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Influenza incidence, household transmission, and prevention among pregnant women, postpartum women, their infants and household contacts in Mali

Author
Ortiz, Nancy

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Influenza incidence, household transmission, and prevention among pregnant women, postpartum women, their infants and household contacts in Mali

By

Nancy Ortiz

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Committee in charge:

Professor Arthur L. Reingold, Chair
Professor Alan Hubbard
Professor Eva Harris

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Nancy Ortiz

Abstract

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Nancy Ortiz

Doctor of Philosophy in Epidemiology

University of California, Berkeley

Professor Arthur L. Reingold, MD, Chair

Pregnant women, infants, and children < 5 years of age are at increased risk of acquiring influenza and its complications. As a result, the World Health Organization recommends that pregnant women and children 6 months to < 5 years of age receive influenza vaccine. However, nearly all of the risk information about influenza in pregnant women, infants, and children comes from temperate regions of the world, where vaccine is plentiful and the disease is seasonal. Very little information exists on influenza in pregnant women and young children in tropical zones, where vaccine coverage is scant and seasonality of influenza transmission is less well understood. Although the highest incidence rates of respiratory illness among children are found in Africa, influenza vaccine use there is rare, and very little research on influenza has been carried out in this region, where co-morbid conditions, such as HIV infection are prevalent, and increase the risk of severe influenza outcomes. Given the limited knowledge of influenza in this region, it is difficult to argue for the use of influenza vaccination.

This dissertation used data collected in Mali from a cohort study of influenza vaccine naïve pregnant and postpartum women and their infants conducted in April 2010 – August 2011 and from a randomized controlled trial of influenza vaccine in pregnant women who were randomized to receive either influenza vaccine or a control vaccine in 2011-14. In both studies, pregnant and postpartum women, their infants, and household contacts < 5 years of age were followed prospectively, and participants were actively assessed for signs of influenza-like-illness (ILI); samples were taken when individuals met the case definition for ILI and tested for influenza by RT-PCR. We also collected a blood smear to exclude cases of ILI due to malaria. In both studies, all households members were followed until the infant turned six months of age. These datasets were used to answer questions regarding influenza and ILI incidence in pregnant women, postpartum women, and infants < 6 months, and household transmission in these groups, as well as predicting the likely impact of modeling influenza vaccine use in the influenza vaccine naïve population.

In Chapter 1, we present results from our analyses that determined the age-adjusted incidence of ILI and laboratory-confirmed influenza (LCI) among pregnant and postpartum women and their newborn infants < 6 months of age in 2010-2011. Although LCI incidence was low, as compared to estimates reported in these same groups in other countries, we found that pregnant and postpartum women < 20 years had the highest rates of LCI, and that rates of LCI decreased with each additional decade of life. We found high rates of ILI in pregnant women, postpartum women and infants. Despite high rates of ILI in postpartum women, rates were higher in pregnant women, and pregnancy was a risk factor for ILI. Women were 2.28 times as likely to develop ILI during pregnancy as during the postpartum period. While pregnant women had a greater risk of LCI than postpartum women, the difference was not statistically significant, possibly due to the low case count we observed in the cohort. We also found that the risk of influenza in adult women was greater in households with a child under the age of five years, compared to households with no children in this age group. Among infants, incidence of LCI and ILI was highest among four and five month olds. The risk of a case of influenza in an infant increased with increasing number of children < 5 years of age residing in the household, although this difference was not statistically significant. Lastly, we found that although we observed few cases of LCI, influenza activity in Mali exhibited a defined bimodal seasonality, with cases peaking in September/October and February.

In Chapter 2, we describe our estimates of the serial interval (SI), the time between primary and secondary cases of influenza in the household, for influenza A (H1N1), influenza A(H3N2), and influenza B viruses. We found that our estimates were, generally, at the shorter end of the range of those previously reported. In this chapter, we also investigated the effect of individual and household factors on the median SI. The presence of cigarette smoking in the household was associated with a shorter SI. SIs were longer in households with moderate and high levels of household crowding. Residing in a household where a pregnant woman had received influenza vaccine during the trial was associated with a shorter SI, although none of the cases of household transmission occurred in pregnant women. Few instances of household transmission involved adults. The age of the index case did not have a statistically significant effect on median SI.

In Chapter 3, we present the predictions from the mathematical modeling analysis in which we used an SEIR compartmental model and ordinary differential equations to model the seasonal peaks of influenza activity in Mali and modeled the likely impact of introducing influenza vaccination in children on the cumulative case count of influenza among children residing with other vulnerable populations at risk for severe influenza-related outcomes (i.e. pregnant, postpartum women and infants). We found that even nominal influenza vaccine coverage (10%), if achieved before transmission began, reduced the total epidemic size considerably, to only one-fourth of the number of cases observed in the same population without vaccination. Our analyses also demonstrated that the timing of vaccination efforts played an important role in the final epidemic size, with implementation of vaccination delayed until after the first case of influenza had appeared resulting in larger projected epidemic sizes than if vaccination had been implemented four weeks before influenza activity began. Vaccinating in late August and the third week of December led to the largest projected reductions in total case counts.

In sum, we found that influenza circulation had a defined bimodal seasonality in Mali, with peak cases occurring in late September/October and February. The risk of influenza in adult women was greater in households with a child < 5 years of age, as compared to households without children in this age group. In our study of household transmission of influenza, we found that presence of cigarette smoking in the household was associated with a shorter SI. The mathematical models suggested that influenza vaccination of children six to fifty-nine months of age administered prior to periods of peak influenza activity (late August and December) may reduce the cumulative number of cases of influenza in children who reside with pregnant and postpartum women and infants under six months of age in Mali.

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Introduction

Influenza illness can range from a mild to a life-threatening illness, and in cases of severe illness, can lead to complications such as hospitalization, the need for intensive care, and even death. Pregnant women, infants < 6 months of age, and children < 5 years of age are at substantially elevated risk of experiencing severe influenza-related outcomes (1). During the influenza H1N1pandemic in 2009, postpartum women also experienced severe illness resulting in hospitalization, suggesting that they may also be at increased risk of severe influenza-related outcomes (2).

Although the efficacy/effectiveness of influenza vaccine varies between seasons and by age group, vaccination can provide protection from influenza illness, by preventing it or reducing the severity of illness. While no influenza vaccine is approved for use in children < 6 months of age, vaccination of pregnant women provides protection from influenza in women during pregnancy, as well during the postpartum period, and it confers maternally-derived protection to their infants (3-5). Influenza vaccine is also efficacious in children 6 – 59 months of age (6, 7). Given the increased risk of severe illness in pregnant women and children 6 – 59 months of age, WHO recommends influenza vaccination for both groups (8). Although influenza causes respiratory illness worldwide, the highest rates of mortality due to respiratory illness in children occur in Africa (9). Despite the efficacy of influenza vaccine, its availability in Africa and its use is very limited, and as of 2014, only one country in sub-Saharan Africa, (South Africa) had national influenza vaccination policies (10, 11).

Much of our understanding of influenza is based on research carried out in high income countries in temperate areas where and vaccine is plentiful; and there are major gaps in our knowledge regarding influenza incidence, illness severity, household transmission, and seasonality in low income, tropical countries, especially in sub-Saharan Africa where influenza vaccine coverage is scant (12, 13). Morbidity and mortality from HIV, malaria and tuberculosis remain very high in many African countries, and public health efforts and scarce available resources have invariably been allocated to mitigate these diseases (14). Because malaria is endemic in most sub-Saharan African countries, patients with a febrile illness are routinely given a diagnosis of malaria and treated accordingly (15). Historically, in many tropical regions, influenza was believed be to nonexistent or negligible until 2009, a novel strain of influenza A (H1N1) circulated globally and demonstrated the pressing need for laboratory diagnostic capacity and surveillance for influenza (16). Around the same time, avian die offs occurred in Africa, drawing further attention to the need for influenza surveillance and research in Africa (14, 17). As a result, WHO, CDC, and other organizations began to work with Ministries of Health on the African continent in an effort to build influenza laboratory diagnostic capacity and establish regional influenza reference laboratories for the detection and characterization of influenza viruses in the African region (18). Even a decade after the pandemic, however, little is known about the epidemiologic features of influenza in sub-Saharan Africa.

In temperate countries, influenza transmission occurs with marked seasonality, coinciding with the winter months, and this seasonality informs the timing of the initiation of influenza vaccination initiation and public health prevention messaging, which begins in the months preceding the first expected cases of influenza (19). Temporal patterns of influenza transmission

in tropical countries are quite heterogenous, with studies in diverse countries exhibiting bimodal peaks of influenza, one annual peak of influenza case activity, or continuous year-round transmission (13, 20-22). Understanding regional patterns of influenza seasonality is critical for the timing of influenza vaccination efforts (23). Given the paucity of information concerning the incidence and transmission of influenza in Africa, it is difficult to argue for the initiation of influenza vaccination programs, develop policy regarding the implementation of such a strategy or establish national influenza control programs.

Despite their increased risk of severe outcomes related to influenza illness, pregnant women and infants have been the subject of relatively few studies of its incidence. A systematic review found fewer than 10 studies using laboratory testing of suspected influenza cases to determine the incidence of influenza in pregnant women, and again, all of these studies were conducted in high or middle-income countries (24). Another systematic review of laboratory-confirmed influenza among infants < 6 months found that limited data were available worldwide, and nearly all of the data came from the U.S or other high-income countries (25). That review concluded that research on influenza in infants < 6 months of age is needed to define the burden of influenza-associated illness in this high-risk group, as well as to quantify the possible impact of maternal influenza vaccination among infants, especially in low income countries, including those in Africa.

In Mali, the site of the current research, has a population that is relatively young, with individuals < 15 years of age comprising nearly half of the population (26). Mali has one of the highest fertility rates in the world, at six births per woman, as well as a very high maternal mortality ratio (587/100,000) (27, 28). Additionally, neonatal mortality is the ninth highest in the world, at 67.6/1,000 lives births, and one in ten children die before their fifth birthday (29).

In temperate regions, pregnant women and infants and young children have been identified as being at high-risk for influenza and severe influenza related-outcomes. Considering that Mali's population is relatively young and that the country has poor overall key health indicators for pregnant women, infants, and children < 5 years of age, research is needed to quantify the incidence of influenza and understand its transmission in these groups in order to inform local decision making regarding the use of influenza vaccine in Mali.

Previous studies have estimated that up to 40% of transmission of influenza virus occurs inside the home (30). However, influenza transmission in low-income countries has not been well investigated, and data from sub-Saharan Africa are lacking. Household transmission studies of influenza in wealthy and middle income countries have implicated children as playing a pivotal role as sources of transmission of influenza in the community (31, 32). Studies have also pointed to increased susceptibility of young children to acquiring influenza in the households setting (21). In Mali, household composition is typically multigenerational and households often include many family units, including many children. Although some studies have found that household crowding plays an important role in household transmission of influenza, others have reported that crowding was associated with a decreased probability of transmission of influenza in the household, while other studies have reported that crowding was associated with increased transmission (33, 34). Given the patterns of household composition in Mali, as well as the

country's young population, understanding the role of children in influenza transmission dynamics in Mali may be informative for guiding influenza prevention strategies there.

Smoking of tobacco products in the household has been associated with an increased risk of respiratory illness in children, including acute respiratory infections, pneumonia, influenza (35). Children residing in households with smokers have been found to be at increased risk of complications related to influenza such as extended hospitalization stays and a greater odds of requiring intensive care (36). In Mali, the majority of smokers are male, and it estimated that between 15 to 30% of men smoke regularly (37, 38). Understanding the effects of cigarette smoke on the household transmission of influenza may help identify behavioral interventions (i.e. smoking cessation) strategies that can be used in tandem with vaccination to reduce the risk of prevent influenza and its complications.

Children disproportionately comprise the majority of influenza cases in wealthy countries, and it is estimated that they are also disproportionately affected in low income countries. In wealthy countries, children are also at increased risk of severe influenza outcomes, and they plausibly are at increased risk of severe influenza outcomes in poorer countries where medical care is less accessible, and influenza vaccine is rarely used. While WHO guidelines recommend that pregnant women and children 6 months to < 5 years of age be given influenza vaccine, only 59% of WHO member states have a national influenza vaccination policy and only one country in Sub-Saharan Africa (South Africa) has established such guidelines (39, 40). Failure to establish and implement such policies may be due to the absence of data on the incidence of influenza and of the resulting severity of illness in Africa, as well as lack of clarity regarding the efficacy of the vaccine in children in this setting. Although very few randomized controlled trials of influenza vaccine have been carried out in children in Africa, one case control study in Kenya estimated that influenza vaccine was effective (VE = 54%) in preventing influenza in children (41, 42). However, evidence from randomized controlled trials in infants and young children in low income countries is lacking, particularly from sub-Saharan Africa.

Compartmental models allow public health practitioners to model the dynamics of infectious disease spread in populations and allow for the simulation of various prevention and mitigation strategies (43). Such modeling exercises can be especially useful in situations in which carrying out interventions studies would be logistically or ethically challenging (e.g. in the evaluation of targeted prevention strategies or at varying levels of an intervention). Therefore, although few efficacy trials have been conducted in Africa, and influenza vaccination use there is rare, mathematical modeling can allow the simulation of the potential impact of using influenza vaccine in children in this setting.

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Chapter 1: Incidence of influenza and influenza-like illness in households of pregnant women, postpartum women and infants under six months of age in Bamako, Mali, 2010-11.

1 Introduction

Pregnant women and young children, particularly infants under six months of age, who are too young to receive influenza vaccination, are at increased risk of experiencing severe influenza-related outcomes, which can lead to hospitalization and even death (1). Additionally, studies conducted in the context of outbreaks of pandemic influenza have demonstrated that postpartum women are at risk of severe illness outcomes as well (2). However, few studies have examined the incidence of laboratory-confirmed influenza (LCI) in pregnant and postpartum women and in young infants, and there is a paucity of information regarding the burden of influenza-related illness in low income countries, particularly those in sub-Saharan Africa (3-7).

Previous studies have demonstrated that influenza vaccination of pregnant women is effective in reducing the risk of influenza in pregnant women and in their infants (8-10). While the WHO recommends that all pregnant women receive influenza vaccination, utilization of influenza vaccination in this population remains limited in low income countries, particularly those in Africa, where patients presenting with a febrile illness are systematically treated for malaria (6, 11). The current study determined the incidence of LCI and influenza-like illness (ILI) among pregnant women, postpartum women, and infants under six months of age in Bamako, Mali, West Africa in 2010-11. Mali is one of the poorest countries in the world and has minimal influenza vaccine utilization. Understanding the burden of influenza-related illness may help inform influenza vaccination implementation in Mali.

While influenza activity in temperate regions is characterized by marked seasonality, with one sharp peak in cases annually that coincides with the winter, in tropical areas, the seasonality of influenza is poorly understood (12-14). Studies of global influenza seasonality have shown that it is quite heterogeneous, with some describing bimodal peaks of influenza, one annual peak in influenza case activity, or continuous year-round transmission (15-19). In African countries that lie closer to the equator, surveillance has shown that influenza patterns are varied and poorly defined (20). Given the observed heterogeneity in the seasonality of influenza in tropical countries, it is important to understand local patterns and transmission dynamics to inform vaccination and prevention policies, so that their delivery can be timed in accordance with local needs (17).

2 Materials and Methods

Study design and participants

Between April 1, 2010 and August 18, 2011, we carried out a prospective cohort study among pregnant and postpartum women and their infants in households from four neighborhoods in Bamako, Mali. Pregnant women were recruited in their third trimester (≥ 28 weeks' gestation).

Mother-infant pairs were eligible for inclusion until the child turned five months old. Multiple pregnant women and/or mother-infant pairs could participate from the same household.

Ethical approval for this research was obtained from the University of Maryland, Baltimore Institutional Review Board, the ethics committee of the Faculté de Medecine, Pharmacie et Odonto-Stomatologie of Mali, the Ministry of Health of Mali, and the Committee for Protection of Human Subjects of the University of California, Berkeley.

Recruitment

Recruitment for the cohort was carried out over 12 months. To maximize the period of observation for the number of individuals in the study, we employed a cross-sectional strategy to recruit study participants and assemble the cohort, as depicted in Appendix Table 1. Pregnant women could be recruited at any time during their third trimester (i.e. beginning in the 7th month of pregnancy). Mother-infant pairs also were enrolled in the study if the infant was ≤ 5 months of age. At baseline (Month 1 in Appendix Table 1), nine separate groups (including women in their 7th (*Group A*), 8th (*Group B*), or 9th month of pregnancy (*Group C*) and mother-infant pairs from newborn (*Group D/d*) to five months of age of the infant (*Group I/i*) were recruited to form the initial cohort. Mother-infant pairs were followed until the child reached six months of age. Therefore, one month after the baseline cohort was assembled (Month 2), *Group (I/i) Mother/Infant pair*, for whom recruitment occurred when the infant was five months of age, aged out of the study, while the other eight groups continued to be followed. This recruitment strategy was employed for 12 months, after which no additional pregnant women were recruited. However, we continued to follow the remaining cohort members for four months after recruitment was completed.

Le Centre pour le Développement des Vaccins du Mali (CVD-Mali) carries out demographic surveillance in two neighborhoods, Banconi and Djikoroni-para in Bamako. Demographic surveillance system (DSS) field workers visit households in the neighborhood every four months and collect information on pregnancies, births, deaths and in- and out-migrations that have occurred during the previous quarter. Households are asked about health seeking behavior and whether they would be willing to be contacted regarding participation in future research projects.

Individuals were recruited for this study from households participating in the DSS, as well as from non-DSS neighborhoods. In neighborhoods participating in the DSS, we utilized the DSS to identify pregnant women and mother-infant pairs that met demographic eligibility criteria, and who had indicated that they were willing to be approached about research studies. We generated a random sample of these households and then visited these homes to describe the study and attempt to enroll eligible participants. In the non-DSS neighborhoods, Mekin Sikoro, Hippodrome, Lafiabougou, and Sébénikoro, study staff identified eligible participants and recruited pregnant women and mother-infant pairs at community health centers (Centre de la Santé Communitaire, "CSComs") during prenatal care and well child visits. In Bamako, pregnant women are encouraged to visit the CSCom for prenatal care, and approximately 90% of women present there for care multiple times during their pregnancy.

Pregnant women and mother-infant pairs were recruited in their homes or at local clinics. Prior to enrollment, study staff explained the study objectives to the prospective participants, discussed

the perceived risks and benefits of participation, and answered questions. The participants either read a printed consent form in French or listened to an audiotaped version of the consent form in Bambara, the local language. If they consented to participate, the individual signed the printed version of the form. Study staff also approached the head of the household to obtain consent for the household to participate in the study. A second consent form was used for this purpose.

Study procedures

We carried out active surveillance for respiratory tract infections, visiting each household weekly throughout the course of the study. At each study visit, we assessed the health of the pregnant or postpartum women and recorded an oral temperature and a history of fever and of symptoms of respiratory illness in the prior seven days. If the woman had given birth to a child since the last study visit, we also recorded the outcome of the pregnancy, which was collected through participant self-report. The health of the infant was also assessed at each study visit. Axillary temperature was recorded at the time of the visit, along with history of fever and of symptoms of respiratory illness in the prior seven days. Participants (either pregnant or postpartum women or infants) exhibiting symptoms of febrile illness who met the case definition for ILI (Appendix) had oropharyngeal and/or nasopharyngeal swabs collected and tested for influenza by RT-PCR. Given that malaria is endemic in Mali, blood smears were also collected to exclude malaria as the cause of the febrile illness. Illnesses in which the malaria smear was positive were excluded from the analyses.

Statistical analyses

Pregnant women, postpartum women and infants born prior to recruitment began to contribute person-time at the time of enrollment. Infants born during the course of the study began to accrue person-time at birth. All study participants stopped contributing person time to the study when the infant in the household aged out of the study (at six months of age) or if lost to follow up. LCI and ILI incidence were calculated as the total number of episodes experienced by each of the three study populations, divided by the total person-time for each of the subgroups, multiplied by 100. We used a Poisson distribution to calculate 95% confidence intervals (CIs) for the incidence rates and general estimating equations with a Poisson distribution to estimate Incidence Rate Ratios (IRRs), which were interpreted as measures of relative risk. We used robust standard errors with an exchangeable working correlation structure to calculate the 95% CIs for the IRRs. Means were compared using Student's *t* test. Data analyses were performed in STATA version 14.0 (Stata Corp., College Station, TX).

3 Results

A total of 2,264 women from 2,070 households were enrolled in the cohort at baseline, including 1,636 pregnant women and 628 postpartum women. In total, 2,112 infants participated in the study, including 641 infants who were enrolled at baseline and 1,471 infants who were enrolled as participating pregnant women gave birth. Table 1 summarizes characteristics of the cohort at baseline. Pregnant women and postpartum women did not differ significantly in age. Although

no data were collected on maternal influenza vaccination status, influenza vaccine utilization is very rare in Mali, so women and infants likely comprised an influenza vaccine naïve population.

Women were observed for a total of 933.3 person-years, including 234.0 person-years of observation time among pregnant women and 719.3 person-years of observation time among postpartum women (Table 2). Infants accrued 720.9 person-years of follow-up time. In pregnant women, the incidence of LCI was 2.9 cases per 100-person-years (95% CI, 1.4–6.2) and the incidence of ILI was 127.3 episodes per 100 person-years (95% CI 113.6–142.6). In postpartum women, we observed a slightly lower incidence of LCI, 2.5 cases per 100-person years (95% CI, 1.5–3.9), and a substantially lower incidence of ILI of 54.9 episodes per 100 person-years (95% CI 49.7–60.6) than in pregnant women (Appendix Supplemental Table 2). Among pregnant women, 2.3% of ILI samples tested positive for influenza, whereas 4.5% of postpartum women with ILI were positive for influenza.

The incidences of LCI and ILI among women were highest among teenage women (i.e. < 20 years of age) among both those who were pregnant and those who were postpartum. In pregnant women, the incidences of both LCI and ILI decreased with increasing decades of age. Among postpartum women, the incidence of ILI also fell which increasing age, following a pattern similar to that observed in pregnant women. The incidence of LCI in postpartum women was similar across age groups, except for the oldest age group of women (40 – 49 years), in which we did not observe any cases of LCI.

Pregnant women were 2.28 times as likely as postpartum women to develop ILI (95% CI 1.89—2.75), after adjusting for age (Appendix Table 3). Although the incidence of LCI was also higher in pregnant women than in postpartum women, this difference was not statistically significant (RR = 1.15, 95% CI, 0.48—2.74) (Table 3). The relative risk of LCI and ILI in women as compared to the reference age group (40-49 years) decreased with each increasing decade of age, although the observed decrease was very small for LCI, and, these differences were not statistically significant.

Among the 2,112 infants followed over time, there were 35 cases of LCI and 1,577 episodes of ILI during the study period. Thirty-three infants had one case of LCI and two infants had two cases of LCI while they were in the study. A total of 666 infants had one episode of ILI, 279 infants had two episodes, and 111 infants had >2 episodes of ILI during the study. The incidence of LCI in infants was 4.8 episodes per 100 person-years (95% CI 3.4 – 6.7) and the incidence of ILI among infants was 218.8 episodes per 100 person-years (95% CI 208.2–229.8). The highest incidence rates of both LCI and ILI were observed in the 5th and 4th months of life, respectively. For ILI, high rates were observed for the entire six-month period, with the highest rates observed in the 5th and 4th months of life, followed by the first two months of life. The incidence rates of LCI and ILI did not differ between male and female children (Table 2; Appendix Supplemental Table 2).

Half of the cohort of infants (50.2%) experienced at least one episode of ILI during the study period (50.2%), of which 2.2% were due to LCI; a similar percent (2.3%) of ILI episodes observed among pregnant women was due to LCI.

At the time of enrollment, we collected data on the number of the children < 5 years of age who were living in the household (Table 4). The mean number of children < 5 years of age per

household was just under one child, 0.82 (range: 0-8) children. Increasing number of children < 5 years of age living in the household was not a significant predictor of a case of LCI among pregnant or postpartum in the household (Table 5). We observed a marginally statistically significant increased risk of LCI among women with one child < 5 years of age living in the household, as compared to women in households without young children, RR =1.05 (95% CI 1.01—1.09). An increasing number of young children residing in a was associated with an household increased risk of LCI among infants; however, this difference was not statistically significant.

In our cohort, we observed that LCI cases peaked semi-annually, with peak influenza activity occurring in September/October 2010 and in February 2011 (Figure 1). Although we observed the cohort for only a sixteen and a half month period, this temporal pattern of LCI activity is consistent with that seen in Mali among participants of a randomized controlled trial of influenza vaccine in 2011-2014, and as well as with the periodicity in influenza activity observed in national surveillance data (i.e. WHO FluNet) in Mali.

4 Discussion

Data on the incidence of laboratory-confirmed influenza among pregnant women are sparse, especially from low and middle income countries (3). In addition, the incidence of LCI among non-pregnant women of childbearing age has not been well defined, making it difficult to examine pregnancy as a risk factor for LCI in this age group.

Pregnant women have been reported to be at increased risk of severe influenza-related outcomes, as have postpartum women; however, these studies were done primarily in high income countries in the setting of an influenza pandemic. Nevertheless, WHO considers pregnant women a priority group for influenza vaccination in low and middle income countries. Our study is among the first to quantify the burden of LCI among cohorts of pregnant and post-partum women in a low-income setting (11). Although the incidence of LCI was low in both groups, we confirmed that influenza was the causative agent for (3.6 %) episodes of ILI among these populations in Mali in 2010-11. Pregnancy was a strong predictor of ILI; pregnant women had an increased risk of ILI as compared to postpartum women. The same trend was observed for LCI, but was not statistically significant, possibly due to the small number of cases of LCI in our cohort.

Our results demonstrate a substantial burden of ILI among pregnant women in a malaria endemic setting, over five times the incidence of ILI found in a cohort of pregnant women in Malawi (21). Additionally, although pregnancy was a strong predictor of ILI, postpartum women also had an very high incidence of ILI, over twice that of ILI among pregnant women in Malawi. A recent study of influenza-related hospitalizations among women of childbearing age women in New Zealand noted increased rates of LCI-related hospitalizations in pregnant women, as compared to non-pregnant women of childbearing age (22). However, the increased rate of hospitalization was not observed among postpartum women as compared to other non-pregnant women. That study was carried out in a high income country, and was among the first studies of influenza among women, of childbearing age that looked at severity of influenza illnesses in pregnancy

and non-pregnancy. This study also found that influenza severity varied by influenza subtype, with the majority of hospitalization due to influenza A (H1N1 and H3N2). Severity also varied by ethnicity, with highest LCI hospitalization rates observed in pregnant women of Maori and Pacific descent, groups with considerable social and economic inequality as compared to individuals of European ancestry in New Zealand (23).

We found that teenage pregnant women had the highest rates of LCI and ILI, as compared to older pregnant women. A multi-year household observation study of influenza in the U.S. that included household members of all ages and sexes found elevated rates of influenza for all participants between 0 - 19 years of age, as compared to adults > 20 years of age (24), although the results were not stratified by sex. We also observed a trend of decreasing risk of LCI and ILI with each additional increase in decade of life among women of childbearing age, although this finding was not statistically significant. We also observed that households that reported having a child under five years of age residing in the household at baseline had a greater risk of having a case of LCI among pregnant or postpartum women in the home, compared to households that did not have a child under the age of five.

Children under the age of five are at increased risk of severe influenza illness and its related, complications, including hospitalization and death. In 2012, the Influenza Working Group for the World Health Organization Strategic Advisory Group of Experts (SAGE) group recommend that the WHO update its recommendation regarding annual seasonal influenza vaccination to include children aged 6 months – 5 years of age . A systematic review and meta-analysis of the global burden of LCI among children < 5 years of age estimated that 28,000–111,500 deaths occur annually in this group due to influenza-associated acute lower respiratory infections (ALRI), with 99% of them occurring in non-industrialized countries (25). The incidence of LCI cases observed in infants in our study more closely approximates the reported incidence among children < 1 year of age estimated for wealthy counties, (52 cases per 1000 person-years, 95% CI, 28-98), than the estimated incidence among similarly aged children in low income countries, of 119 cases per 1000-person years (95% CI, 77-186). However, it is important to note that limited data are available on LCI in infants, and there is great heterogeneity in the observed incidence by influenza season and also by region of the world. Most studies that contributed to the global estimates of LCI were based on cases detected in a small number of cohorts followed over time. While the higher incidence rates have been in children < 1 year of age, no prior studies have calculated age specific estimates for infants < 6 months of age. We observed the highest incidence of LCI in the 4th and the 5th month of life, followed by the first two months of life. The crude influenza incidence we observed was 4.8 cases per 100 person-years among all infants 0-5 months of age (95% CI 3.4 — 6.7), approximately half of the incidence of LCI reported among infants of the same age in a birth cohort from Nicaragua in 2011-13 (7).

Although infants < 6 months of age are at greatest risk for severe influenza illness, there have been relatively few studies of influenza in this age group, especially in resource-limited settings. Nearly all of the available data have been gathered from wealthy countries. In addition, relatively few individuals with ILI seek care, and therefore relying on studies using passive surveillance of identify cases of ILI will lead to an underestimate of the LCI incidence, as well as bias towards more severe cases. While influenza vaccine is not approved for use in children < 6 months of age,

influenza immunization of pregnant women has been shown to confer protection to their newborns (10, 26).

Our study demonstrated that older infants (i.e. 5th and 4th month of age) had the highest rates of both LCI and ILI among infants < 6 months of age; this same age pattern was observed for severe acute respiratory infection (SARI) incidence among a cohort of infants in Mongolia (27). Interestingly, a randomized controlled trial of influenza vaccine in pregnant women in Mali found that vaccination conferred robust protection to the infant for the first four months of life, after which vaccine efficacy dropped markedly (26). Other studies have observed higher rates of LCI and acute respiratory infections (ARI) in 6-12 month olds, as compared to infants between 0-6 months of age, and it postulated that this might possibly due to diminishing maternally derived passive conferred immunity (7, 28). It is possible that the higher rates of LCI we observed in 4th and 5th months of age (as compared to younger infants) in our influenza naïve study population may be due, in part, to the loss of that immunity.

We also observed a trend of increasing risk of having a case of influenza in an infant with an increasing number of children under age five residing in the household, although this trend did not reach statistical significance. Although it was not possible in our cohort to ascertain whether cases of influenza occurred due to household transmission from other children residing in the home, prior household studies of influenza have demonstrated that household transmission was more frequent in households with lower mean age of inhabitants and that the risk of secondary infection was highest among children < 9 years of age (29). Another study of households found that children were more likely than adults to transmit influenza to others living in the household (30).

We found a substantial burden of ILI among infants < 6 months of age throughout the first six months of life. The unadjusted incidence of ILI in infants < 6 months was over 2.5 times that reported among two-year olds (the age group with the highest incidence) in a cohort of children aged 2-11 years in Nicaragua and over 10 times greater than that seen in nine year-olds (who had the lowest incidence) in the same cohort (31). Although our study occurred during a different influenza season, and among a younger cohort of children, the infants we followed had a markedly higher incidence of ILI compared to older children.

Mali has the fourth highest fertility rate in the world, with 6.1 births per woman (32). Given the large average number of children per woman and that it is cultural norm in Mali for multiple nuclear families to reside together in a single extended household, multiple pregnant and postpartum women, and their infants and small children often reside together, which can lead to increased transmission of ILI and LCI among groups at high-risk of influenza outcomes. Use of influenza vaccination in Mali to date has been virtually nonexistent. The vaccine is not widely available and individuals must pay out of pocket to receive it. Given the risk of severe outcomes among pregnant women and infants, influenza of pregnant women should be considered as a worthwhile preventative measure to provide protection for women and infants, possibly prioritizing pregnant women who live in households with large numbers of children under the age of five.

Influenza activity in our study peaked during September/October 2010 and February 2011. The periodicity we observed in LCI among pregnant women, postpartum women and infants, was similar to that observed among these groups as part of randomized controlled trial in Mali (2011-14) and is further substantiated by influenza circulation pattern reports produced by WHO Flunet from the influenza national surveillance in Mali. Given that influenza vaccine is more effective when provided before influenza cases begin to occur, the administration of influenza vaccine a few weeks in advance of the onset of influenza circulation, possibly in August and late December, may prevent more cases of LCI than if influenza vaccine were provided after influenza activity began in Mali (33, 34).

The strengths of the current study include its prospective design and the frequent follow up of participants to identify episodes of ILI. Additionally, the recruitment strategy we employed allowed us to accrue a considerable amount of person-time for each sub-group in the study population, despite the fact that the study took place over a relatively short period of calendar time. Additionally, testing for malaria allowed us to exclude it as a cause of febrile illness in those with ILI episodes and to calculate non-malarial ILI in a malaria endemic region. We followed women postpartum, which allowed us to calculate the incidence of LCI in non-pregnant women of childbearing age and allowed to us assess pregnancy as a risk factor for LCI. To our knowledge ours is the first study to provide age-specific rates of LCI and ILI among a cohort of pregnant and postpartum women from a low income country.

We observed a substantial burden of ILI, but, the overwhelming majority of illnesses were not due to influenza. Additional research is necessary to identify the other causes of ILI in such settings in order to identify areas for prevention for ILI not caused by influenza.

Tables and Figures:

Table 1: Baseline Characteristics of women and infant cohort members in Bamako, Mali 2010-11			
Women			
Characteristic	Pregnant Women n = (1,636)	Postpartum Women (n = 628)	p value*
Age, years			
13-19	357 (21.8%)	125 (19.9%)	
20-29	867 (53.0%)	338 (53.8%)	
30-39	321 (19.6%)	118 (18.7%)	
40-49	16 (0.9%)	13 (2.0%)	
Missing	75 (4.5%)	34 (5.5%)	
Mean age (SD), years	24.5 (5.7)	24.8 (5.9)	0.30^
Infants, (30.3 % all infants), n = (641)			
Characteristic	All infants		
Sex			
Male	295 (46%)		
Female	346 (54%)		
Age, months			
<1	290 (45.2%)		
1	112 (17.4 %)		
2	77 (12.0%)		
3	87 (13.5%)		
4	58 (9.0%)		
5	17 (2.6%)		
Mean age (SD), months	1.73 (1.56)		

^Determined by T-test

SD = standard deviation

Table 2: Incidence of influenza and in cohort of pregnant, postpartum women and infants < 6 months of age, Bamako, Mali 2010-11

	Person-years†	Influenza episodes*	Incidence/100 person-years§	95% CI‡
Pregnant Women	**231.6**	**7**	**3.0**	**1.4 — 6.4**
Characteristic				
Age categories, y				
13-19	47.0	2	4.3	1.1—17.0
20-29	127.6	4	3.1	1.2 — 8.4
30-39	48.2	1	2.1	0.3—14.7
40-49	2.1	0	0	-
Postpartum Women	**701.7**	**18**	**2.6**	1.6—4.1
Characteristic				
Age categories, y				
13-19	143.8	4	2.8	1.0—7.4
20-29	384.4	10	2.6	1.4—4.8
30-39	144.5	4	2.8	1.0—7.4
40-49	8.1	0	0.0	—
Infants	**720.9**	**35**	**4.8**	**3.4 — 6.7**
Characteristics				
Sex				
F	335.0	16	4.7	2.9 — 7.7
M	370.1	19	5.1	3.2 —8.0
Age, months				
<1	131.2	3	2.2	0.7 — 7.0
1	132.9	3	2.2	0.7— 6.9
2	127.0	7	5.5	2.6— 11.5
3	120.1	2	1.6	0.4 — 6.6
4	111.8	10	8.9	4.8 — 16.6
5	97.5	10	10.2	5.5—19.0

‡CI = confidence interval

†Person-years were determined by dividing the total number of person-days by 365.25.

§Incidence is presented in cases/100 person-years. Incidence is presented in cases/100 person-years.

Table 3: Risk factors for influenza in cohort of pregnant, postpartum women and infants < 6 months of age, Bamako, Mali 2010-11

Women				
Characteristics	**Crude RR†**	**95% CI**	**Adjusted RR†‡**	**95% CI**
Pregnancy Status				
Pregnant	1.15	0.48—2.74	1.14	0.48—2.73
Postpartum	Ref	—	Ref	—
Age categories, y				
13-19	1.14	0.35—3.63	1.14	0.35—3.64
20-29	1.07	0.39—2.91	1.08	0.39—2.92
30-39	Ref	—	Ref	—
40-49	1.88E-12	5.10e-13—6.93e-12	1.39E-11	3.81e-12—5.06e-11
Infants				
Characteristics				
Sex				
M	1.12	0.5—2.2	1.08	0.5 —2.1
F	Ref	—	Ref	—
Age, months				
<1	1.26	0.20—7.87	1.29	0.20—8.05
1	1.26	0.21—7.42	1.27	0.21—7.52
2	3.24	0.69—15.20	3.26	0.69—15.25
3	Ref	—	Ref	—
4	5.36	1.22—23.45	5.34	1.22—23.39
5	9.05	1.95—41.87	8.95	1.93—41.32

*RR, relative risk; CI, confidence interval; Ref, reference.
†The measure of RR used is the incidence rate ratio.
‡Multivariate models included age and pregnancy status for adults and sex and age for infants.

Table 4: Baseline household characteristics of cohort of pregnant women, postpartum women and infants in Bamako, Mali, 2010-11

Characteristic	Households, n = (2,070)
Number of children < 5 years living in the household	
0	443 (21.4%)
1	835 (40.3%)
2+	160 (7.8%)
Missing	632 (30.5%)
Mean number of children (SD), counts	0.82 (0.71)

Table 5: Household risk factors for influenza among women, postpartum women and infants in Bamako, Mali 2010-11*

All women

Characteristic	Crude RR†	95% CI
Number of children < 5 years living in the household		
0	Ref	—
1	1.05	1.01—1.09
2+	1.02	0.95— 1.08

Infants

Characteristic		
Number of children < 5 years living in the household		
0	Ref	—
1	1.34	0.47—3.78
2+	2.47	0.71— 8.57

*RR, relative risk; CI, confidence interval; Ref, reference.
†The measure of RR used is the incidence rate ratio.
‡Models included number of children under five years living in the household.

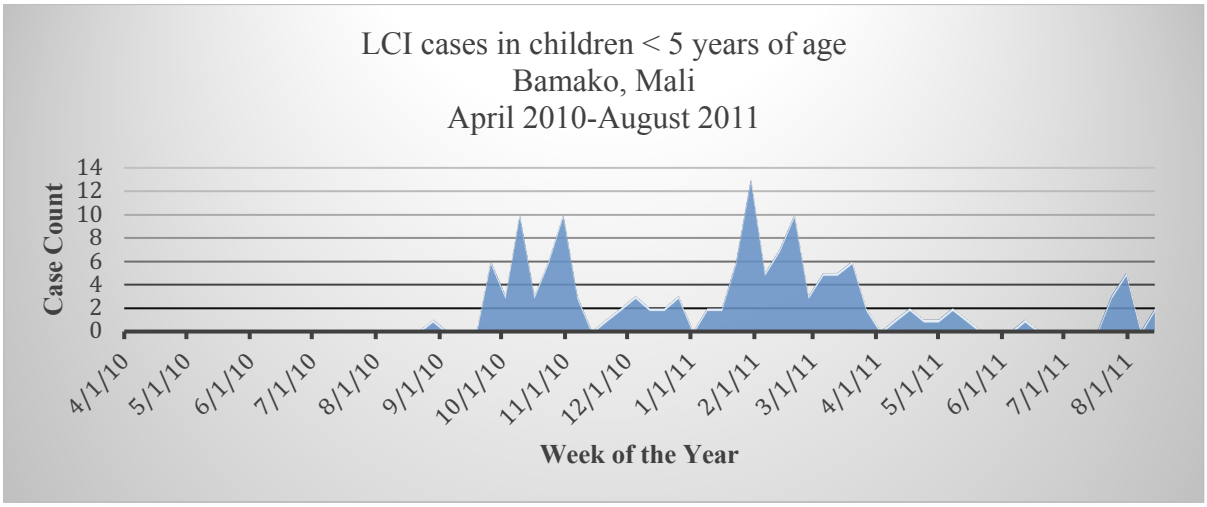

Figure 1: Weekly case counts for laboratory-confirmed influenza (LCI) in children < 5 years of age, Bamako Mali, 2010-2011.

Appendix

	Study Recruitment and Follow Up by Month																
Group	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**
Pregnant: 7 months	A	J	K	L	M	N	O	P	Q	R	S	T					
Pregnant: 8 months	B	A	J	K	L	M	N	O	P	Q	R	S	T				
Pregnant: 9 months	C	B	A	J	K	L	M	N	O	P	Q	R	S	T			
Mother/ Infant: < 1 month	D/d	C/d	B/b	A/a	J/j	K/k	L/l	M/m	N/n	O/o	P/p	Q/q	R/r	S/s	T/t		
Mother/ Infant: 1 < 2 months	E/e	D/d	C/c	B/b	A/a	J/j	K/k	L/l	M/m	N/n	O/o	P/p	Q/q	R/r	S/s	T/t	
Mother/ Infant: 2 < 3 months	F/f	E/e	D/d	C/c	B/b	A/a	J/j	K/k	L/l	M/m	N/n	O/o	P/p	Q/q	R/r	S/s	T/t
Mother/ Infant: 3 < 4 months	G/g	F/f	E/e	D/d	C/c	B/b	A/a	J/j	K/k	L/l	M/m	N/n	O/o	P/p	Q/q	R/r	S/s
Mother/ Infant: 4 < 5 months	H/h	G/g	F/f	E/e	D/d	C/c	B/b	A/a	J/j	K/k	L/l	M/m	N/n	O/o	P/p	Q/q	R/r
Mother/ Infant: 5 < 6 months	I/i	H/h	G/g	F/f	E/e	D/d	C/c	B/b	A/a	J/j	K/k	L/l	M/m	N/n	O/o	P/p	Q/q

Table 1: Study Recruitment and Follow Up Schedule by Month

Newly recruited participant(s)

Participant(s) in follow up

ILI Case Definition for pregnant and postpartum women:

Women met the ILI criteria if the following were observed by the examining physician or were classified by the physician as having:

 - Onset of fever (oral temperature >38^{O}C) < 7 days duration AND

 - Cough or sore throat AND

 - Absence of other diagnoses

OR

 - Onset of feverish feeling < 7 days duration AND

 - Cough or sore throat or chest pain on breathing in AND

 - Absence of other diagnoses

ILI Case Definition for infants:

An infant met the case definition two of the following conditions, as reported by the mother/caretaker or observed by a clinician:

 - Fever without an apparent source, documented by a clinician's measurement to be an axillary temperature >38°C or maternal perception of fever and administration of antipyretic in previous eight hours

* No source means that there is no apparent cause for the fever such as soft tissue infection, although generalized symptoms, such as irritability, loss of appetite, and/or lethargy may be present;

Or

 - Fever (as defined below)* and acute respiratory infection.

As defined as ANY of the following on the same or consecutive days: runny nose, nasal congestion, cough, difficulty breathing, pus draining from an ear or wheezing;

AND

□ - > 7 days since last reported fever

Fever was defined as any of the following:

□ - Mother's perception that the infant had fever during the previous 24 hours

□ - Mother measured the infant's temperature as >38^{O}C during the previous 24 hours

□ - Clinician or study staff measured the infant's temperature to be >38^{O}C

□ - Maternal perception of fever and administration of antipyretic in previous eight hours

Supplemental Table 1: Incidence of influenza-like illness (ILI) and in cohort of pregnant, postpartum women and infants < 6 months of age, Bamako, Mali 2010-11

Characteristic	Person-years†	ILI episodes*	Incidence/100 person-years§	95% CI‡
Pregnant Women	**231.6**	**298**	**128.6**	**114.8—144.1**
Age categories, y				
13-19	47.0	69	146.7	115.9—185.7
20-29	127.6	167	130.9	112.5—152.3
30-39	48.2	59	122.5	94.8—138.1
40-49	2.1	2	95.0	23.8— 379.8
Postpartum Women	**701.7**	**392**	**55.9**	**50.6— 61.7**
Characteristic				
Age categories, y				
13-19	143.8	86	59.9	48.4—73.9
20-29	384.4	225	58.5	51.4— 66.7
30-39	144.5	77	53.3	42.6—66.6
40-49	8.1	3	37.2	12.0—115.3
Infants	**720.9**	**1577**	**218.8**	**208.2—229.8**
Characteristics				
Sex				
M	370.1	860	232.4	217.3—248.4
F	335.0	716	213.7	198.6—229.9
Age, months				
<1	131.2	282	214.9	191.2—241.5
1	132.9	293	220.3	196.5—247.1
2	127.0	244	192.0	169.4—217.7
3	120.1	238	198.0	174.4—224.9
4	111.8	264	235.9	209.1—266.1
5	97.5	256	262.3	232.0—296.4

†Person-years were determined by dividing the total number of person-days by 365.25.

*ILI, influenza-like illness

‡CI = confidence interval

§Incidence is presented in cases/100 person-years.

Suplemental Table 2: Risk factors for influenza-like illness in cohort of pregnant, postpartum women and infants < 6 months of age, Bamako, Mali 2010-11*

Women				
Characteristics	**Crude RR†**	**95% CI**	**Adjusted RR†‡**	**95% CI**
Pregnancy Status				
Pregnant	2.28	1.89—2.75	2.28	1.89—2.75
Postpartum	Ref	—	Ref	—
Age categories, y				
13-19	1.55	0.64—3.75	1.46	0.63— 3.40
20-29	1.34	0.55—3.22	1.26	0.54—2.92
30-39	1.33	0.55—3.24	1.25	0.54—2.93
40-49	Ref	—	—	—
Infants				
Characteristics				
Sex				
M	1.07	0.98—1.17	1.07	0.98—1.16
F	Ref	—	Ref	—
Age, months				
<1	1.09	0.93—1.28	1.12	0.96—1.31
1	1.11	0.95—1.31	1.12	0.96 — 1.32
2	0.97	0.82—1.15	0.98	.83—1.15
3	Ref	—	Ref	Ref
4	1.18	1.00—1.40	1.19	1.01—1.39
5	2.00	1.69—2.37	2.00	1.68—2.36

*RR, relative risk; CI, confidence interval; Ref, reference.

†The measure of RR used is the incidence rate ratio.

‡Multivariate models included age and pregnancy status for adults and sex and age for infants.

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Chapter 2: Estimating the serial interval of household transmission of laboratory-confirmed influenza within households of pregnant women, postpartum women, infants under six months of age and their household contacts in Bamako, Mali.

1 Introduction

Pregnant and postpartum women, and infants and children < 5 years of age are at elevated risk for either influenza illness, severe influenza-related complications, or both (1-3). It is estimated that approximately 40% of household transmission of influenza occurs in the home (4, 5). However, aside from studies that have focused on the role of children in household transmission of influenza, very few studies have explored influenza transmission dynamics within households in which other high-risk populations (i.e. infants, and pregnant and postpartum women) reside. Additionally, there are limited data on household transmission of influenza within households in low income countries, particularly in Sub-Saharan Africa, where only a handful of household studies of influenza have been conducted (6, 7).

Understanding influenza transmission parameters is critical for modeling the likely impact of prevention measures on seasonal influenza, as well as mitigation strategies for curbing outbreaks (8). The serial interval (SI), the time from symptom onset in a primary case of influenza to the onset of symptoms in a secondary case, is a critical parameter for modeling the spread of influenza (9). The majority of influenza SIs have been estimated for pandemic influenza, and few studies of the SI have reported estimates for influenza A(H3N2) or influenza B (8).
The SI for influenza has been estimated primarily from households in industrialized countries; although SIs have been reported from middle income countries, estimates from low income countries are scarce (10-12). Household composition in low income countries differs substantially from that in industrialized countries; many households in low income countries are multi-generational and include multiple family units, as well as a larger number of children, due to higher fertility rates in women. SI estimates vary between influenza viruses (13). For influenza A(H1N1), a systematic review reported a range between 1.9 – 6 days for the SI (14). The same review reported a narrower range of SIs, 2.2 – 4 days, for influenza A(H3N2). The reported range of SI estimates for influenza B is even narrower, at 3.4 – 3.7 days, although very few studies have reported SI estimates for influenza B virus (10, 15, 16).

Heterogeneity in the observed SIs for influenza viruses may be due, in part, to a number of factors, including the incubation period, the infectivity of the primary case in the household (i.e. viral shedding), susceptibility of the contacts to influenza, and contact patterns in the household (5, 10, 17). Because the SI is affected by a number of external factors that may differ between populations and settings (e.g. schools vs. households), it is important to estimate the SI in various environments, in order to better establish the transmission parameters used to model the effects of interventions in those settings, especially among groups at risk of severe influenza illness in low resource countries. Understanding the factors that contribute to the heterogeneity in the SI among pregnant, postpartum women, infants and children may allow for better parameterization

of the mathematical models that examine the effect of influenza interventions in these groups (18).

2 Materials and Methods

Study Design

We used data from a randomized controlled trial of influenza vaccine in pregnant women conducted in Bamako, Mali during 2011-2014. The study design and recruitment have been described previously (19, 20). Women in their third trimester (≥ 28 weeks gestational age) of pregnancy were recruited to participate in the trial. Study participation was limited to one pregnant woman per household. The pregnant women were block randomized to receive either inactivated trivalent influenza vaccine or a control vaccine, quadrivalent meningococcal conjugate vaccine. At enrollment, household contacts < 5 years of age were enumerated and recruited. If the household was comprised of more than five eligible children, the youngest five eligible children were enrolled. Study staff carried out active surveillance for febrile respiratory illness, visiting households weekly to assess signs of influenza-like illness (ILI). They collected oropharyngeal and nasopharyngeal samples from participants who met the case definition of ILI (Supplement), and tested them for influenza by RT-PCR. Households were followed from the date of vaccination of the pregnant woman through the child's birth and until the infant aged out of the study upon reaching six months of age. The enrolled household contacts (i.e. the postpartum women and enrolled children < 5 years of age) were followed for the same period of time. All household participants exited the study at the same time, either at study completion, when the infant born to the woman who had received either the influenza or control vaccine aged out (at six months of age), in the event of the index infant's death, if the household moved out of the study area, or if the household no longer wished to participate.

Statistical analyses

Primary cases of laboratory-confirmed influenza (LCI) in the household were considered index cases. Household contacts of index cases were considered secondary cases if they acquired the same subtype of LCI within seven days of the index case. The SI was defined as the time between the first day an index case reported fever or respiratory symptoms (i.e. nasal congestion, cough) and the day these symptoms appeared in the secondary case of LCI in the household (21, 22). In our study, household transmission was considered to have occurred when two cases of LCI occurred within seven days of one another. We limited our analysis to the primary and secondary cases of LCI in the household and excluded tertiary and quaternary cases from our analyses. However, we included multiple secondary LCI cases if the symptoms in these individuals began on the same day. Households with two co-primary LCI cases (i.e. with onset of symptoms on the same day) were excluded from the analyses. Households could have recurrent events of household transmission if the second instance of household transmission occurred more than two weeks after the previous instance of influenza transmission.

We fitted parametric models to the data assuming Weibull and lognormal distributions. We compared model fit with the Akaike Information Criterion (AIC) and found that a lognormal

distribution provided a better fit, as indicated by the lower AIC value for this model. Mean SIs were estimated from the fitted lognormal model for each influenza subtype. We used accelerated failure time models with a lognormal distribution to carry out multivariable analysis to examine the relationship between individual and household characteristics and their effect on SI, specifically age of the index and of the secondary case, the subtype of influenza virus, and household factors (i.e. household crowding, smoking in the household and randomization of the pregnant woman in the household to receive influenza vaccine vs. the control vaccine). We interpreted the transformed model coefficients for the covariates as the acceleration factor (AF), the increase or decrease of the median SI.

Age of the index case was grouped into two categories (0 < 6 months) and (6 months < 5 years), as was the age of the secondary case. Influenza types were categorized into influenza A(H1N1), A(H3N2) and influenza B. Household crowding was calculated as the ratio of the number of people residing in the household to the number of rooms used for sleeping in the household, grouped as follows: low— households with two or fewer people sleeping per room, medium— households with between two and 3.5 people sleeping per room, and high—households with > 3.5 people sleeping per room. Household smoking was a binary variable that referred to whether someone in the household smoked. We performed the data analysis in STATA version 14.0 (Stata Corp., College Station, TX).

3 Results

A total of thirty-six households had multiple LCI cases in which symptom onsets in the index and secondary cases occurred within seven days of each other. However, in six of these households, onset of ILI symptoms began on the same day in both cases, and these were considered co-primary cases. These households experienced no subsequent cases of household transmission within seven days of the co-primary cases becoming symptomatic and were excluded from the analysis. Among the remaining thirty households, household transmission of the same influenza virus occurred in a total of twenty-six households, producing a total of thirty secondary transmission events.

We estimated an overall influenza SI of 1.91 days (95% CI, 1.62 – 2.20 days) (Table 1). For influenza A viruses, we estimated an SI of 1.77 days for influenza A (H1N1) (95% CI, 1.53 – 2.00 days) and 2.41 days for (H3N2) (95% CI, 1.01 – 3.83 days) The SI for influenza B virus was 1.83 days (95% CI, 0.11 – 3.56 days). We calculated the confidence intervals using a parametric bootstrap.

In a univariate model that included only influenza A (H1N1 and H3N2) and influenza B, the mean SIs for influenza A(H3N2) and influenza B were not statistically significantly different from the reference, influenza A(H1N1) (Table 1). Very few (four) instances of LCI transmission in a household involved adults as either the first or secondary case in the household. Interestingly, in those four transmission events in which adults did play a role, all cases occurred in postpartum women; no pregnant women were involved in transmission events in the household. Because so few instances of household transmission involved adults, when we examined the effect of the ages of the index case and secondary case on median SI, we did not have sufficient instances among

the adults to include them as an age category in either covariate. SIs were nearly identical when the index case was an infant < 6 months of age and when the index case was a child between 6 months < 5 years of age. In our model, SIs were 24% shorter when the secondary case occurred in an infant as compared to in a 6 month to < 5 year old child (AF = 0.76, 95% CI, 0.48–1.18). However, neither the age of the index case nor the age of the secondary case had a statistically significant effect on the length of the SI.

The SI was longer in households with medium and high levels of household crowding, relative to households with a low level of household crowding. The longest median SI was in households with a medium level of crowding, which was 79% longer than the median SI in households with a low level of household crowding, (Acceleration Factor (AF) = 1.79, 95% CI, 1.12—2.84). The SI was also 30% longer in households with a high level of crowding, as compared to the SI in households with a low level of crowding, although this difference was not statistically significant, possibly due to the small number of households in the high crowding category. When we combined households with medium and high levels of crowding and compared them to those with a low level of household crowding, the median SI was 62% longer in households with a high level of crowding, as compared to the median SI in households with a lower level of crowding, with the difference being statistically significant (results not shown). We found that in households with smokers, the median SI was 44% shorter than that in households without a smoker (AF = 0.56, 95% CI, 0.38—0.81). We also observed a difference in the SI between vaccine study arms, with households in which the pregnant woman received influenza vaccine having a 43% shorter median SI than the SI in the households where the pregnant woman received the control vaccine (AF = 0.57, 95% CI, 0.31—0.79). However, as noted above, no household transmission of influenza involved transmission to or from a pregnant woman.

4 Discussion

We estimated SIs for three influenza viruses among households of pregnant women participating in a randomized controlled trial of influenza vaccine in Mali. We found that the influenza SIs were, in general, shorter than those previously reported. Our estimate of the SI for influenza A(H1N1), 1.77 days, was shorter than estimates reported in prior studies, which ranged from 1.9-6 days (14). However, nearly all of the index and secondary cases of LCI in our study population occurred in children < 5 years of age. One prior household study of influenza that exclusively enrolled pediatric index cases found that the SIs were shorter when secondary cases were in children (10). Therefore, it is possible that the SIs we found were shorter because nearly all of the primary and secondary cases occurred in young children. Few prior estimates of SIs for influenza have been reported from tropical regions of Africa. However, investigators studying an outbreak of H1N1 influenza among students in a school in Ghana reported an SI of 2.0 days. Our results are consistent, as we reported a short SI for influenza A (H1N1); findings from both studies may possibly be explained by the influence of air temperature, humidity, and precipitation on the SI (23).

Our estimate of the mean SI for influenza A(H3N2) (2.4 days) is consistent with previously published estimates (2.2–2.4 days). There have been few estimates of the SI for influenza B virus, although those reported have been significantly longer than those reported for influenza A

(10, 17). Our estimate of the SI for influenza B (1.83 days) was much shorter than estimates previously reported (3.4 – 3.7 days). Although just a handful of studies have reported SIs for influenza B, those estimates have been fairly consistent. We observed few cases of influenza B in our study population, and our estimate may be biased if the transmission events we observed involved individuals who had increased susceptibility and whose development of influenza B was accelerated as a result.

We also examined the effect of individual characteristics (e.g. ages of index and secondary cases) and household factors (e.g. household crowding, smoking, and influenza vaccination of the pregnant women in the household) on SI. We found no statistically significant effect of age on SI. Preschool-aged (2 < 5 years of age) and school aged-children (i.e. > 5 years of age) have been implicated in driving influenza transmission in households, i.e. contact with these young children increases the likelihood of acquiring influenza. One prior household study found that preschool-aged children were more likely to acquire influenza and to transmit it to others, compared to adults (24). A study of shedding of influenza virus in households in Nicaragua found that children exhibited a longer period of viral shedding, as compared to adults. This shedding was observed before the children became symptomatic, while they were ill and after symptoms had abated, suggesting that children have a longer period of infectiousness than adults, and, therefore, a longer window in which to transmit influenza to others (25). Although the age of the index and secondary cases did not have a statistically significant effect on SI in our study, it important to note that infants and other children < 5 years of age comprised 86% of the index and secondary cases. Very few instances of transmission in the households we studied involved adults. One study of heterogeneity in the SI of influenza in households found the ages of the index and secondary case were associated with the length of SI, but that study followed children who were older than those in our study and included adults in the analyses; they reported that SIs were shorter when the index case was a young child (0-2 years of age) as compared to an older child (11-14 years) and when secondary cases occurred in children < 15 years of age as compared to adults (10). Because of the small number of instances in our study of household transmission involving adults, we limited our analysis of the effect of age on SI to infants and children < 5 years of age. It may well be that SIs among infants and children < 5 years of age are very similar, which might partially explain why we did not detect an age effect on SI.

Our study found that relative to households with low crowding, SIs in households with medium and high crowding were longer. Most prior studies of household transmission of influenza have focused on calculating secondary attack rates and have compared households in which secondary transmission of influenza occurred to those households in which it did not. The results from studies that have examined household crowding as a risk factor for household transmission have been mixed, with some studies reporting that household crowding was associated with household transmission of influenza and ILI, and others reporting that crowding was associated with reduced household transmission of influenza (7, 26). To our knowledge, ours is the first study to examine the effect of household crowding on the time between the onsets of cases when household transmission has occurred. We found that when household transmission occurred, increased household crowding was associated with a longer SI. Our definition of crowding was constructed based on the number of individuals who slept in the same room; a possible explanation for our finding that the SI was longer in individuals in households with increased

crowding might be that they spend more time outdoors, rather than in the home, and as a result have less contact with one another, and that this reduced contact rate influences the time between the onsets of cases of LCI in the household.

Smoking in the household was associated with a shorter SI when influenza was transmitted in the household. The presence of smoking in the household has been studied in relation to secondary attack rates of influenza in the home, but it has not previously been examined as a factor contributing to the heterogeneity in the time between primary and secondary cases of LCI in a household. Smoking has been associated with an increased risk of respiratory tract infections, caused by respiratory syncytial virus, and influenza, and with an increased risk of pneumonia, as well as with having more severe respiratory illnesses (27, 28). In a study of children hospitalized with pneumonia in the U.S., children with two or more smokers in the home had longer hospitalizations and a greater odds of requiring intensive care (29). In studies of pandemic and seasonal influenza, smokers were more likely to develop influenza than nonsmokers, as well as to experience more severe illness and influenza-related hospitalizations (30, 31). We did not collect information regarding the number of smokers in the household or their demographic characteristics, so we do not know if the adults involved in household transmission were smokers or if the smokers in the household were other adults who were not enrolled in the cohort and being followed. One prior study of influenza in the United States found no association between having a current smoker in the household and secondary transmission of influenza in the household (17, 32). Although its findings were not statistically significant, another study also found an increased hazard of household transmission of influenza related to having a current smoker as a household contact (24). Studies of household transmission of influenza have not found an association between the presence of smoking in the household and secondary attack rates of influenza. However, other studies have demonstrated that exposure to tobacco smoke is associated with more severe influenza illness, as evidenced by longer hospitalizations and an increased risk of intensive care in children hospitalized for influenza (29). Although smoking in the household has not previously been associated with household transmission of influenza, we found that smoking was associated with a shorter SI between illness events when secondary transmission of influenza in the household occurred. It is possible that exposure to environmental tobacco smoke increases a child's susceptibility to influenza infection and accelerates the time between onset in the primary and secondary cases in the home.

Shorter SIs were found in households in which a pregnant woman had received influenza vaccine than in households in which the pregnant woman had received the control vaccine. However, in none of the households were pregnant women involved in transmission of influenza virus; index cases among adults occurred exclusively in postpartum women among those randomized to received the control vaccine. In households in which influenza transmission occurred, individuals involved in the transmission had not receive influenza vaccine themselves. A prior study among households in which one individual (a pregnant woman) had been randomized to receive either influenza vaccine or a control vaccine, found no statistically significant difference in household transmission of influenza within households in which the pregnant woman received influenza vaccine, compared to households in which the pregnant woman received the control vaccine (7). In a cluster randomized trial in Hong Kong in which one child in the household was randomized to influenza vaccine or placebo, the infection probability of influenza among unvaccinated household members in households randomized to influenza vaccine was only 5%

lower than that in unvaccinated individuals in households that were randomized to the control vaccine (33). These results suggest that the vaccination of one individual in the household may prevent very few, if any, secondary cases of influenza in unvaccinated household contacts, once influenza has been introduced into the household. Given that vaccination of one individual in the household does not seem to prevent secondary household transmission of influenza in the household, it seems unlikely that residing in a household in which a pregnant woman had received influenza vaccine would play a role in shortening the household SI of influenza. It is possible that the effect that we observed was due to chance, given that none of the transmission events occurred in women who had received influenza vaccine.

The prospective nature of our study design and the accompanying active surveillance for ILI may have allowed us to capture less severe cases of LCI than studies that relied on a case-ascertainment design, in which individuals (and their households) are enrolled in a study only after a symptomatic household member seeks treatment for influenza. Household SIs for influenza have been estimated in two countries in Africa (South Africa and Kenya) (6, 12) that are categorized as middle income countries. To our knowledge, ours is the first study to estimate the SI for influenza among households in a low income country in Africa. We estimated the SI for influenza viruses in a study population comprised of individuals at increased risk for severe influenza-related outcomes in one of the poorest countries in the world. These SIs may allow for better parametrization of models that examine the possible effects of interventions used in households with infants and children < 5 years of age in such settings.

Table 1

Table 1 Crude and Adjusted Serial Intervals for Influenza Virus Infection in 26 Households in Bamako, Mali, 2011-14.

Characteristic	No.	Mean Crude Serial Interval, Days (SD)	Univariable Model AF*	95% CI‡	Multivariable Model AF*	95% CI‡
Influenza virus type		**2.00 (1.72)**				
Influenza A (H1N1)	15	1.80 (1.14)	1.00	Ref	1.00	Ref
Seasonal Influenza A (H3N2)	9	2.44 (2.35)	1.13	0.67—1.91	1.10	0.69—1.76
Seasonal Influenza B	6	1.83 (2.04)	0.86	0.48—1.57	0.96	0.60—1.51
Index case, age						
0 to < 6 months	7				1.00	Ref
6 months to < 5 years	19				0.76	0.48—1.18
Secondary case, age						
0 to < 6 months	10				1.00	Ref
6 months to < 5 years	18				1.00	0.60—1.67
Level of household crowding						
Low	9				1.00	Ref
Medium	13				1.79	1.12—2.84
High	4				1.30	0.73—2.33
Cigarette smoking in the household						
Yes	10				0.56	0.38— 0.81
No	16				1.00	Ref
Influenza vaccination in a pregnant woman						
Yes	14				0.57	0.37—0.88
No	12				1.00	Ref

*The Acceleration Factor (AF) represents the proportional increase (AF > 1) or decrease (AF < 1) in median household serial interval associated which each charact

‡CI = confidence interval

Appendix:

ILI Case Definition for pregnant and postpartum women:

Women met the ILI criteria if the following were observed by the examining physician or were classified by the physician as having:

 - Onset of fever (oral temperature >38^{o}C) < 7 days duration AND

 - Cough or sore throat AND

 - Absence of other diagnoses

OR

 - Onset of feverish feeling < 7 days duration AND

 - Cough or sore throat or chest pain on breathing in AND

 - Absence of other diagnoses

ILI Case Definition for infants:

An infant met the case definition two of the following conditions, as reported by the mother/caretaker or observed by a clinician:

 - Fever without an apparent source, documented by a clinician's measurement to be an axillary temperature >38°C or maternal perception of fever and administration of antipyretic in previous eight hours

* No source means that there is no apparent cause for the fever such as soft tissue infection, although generalized symptoms, such as irritability, loss of appetite, and/or lethargy may be present;

Or

 - Fever (as defined below)* and acute respiratory infection.

As defined as ANY of the following on the same or consecutive days: runny nose, nasal congestion, cough, difficulty breathing, pus draining from an ear or wheezing;

AND

 - > 7 days since last reported fever

Fever was defined as any of the following:

 - Mother's perception that the infant had fever during the previous 24 hours

 - Mother measured the infant's temperature as >38^{O}C during the previous 24 hours

 - Clinician or study staff measured the infant's temperature to be >38^{O}C

 - Maternal perception of fever and administration of antipyretic in previous eight hours

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Chapter 3: Modeling the impact of the use of influenza vaccine in children under five years of age on the cumulative case count of influenza in Mali.

1 Introduction

In Mali, approximately one in ten children die before their fifth birthday, and despite gains over the last decade, the country has one of the highest under five mortality rates in the world (1). Despite causing high rates of respiratory illness among children around the world, influenza has been poorly studied in Africa ((2, 3)). The World Health Organization recommends prioritizing influenza vaccination of children under five years of age, as well as other groups at high risk for severe influenza-related outcomes, such as pregnant women, the elderly and the immunocompromised (4). Despite this recommendation, influenza vaccine utilization in Africa remains very limited (< 0.5 – 2% of total population) (5).

In temperate regions of the world, preschool and school-aged children have been implicated as driving influenza transmission, both in the community and in households, which is supported by their higher rates of influenza illness and longer viral shedding. Children also have extensive social contacts and exposure to many other children, in school and in day care, which facilitates the spread of influenza (6, 7). In the U.S., one study estimated that 30% of infants acquire influenza in their first year of life. Findings from this study also suggested that young household contacts may pose an increased risk to infants, as 60% of infants with older siblings developed influenza in their first year of life (8). As reported in Chapter 2, among Malian households of pregnant women, postpartum women, infants and their households contacts under five years of age, we found that the majority of index and secondary influenza cases were observed in children six month to fifty-nine months of age.

Although influenza vaccine efficacy varies every season, influenza vaccination in children has been demonstrated to be efficacious, but vaccination of one child has not been demonstrated to be protective against transmission of laboratory-confirmed influenza (LCI) in the household, once influenza has been introduced. Another study found fewer cases of LCI in households in which a pregnant women had received influenza vaccine, although the difference in incidence rates was not statistically significant (9). These findings suggest that vaccinating only one individual in the household against influenza does not prevent household transmission among unvaccinated household contacts. However, influenza vaccination may provide direct protection to the individual in the household who received the vaccine, which may reduce the likelihood of introducing influenza in the home by reducing the number of susceptible individuals.

Although imperfect, influenza vaccination is the most effective measure available to prevent influenza (10). Observational and experimental studies of the impact of vaccination and other mitigation strategies can be costly and time-consuming. Predictive modeling allows for rapid assessment of the likely impact of various control strategies used to curb epidemics of infectious diseases, such as isolation of cases, social distancing (e.g. school closures), vaccination, and the use of antivirals, which can potentially inform decision-making regarding which mitigation strategies to implement. Mathematical modeling can yield predictions regarding the potential effectiveness of vaccination under various scenarios that may be particularly useful for modeling strategies in low resource settings, where interventional trials may be logistically very

challenging to undertake, as well as constrained by ethical concerns. Models can be adapted to include specific characteristics of these settings, for example including an exponential age structure, which is more representative of population age structures in low income countries than the rectangular age structure observed in wealthy countries. Furthermore, these models make it feasible to examine the impact of the implementation of prevention and control measures under various conditions, such as evaluating the timing and the duration of control measures (e.g. isolation for 18 days versus 21 days) and targeted interventional strategies, such as prioritizing the elderly for the provision of antiviral medications. Modeling also makes it possible to quickly evaluate the likely impact of mass-vaccination and model the effectiveness of control measures when implemented with varying levels of coverage.

2 Materials and Methods

Study Design

Our dataset consisted of sixteen and a half months (April 1, 2010 – August, 18 2011) of influenza surveillance data from a cohort of pregnant women, postpartum women, their infants under six months of age, and their household contacts between six months and fifty-nine months of age (Appendix Figure 1). The study design has been described previously (Chapter 1). During the study period, we observed two peaks of influenza activity, the timing of which were consistent with temporal trends of influenza circulation previously observed in Mali (11, 12). During the largest peak of influenza activity, which occurred in February 2011, 12% of cases occurred among pregnant and postpartum women, 12% occurred among infants under six months of age, and 76% occurred in children between six and fifty-nine months of age. These proportions were similar during the smaller peak of influenza activity that began in September 2010. We carried out a mathematical modeling analysis to model the impact of introducing influenza vaccination of the children on the cumulative case count of LCI during peak influenza activity during the study period.

Analyses

We used a deterministic compartmental model to examine the impact of the direct effect of vaccinating children with trivalent inactivated influenza vaccine (TIV) on the LCI case count during the period of peak influenza activity in 2010-11 (Figure 2). A closed SEIR compartmental model was used to account for the latent period of influenza, the short duration of peak activity, and the fact that we were examining LCI cases in households. In the model, the "S" compartment represents the individuals susceptible to influenza at time(t), the "E" stands for those who are pre-infectious, the "I" compartment represents those who are infectious, and the "R" indicates those who have recovered (13). Analyses were conducted in Microsoft Excel and Berkeley Madonna software.

We calculated R_0, the average number of secondary cases produced, given infection in a completely susceptible population, for the largest peak of influenza activity observed from our

empirical data (14). We began by organizing the data to obtain weekly LCI counts and using linear regression to calculate *a*, the period of exponential growth for the observation period. We then calculated R_0, utilizing the equation for the SEIR model, where *D* is the duration of infectiousness and *F* is the average duration of the pre-infectious period (15). We utilized the serial interval of 1.91 days that we calculated from the data in our study of influenza in households in Mali carried out in 2011-14 (Chapter 2). We drew the influenza latency parameter, one day, from the literature (16):

SEIR Model: Influenza R_0 (February 2011) = $(1+aD)(1+aF)$ = 1.27

We derived the effective contact rate, β, from R_0, as it is inversely proportional to the growth rate in the SEIR model (14). We developed ordinary differential equations (ODEs) for each of the compartments (Figure 2) to model the peak of influenza activity that occurred in the absence of vaccination and compared it to the observed data. We utilized the Curve Fit function to examine how well the model predictions fit the observed data and observed a good model fit, as indicated by the low root-mean-square-error. Curve fit also provided updated parameters that improved the fit of the model to the data.

We adapted our ODEs to incorporate a pulse function for vaccination at three levels: 100% of the population, 2011 U.S. influenza vaccination coverage for children (46.9%) and nominal coverage (10%), with the best fitting model parameters as well as those drawn from published prior studies, which assumed an influenza vaccine efficacy = 54.9% (Table 1). Vaccination timing was incorporated four weeks before the epidemic growth of peak activity, which coincided with the third week of December 2010 for the largest peak of activity observed in Mali; however, we also varied the timing of the implementation of vaccination to examine the effect of vaccination timing on the overall size of the epidemic.

3 Results

We compared influenza incidence in children (six months to fifty-nine months of age) in the absence of vaccination to the incidence expected in the same children had they been vaccinated with influenza vaccine at 46.9% coverage, the U.S. influenza vaccine coverage level in children in (2010-11). When vaccination in children aged six to fifty-nine months was introduced at that level of coverage, influenza incidence declined sharply immediately, and cases ceased to occur after the twentieth week of the year (Appendix: Figure 3). In the absence of vaccination (left graph), cases continued to occur for nearly twice as long. Without vaccination, the peak of influenza activity observed in the influenza vaccine naïve population began to decline in the absence of any intervention over the course of several weeks, likely because the epidemic had reached the Herd Immunity Threshold (HIT). Given that the R_0 for that year of seasonal influenza was quite low (i.e. R_0 = 1.27), the HIT was reached when a relatively small proportion of individuals had experienced influenza.

We also examined the cumulative number of cases that occurred during the peak of influenza activity, as compared to the cumulative case count under three scenarios of differing levels of influenza vaccination coverage in the children (Figure 4). Without vaccination, in a population of 1,512 children, we observed seventy-five cases of influenza. In the same size population of children with vaccination of all children, the cumulative case count would drop to approximately three cases during the period of peak influenza activity, resulting in nearly a (96%) reduction in total cases. Because vaccinating all of the children would be difficult to achieve, we also examined the impact of vaccinating a proportion of children similar to the level of influenza vaccination coverage achieved in the U.S. (46.9%) in the 2010-11 influenza season. We found that vaccinating just under half of the children would result in a 93% reduction in the cumulative number of cases (five would be expected), compared to the total case count observed without influenza vaccine. Given that the U.S. achieves 46.9% coverage due to a robust infrastructure in which vaccines are available in many settings (e.g. pharmacies and in local public health vaccination campaigns), however, it is not reasonable to assume that Mali would easily be able to achieve the same level of coverage, given the more limited access to prevention services. Therefore, we examined the effect of introducing vaccination at only 10% coverage of the children in the population with an influenza vaccine of the same efficacy, 54.9%. Even at this nominal level of influenza coverage, we observed that cumulative influenza cases would be reduced by 72% (twenty-one cases would be expected). Overall, modeling demonstrated that a greater percentage of children vaccinated resulted in a greater reduction in the number of cumulative cases during the period of peak influenza activity.

Our models demonstrated that the timing of vaccination also played an important role in the epidemic size. The above total case counts expected at varying levels of vaccination coverage all assumed that vaccination was carried out four weeks in advance of the first case of influenza for the season. When vaccination efforts were delayed until one week after the first case of influenza had appeared, cumulative case counts were, as expected, larger for all vaccination coverage levels, and the number of cases increased with each additional week of delay in initiating vaccination. For example, at 10% influenza vaccination coverage among children, with vaccination at four weeks before the start of the influenza season, we projected a 72% reduction in the total case count. With a delay in vaccination, until three weeks after the onset of the first case, the expected reduction in the total number of cases decreased to 62%, and with a three week delay, the expected reduction in the cumulative number of cases was only 58% (Figure 5). While vaccination coverage level had a greater impact on the total case count than when influenza vaccination was initiated, modeling demonstrated that a greater reduction in total cases would be expected when vaccination occurred prior to the first case of influenza. This trend was observed at all levels of vaccination coverage.

The trends regarding the reduction in the expected case counts observed under all three vaccination coverage levels and vaccination timing scenarios were consistent with the results observed when we modeled the use of influenza vaccine in children using data from the second peak of influenza activity in Mali, (September–October 2010, data not shown). This peak, in 2010, was smaller, with only fifty-two cases, and vaccination during the final week of August yielded the biggest reductions in the total number of cases.

4 Discussion

We modeled the impact of introducing influenza vaccination in children (six to fifty-nine month olds), on the cumulative influenza case count during the largest peak of influenza activity in 2011 in Bamako, Mali at three levels of vaccination coverage among a population of children who were household contacts of pregnant women, postpartum women and infants under six months of age. The models demonstrated that introducing vaccination into this population of Malian children may have reduced the number of influenza cases substantially, 72% of cases, even with only 10% of the children in the study population being vaccinated. Additionally, the models demonstrated that vaccinating the children in advance of the first case of influenza, in December, resulted in lower peak activity than delaying the vaccination of the children until after the first case appeared.

Although vaccination coverage levels had a greater impact on the final size of the epidemic, the timing of vaccination efforts also had an impact on the cumulative count of influenza cases, with delayed vaccination leading to a larger case count than if vaccination had been implemented four weeks in advance of the first case. This trend persisted for each level of vaccination coverage and for both peaks of influenza activity.

Young children are drivers of household transmission of influenza. In Mali, households are multigenerational and are often comprised of multiple nuclear families. Because, on average, Malian women have six children in their lifetime, households are often large and include many small children. Randomized trials have demonstrated that influenza vaccine prevents both influenza A and B in children, even in years with a considerable mismatch between the influenza strains included in the vaccine and circulating influenza strains (18-20). Our modeling analysis demonstrated that cases of influenza would have been reduced significantly among young children residing with infants < 6 months of age, pregnant women and postpartum women in Mali in 2011, even with minimal influenza vaccine coverage. While we did not model the indirect effects on other members of the households, vaccinating young children living with other vulnerable populations who may have frequent contact with each other may minimize the likelihood that influenza will be introduced into the household, thereby potentially providing some indirect protection to others with whom they reside.

Randomized controlled trials of influenza vaccine in low resource countries have been limited in number, and none have been conducted among children in Africa in the age group we modeled. The value of influenza vaccine efficacy we used in the model came from a case control study among children in Kenya; we applied this vaccine efficacy to children six months to five years of age for a twelve month period for the 2010-11 season. Our analysis modeled the use of trivalent inactivated influenza vaccine (TIV), which was the vaccine available during the 2010-11 influenza season. However, in 2012, quadrivalent influenza vaccines, which added protection against a second influenza B lineage, were licensed in the U.S (21). A randomized controlled trial carried out in Europe, the U.S., and the Philippines among children 3 – 17 years of age found a high level of immunogenicity to the additional strain of influenza B in children who received QIV, compared to those who received TIV, which suggests that QIV may provide more protection against influenza B (22).

Households with infants in our study were observed for a maximum of six months. The peaks of influenza activity observed over the study period were separated by six months, and very few households were observed over both peaks, which led us to analyze the periods separately, as we could not evaluate the effect of influenza vaccine over two periods of peak influenza activity. Another limitation of our study includes the fact that we had only one year of data. Data for multiple years would have allowed us to include possible waning of immunity in our equations and model influenza vaccination with a SEIRS compartmental model. In addition, we assumed a closed study population, while modeling that incorporates an open population with additional groups in the households (i.e. the elderly, the immunocompromised, etc.) would have been more generalizable.

We did not observe sufficient temporal clustering of cases among pregnant women, postpartum women and infants to model the impact of vaccinating pregnant women with influenza vaccine on cumulative case count among these groups. Aside from vaccinating pregnant women, which has been demonstrated to confer partial immunity against influenza to their infants, vaccination of an infant's household contacts may be an alternative prevention strategy to consider, given that another possible protective measure, isolation, is not feasible and antivirals, such as oseltamivir, which is approved in the U.S. for children as young as two weeks of age, is not available in Mali (23-25).

In 2019, Mali announced reforms to its national healthcare system that will provide free primary healthcare for pregnant women and children under five years of age (26). These reforms will be rolled out incrementally, with the system expected to be fully in place by 2022. Up until now, Mali's health system has been one in which individuals bear the cost of their healthcare, and individuals seldom seek out primary care. Improving access to primary care should increase the likelihood that children will adhere to a routine vaccination schedule, and if health- seeking behavior shifts to one in which children are regularly brought in for well child visits, it may be possible to vaccinate children between six and fifty-nine months with seasonal influenza vaccine.

Tables and Figures:

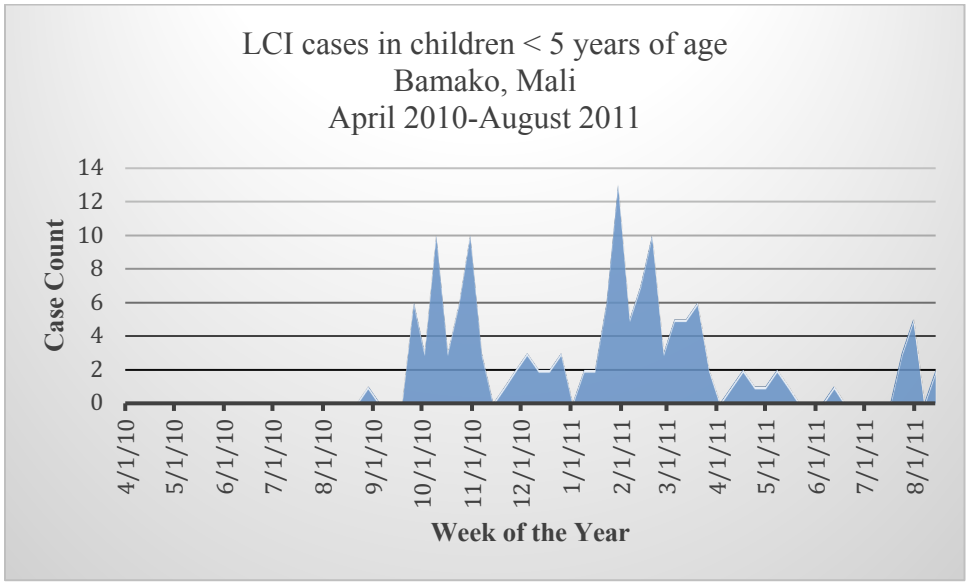
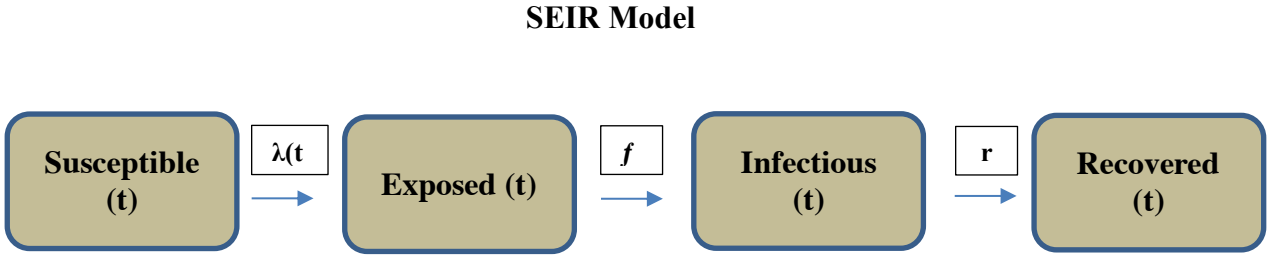


Figure 1: Weekly case counts for laboratory-confirmed influenza (LCI) in children < 5 years of age, Bamako Mali, 2010-2011.

Figure 2: SEIR model and ordinary differential equations in the absence of influenza vaccination



Equations:

dS/dt = -β(I/N)S - pulse(Inf0,t0,1000)

dE/dt = -β(I/N)S - *f*(E) pulse(Inf0,t0,1000)

dI/dt = *f*(E) – r(I)

dR/dt = r(I)

Parameters:

λ(t) = Beta(I/N) = force of infection

β = rate at which two specific individuals come into effective contact per unit time

f = rate of progression from latent to infectious

r = recovery rate

Incidence = f*E

Table 1: Parameters Drawn from the Literature

Parameter	Estimate	Citation
Latent Period	1 day	(16)
Influenza vaccine coverage among children in the U.S. (2011)	46.9%	(27)
Influenza vaccine efficacy in children 2011, Kenya	54.9% - among children 6 months – 5 years of age	(28)

Table 2: Equations and parameters for introduction of influenza vaccination

Equations:

$d/dt(S) = -\beta(I/N)S - pulse(Inf0,t0,1000) - pulse(S(vaccP*eff), tv, 1000)$

$d/dt(E) = \beta(I/N)S - f*E + pulse(Inf0,t0,1000)$

$d/dt(I) = f*E - rec*I$

$d/dt(R) = rec*I + pulse(S*vaccP*eff, tv, 1000)$

Parameters:

tv = -2, timing of vaccination, varied by increments of one week when examining the timing of the implementation of vaccination

t0 = 2, time at which first case of influenza occurs

vaccP = (varied by coverage levels modeled: vaccP =1.000 for 100% vaccination proportion vaccinated, vaccP = 0.469 for U.S. comparison, and vaccP = 0.100 for low uptake scenario of 10%)

eff = 0.549, vaccine efficacy for TIV during 2010-11 in children

Figure 3: Comparison of observed incidence of influenza (Left) and incidence with U.S. level vaccination (46.9%) coverage (Right) .

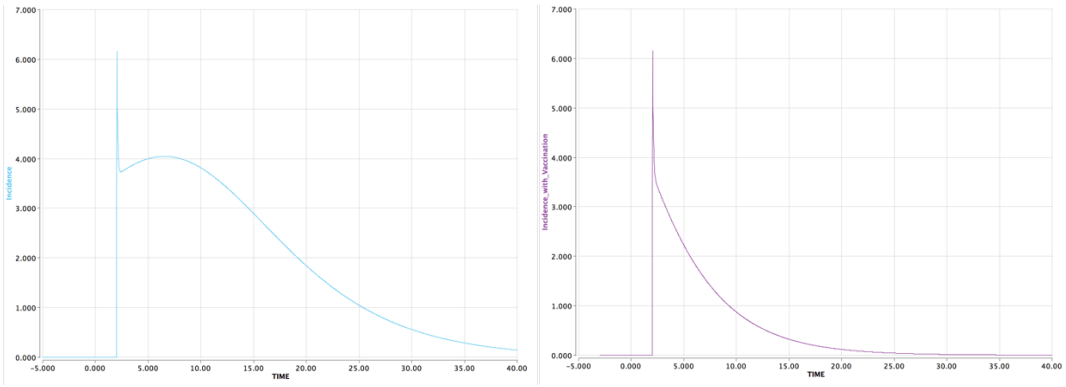


Figure 4: Cumulative cases expected among cohort of Malian children in 2011 with 100%, 46.9%, and 10% influenza vaccination coverage.

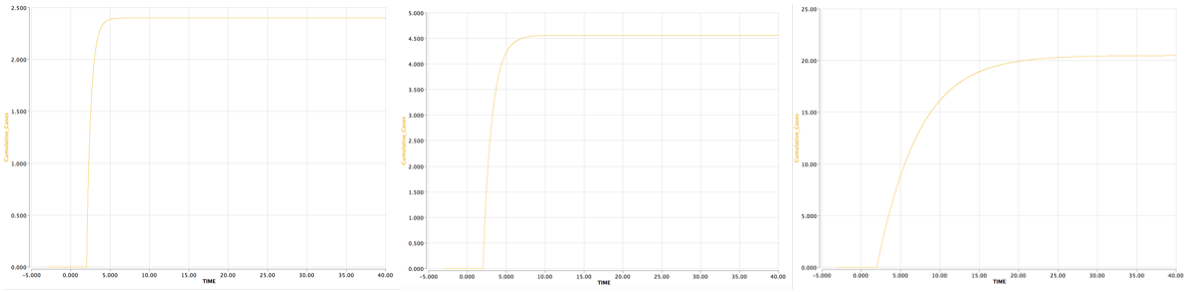
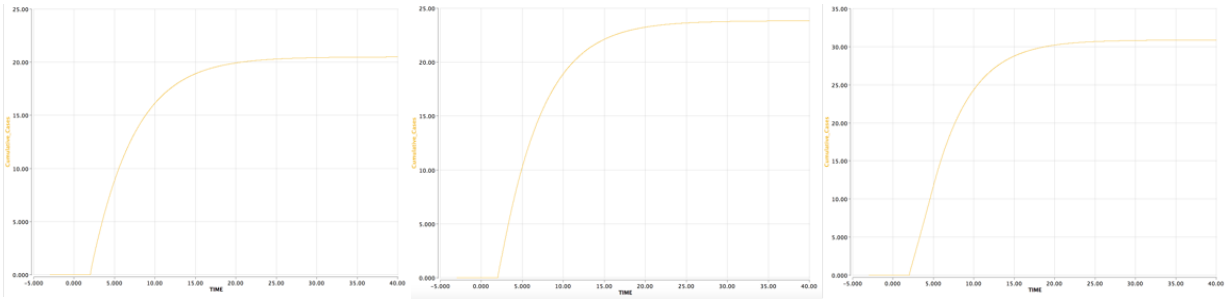


Figure 5: Cumulative cases expected among a cohort of Malian children in 2011 at 10% influenza vaccination coverage with vaccination implementation 4 weeks prior to the start of influenza season, 1 week after influenza activity began, and 3 weeks after influenza transmission began.



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Conclusion

This dissertation demonstrated that influenza circulated among pregnant and postpartum women and their infants in Mali during the study period, with peak activity in these groups occurring twice a year, in September/October and February. Although we followed our cohort for only sixteen months, the seasonality we observed is consistent with temporal influenza patterns captured by the WHO FluNet global surveillance system, as well as with influenza activity patterns observed in pregnant women, postpartum women, and infants in Mali in 2011-14. However, the incidence of LCI we observed among pregnant, and infants was lower than that reported in temperate regions, as well as lower than that previously reported for infants in other tropical countries, despite the frequency of follow up and laboratory testing for influenza. The incidence and severity of influenza vary annually, and given that we observed our cohort for only a little over one calendar year, it is possible that the incidence we observed is reflective of a year with low influenza activity. Additional studies that actively follow pregnant women and infants for multiple years should be designed and funded to capture multiple influenza seasons and may provide estimates that capture the expected variability in the incidence of influenza in these groups in Mali.

As expected, we observed a considerable burden of ILI and febrile illness among pregnant and postpartum women and infants that was not due to influenza or malaria. While pregnancy was a risk factor for ILI in the women we studied, the incidence of ILI in postpartum women was very high, four times greater than that observed in pregnant women in Malawi, another malaria endemic country. A prior study in Mali found a very high incidence rate of Respiratory Syncytial Virus (RSV) infections among infants whose mothers had participated in a randomized trial of influenza vaccine (1), with cases of RSV peaking at five months of age, mirroring the age-related incidence patterns we observed for influenza. In a study of RSV among families, fever was present in household members with RSV illness in only 5-27% of cases, suggesting that fever is not a symptom that is always experienced by individuals with RSV illness (2, 3). Our case definition of ILI required that fever be present; however, given the high incidence rate of RSV in infants in Mali that met the case definition of ILI, it is likely that RSV was responsible for some of the ILI episodes we observed in the infants in the cohort. A multi-year study of RSV in Nepal among pregnant and postpartum women that tested women with ILI for RSV found that RSV was rare (it was detected in only 0.4% of ILI episodes in Nepal); in 57% of the postpartum women with RSV infection, their infant also tested positive for RSV, suggesting transmission between mother-infant pairs (4). A similar study carried out in pregnant and postpartum women in Mongolia reported that RSV was rare in pregnant women (5). Although RSV infection was uncommon in pregnant and postpartum women in Nepal and Mongolia, it is possible that RSV may be responsible for some of the ILI episodes we observed in Mali, given the high incidence of RSV observed there among infants. Additional research is warranted to find the causative agent(s) for the ILI observed in pregnant and postpartum women in Mali, in order to guide the development and implementation of effective prevention measures.

A sizeable proportion of influenza transmission is believed to occur in the home, although the evidence to support this conclusion is drawn from studies of household transmission carried out in wealthy countries with temperate climates. Understanding the dynamics of household transmission can provide important insight concerning the factors related to the spread of

influenza in the home and inform prevention and control strategies. Influenza transmission in households in Africa has been poorly studied, and there are limited data on the frequency or dynamics of household transmission of influenza there, especially among populations at high-risk for severe influenza illness (e.g. pregnant women, infants, and children <5 years). Quantifying the serial interval (SI) allows us to understand how quickly influenza spreads in the household and can yield a transmission parameter necessary to model the possible impact of influenza vaccination. In Chapter 2, we estimated the SI of influenza and examined factors that may affect the time interval between primary and secondary cases of LCI in the household. The SI estimates we found for influenza were at the lower end of the ranges previously reported for influenza A(H1N1), and influenza B, as well as for our overall influenza SI estimate. While our estimate of the overall influenza SI (1.91 days) is relatively short, it is consistent with the SI observed in the only other study to measure it in West Africa (2 days), suggesting that perhaps local climatic factors, such as precipitation, and humidity, may play a role in the time interval between primary and secondary cases of influenza in the home. Further studies that provide estimates of the SI for influenza in this region may clarify whether such factors are related to the shorter SIs observed there. Another possible explanation for the short SI may be that influenza transmission in our study involved almost exclusively children < 5 years of age, including infants, and very few cases involved adults. One prior study found that when secondary cases of influenza occurred in children, the SIs were shorter than when they involved adults, and that study also found that increased contact with an index case when the index case occurred in a child was associated with a shorter SI (6). Children < 5 years of age accounted for over 85% of the individuals involved in household transmission in our study. Studies of contact patterns among individuals have demonstrated that people have the highest contact rates with individuals of their own age. Therefore, it is possible that the shorter SIs we observed were due, in part, to primary and secondary cases of influenza in the household occurring in children.

We also found that the presence of cigarette smoking in the household was associated with a shorter SI, suggesting that environmental cigarette smoke exposure in the home may shorten the time interval between cases of LCI in this setting. Cigarette smoke exposure in the household has been associated with an increased risk of influenza and of pneumonia, as well as other respiratory infections in children, and it is also associated with an increased risk of illness severity, as evidenced by longer influenza-related hospital stays and a higher odds of requiring intensive care during hospitalization. In adults, cigarette smoking is associated with influenza illness severity. Because the majority of smokers in Mali are men, future household studies of influenza should include them, and information regarding smoking habits should be collected. Such studies will allow evaluation of whether men experience more influenza in Mali than women, in whom smoking is rare, and will also lead to a better understanding of men's role in household transmission of influenza, as well as the effect of frequency of cigarette smoking on household transmission of influenza. To our knowledge, ours is one of only two studies that has examined factors that may help explain heterogeneity in estimates of influenza SIs. Additional research on factors that may increase or reduce the interval between cases of influenza in a household may provide insight concerning the transmission dynamics that facilitate the spread of influenza. Gaining a better understanding of the factors that accelerate the spread of influenza may yield insights into transmission that lead to interventions, such as encouraging smoking cessation during periods of increased influenza activity.

In Chapter 3, our analysis modeling the use of influenza vaccination among a group of influenza naïve children between 6 to 59 months of age during the largest peak of influenza activity, predicted that even modest influenza vaccine use in this age group may have reduced the cumulative case count of LCI in this age group considerably. We modeled influenza vaccination use in children at three levels of coverage and predicted a nearly 75% reduction in the number of LCI cases with a minimal level of vaccination coverage (10%) in the study population. Although level of vaccine coverage had the greatest impact on the final case count, we found that the timing of the introduction of influenza vaccine also influenced the total number of cases predicted. When we modeled the use of influenza vaccine in children four weeks before the peak of influenza cases observed during the study period, the model predicted the largest reduction in total cumulative case count. Modeling the initiation of influenza vaccine use in the same children one week after the first cases of influenza had appeared predicted a substantially larger case count than that we would have observed if vaccination had occurred four weeks prior to the start of influenza activity. We also observed that each additional week of delay in implementation of influenza vaccine resulted in larger cumulative numbers of cases at the end of the peak of influenza activity. The results of the modeling analyses suggest that giving children influenza vaccine has the potential to prevent a significant number of the influenza cases observed during the peak of influenza activity in Mali, even at nominal levels of vaccine coverage.

While other studies have demonstrated that vaccinating one person in the household with influenza vaccination does was not associated with a reduction in household transmission after a case in the household has occurred, vaccinating one individual will confer protection to the individual who received the vaccine, which will reduce the number of susceptible individuals in the household, and therefore may reduce the likelihood of introducing influenza into the home. Randomized controlled trails of influenza vaccine in children are necessary for determining vaccine efficacy in this group. Few such studies have been carried out in children in low income countries. In our analysis, we used the vaccine effectiveness of influenza calculated in a case-control study in Kenya. Although vaccinating one individual in the household does not prevent household transmission, because most influenza is believed to be introduced into the home by a child, examining the effect of vaccinating multiple children in the home may generate new information regarding this strategy's potential to prevent transmission in the home, which may be particularly useful in informing decisions about prioritizing the use of influenza vaccination when vaccine availability is scarce, especially in countries like Mali, where most households include multiple children.

Our findings in Chapter 1 suggest that there is an increased risk of LCI in pregnant women in households with a child < 5 years of age, compared to households without a child of that age in the home; we also observed an increased risk of LCI in infants in households with an increasing number of children residing in the home, although this finding was not statistically significant, possibly due to the small number of LCI cases in this study. While influenza vaccination in pregnant women can provide protection to the woman during pregnancy and in postpartum interval, as well confer protection her infant, because vaccinating one individual does not prevent household transmission, studies in which multiple individuals, particularly children, receive influenza vaccine can provide insight concerning the efficacy and effectiveness of influenza vaccine, as well whether vaccinating multiple children reduces the risk of household

transmission. This approach may provide the evidence needed to argue for prioritizing use of influenza vaccine in children as an prevention strategy to consider in low resource settings if influenza vaccine availability is limited.

In 2019, Mali passed a referendum to provide free preventive care to pregnant women and children < 5 years of age that is expected to be fully implemented by 2022 (7). While the implementation of an influenza vaccination strategy may have been challenging to undertake in a setting in which individuals, on average, presented for care only once every three years, it is possible that the provision of seasonal influenza vaccine in pregnant women and children may be feasible once access to preventative care is expanded in Mali.

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