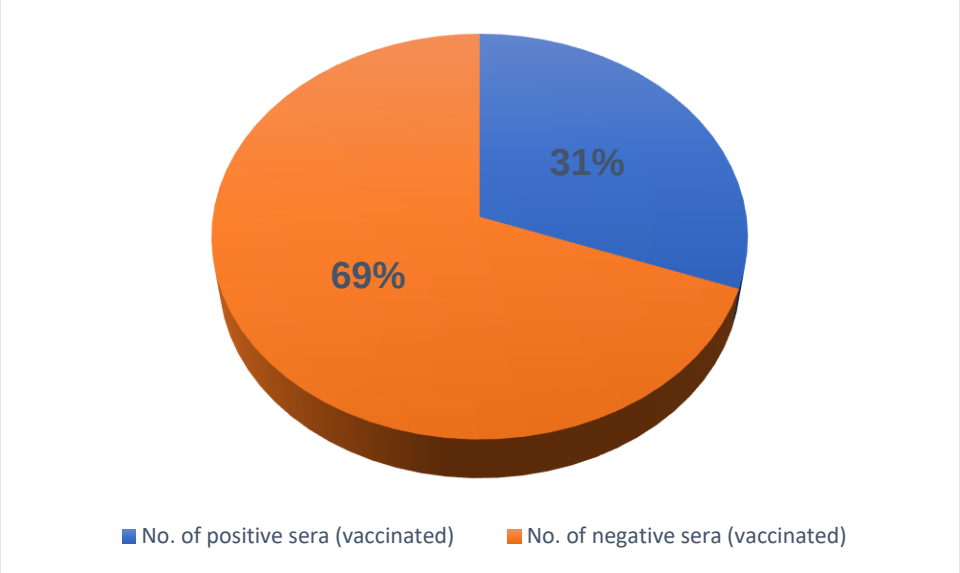

Figure 1: Measles Sero-prevalence in vaccinated population age 1-5 years

17,000 in 2021(Muzzamil et al. 2023). The projected number of new cases estimated between November 2022 and April 2023 is approximately 6,426 (Muzzamil et al. 2023; Minta and Report 2023). Millions of children die from treatable and preventable diseases annually and vaccination plays a crucial role in saving lives. Specifically, measles vaccines alone are believed to have prevented more than 21 million deaths from 2000 to 2017(Khan et al. 2023) . Nonetheless, the risk of measles outbreak persists when individuals travel there where measles is endemic or citizens of such areas move globally(Khalil et al. 2005).

A measles-specific seroprevalence study is an important tool for assessing the level of immunity within a population against vaccine-preventable diseases such as measles. By analyzing the presence of specific antibodies in blood samples, researchers can determine the proportion of individuals who are immune to the disease due to vaccination or prior infection. When combined with computer modeling, sero-epidemiologic studies can provide valuable insights into the dynamics of measles transmission within a population. This approach allows researchers to identify pockets of susceptible individuals, particularly children, and assess the potential risk of outbreaks. By analyzing age-specific immunity levels, the study can also highlight changes in the risk of infection across different age groups(Wilson et al. 2012). Serosurveillance studies, which evaluate vaccine-preventable diseases, estimate the percentage of individuals in a population possessing antibodies in their blood. These antibodies indicate previous exposure to infectious agents and viruses. Researchers can identify how many individuals are infected with a specific virus through the detection of antibodies(de Coronado, Remennik, and Elkin 2023).

Sero-surveillance is, therefore, the best method for measuring immunity in a whole population, and complements disease survey. Health authorities always look forward to complete information and the status of the vaccinated population. Therefore, serological studies give comprehensive knowledge about the status of immunity and virus transmission. Disease surveys that give antibodies titer, rather than presence or absence; provide further information on the potential for long-term protection, likely distribution, and re-occurrence of measles virus.

More alarmingly, the measles-susceptibility of children residing in rural areas (a major hub of

measles outbreaks) is only imagination at the moment. There are no data to delineate the efficacy of measles vaccination in most rural areas of the country. In this context, it is highly relevant to undertake population-based surveillance studies to identify the measles-susceptible population in Pakistan so that appropriate actions can be designed by relevant authorities. We therefore investigated the measles-susceptibility of vaccinated children in the district Qambar Shahdadkot (QSK).

2. Material and Methods

2.1. Ethical Considerations

This population-based study was reviewed and approved by the Institutional Review Board at the Dow University of Health Sciences, Karachi, and Ref.no: IRB-516/DUHS/-14.

2.2. Study Population, Design and Data Collection

The present study is an age-stratified serological survey that was carried out for three months from October to December 2014. Randomly serum sample collection in the population was used. This study was conducted at the Dow University of Health Sciences, Karachi. After obtaining approval from the institutional review board of the university, a team of researchers (including a physician and four phlebotomists) visited the district QSK for recruitment and collection of samples. District QSK is composed of >200 villages. Of these, we recruited children from four villages of the district QSK namely Long Khan Khoso, Mirza Khan Jagirani, Nizam-ud-Din Khoso, and Habit Khan Magsi villages. An eligible-for-study child was defined as any child aged between 1 and 5 years, vaccinated for measles, and with no current history of measles infection. With the cooperation of the local residents, a day-long camp was organized in a primary school. Announcements were made via mosques and word of mouth by local residents regarding the inclusion criteria for the study. At the camp, a total of 186 children were found eligible, while 13 parents refused to consent. Therefore, the total number of recruited children in the study was 173. Whenever an eligible child was found, the parent/guardian/caretaker explained the study protocol and was asked to sign the informed consent. A questionnaire to read out to them in the language of their communication (mostly Sindhi) to record socio-demographic details. The low-income group is defined as those with limited financial resources, the middle-income group falls between low and high-income brackets, and the high-income group comprises individuals with substantial financial resources. In terms of vaccination history, we always inquired about the availability of a vaccination card. However, in the absence of available vaccination cards, the subject was excluded from the study.

2.3. Collection of Blood and Quantification of Measles Specific IgG Antibodies

After informed consent and questionnaire filling, 5ml of venous blood was collected and the tubes were kept in 4C overnight. The next day, blood samples were centrifuged and the sera (supernatant) were collected and stored at -20C until further use. Measles-specific IgG antibodies were quantified using an ELISA (DIA Source, Measles IgG specific) kit.

2.4. Statistical Analyses

Data were entered in SPSS version 20 by three authors (ZH, MAQ, and SS) and were checked for accuracy. In case of any discrepancy, a consensus was sought by looking at the raw data (hand-filled questionnaires). Chi-square and t-tests were used to analyze categorical and continuous variables respectively. In order to investigate/identify possible factors associated with the levels of measles-specific IgG antibodies, multiple logistic regression analysis was used. A p-value of <0.05 was considered as significant for all the analyses described herein.

3. Results

Demographic details of the children recruited in the study are shown in (**Table 1**). The average age of children in our study was 41.3 months (SD=15.7),

Table 1: Factors associated with measles susceptibility in children living in district Qamber Shahdadkot of Sindh, Pakistan

Variables	Measles antibodies present, frequency (%)	Measles antibodies absent, frequency (%)	Crude OR (95% CI)	Adjusted OR (95% CI)
Overall	51 (30.2)	118 (69.8)		
Gender				
Male	24 (14.2)	64 (37.9)	1	1
Female	27 (15.9)	54 (32)	0.75 (0.38, 1.44)	0.83 (0.41,1.68)
Age in months				
12 ≤ 24	10 (5.9)	31 (18.3)	1	1
>24 ≤ 36	14 (8.4)	24 (14.2)	0.71 (0.27, 1.81)	0.75(0.27, 2.12)
>36 ≤ 48	12 (7.1)	30 (17.7)	1.28 (0.52, 3.15)	1.22 (0.47, 3.1)
>48 ≤ 60	15 (8.9)	33 (19.5)	0.88 (0.35, 2.17)	0.69 (0.25, 1.8)
Mother's education				
No formal education	47 (27.8)	96 (56.8)	1	1
≤ 10 years	0 (0)	2 (1.2)	2.44 (0.67, 8.87)	3.87 (0.75, 20.1)
11-14 years	1 (0.6)	5 (2.9)	0.00	0.00
≥ 14 years	3 (1.8)	15 (8.9)	1.0 (0.084, 11.93)	1.97 (0.13, 29.5)
Father's education				
No formal education	7 (4.1)	17 (10.1)	1	1
≤ 10 years	10 (5.9)	10 (5.9)	1.27 (0.411, 3.96)	0.55 (0.14, 2.18)
11-14 years	24 (14.2)	60 (35.6)	3.10 (1.1, 9.59)	1.39 (0.35, 5.52)
≥ 14 years	10 (5.9)	31 (18.3)	1.24 (0.52, 2.91)	0.55 (0.18, 1.72)
Socio-economic status				
Low	50 (29.6)	106 (62.7)	1	1
Middle	1 (0.6)	12 (7.1)	5.66 (0.71, 44.7)	6.7 (0.71, 64.13)
High	0	0	-	-
Place of birth				
Home	49 (29)	114 (67.4)	1	1
Hospital	2 (1.2)	4 (2.4)	1.16 (0.21, 6.56)	3.93 (0.43, 35.7)

*Omnibus tests of model co-efficient and multi-variate regression analyses showed that none of the independent variables significantly contributed to the odds of "not being measles susceptible" compared to the measles-susceptible children, i.e., p-value>0.05 for all independent variables.

Notably, the Cox & Snell and Nagelkerke R2 values indicate that only 8-11% of the variability in measles susceptibility is explained by the independent variables described in this table, suggesting more important players not investigated in the study, such as the quality of the vaccines used.

with almost equal distribution of males (n=88) and females (n=81). All children were vaccinated according to their parents and vaccination cards were available for all of the children investigated in this study.

Alarmingly, measles-specific IgG antibodies were detected in only 53 (30.6%) of the children while a total of 120 (69.4%) of the vaccinated children were negative for protective antibody titers making

them highly susceptible to measles attacks. Shown in (**Figure 1**).

In order to investigate any possible factors associated with measles susceptibility in these children, we performed multivariate logistic regression analyses using "measles-susceptibility" as the dependent while gender, mother's education, father's education, socio-economic status, age of children, and place of birth as

Table 2: Distribution and prevalence of measles IgG antibodies according to sex in different

Age Group (Years)	Vaccinated Males			Vaccinated Females		
	Sero +ve (%)	Sero -ve (%)	Total No.	Sero +ve (%)	Sero -ve (%)	Total No.
1 – 2	20.8 (5)	79.2 (19)	24	27.8 (5)	72.2 (13)	18
2 – 3	37.5 (6)	62.5 (10)	16	37.5 (9)	62.5 (15)	24
3 – 4	27.8 (5)	72.2 (13)	18	32.0 (8)	68.0 (17)	25
4 – 5	26.7 (8)	73.3 (22)	30	38.9 (7)	61.1 (11)	18
Total	32.9 (24)	72.7 (64)	88	34.1(29)	65.9 (56)	85

predictor/independent variables. Interestingly, none of the independent variables were found responsible for measles susceptibility in our study. Moreover, R^2 values using various models (Cox & Snell and Nagelkerke) showed that only 8%-11% of the variability could be explained in our study, strongly suggesting that more important players (such as quality of vaccination) may be responsible for measles susceptibility in this area of Pakistan. Children were divided into four groups 1-2, 2-3, 3-4, 4-5 years old. In the first group 1-2 years old male children 20% were seropositivity, and 79% were seronegativity. In 2nd group of children 6(37.5 %) male children were sero positive, 10(62.5%) male were seronegative, 9(37.5%) female were seropositive, 15(62.5%) were female sero negative, In 3rd group of children, 5(27.8%) male were sero positive,13(72.2%) male were sero negative however in female seropositivity were 8(32%), seronegativity were 17(68.8%). In our last group shown in **Table 2**. Male seropositive and sero negative were 8(26.7%), and 22(73.3%), as in female sero positive samples were 7(38.9%), and sero negative were11 (61.1%).

4. Discussion

This serological study examined the seroprevalence of Measles antibodies in vaccinated children of the population following the national measles vaccination program which used a dosing regimen. Despite the annual organization of free immunization campaigns by the Expanded Program of Immunization (EPI) and the Ministry of Health Pakistan (MOH), the incidence of measles continues to be reported every year, highlighting the ongoing need for vaccination as the primary prevention method against this highly contagious and infectious disease. Several studies were carried out in Pakistan but their approach was on 1 to 17 years in vaccinated as well as non-vaccinated in urban areas according to that study seropositive status was 79% against measles disease(Channa et al. 2012). The present study is the first which is conducted in vaccinated children of 1 to 5 years old children of the rural population of Sindh district qambar-shahdadkot, Pakistan considering the possible association between age, sex, and immunological response to vaccination.

Measles IgG antibodies seropositivity ratio in children amongst the vaccinated population is 53 (30.6%), sero negative 120 (69.4%) out of 173. Although the threat of getting infected by measles is elevated yet within un-vaccinated inhabitants, the augmented outbreak within vaccinated populations elevates substantial questions on our

immunization plan. The reason of measles outbreaks in spite of widespread immunization demands considerable focus and concentration(Gastañaduy et al. 2021). The increase in reported cases of measles in various countries, including both developed and developing nations, is a cause for concern and has been attributed to several factors. One of the primary reasons for the resurgence of measles is vaccine refusal and hesitancy, which has led to suboptimal vaccination coverage in many communities(Wilder-Smith, Qureshi, and Health 2020). The findings indicate a limited seropositivity response following vaccination in the Qamber Shahdadkot district. Measles virus infection is a highly contagious disease transmitted through droplets from one person to another. Each year, outbreaks claim the lives of numerous innocent children due to inadequate diagnosis and treatment options. In Pakistan, low measles seropositivity rates are influenced by multiple factors. Vaccine efficacy plays a crucial role, with variations arising from issues like improper storage, transportation, and administration practices, impacting the vaccine's ability to elicit an adequate immune response.

The World Health Organization (WHO) has highlighted the impact of vaccine hesitancy as a significant contributor to the rise in measles cases. This hesitancy is often fuelled by misinformation, mistrust in vaccines, and concerns about potential side effects. As a result, some individuals or communities choose not to vaccinate themselves or their children, leading to a decline in overall immunity and leaving populations vulnerable to measles outbreaks(Naso 2020).The resurgence of measles poses a significant public health threat, as the disease can lead to severe complications, especially in vulnerable populations such as infants, the elderly, and individuals with compromised immune systems(Gastanaduy et al. 2018). To address this global health challenge, efforts to combat vaccine hesitancy, improve vaccination coverage, and strengthen healthcare systems are essential. Public health authorities, healthcare providers, and communities must work together to promote the importance of vaccination, address misinformation, and ensure equitable access to immunization services. Additionally, ongoing surveillance, outbreak response, and international collaboration are crucial in controlling and ultimately eliminating measles as a public health threat.

There is a dire need for the EPI related representatives into the deficiencies in the system especially in the territories of KPK, Baluchistan, and Sindh(Ali and Rais 2021). To the best of our knowledge, this sero-surveillance study was conducted for the first time in rural areas of Sindh. Therefore sero-surveillance is the best method to monitor vaccine status in the population and it is recommended at the provincial level to monitor the actual level of protective antibody titers and evaluate country's vaccination status(Pradhan et al. 2020). According to susceptibility profile for a particular country can be estimated by disease incidence data and vaccination coverage, but the most direct evidence comes from seroprevalence studies(Cutts et al. 2020). Vaccines have important benefits at the individual, community, and socio-economical levels for children. Despite this Immunization programs often fail to protect children from measles(Machado et al. 2021). The results show high seronegativity, and thus very high susceptibility, amongst vaccinated children of district QSK, indicating a lack of effective measles vaccination in the district. It is therefore very important that relevant authorities undertake urgent actions to prevent further measles outbreaks in this region.

5. Conclusion:

The average seropositive prevalence for measles vaccine in male children is lower than in female children, and seronegative is higher in 1-2 years age group (as our study designed). The seropositive ratio is not dependent on age with increasing age groups such as 20.2 % in 1-2 37.5%

observed in group two (2-3) and 27.8% in group three (3-4 years), 26.7% in group four (4 to 5) respectively. In this study, we argue that immunization programs against measles disease should be more effective and furthermore, that action should be taken against policymakers to ensure that the benefits of vaccination are fully realized. Policymakers should work more effectively on data collection and analysis, ensuring engaging healthcare professionals, changing public perceptions of vaccination, and integrating vaccination into primary education school systems.

Conflict of Interest

The authors declare that they have no competing interests.

Funding

This research received no external funding.

Study Approval

This study was approved by the Institutional Review Board at the Dow University of Health Sciences, Karachi, and Ref.no: IRB-516/DUHS/-14.

Consent Forms

The consent form was taken from the parent and guardian.

Authors Contribution

ZH conceptualized the study and wrote the final manuscript, SS critically reviewed and edited the manuscript, MSQ critically reviewed the manuscript and made editing and language improvements. AIK, ZH, and AF helped with the analysis and figures, and SS was responsible for the supervision, critical review, and finalized manuscript writing.

Data Availability

Data is available upon reasonable request from the corresponding author.

Acknowledgment

The corresponding author thanks all the coauthors and laboratory fellows for their help during this project.

References:

Ali, Sameen A Mohsin, and Rasul Bakhsh %J South Asia: Journal of South Asian Studies Rais. 2021. "Pakistan's Health-Care System: A Case of Elite Capture." 44 (6): 1206-1228.

Channa, Rafique Ahmad, Shahzad Hussain, Nisar Ahmad Kanher, Farnaz Malik, M Memon, Sidra Mahmood, Fahdiya Yasin, Amina Wajid, Shazia Shafaat, Rashid %J African Journal of Pharmacy Mahmood, and Pharmacology. 2012. "Sero-surveillance of measles amongst vaccinated and non-vaccinated children: An age stratified population based survey in Pakistan." 6 (24): 1713-1718.

Cutts, FT, E Dansereau, MJ Ferrari, M Hanson, KA McCarthy, CJE Metcalf, S Takahashi, AJ Tatem, N Thakkar, and S %J Vaccine Truelove. 2020. "Using models to shape measles control and elimination strategies in low-and middle-income countries: a review of recent applications." 38 (5): 979-992.

Dabbagh, Alya, Rebecca L Laws, Claudia Steulet, Laure Dumolard, Mick N Mulders, Katrina Kretsinger, James P Alexander, Paul A Rota, James L %J Morbidity Goodson, and Mortality Weekly Report. 2018. "Progress toward regional measles elimination—worldwide, 2000–2017." 67 (47): 1323.

de Coronado, Sherri, Lyubov Remennik, and Peter L Elkin. 2023. "National Cancer Institute Thesaurus (NCIt)." In Terminology, Ontology and their Implementations, 395-441. Springer.

Gastanaduy, Paul A, Emily Banerjee, Chas DeBolt, Pamela Bravo-Alcántara, Samia A Samad, Desiree Pastor, Paul A Rota, Manisha

Patel, Natasha S Crowcroft, David N %J Human vaccines Durrheim, and immunotherapeutics. 2018. "Public health responses during measles outbreaks in elimination settings: Strategies and challenges." 14 (9): 2222-2238.

Gastañaduy, Paul A, James L Goodson, Lakshmi Panagiotakopoulos, Paul A Rota, Walt A Orenstein, and Manisha %J The Journal of Infectious Diseases Patel. 2021. "Measles in the 21st century: progress toward achieving and sustaining elimination." 224 (Supplement_4): S420-S428.

Greenwood, Brian %J Philosophical Transactions of the Royal Society B: Biological Sciences. 2014. "The contribution of vaccination to global health: past, present and future." 369 (1645): 20130433.

Khalil, Mohamed KM, Yagob Y Al-Mazrou, Mansour N AlHowasi, and Mohamed %J Annals of Saudi Medicine Al-Jeffri. 2005. "Measles in Saudi Arabia: from control to elimination." 25 (4): 324-328.

Khan, Amna, Shehzad Saleem, Alishba Rasool, Mahnoor Khan, Marina Akhtar, Irfan Munir, Saira %J Journal of Society of Prevention Afzal, Advocacy, and Research KEMU. 2023. "Seroprevalence of Vaccine Preventable Diseases among Children." 2 (3): 183-190.

Machado, Amanda Alberga, Sarah A Edwards, Melissa Mueller, and Vineet %J Vaccine Saini. 2021. "Effective interventions to increase routine childhood immunization coverage in low socioeconomic status communities in developed countries: A systematic review and critical appraisal of peer-reviewed literature." 39 (22): 2938-2964.

Minta, Anna A %J MMWR. Morbidity, and Mortality Weekly Report. 2023. "Progress Toward Measles Elimination—Worldwide, 2000–2022." 72.

Muzzamil, Muhammad, Farwa Fatima, Rabia Baloch, Shahkamal Hashmi, and Abdullah %J IJS Global Health Malikzai. 2023. Under-5 children in Pakistan are disproportionately affected by the alarming increase in measles cases. LWW.

Naso, James. 2020. "Vaccine hesitancy: exploring its causes and solutions." Boston University.

Pradhan, Sanchita Roy, Sutapa Mahata, Dipanwita Ghosh, Pranab Kumar Sahoo, Sinjini Sarkar, Ranita Pal, and Vilas D Nasare. 2020. Human Papillomavirus Infections in Pregnant Women and Its Impact on Pregnancy Outcomes: Possible Mechanism of Self-Clearance. IntechOpen.

Sheikh, Sana, Asad Ali, Anita KM Zaidi, Ajmal Agha, Asif Khowaja, Salim Allana, Shahida Qureshi, and Iqbal %J Vaccine Azam. 2011. "Measles susceptibility in children in Karachi, Pakistan." 29 (18): 3419-3423.

Wilder-Smith, Annika B, Kaveri %J Journal of epidemiology Qureshi, and global health. 2020. "Resurgence of measles in Europe: a systematic review on parental attitudes and beliefs of measles vaccine." 10 (1): 46.

Wilson, Sarah E, Shelley L Deeks, Todd F Hatchette, and Natasha S %J Cmaj Crowcroft. 2012. "The role of seroepidemiology in the comprehensive surveillance of vaccine-preventable diseases." 184 (1): E70-E76.