**INTRODUCTION**

The sustainability of vaccination programs depends on understanding risk of vaccine-preventable illnesses and benefits of vaccination. Pregnant women are at increased risk of influenza hospitalization,\(^1\) adverse birth outcomes,\(^2,3\) and death as a result of influenza infection. Their disproportionate risk of influenza complications is attributed to anatomical, physiological, and immune changes during pregnancy.\(^1\)
The 2016–2017 influenza season in the Americas was associated with an annual peak in late December—early January. In 2016, important gaps in knowledge about risk of influenza illnesses among pregnant women were identified. Such gaps in knowledge threaten the sustainability of newly established vaccination programs. For example, pregnant women seem to underutilize vaccines because they and their providers are often unaware of illness risks and uncertain about the benefits of vaccination. This is reflected in the modest 2018 influenza vaccination coverage among pregnant women in 22 of the 45 PAHO countries reporting such data in the Joint Reporting Form (median 72.5%, interquartile range 38%–82%).

To address a gap in knowledge about the risk of influenza, and better understand the benefits of influenza vaccination, we established mother/baby cohorts in Panama and El Salvador. Our primary objective was to quantify rates of influenza illnesses among pregnant women and their offspring in each country. As secondary objectives, we quantified rates of rhinovirus, parainfluenza 1–3, respiratory syncytial viruses (RSV), human metapneumovirus, and adenovirus. In this manuscript we present pregnant women’s risk of acute respiratory illnesses (ARI) by viral etiology. While we were not powered to estimate vaccine effectiveness, we quantify and time vaccine coverage in relation to the timing of influenza epidemics. Influenza illness and vaccination knowledge, attitudes, and practices among pregnant women are part of a separate investigation.

2 METHODS

2.1 Study population

In 2014, we established a multi-center longitudinal prospective cohort study of pregnant women in two primary health care centers in Panama (i.e., Tocumén and 24 de Diciembre Health Centers) and two in El Salvador (i.e., Tomas Piñeda and San Rafael Health Units). The cohort was named INFLUMIKA for its Spanish language acronym (i.e., Influenza maternal e Infantil y Zika). We chose these four peri-urban health centers because their staff managed prenatal care for 300–400 women per year and provided influenza vaccination free-of-charge as part of prenatal care. We sought to enroll all pregnant women aged 15 or older who resided in the catchment of these health centers and were willing and able to provide written informed consent to participate.

2.2 Patient involvement

Upon enrollment, study staff documented demographic, socioeconomic, and clinical histories using standardized survey instruments into an open-source software (OpenClinica, LLC, Waltham, MA, USA). Information about ultrasound gestational age dates and vaccination was updated during subsequent pre-natal visits. When feasible, self-reports were compared with medical records. Participants were instructed to contact study staff if they developed any ARI symptoms including cough, sore throat, or rhinorrhea. Participants were also called and/or texted each week throughout their pregnancy to identify ARI. Women with new onset measured fever of ≥38°C or subjective fever and ARI within 7 days of contact were asked to come to the clinic for respiratory virus testing. If women were unreachable for two consecutive prenatal check-ups or after three phone calls and/or texts, they were considered lost to follow-up.

2.3 Laboratory testing for core outcome

Swabs were transported at 2–8°C to the national reference laboratories within 24–48 h of sample collection. Gorgas Memorial Institute for Health Studies in Panama or the Laboratorio Nacional de Referencia in El Salvador tested specimens through reverse-transcription polymerase chain reaction to identify respiratory virus RNA using a protocol and primers and probes provided by the US Centers for Disease Control and Prevention (CDC).

2.4 Data management and analysis

We estimated the incidence of respiratory viruses during pregnancy using two risk periods. First, we divided the number of ARI and the number of laboratory-confirmation of each virus by the total person-time mothers contributed to study. Second, we divided the number of laboratory-confirmation of each virus by the person-time mothers contributed to the study during weeks that virus was circulating nationally as detected by that country’s National Influenza Centers (NICs). We subtracted 2 weeks from this risk period for every laboratory-confirmed illness because we presumed convalescing women would not be susceptible to a new infection with the same virus within that time. We also assumed ARI were separate events if they occurred after >14-day symptom-free period.

We estimated the proportion of women with ARI, but without respiratory swabs, that could have tested positive for each virus. To estimate such missed detections, we multiplied the number of women with untested ARI by the proportion of persons aged 15–49 years with respiratory swabs that tested positive for that virus in that country’s national surveillance systems during that week. In doing so, we assumed that untested women with ARI in the Panama and El Salvador cohorts would have been as likely to test positive for those respiratory viruses as persons aged 15–49 years identified through each country’s national surveillance system. We adjusted by the proportion of influenza detections among afebrile vs. febrile adults with ARI (i.e., 0.24) because untested women were typically afebrile, while those tested were febrile. To partially account for uncertainty, we incorporated the variance in the proportion of persons aged 15–49 years testing positive for each virus per week among all tested and the variance in the proportion testing positive for influenza among afebrile vs. febrile persons. To assess whether women
had been vaccinated against influenza before the start of same-year influenza epidemics, we defined epidemics as the first week when the proportion of samples testing positive for influenza was above the annual median for ≥3 consecutive weeks. We used STATA SE 15.1 (StataCorp., College Station, TX, USA) for all statistical analyses.

3 | RESULTS

3.1 | Enrollment, demographics and follow

During October 11, 2014 to April 10, 2017, we approached 2604 pregnant women and enrolled 2556 (98%) (Table 1); 1051 in El Salvador contributed 464 person-years (py) and 1505 women in Panama contributed 533 py (Table 2). In El Salvador, women were at risk for influenza during 297 py (64%) of 464 py and in Panama during 293 py (55%) of 533 py. Women were more frequently primigravidae in El Salvador (487, 47%) and had <7th grade education (265, 28%) and <$400 USD income per month (741, 89%) when compared to women in Panama (423, 28%, 212, 18%, and 335, 38%, respectively, P < 0.0001). Women in Panama had a maximum vaccination coverage of 66% and only 13% were vaccinated against influenza before the start of the influenza epidemic period (Figure 1a). Similarly, women in El Salvador had a maximum vaccination coverage of 66% and only 13% were vaccinated against influenza before the start of the influenza epidemic period (Figure 1b). On average, pregnant women were vaccinated at 17 weeks gestation (IQR 11–25).

### Table 1 Characteristics of pregnant women at enrolment in the INFLUMIKA cohort

|                        | El Salvador | Panama | P value | Total     |
|------------------------|-------------|--------|---------|-----------|
| n                      | 1051        | 1505   |         | 2556      |
| Age, median (interquartile) | 23 (19–27)  | 24 (20–27) | 0.012   | 401 (15.7%) |
| Race                   |             |        |         |           |
| Indigenous             | 1 (0.1%)    | 400 (26.6%) | <0.001 | 401 (15.7%) |
| Mestizo                | 1049 (99.8%)| 758 (50.4%) |        | 1807 (70.7%) |
| White                  | 0 (0%)      | 247 (16.4%) |        | 247 (9.7%)     |
| Black                  | 1 (0.1%)    | 97 (6.4%)    |        | 98 (3.8%)      |
| Other                  | 0 (0%)      | 3 (0.2%)    |        | 3 (0.1%)       |
| Primary school or less | 265 (28.3%) | 212 (17.7%) | <0.001 | 477 (22.3%) |
| Income <$400 USD       | 741 (88.8%) | 335 (37.8%) | <0.001 | 1076 (62.6%) |
| Drinks alcohol         | 5 (0.5%)    | 46 (3.1%)   | <0.001 | 51 (2.0%)      |
| Smoker                 | 6 (0.6%)    | 12 (0.8%)   | 0.633  | 18 (0.7%)      |
| Pre-existing condition | 42 (4%)     | 171 (11.6%) | <0.001 | 213 (8.4%)     |
| Asthma                 | 19 (1.8%)   | 59 (3.9%)   | 0.002  | 78 (3.0%)      |
| Hypertension           | 6 (0.6%)    | 27 (1.8%)   | 0.007  | 33 (1.3%)      |
| Kidney disease         | 0 (0%)      | 5 (0.3%)    | 0.082  | 5 (0.2%)       |
| Heart disease          | 1 (0.1%)    | 3 (2.0%)    | 0.648  | 4 (0.2%)       |
| Diabetes               | 3 (0.3%)    | 2 (0.1%)    | 0.407  | 5 (0.2%)       |
| Cancer                 | 0 (0%)      | 2 (0.1%)    | 0.516  | 2 (0.1%)       |
| HIV                    | 0 (0%)      | 2 (0.1%)    | 0.516  | 2 (0.1%)       |
| Primigravid            | 487 (46.5%) | 423 (28.2%) | <0.001 | 910 (35.7%)   |
| Influenza vaccines     |             |         |         |           |
| Ever vaccinated        | 693 (65.9%) | 1232 (81.9%) | <0.001 | 1925 (75.3%) |
| During pregnancy       | 625 (59.5%) | 1074 (71.4%) | <0.001 | 1699 (66.5%) |
| Before influenza epidemics | 133 (12.6%) | 652 (43.3%)  | <0.001 | 785 (30.7%)   |

INFLUMIKA is the Spanish language acronym of the cohort (i.e., Influenza maternal e Infantil y Zika).

Typical influenza epidemic period April-September.¹⁵
to the hospital in El Salvador for the management of asthma exacerbations. One woman referred to the hospital had laboratory-confirmed RSV and the second was untested but had illness when RSV was predominant.

### 3.3 Viruses detected among tested ARI cases

Viral RNA was detected in 98 (50%) of 196 samples (Table 2). The most common detections were rhinovirus (n = 42, 21%), influenza (21, 11%), RSV (14, 7%), parainfluenza 3 (9, 5%), human metapneumovirus (4, 2%), parainfluenza 1 (3, 2%), and parainfluenza 2 (5, 2%). None tested positive for adenovirus (Table 3). Only two samples had co-detections, one with respiratory syncytial virus and parainfluenza-2 and another one with respiratory syncytial virus and rhinovirus. The majority of influenza positive samples were A(H3N2) (n = 16, 76%), followed by influenza B (n = 3, 14%), and influenza A(H1N1)pdm09 (n = 2, 10%). More than ¾ of influenza detections (16, 76%) occurred during the typical April through September southern hemisphere influenza season.15
FIGURE 1  Influenza vaccinations among pregnant women in the INFLUMIKA cohort and proportion of influenza detections Panama and El Salvador National Influenza Centres, April 2015–August, 2017. NIC, National Influenza Centre; ARI, Acute respiratory illnesses
Estimated etiology of ARI among untested cases

Women in El Salvador had 67 (31%) of 216 ARI unsampled and women in Panama had 201 (81%) of 248 ARI unsampled (Table 2). Using El Salvador’s national surveillance findings (Figure 1), we attributed 1.9 (95% confidence interval [CI] 0.0–3.7) additional ARI to influenza, 1.7 (95% CI 1.3–2.1) to rhinoviruses, 0.2 (95% CI 0.0–0.4) to parainfluenza viruses, 0.8 (95% CI 0.5–1.1) to RSV, and 0.1 (95% CI 0.0–0.3) to human metapneumovirus. Similarly, in Panama, we attributed 6.5 (95% CI 5.8–7.2) additional ARI to influenza, 28.0 (95% CI 25.1–30.9) to rhinoviruses, 6.0 (95% CI 4.5–7.4) to parainfluenza viruses, 2.9 (95% CI 2.1–3.8) to RSV, and 2.2 (95% CI 1.3–3.1) to human metapneumovirus.

Incidence of ARI and ARI by etiology

The febrile ARI incidence rate (19.7/100 py) was 42% of the incidence for all ARI (46.5/100 py) (Table 2). The incidence of laboratory-confirmed influenza during the entire calendar follow-up period was 2.1/100 py (1.5/100 py in El Salvador and 0.6/100 py in Panama). The incidence of influenza adjusted for untested ARI (i.e., laboratory-confirmed influenza plus ARI attributable to influenza) during epidemic weeks when influenza was nationally detectable, was 5.0/100 py (95% CI 4.5–5.4/100 py) (Figure 2); 5.7/100 py (95% CI 5.0–6.3/100 py) in El Salvador and 4.3/100 py (95% CI 4.0–4.5/100 py) in Panama. Similarly, the adjusted incidence of rhinovirus during weeks when this virus was detectable nationally was 7.3/100 py (95% CI 7.0–7.7/100 py), parainfluenza viruses 3.3/100 py (95% CI 3.1–3.5/100 py), RSV 2.4/100 py (95% CI 2.2–2.5/100 py), and human metapneumovirus 0.9/100 py (95% CI 0.8–1.1/100 py). Based on the only laboratory-confirmed RSV hospitalization, the RSV hospitalization rate in El Salvador was 3.0/1000 py.

### TABLE 3 Signs, symptoms, and bronchopneumonia diagnosis associated with acute respiratory illnesses (ARI) in the INFLUMIKA cohort

| Condition                        | El Salvador | Panama | P-value   | Total |
|----------------------------------|-------------|--------|-----------|-------|
| Acute respiratory illnesses (ARI)| 216         | 248    | <0.001    | 464   |
| Cough                            | 167 (15.9%) | 139 (9.2%) | <0.001    | 306 (12.0%) |
| Rhinorrhea                       | 166 (15.8%) | 145 (9.6%) | <0.001    | 311 (12.2%) |
| Subjective fever                 | 133 (12.7%) | 60 (4.0%)  | <0.001    | 193 (7.6%) |
| Sore throat                       | 91 (8.7%)   | 82 (5.4%)  | 0.002     | 173 (6.8%) |
| Headache                         | 41 (3.9%)   | 69 (4.6%)  | 0.429     | 110 (4.3%) |
| Fatigue                          | 21 (2.0%)   | 25 (1.7%)  | 0.548     | 46 (1.8%) |
| Shortness of breath              | 11 (1.1%)   | 32 (2.1%)  | 0.042     | 43 (1.7%) |
| Myalgias                         | 3 (0.3%)    | 19 (1.3%)  | 0.008     | 22 (0.9%) |
| Prostration                      | 0 (0%)      | 3 (0.2%)   | 0.273     | 3 (0.1%) |
| Bronchopneumonia                 | 4 (0.4%)    | 0 (0%)     | 0.028     | 4 (0.2%) |

**Note:** INFLUMIKA is the Spanish language acronym of the cohort (i.e., Influenza maternal e Infantil y Zika).
influenza. The incidence of influenza was second only to rhinovirus among cohort women. CDC encourages persons to avoid exposure to respiratory viruses through the use of non-pharmaceutical interventions. These include frequent handwashing, avoiding close contact with sick persons, and during epidemic periods, physical distancing, and mask-wearing in public. Influenza specifically can be safely and effectively prevented\textsuperscript{14} through vaccination.\textsuperscript{7,17} Influenza illness increases pregnant women’s risk of hospitalization, pregnancy loss,\textsuperscript{18} and premature delivery.\textsuperscript{19} While there were insufficient women in our study to assess such outcomes, influenza during pregnancy was associated with late pregnancy loss and reduction in mean birthweight in a concurrent three-country pregnant women cohort.\textsuperscript{18}

Conversely, maternal influenza vaccination decreases the risk of influenza illness complications and passively protects infants from influenza during the first months of life.\textsuperscript{6,7,16} Maternal immunization is important for infants because those younger than 6 months are still developing their immune system and ineligible for influenza vaccination. Our results reaffirm Panama and El Salvador’s decisions to prioritize pregnant women for influenza vaccination and to annually invest in government-purchased influenza vaccines for administration free-of-charge during prenatal care. While we were underpowered to quantify vaccine effectiveness, it is noteworthy that pregnant women in Panama were more likely to be vaccinated against influenza and ecologically had lower rates of influenza than women in El Salvador.

Our influenza rates were similar to those reported among pregnant women in China (3.8/100 py),\textsuperscript{20} Nepal (5.0/100 py),\textsuperscript{7} and South Africa (3.6/100 py).\textsuperscript{22} Our rates were also similar to symptomatic persons aged 18–49 years in the U.S (5.3 patient-years).\textsuperscript{22} Our El Salvador rates (5.7 [5.0–6.3]/100 py) were also like Peru’s (6.5 [4.5–9.5]/100 py)\textsuperscript{18} possibly because both countries are middle-income and have modest vaccination coverage when compared to higher-income Panama. While our Panama and El Salvador cohorts were neither designed nor powered to examine associations between influenza and birth outcomes, the Peru study was part of three-country cohort which identified adverse birth outcomes associated with influenza ARI.\textsuperscript{18} It is therefore likely that pregnant women with influenza in El Salvador and Panama may have also been at higher risk of pregnancy loss and lower weight neonates when compared to uninfected women.

The self-reported vaccination coverage among study women in 2015 was like that estimated through PAHO’s administrative methods in a 2014 publication (i.e., 50%–70%).\textsuperscript{23} However, <43% of influenza vaccinations occurred before the start of the influenza epidemics in Salvador and Panama. Furthermore, influenza vaccination steadily decreased in both countries during 2015–2017 (Figure 1). Such findings raise concerns about these programs’ sustainability. The sustainability of vaccination programs is likely influenced by multiple factors, including population awareness of risk of influenza illness,\textsuperscript{12} confidence in the value of mitigation measures, and competing health priorities. Until now, obstetricians in Central and South America would not have had sub-regional influenza burden estimates from pregnant women to inform risk perception and reaffirm commitment to vaccination guidelines. Our study findings can therefore be used to develop risk communication messages for providers\textsuperscript{24} and pregnant women to urge timely vaccination before the start of each influenza season.

Our study had noteworthy strengths; we conducted a prospective 3-year, two-country, active surveillance cohort. We estimated the incidence of influenza using sensitive molecular diagnostics to confirm influenza illness and previously used methods to correct for under-ascertainment.\textsuperscript{25} We then reported findings following STROBE guidelines.

Nevertheless, our study also had noteworthy limitations. We had insufficient resources to follow women more frequently than once a week. We asked women to present for swabbing only if they developed fever, when specimens would have been most likely to test positive for influenza. This strategy missed opportunities to laboratory-confirm viral etiologies. Under-ascertainment occurred more frequently in Panama, where women seemed less likely to report subjective fever than in El Salvador. Second, while Panama’s and El Salvador’s reference laboratories collected influenza and RSV data, which we used to determine risk periods, neither country collected parainfluenza data stratified by type and El Salvador did not collect human metapneumovirus and rhinovirus data during the entire study period. Therefore, we present aggregate parainfluenza rates and used the proportion of Panama’s human metapneumovirus and rhinovirus risk weeks (79% of risk period) to estimate El Salvador risk weeks during 2016 and 2017. Last, while we are reassured that our findings are like those of other countries,\textsuperscript{18} it is improbable that rates are representative of pregnant women in all tropical middle-income countries.

Pregnant women in Panama and El Salvador frequently had ARI and more than one in ten febrile ARI were attributable to influenza. Our findings suggest the value of Panama’s and El Salvador’s investment in vaccination to protect pregnant women and their infants from influenza illnesses. During 2014–2018, however, less than half of the cohort of women in Panama had been vaccinated against influenza before the start of annual influenza epidemics. Indeed, less than one in five had been vaccinated in El Salvador, where influenza rates were higher than those of Panama. Pregnant women should receive inactivated influenza vaccines as soon as these become available at the start of each epidemic.\textsuperscript{26} The influenza season starts approximately in April in countries with a southern hemisphere epidemic pattern and in October in countries with a northern hemisphere epidemic pattern.\textsuperscript{27}

As of 2021, Panama and El Salvador offer southern hemisphere formulation influenza vaccines to pregnant free-of-charge starting approximately April each year, but the lack of electronic vaccination registries makes it difficult to determine whether vaccine coverage has improved since 2014–2018. Such findings suggest Panama and El Salvador might optimize the benefit of their investments through World Health Organization-recommended post-introduction evaluations of their influenza vaccination programs.\textsuperscript{28} The frequency of ARI with other respiratory viruses also suggests the value of promoting self-protection through non-pharmaceutical interventions as has been successfully done during the COVID-19 pandemic.\textsuperscript{29}
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CONFLICTS OF INTEREST
The authors have no conflict of interest.

AUTHOR CONTRIBUTIONS
EAB designed the study, assisted with protocol development, data cleaning, analyses, and manuscript writing. RG assisted with study design, drafted protocol, managed field teams during data collection, and assisted with interpretation of study findings. AC and VC assisted with data collection, cleaning, and interpretation. DF and JMP led laboratory testing and interpretation. RD, RF, JA led field teams in El Salvador and assisted with data interpretation and manuscript development. AH assisted with interpretation of study findings and manuscript review.

ORCID
Eduardo Aziz-Baumgartner https://orcid.org/0000-0001-8038-0530
Vic Veguilla https://orcid.org/0000-0002-0610-4316
Arlene Calvo https://orcid.org/0000-0002-6321-3674
Rhina Dominguez https://orcid.org/0000-0001-7860-0602
Aron J. Hall https://orcid.org/0000-0001-7250-1013
Rosalba González https://orcid.org/0000-0001-8040-036X

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