博士論文

(PhD thesis)

Burden of Influenza and Respiratory Syncytial Virus Infection in Pregnant Women and Infants under 6 months in Mongolia
(モンゴル国での妊婦および生後6ヶ月児でのインフルエンザおよびRSウイルス感染症の疾病負荷に関する疫学研究)

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2. SUMMARY

Background: Pregnant women and infants under 6 months old are at risk of influenza-related complications. Limited community-based data exists on the burden of respiratory viruses for both groups.

Objectives: To determine the incidence and risk factors of influenza-like illness (ILI), severe acute respiratory infection (sARI) and virus-positive cases (influenza A, influenza B and RSV) among pregnant women and infants under 6 months.

Design: Prospective and observational open cohort study.

Participants: All pregnant women and children less than 24 weeks old who reside in Baganuur district (バガヌール地区), Mongolia from October 1st 2013 - April 30th 2014 and October 1st 2014 - April 30th 2015 (two consecutive influenza seasons).

Data collection: ILI and sARI cases were identified by scheduled follow-up calls twice a week. For those identified, clinical information was collected and influenza and Respiratory Syncytial Virus (RSV) were tested by point-of-care test kits (QuickNavi^{TM} – Flu + RSV, Denka Seiken Co. Ltd, Japan).

Statistical analysis: Overall and stratified (by trimester for pregnant women and age group for infants) incidence rates were calculated. Cox proportional hazard regression was used to analyze the risk factors of infection.

Results: A total of 1260 unvaccinated pregnant women were enrolled, whereby 174 (13.8%) ILI and 2 (0.2%) sARI cases were detected. From all tested ILI (94.8%) and sARI (100%) cases, 26 ILI and 1 sARI case tested positive for influenza A. The overall incidence rates for ILI, sARI

and influenza A were 11.8 [95% confidence interval (C.I): 11.2 – 12.4], 0.1 [95%C.I: 0.0 – 0.4], and 1.7 [95% C.I: 1.5 – 1.9] per 1,000 person-days, respectively. Incidence rates and adjusted hazard ratios for ILI and influenza A were lowest in the third trimester. Pregnant women with co-morbidity were 1.4 times more likely to develop an ILI episode [Adj.HR: 1.4 (95%C.I: 1.1–1.9)].

A total of 1304 infants under 6 months were enrolled, whereby 246 (18.9%) ILI and 255 (19.6%) sARI cases were detected. The overall ILI and sARI incidence rates were 15.2 [95%C.I: 14.5 – 15.8] and 20.5 [95%C.I: 19.7 – 21.3] per 1,000 person-days, respectively. Among the tested ILI (77.6%) and sARI (30.6%) cases, the overall positivity rates for influenza A, influenza B and RSV were 6.3%, 1.1%, and 9.3%, respectively. Positivity rates of influenza A and RSV tend to increase with age. sARI cases were 1.4 times more likely to be male [Adj.HR: 1.4 (95%C.I: 1.1–1.8)]. From all influenza A and RSV positive infant cases, 11.8% and 68.0% were respectively identified among sARI hospitalized cases.

Conclusion: Low overall influenza A burden in both groups was observed, though underestimation was likely due to point-of-care tests used. For infants, RSV burden was more significant than influenza A. These findings would be useful for establishing control strategies for influenza and RSV in Mongolia.

3. BACKGROUND

3.1. BASIC INTRODUCTION TO INFLUENZA VIRUS

Influenza viruses are enveloped, single-stranded, negative sense, segmented, ribonucleic acid (RNA) viruses belonging to the *Orthomyxoviridae* family [1]. They are classified into three genera or types (A, B, and C). Genus Influenza virus A has one species, influenza A. It includes all avian and equine influenza viruses, and most influenza viruses of swine. This virus can be classified into subtypes, based on the surface glycoproteins: haemagglutinin (HA), and neuramindase (NA). There are 16 HA (H1-16) and 9 NA (N1-9) recognized subtypes. Genus Influenza virus B has one species, influenza B, which is less common than influenza A and infects mainly humans. It can be divided into two antigenically and genetically distinct lineages: B/Yamagata and B/Victoria. Influenza B mainly causes disease for school-aged children [2]. Lastly, genus Influenza virus C has one species, influenza C, which is the least common than the other types. It usually causes mild disease in children, and it can also infect dogs and pigs. Out of the three types, Influenza A and B viruses are known to cause influenza epidemics in humans, leading to significant morbidity and mortality. Giving the rich source of host reservoirs mainly from birds and swine, influenza A viruses are able to cause epidemics and pandemics through re-assortment and mutation processes. The five known influenza pandemics occurred in 1918 A(H1N1) Spanish flu, 1957 A(H2N2) Asian flu, 1968 A(H3N2) Hong Kong flu, 1977 A(H1N1) Russian flu and 2009 A(H1N1) Swine flu. Influenza B, on the other hand, has lesser chance of causing pandemics.

The risk groups for influenza who are recommended to get vaccinated include those who are at risk of developing severe disease (children < 5 years old, pregnant women, the elderly, and individuals with underlying medical conditions such as HIV/AIDS, asthma and chronic

heart or lung diseases), and those at risk of getting exposed to the virus (health-care workers) [3].

3.2. Basic Introduction to Respiratory Syncytial Virus (RSV)

Human RSV is a non-segmented negative-sense, single-stranded, enveloped RNA virus belonging to the *Paramyxoviridae* family, genus *Pneumovirus* genus and *Pneumovirinae* subfamily [4]. It is divided into two major antigenic subgroups, A and B that tend to co-circulate in a given season. Humans are the only host for RSV. As there is incomplete immunity upon RSV infection, multiple re-infections can occur throughout life. It is known that most children will experience at least one RSV infection by the age of two years old [5]. Primary infection with RSV is usually symptomatic, and the disease severity decreases with each subsequent exposures.

The risk factors for severe RSV disease include medical conditions like premature birth, having chronic lung disease of prematurity, neuromuscular disease, congenital abnormalities of the airway, cystic fibrosis and immuno-deficiencies [5]. There are also social, demographic, and environmental risk factors: age < 6 months, young siblings in the household, daycare attendance, low birth-weight, exposure to tobacco or other air pollutants, family history of asthma, and multiple births [5].

3.3. Influenza and RSV Burden for Pregnant Women

Pregnant women are known to be at high risk of influenza-associated morbidity and mortality, particularly during the pandemic periods. During the 1918 A(H1N1) Spanish flu pandemic in the United States, 27% of the pregnant women died from pneumonia [6]. A case series study in Minnesota reported that 1957 A(H2N2) Asian flu pandemic accounted for 19.2% of all deaths among pregnant women. Also, half of all women of reproductive age

who died during this period were pregnant [7]. Similar trend can also be seen with the recent influenza pandemic (H1N1) 2009, where pregnancy is associated with increased risk of influenza-associated hospitalization and death [8, 9]. Pregnant women with A(H1N1)pdm09 infection were about seven times more likely to be hospitalized and two times more likely to die, when compared to non-pregnant women of reproductive age [8].

There were also reports on increased risk of influenza-associated hospitalization during the inter-pandemic period, especially among pregnant women with co-morbidity [10] and asthma [11, 12]. Women in the third trimester also have increased risk of influenza-associated hospitalization, particularly if they have co-morbidities [13, 14]. When compared to post-partum women, women in their third trimester of pregnancy were 3 – 4 times more likely to be hospitalized for an acute cardiopulmonary illness during the influenza season [14]. On the other hand, studies using databases from Kaiser Permanente Health Plan in Oregon [15] and Northern California [16] reported low hospitalization rates for pregnant women during the 1975-79 and 1997-2002 seasons, respectively. During the inter-pandemic period, there is also one study that investigated the occurrence of influenza-like illness (ILI) episodes at each pregnancy stage [17]. They found increasing risk of developing ILI with increasing trimester stage, with highest risk during the post-partum period.

Influenza infection during pregnancy is also associated with poor pregnancy outcomes, in both pandemic [6, 7, 9] and inter-pandemic [18, 19] periods. Such outcomes include spontaneous abortion, preterm birth, fetal distress, caesarean delivery, small for gestational age, and low birth weight. During the 1918 H1N1 influenza pandemic, half of the cases who developed pneumonia had spontaneous abortion [6]. Pre-term birth and emergency caesarean deliveries were also reported during the pandemic (H1N1) 2009 [9].

Unlike influenza, RSV infection among pregnant women is not much explored. To my knowledge, there has been no such study ever conducted previously. This is not unusual, as RSV disease severity is known to be low among young adults, whom must have already been exposed to RSV at a young age. RSV infection among young working adults was previously studied, but the attack rate was lower and symptom severity was milder than those for infants [20].

3.4. Influenza and RSV Burden for Infants under 6 months

The burden of acute lower respiratory infections due to influenza virus [21] and RSV [22] is known to be high globally among children < 5 years old. Globally, RSV is the most common cause of childhood acute lower respiratory infections (ALRI) and major cause of hospitalizations due to severe ALRI [23]. Infants under 6 months were found to have relatively high incidence of influenza in Bangladesh [24] and the United States [25], as well as RSV in Kenya [26].

Hospitalization rates for severe acute respiratory infection (sARI) are also known to be high globally among children < 5 years old [23]. Among these children, those under 6 months old have high hospitalization rates related to influenza and RSV. In the United States, the influenza-related hospitalization rate was estimated to be 4.5 per 1,000 children under 6 months old [27], while that due to RSV was 16.9 per 1,000 children [28]. Healthy infants and young children are also still at risk of either influenza- [29] or RSV- [28, 30] related hospitalizations.

Young male infants are known to be at risk of sARI, leading to hospitalizations [23, 31, 32]. Male gender is also a known risk factor of severe RSV disease [33]. When compared to females, young male infants tend to have reduced airway function [34, 35] and also tend to

be more susceptible to infection [36]. The former was also reported for young males who were born full term [34, 35] and premature [37].

Although young children who were born premature and/or born with low birth weight are at risk of severe RSV-related disease [5], there are also reports on RSV burden among previously healthy young children [28, 30]. These studies enrolled infants from two different settings: hospital and outpatient [28], and randomized clinical trial [30]. This suggests that intervention strategies for RSV should consider both high-risk and healthy children.

3.5. Physiological and Immunological Changes during Pregnancy

Anatomic and physiological changes during normal pregnancy and they occur at the cardiopulmonary, immunological and hormonal levels [38, 39]. At the cardiopulmonary level, the diaphragm is elevated to accommodate the uterus, which results in up to 40% increase in tidal volume and up to 20% decrease in the functional residual capacity in the later stages of pregnancy. These changes, along with an increase in in oxygen consumption, result in a predisposition to respiratory infections and severe disease during the later stages of pregnancy [40].

At the immunological and hormonal levels, the body goes into a unique immuno-modulated state whereby different immune responses occur depending on the stages of pregnancy [41]. In the early stages of pregnancy, uterine natural killer (uNK) cells and uterine dendritic cells (uDC) are necessary for blastocyst implantation and placentation processes to occur successfully. Thus, elevated numbers of NK cells and DC can be observed at least during the first trimester. In fact, studies have shown that NK cells begin to decrease in the blood circulation after 20-week gestation [42, 43]. NK cells were found to be important for immune response to influenza [44]. After 20-week gestation, levels of NK and T cells (particularly

CD4+ and CD8+) decrease from the second half of the pregnancy period [42]. Reduced levels of highly inflammatory Th1 cytokine release also occur during the later stages of pregnancy [43]. This dampening of the pro-inflammatory response, particularly NK cells, results in viral clearance delay of influenza infections, which leads to an increased risk of severe illness [43].

In contrast, defensive immune responses (via phagocytic cells and α-defensins 1-3) were found to be elevated from the first trimester until at least 35-weeks gestation [42]. High levels of estrogen and progesterone also mediates these processes by suppressing pro-inflammatory responses to infections and stimulating anti-inflammatory responses involved in defensive immunity [45]. Notably, the levels of these sex hormones surge from the first trimester and peak in the third [43]. Thus, the immune system is capable of preventing the establishment of viral infections as pregnancy progresses. But once the infection is established, the changes in the pulmonary physiology [40] and the reduced capacity to clear the infection [43] leads to increased risk of developing severe illness.

The dampening of the pro-inflammatory responses in the later stages of pregnancy is evidently seen among pregnant women with auto-immune diseases like Rheumatoid Arthritis (RA), Multiple Sclerosis (MS) and Systemic Lupus Erythematosus (SLE). These auto-immune diseases tend to have increased activity (or flares) during the early pregnancy stages, but decreased during the third trimester [46]. This tendency also correlates strongly with estrogen levels [43]. Notably, high levels of both sex hormones are associated with improvements to pulmonary function in pregnant asthmatic women [47].

3.6. Immune Responses of Infants under 6 months

Infants are thought be dependent on the innate immune system for protection against infections [48], whereby anti-inflammatory responses play a more dominant role. Directly

vaccinating infants under 6 months with the influenza vaccine is not allowed because their adaptive immune system is not mature enough to develop adequate immune response. Hence, one option to protect them against influenza is to acquire the influenza antibodies from the mother *in utero.* Infants who had acquired maternal antibodies against influenza [49, 50] and RSV [51] were reported to be protected from severe illness and had delayed symptom onset.

3.7. Maternal Vaccination for Influenza and RSV

Maternal influenza vaccination is one of the safe and cost-effective ways of protecting both pregnant women as well as infants under 6 months [52, 53]. There is evidence that maternal influenza vaccination can induce a strong immune response to protect both mother and the unborn fetus. It was found to reduce influenza illness for both pregnant women [54-56] and infants under 6 months [25, 56, 57], and also reduce hospitalizations for infants under 6 months [57-59]. In a randomized study in Bangladesh [56], maternal influenza vaccination reduced 63% of laboratory-confirmed influenza illness in infants under 6 months. It also reduced 29% and 36% of febrile respiratory illness in infants under 6 months and pregnant women, respectively. Another study found an association between maternal influenza vaccine use and reduced likelihood of premature and small for gestational age births [60]. No serious adverse effects have been reported for influenza vaccination during pregnancy [61], though there were limited information available on the safety of vaccination during the first trimester [62]. Also, vaccine effectiveness is similar between pregnant women and other adults [54]. The WHO Strategic Advisory Group of Experts (SAGE) on immunization recommended that pregnant women should be vaccinated against influenza at any pregnancy stage [3].

RSV vaccines are currently not available for any age groups, but they are now under development for four targeted groups, two of which are the infants under 6 months and pregnant women [63]. However, vaccine development proves to be a challenge for each group. On one hand, there is a concern on how vaccine may respond among infants under 6 months who have immature immune system and maternal antibodies. On the other hand, there is also a probable risk of adverse fetal outcomes for pregnant women who get vaccinated. Nonetheless, when it is made available and safety for both mother and fetus are assured, maternal RSV vaccination could be one useful strategy to prevent severe disease among infants under 6 months [64, 65]. Subunit vaccines are being considered for pregnant women and are currently on development [63]. Another good option is to vaccinate school-age children as it was shown to be effective in both field [66] and modeling [67] studies.

Currently, the only available preventive measure against RSV infection among infants under 6 months is the monoclonal antibody, palivizumab [65]. However, it is expensive to administer, has to be administered monthly throughout the RSV season, and does not always prevent serious RSV disease [65, 68].

3.8. Research Gaps and Study Significance

One important research gap is that there is currently limited information on the burden of respiratory viruses for both pregnant women and infants under 6 months at the community level. As outlined above, it is known that pregnant women have high morbidity and mortality rates due to influenza and that the highest impact was observed during pandemics and also on those in the third trimester. However, this is based mainly on hospital data, which could be biased toward severe cases. To my knowledge, only two studies reported on ILI episodes at the clinic level for pregnant women [13, 17]. Thus, including data at the community level

would enable us to see the overall picture of the true disease burden. A similar argument can also be made for infants under 6 months. Like pregnant women, high morbidity and mortality rates were also reported for both influenza and RSV but most population-based studies relied on hospital data. There were also quite few similar studies on infants under 6 months for influenza infections (in Bangladesh [56] and United States [25]) and RSV infections (in Kenya [26]).

For pregnant women, there are a lot of epidemiological reports of high risk to severe influenza-associated illness, but not for susceptibility to influenza infections. There is also few data on whether pregnant women would be more susceptible to infection at a particular trimester stage. Information on the infection risk of pregnant women in the first trimester is also lacking [62]. Thus, data collected from this study would allow us to investigate on any differences in the susceptibility risk between all three trimesters.

In addition, the study results would also benefit Mongolia. There are a few reasons why Mongolia is chosen as the study site. First, maternal and child health is currently one of the important health issues in Mongolia due to its high birth rate and population growth. Between 2005 and 2014, the number of live births increased at an average rate of 6.4% [69, 70]. In 2014, the estimated annual birth rate is 20.9 per 1,000 persons [71]. Second, it was previously shown that children < 5 years old in Mongolia have the highest incidence of influenza-associated illness [72]. Third, the burden among pregnant women has never been assessed in the country, even though 24.1% of all influenza A(H1N1)pdm09-confirmed deaths in Mongolia were pregnant [73]. During the study period, the country's national influenza vaccination policy did not include pregnant women as one of the recommended vaccine recipients [74]. Therefore, the study findings can thus help inform on any disease

burden from influenza and/or RSV among these two growing population groups residing in semi-urban areas of Mongolia.

4. STUDY OBJECTIVES

The study objectives are to determine the incidence and risk factors of influenza-like illness (ILI), severe acute respiratory infection (sARI) and virus-positive cases (influenza A, influenza B and RSV infection) among pregnant women and infants under 6 months.

5. Materials and Methods

5.1. Study Site

Baganuur is a semi-urban district located at about 130 km away from the central part of Ulaanbaatar, Mongolia's capital city (Fig. 1). In 2013, it had an estimated population of 27,440. Medical services are provided mainly by one district hospital and four primary healthcare centers called Family General Practitioners (FGPs). The study was conducted in this district for two consecutive influenza seasons (October 1st 2013 - April 30th 2014 and October 1st 2014 - April 30th 2015).

5.2. Data Collection

In Mongolia, all pregnant women and children < 2 years old are required to register and undergo medical check-ups at their FGPs, which are assigned based on their official residential address. All four FGPs in the district were used as the study entry point. All residents who registered at the FGPs and met the inclusion criteria (being pregnant or aged < 24 weeks during the study period) were enrolled. To include those who had already registered and those newly registered during the study period, enrolment was carried out continuously throughout the whole study period. Follow-up ends if any of the following occurred: a woman was no longer pregnant, a child reached 24 weeks of age, or any participant moved away from Baganuur district.

At the time of enrolment, a baseline questionnaire was administered to all participants to collect their demographic information. Prenatal information and household member demographics were also collected from pregnant women for both seasons. For infants under 6 months, information on birthweight, gestational age at delivery and presence of birth

defects were collected for both seasons, while the household member demographics were collected only during the 2014/15 season. Household members who were < 18 years old were categorized as: young child (until 2 years), kindergarten-age (2 – 5 years), and school-age (6 – 17 years).

For pregnant women, an obstetrician in the district hospital on their first check-up carries out initial antenatal examination, and the results are recorded in the antenatal check-up record book. This book served as the major source of baseline data in this study. The obstetrician classifies all pregnant women as either normal or high risk to indicate whether she would undergo routine or extensive antenatal check-ups, respectively. The first day of the last menstruation was used to determine the start date of pregnancy and the trimester stages were defined as: first (0 - 13 weeks), second (14 - 26 weeks), and third (27 weeks and above). Baseline height and weight information were also collected to calculate the body mass index (BMI). Pregnant women were defined as with co-morbidity if she, at the time of the antenatal examination, has any of the following medical conditions that pre-disposes them to influenza-related complications [75]: pulmonary, cardiovascular, renal, and metabolic conditions.

For infants under 6 months, low birthweight was defined as those born weighing < 2500g. Three categories were defined for term of pregnancy [76, 77]: preterm, early term, and full term.

The primary outcome in this study was the occurrence of a case positive for either influenza A, influenza B or RSV. The secondary outcome was the occurrence of an ILI or a sARI case. An ILI case was defined as the sudden onset of fever of ≥38°C, or with history of fever, and

296 cough within the last 7 days. A sARI case was defined as having ILI symptoms and requires
297 hospitalization.

298 Throughout the study period, scheduled follow-up calls were carried out twice a week (one
299 call every 2 - 5 days) to actively identify any ILI episodes among the study participants. When
300 an ILI episode was identified, a timely home visit was made to interview him/her with a case
301 report form and to collect a nasopharyngeal swab for on-site testing with a point-of-care test
302 kit (QuickNavi^{TM} – Flu + RSV, Denka Seiken Co. Ltd). This commercially available kit enables
303 simultaneous detection of influenza A, influenza B and RSV. In the subsequent two follow-
304 ups calls, the resolution of ILI symptoms was also recorded. If the ILI case had recovered
305 from symptoms on the day of follow-up call, that date was used as the date of symptom
306 resolution.

307 At the district hospital, information on sARI episodes (admission and discharge dates,
308 symptoms presented, clinical indicators, diagnosis at discharge and outcome, and point-of-
309 care test results) was collected. There were some cases who initially visited the FGP for
310 testing, then later referred for hospitalization. An episode whose interval between FGP visit
311 and hospital admission is < 7 days was considered as the same illness episode.

312 The district hospital and all four FGPs in Baganuur are also a part of the national ILI
313 surveillance program in Mongolia. Nasopharyngeal swabs were collected randomly from ILI
314 cases and routinely tested for influenza virus using real-time polymerase chain reaction
315 (PCR). Some randomly selected samples were further tested for other respiratory viruses,
316 including RSV. According to this surveillance [78], three influenza strains [A(H1N1)pdm09,
317 A(H3N2), and B] were detected during the 2013/14 season, while A(H3N2) and RSV were
318 detected during the 2014/15 season (Fig. 2). Some of the study participants (5.6%), who

missed testing by the study's kits, were instead tested under this surveillance. These results were also collected and combined into the analyses.

All field staff involved had been trained with participant interviewing as well as sample collecting, testing and result interpretation. The field supervisor monitored all activity from data collection to encoding.

5.3. Ethical Approval

Ethical approval for this study was obtained from the Ethics Committee of Tohoku University, Graduate School of Medicine, Sendai, Japan (2013-1-253), the Scientific Committee of National Center of Communicable Diseases, Mongolia (14/126), and the Ministry of Health, Mongolia (2014-02). Informed consent was obtained from eligible participants or their parents/guardians before study enrolment as well as from identified ILI and sARI cases before data and sample collection.

5.4. Statistical Analysis

Overall incidence rates (IR) were derived by dividing the actual number of cases detected with the total person-days at risk, and 95% confidence intervals (C.I) were calculated using exact methods. For participants with ILI or sARI, the symptomatic period was considered as days not-at-risk of infection and therefore not included in the denominator. For those with missing symptom resolution dates, I set 7 days as the duration of symptoms, based on previous studies on adults [79] and children < 1 year old [80]. For sARI cases, the symptomatic period was defined as the number of hospitalized days.

I also investigated the risk factors of getting ILI, sARI and virus positive cases. Participants who did not have ILI symptoms (non-ILI group) were initially compared univariately with the

ILI cases and also with each virus positive cases. sARI cases were only investigated for infants under 6 months, and they were compared univariately with those who were not hospitalized for sARI (non-sARI group). Parametric (Student's *t* and chi-square) and non-parametric (Wilcoxon rank sum and Fisher's exact) tests were used whenever appropriate. Factors with a *p*-value of ≤ 0.1 and deemed relevant for acquiring illness episodes were then included in the Cox proportional hazards (PH) regression model. In all models runs, non-ILI or non-sARI were used as the comparison group. Participants with multiple episodes within each season were also accounted for, in both un-adjusted and adjusted models. To ensure that the model assumptions were met, diagnostic tests on the proportional hazards assumption were done and the residuals were also checked. For pregnant women, all covariates except age at enrolment were categorically included in the final Cox PH model. Alternative model runs using categorized age quartiles were consistent with the main results reported. For infants under 6 months, all covariates included were categorical. A *p*-value of < 0.05 was considered statistically significant.

I hypothesize that trimester stage (for pregnant women) and age group (for infants under 6 months) could contribute to differences in infection risk. Therefore, I also stratified the IR calculations accordingly and included them as the time-dependent variable in the Cox PH model. Microsoft Excel and R software, version 3.2.0 [81] were used for all analyses.

6. RESULTS

6.1. DISTRIBUTION OF ILI AND SARI CASES, AND CIRCULATING VIRUSES IN BOTH POPULATIONS

Figure 3 shows the weekly number of ILI and sARI cases in pregnant women and infants under 6 months during the study period. Most ILI and sARI cases tend to occur between November to March, coinciding with winter months in Mongolia. The temporal trend of sARI correlated with that of ILI in infants ($r^2 = 0.71$) while there were only 2 sARI cases observed in pregnant women.

Figure 4 shows the weekly number of virus positives detected in the two populations. In general, positive cases were detected mainly from January to February, coinciding with the high activities of both ILI and sARI. All three targeted viruses were detected during the 2013/14 season, while influenza B virus was not detected during the 2014/15 season. This was quite consistent with data from the national ILI surveillance, except that RSV was not detected during the 2013/14 season (Fig. 2). There were periods of limited point-of-care test kit availability at the beginning of both seasons and early December in the 2014/15 season. Hence, virus testing was compromised during those periods.

6.2. INCIDENCE RATES OF ILI, SARI AND VIRUS POSITIVES FOR PREGNANT WOMEN

We individually enrolled 1,260 pregnant women and they were followed for a total of 120,887 person-days. The population characteristics were quite different for the two seasons (Table 1). Briefly, the 2014/15 season cohort tend to be more educated, employed, overweight, enrolled at an earlier gestational age, and did not have co-morbidities. The median age at enrolment was 27 years (range: 16 – 44) and 43.0% were enrolled during the

first trimester. Among all pregnant women identified, only seven refused to join the study. None was lost to follow-up and none had received the influenza vaccine.

A total of 174 ILI episodes (13.8%) were detected from 160 pregnant women. There were 12 and 2 women with 2 and 3 ILI episodes respectively, during the same influenza season. Among the ILI cases with recorded symptom resolution dates (85.6%), the mean interval to resolution was 8.1 days (range: 3 - 20). The overall IR for ILI was 11.8 per 1,000 person-days (95% C.I: 11.2 – 12.4) (Table 2a).

Among all tested ILI cases (n = 165), 26 (15.8%) tested positive for influenza A, 2 (1.2%) for influenza B, and 4 (2.4%) for RSV. This gives an overall IR of 1.7 per 1,000 person-days (95% C.I: 1.5 – 1.9) for influenza A, 0.1 per 1,000 person-days (95% C.I: 0.1 – 0.2) for influenza B, and 0.3 per 1,000 person-days (95% C.I: 0.2 – 0.4) for RSV (Table 2b). The majority of samples (96.0%) were tested within 5 days after symptom onset. During the 2014/15 season, 2 women tested positive for both influenza A and RSV from separate ILI episodes.

Two sARI cases were also detected, giving an overall sARI incidence rate of 0.1 per 1,000 person-days (95% C.I: 0.04 – 0.4). One case tested negative in the 2014/15 season during her second trimester, and another was positive for influenza A during the 2013/14 season during her first trimester. No virus co-detection and deaths were observed during the study period.

IRs for ILI and influenza A were the lowest in the third trimester (Table 2). Sensitivity analysis using total person-days only in January and February also revealed the same trend as the main result. This analysis was done because all influenza A positive cases were detected only in the latter months. Similar trend was also observed in the risk factor analysis for both ILI (Table 3a) and influenza A (Table 3b). When compared to the first trimester, pregnant women in the third trimester were 78% less likely to have influenza A detected, after

adjusting for age and presence of prior pregnancy [Adjusted hazard ratio (Adj. HR): 0.22 (95% C.I: 0.07 – 0.73)]. Pregnant women were also 50% [Adj. HR: 0.50 (95% C.I: 0.34 – 0.75)] and 63% [Adj. HR: 0.37 (95% C.I: 0.25 – 0.55)] less likely to develop an ILI episode in the second and third trimesters, respectively, when compared to the first trimester. In addition, pregnant women who have any co-morbidity were 43% more likely to develop an ILI episode [Adj. HR: 1.43 (95% C.I: 1.06 – 1.94)].

6.3. ILI AND sARI INCIDENCE, AND VIRUS POSITIVITY RATES FOR INFANTS UNDER 6 MONTHS

We individually enrolled 1,304 infants under 6 months and they were followed for a total of 122,344 person-days. They were mainly healthy, whereby 88.4% were born full term, 2.9% had low birthweight, and 3.5% were born with a certain birth defect. Their baseline characteristics were similar for both seasons, except that there were significantly more full term infants in the 2014/15 season (Table 4). The median age at enrolment was 12 days (range: 0 – 167). During the 2013/14 season, 99.9% were breastfed for 6 months. Almost all identified infants participated in this study (99.0%) and none was lost to follow-up.

A total of 246 ILI episodes (18.9%) were detected from 201 infants under 6 months. There were 37, 7 and 1 infants with 2, 3, and 4 ILI episodes, respectively, during the same influenza season. Among the ILI cases with recorded symptom resolution dates (82.2%), the mean interval to resolution was 7.5 days (range: 1 - 14). The overall ILI IR was 15.2 per 1,000 person-days (95% C.I: 14.5 – 15.8) (Table 5a). From all tested ILI cases (n = 191), 15 (7.9%) influenza A, 3 (1.6%) influenza B and 8 (4.2%) RSV cases were detected. Most samples were tested within 5 days after symptom onset (98.8%). During the 2013/14 season, RSV was detected twice (on separate occasions) from one female infant of low birthweight and did not result in sARI hospitalization.

A total of 255 sARI hospitalized cases (19.6%) were detected from 218 infants under 6 months, giving an overall sARI IR of 20.5 per 1,000 person-days (95% C.I: 19.7 – 21.3) (Table 5a). There were 30 and 8 infants who were hospitalized for sARI twice and thrice, respectively, during the same influenza season. Also, 36 (14.1%) of them had prior visit to the FGP for the same illness episode. The mean length of hospital stay was 8.9 days (range: 0 – 33). For all sARI cases, 74.3% were admitted within 5 days after symptom onset. A total of 23 (18.3%) and 55 (42.6%) cases were tested during the 2013/14 and 2014/15 seasons, respectively. This gives an overall sARI testing rate of 30.6%. During the 2013/14 season, 4 (17.4%) tested positive for RSV and 2 (8.7%) for influenza A while 13 (23.6%) RSV and no influenza A cases were detected during the 2014/15 season. No influenza B positive sARI cases were detected from either season. No virus co-detection and deaths were also observed during the study period.

Overall, a total of 17 (6.3%) influenza A, 3 (1.1%) influenza B, and 25 (9.3%) RSV cases were detected among the tested ILI and sARI samples (n = 269) (Table 5b). Among the total positive cases detected, 2 (11.8%) influenza A and 17 (68.0%) RSV cases were detected among hospitalized cases with sARI. After stratification by age group, 16 – 24 weeks old infants had the highest ILI IR [26.8 per 1,000 person-days (95% C.I: 25.2 – 28.5)] and sARI IR [25.8 per 1,000 person-days (95% C.I: 24.2 – 27.4)] (Table 5a). The same trend was also observed in the positivity rates of influenza A and RSV (Table 5b). The increase in the RSV positivity rate started from the 8 – 15.9 weeks old group. Also, sARI cases were 1.4 times more likely to be male, when compared to non-sARI [Adj. HR: 1.40 (95% C.I: 1.07 – 1.83)] (Table 6).

7. DISCUSSION

A cohort of unvaccinated pregnant women and infants under 6 months was prospectively followed to actively detect acute respiratory illnesses for two consecutive influenza seasons. With its high participation rate (about 99% for both populations) and no loss to follow up, this cohort arguably provides an accurate picture in Baganuur. Among pregnant women, I observed low overall IR for influenza A. Both ILI and influenza A IR were the lowest in the third trimester. There were also few sARI and influenza A positive hospitalized cases. Also, pregnant women with co-morbidity have about 1.4 times higher risk of developing an ILI episode. Among infants under 6 months, I observed moderately high ILI and sARI IRs. Older infants (16 – 24 weeks old) had higher ILI IR and positivity rates (of influenza A and RSV), while younger male infants (0 – 7.9 weeks old) had higher sARI IR. Among all influenza A and RSV positive infant cases, 11.8% and 68.0% were respectively identified among hospitalized cases with sARI.

One study in the United States [13] reported both ILI and sARI IR for pregnant women in all three trimesters and they observed high IRs, especially for sARI. Both ILI and sARI IRs in the study are also higher than that previously reported for the reproductive-age population (15 – 49 years) in Baganuur [82] and Mongolia [72], though it could possibly be due to active case search in the study. Expanding this study to include non-pregnant women of reproductive-age would allow us to see any impact of pregnancy in acquiring ILI and sARI. These Mongolian studies also reported incidence rates for young children, though aggregates of 0 – 11 months were used. Here, ILI IR in this study is higher than that reported in Baganuur [82]. Relatively high infant ILI IRs were also previously reported from Bangladesh [56] and the United States [25]. Nevertheless, caution should be taken for interpreting these

direct comparisons due to differences in case detection methods, study season, and health-seeking tendencies, that is between women with and without pregnancy, as well as between younger and older infants.

The same study from Mongolia [72] also reported influenza positive rates by age group. The overall influenza positive rates for both ILI and sARI cases were lowest in the 0 - 11 month age group and highest in the reproductive age groups (16 – 44 years). My study findings for infants under 6 months were consistent with the former, as more RSV positives than influenza were detected. The latter was also consistent with this study as pregnant women were mainly positive for influenza A. RSV infection was also documented for young working adults [20], though the attack rate was lower and symptom severity was milder than those of infants. Despite the fact that influenza B can affect people of all ages, only few influenza B cases were detected in both pregnant women and infants, presumably reflecting the circulation of influenza virus in the community.

Both IR and adjusted HR of influenza A were lowest for pregnant women in the third trimester, which could be due to differences in virus exposure at each trimester stage. Studies in the United States have shown that women in the third trimester tend to engage in lesser leisure activities [83] and spend more time at home [84]. Although it is uncertain whether both tendencies are also observed among Mongolians, it can lead to less interaction with others in the community, thus decreasing the probability of acquiring respiratory infections. Notably, I also observed the same finding in the risk factor analysis for ILI, after accounting for the presence of co-morbidity as well as possible transmission in the household (presence of kindergarten-age child) and workplace (employment status). This suggests that settings other than household and workplace may contribute to a certain risk

494 of acquiring ILI and possibly influenza A infections. However, my finding contradicts with that
495 in a previous study, which showed high IR of influenza A in the third trimester [17].
496 Experimental studies have reported that the immune system switch to defensive mode
497 during pregnancy [42]. Defensive immune responses (via phagocytic cells and α-defensins 1-
498 3) are elevated throughout the pregnancy period [42]. Estrogen and progesterone levels,
499 which surge from the first trimester [43], further strengthens this by stimulating anti-
500 inflammatory responses [45]. This means that the immune system is capable of preventing
501 the establishment of viral infections as pregnancy progresses, and that there is little
502 immunological basis for increasing susceptibility to influenza infection [38]. Hence, my study
503 finding is in fact more consistent to what is known with the immunological changes during
504 pregnancy.

505 Low sARI IR among pregnant women was observed and only one was positive for influenza A.
506 This observation is in line with some studies [15, 16], but there are also many reports of
507 increased influenza-associated hospitalization rates during the inter-pandemic periods [10-
508 14, 18]. My study findings could firstly be due to the relatively mild influenza seasons.
509 Antigenic changes to the circulating strains seemed limited during both seasons under study,
510 as the WHO-recommended vaccine strains were the same [85]. Thus, the enrolled pregnant
511 women may have already had the infection to a similar strain prior to the current pregnancy.
512 Secondly, it could also be the low sample size of this study, which makes it difficult to detect
513 severe illnesses that occur at a low rate.

514 It was also observed that pregnant women with co-morbidity had 1.4 times higher risk of
515 developing an ILI episode, a finding which is consistent with previous studies [13]. Although
516 this HR estimate was for both seasons, additional analysis for each season revealed

517 significance only for the 2014/15 season (Table 9). When compared to the 2013/14 season,
518 the 2014/15 season had a lower proportion of pregnant women with co-morbidity enrolled
519 and influenza A activity was also higher. This thus suggests that having co-morbidity is in fact
520 a risk factor of developing ILI symptoms associated with influenza among pregnant women.
521 Of note, this finding needs to be interpreted with caution. In the antenatal care book, each
522 comorbidity condition was broadly categorized to include a broad list of acute and chronic
523 medical conditions listed under the International Classification of Diseases (ICD-10). We
524 could not get further details on these conditions as they were recorded in another record
525 book. Hence, it is likely that pregnant women whom I labeled as with co-morbidity include
526 those with acute conditions.

527 The striking demographic differences between the pregnant women population for both
528 seasons is unexpected but unlikely due to any sampling or selection bias. This is because all
529 identifiable pregnant women were enrolled into this study. Some women, who are Baganuur
530 residents but work/study outside Baganuur, may choose to stay in the district during their
531 pregnancy. This is probable because their assigned FGP for antenatal checkup was based on
532 their residential address. This tendency could also vary between seasons.

533 The ILI and sARI IR for infants under 6 months were the highest among the 16 – 24 weeks old
534 group and similar trend was also observed in the positivity rates of both influenza A and RSV.
535 In fact, 100% and 82.4% of the influenza A and RSV positive sARI cases, respectively, were
536 found in those aged 8 weeks or older. Notably, this finding for RSV is consistent with that in
537 Kenya [86]. This could be explained by the waning of passively acquired maternal antibodies.
538 Infants under 6 months who had acquired maternal antibodies against influenza [49, 50] and
539 RSV [51] were reported to be protected from severe illness and had delayed symptom onset.

Also, protection from maternal antibodies tends to wane after the first 2 - 3 months of life. Previous studies reported the half-life of passively-acquired maternal antibodies to be 21 – 53 days against influenza [49, 50, 87, 88] and 26 – 78 days against RSV [89-91].

sARI IR was also high among the 0 – 7.9 weeks old infants, giving the impression of low IR among the 8 – 15.9 weeks old. However, this could be falsely elevated due to two reasons. First, young infants tend to exhibit signs of breathing difficulties, regardless of infection severity. Thus, parents/guardians tend to bring them directly to the hospital for immediate care. In fact, only 38 sARI cases (14.8%) were referred to the hospital from the FGP; hence suggesting a small number of 'true' sARI cases. Second, Mongolia follows the integrated management of childhood illness (IMCI) guidelines, whereby infants < 2 months old with fever are to be directly admitted to the hospital as a sARI case [92]. Hence, the high tendency to seek healthcare at the hospital and the implementation of the IMCI guidelines could explain the high sARI IR among younger infants. This could also be one reason why the overall IR for sARI was higher than that of ILI.

It was also observed that 68.0% of RSV-positives detected were hospitalized with sARI, which is highly disproportionate when compared to that of influenza A (11.8%). This tendency is consistent with previous studies on hospitalized infants in the United States [27, 28, 31] and Thailand [32]. This data indicates that RSV caused more severe illness that required hospitalization among infants under 6 months in Baganuur. As the testing rate was low, particularly for sARI cases, it means that the actual proportion of hospitalized cases for both viruses could be much higher.

Infant sARI cases were 1.4 times more likely to be males, a trend that is consistent with previous reports [23] and also seen with RSV-associated hospitalizations [33]. When

563 compared to females, young male infants tend to have reduced airway function [34, 35] and
564 also tend to be more susceptible to infection [36]. In addition, all the RSV positive sARI cases
565 were born full term. This means that RSV disease can also affect healthy infants, a finding in
566 line with previous studies [28, 30].

567 Some study limitations need to be addressed. First, point-of-care test was the main
568 diagnostic method used, which could lower the sensitivity of virus detection. A meta-analysis
569 on influenza test kit evaluation studies reported a pooled sensitivity and specificity of 62.3%
570 and 98.2%, respectively [93]. Thus, the incidence and positivity rates reported in this study
571 are an underestimate, possibly by about 40%. For RSV, antigen detection methods are
572 known to be considerably less sensitive particularly for adults [94]. Point-of-care test kits
573 were used mainly due to the limited laboratory capacity to test samples with real-time PCR.
574 It also allowed us to simultaneously test for RSV, as the local laboratory only tests routinely
575 for influenza. In order to ensure higher viral loads in the samples taken, efforts were made
576 to identify cases within 5 days of symptom onset, and to test them as soon as possible upon
577 identification. Second, the overall testing rate was low, particularly for the infant sARI cases
578 (30.6%). This remains to be a major limitation even though the sARI testing rate improved
579 from 18.1% in the 2013/14 season to 42.6% in the 2014/15 season. Hence, the positive case
580 numbers reported for the infants under 6 months are an underestimate. While one major
581 reason for the low testing rate was the limited test kits supply during some study periods,
582 there were also a certain percentage of infants whose sampling was refused by their
583 parents/guardians. Unfortunately, we did not collect detailed data for this. Third, as a
584 consequence of using point-of-care test kits, I also could not provide the IR by influenza
585 subtype and RSV genotype. Fourth, we did not assess the use of non-pharmaceutical
586 interventions, such as hand washing and facemask usage, in our study population. This could

possibly affect the risk factor analysis results. Fifth, I did not detect enough virus-positive cases from both seasons to assess their risk factors. This could merely reflect the low influenza and RSV activities in this community and thus caution must be taken to extrapolate these findings to other areas in Mongolia. Sixth, dates of follow-up and hospitalization were used to determine the symptomatic period for ILI and sARI IR calculations, respectively. These dates do not strictly reflect on each clinical course and may affect the calculated IRs. Lastly, household demographic information was collected among infants only for the 2014/15 season. As a result, these data were excluded from the risk factor analyses for both seasons.

8. CONCLUSION

I reported the results of a prospective, observational open cohort study conducted in a community setting for two targeted high-risk groups, during two consecutive influenza seasons in Mongolia. I observed a low overall influenza A burden for pregnant women in terms of incidence and hospitalization rates, though underestimation was likely due to point-of-care tests used. One surprising finding is the low influenza A incidence rate among the third trimester women, despite their particularly high risk of developing influenza-related severe illness. While for infants under 6 months, the incidence of ILI and sARI was moderately high. An important note here is the high number of RSV positives who got hospitalized when compared to that of influenza A, despite the low testing rate. Including additional data from subsequent influenza seasons would help to ascertain these findings. Despite its limitations, my study findings add into the currently limited knowledge on the burden of seasonal influenza for both groups. This is also the first study of its kind in Mongolia, a developing country where maternal and child health is an important health topic.

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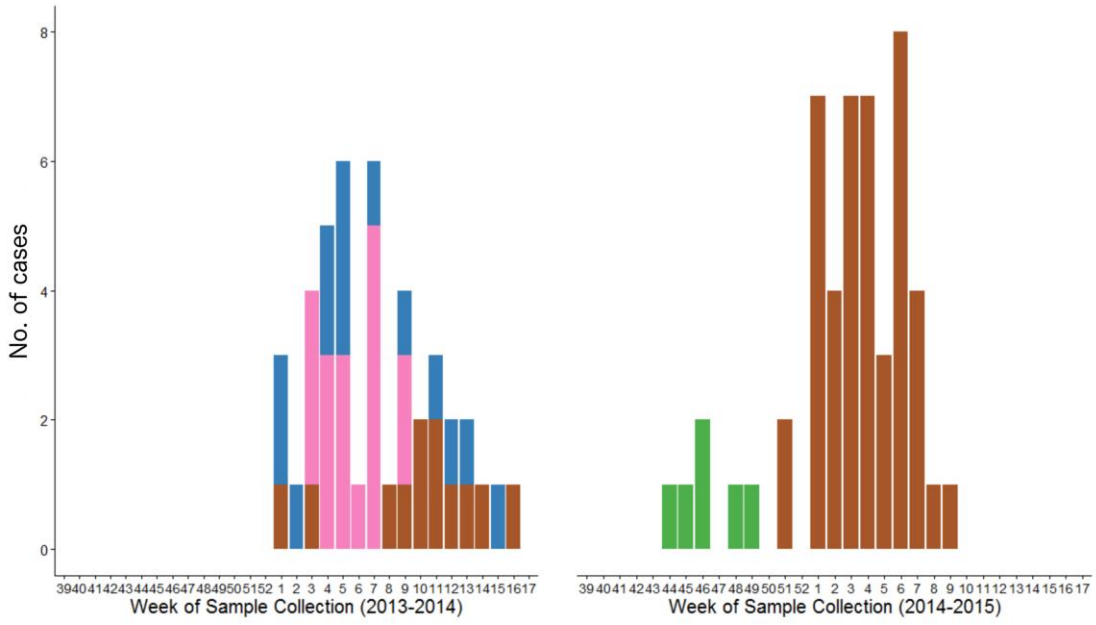
11. FIGURES

Fig 1. Location of the study site, Baganuur (バガヌール地区)

858 

859 **Fig 2.** The weekly number of positive cases detected in Baganuur district for 2013/14 (left)
860 and 2014/15 (right) seasons, based on the national ILI surveillance [78]. The bars show the
861 number of positive cases for influenza A(H3N2) [brown], influenza A(H1N1)pdm09 [pink],
862 influenza B [blue] and RSV [green]. Three influenza strains were detected during the 2013/14
863 season, while influenza A(H3N2) and RSV were detected during the 2014/15 season.

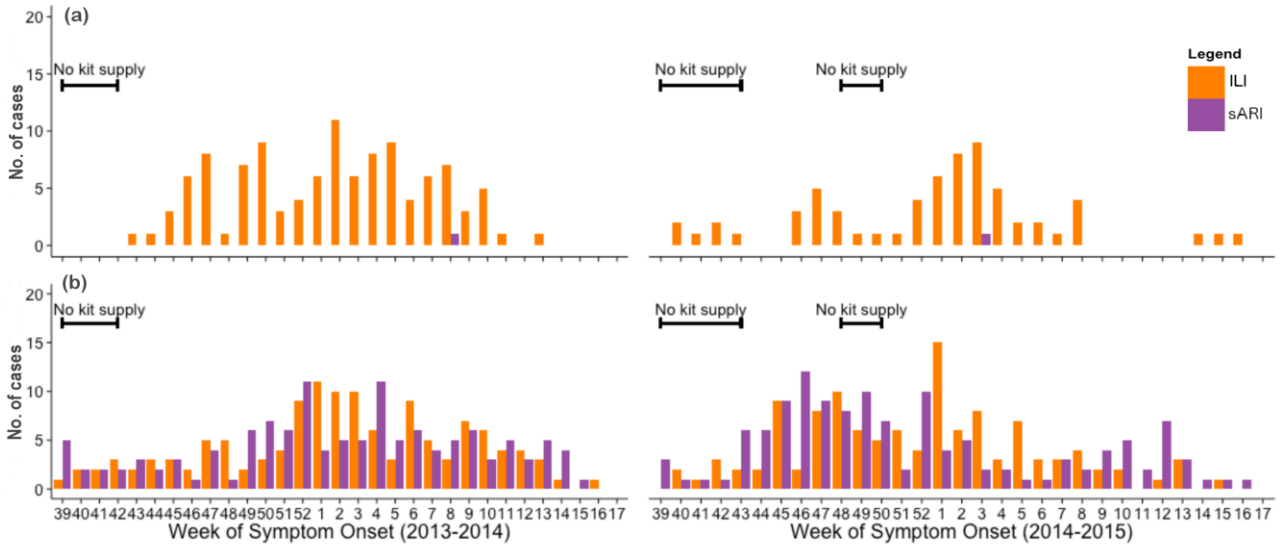
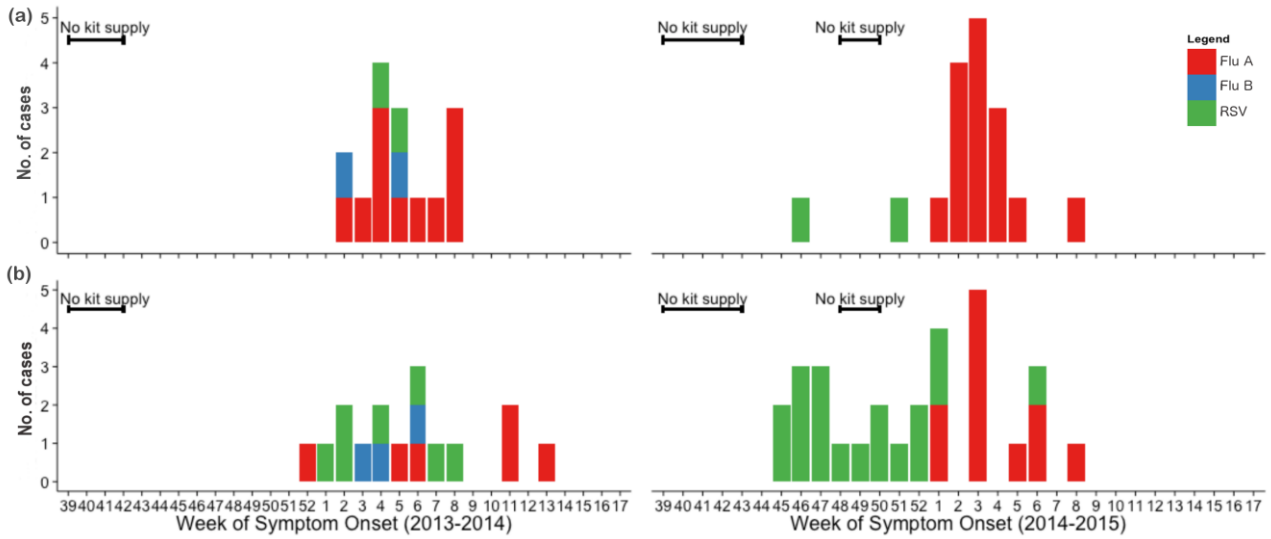


Fig 3. The weekly number of influenza-like illness (ILI) [orange] and severe acute respiratory infections (sARI) [purple] cases during the 2013/14 (left) and 2014/15 (right) seasons, for pregnant women (a) and infants under 6 months (b). The black lines indicate periods of limited point-of-care test kit supply, thus testing was compromised in those weeks.



869

870 **Fig 4.** The weekly number of virus positives detected from ILI and sARI cases during the
871 2013/14 (left) and 2014/15 (right) seasons, for pregnant women (a) and infants under 6
872 months (b). The bars show the number of positive cases for influenza A (red), influenza B
873 (blue) and RSV (green). The black lines indicate periods of limited point-of-care test kit
874 supply, thus testing was compromised in those weeks. The overall testing rate for ILI and
875 sARI cases are 94.8% and 100%, respectively, for pregnant women and 77.6% and 30.6%,
876 respectively, for infants under 6 months.

877

12. TABLES

Table 1. Baseline characteristics of the pregnant women cohort and its comparison between two seasons.

Population characteristic		Total cohort (%)	2013/14 season (%)	2014/15 season (%)	p-value
No. of pregnant women enrolled		1260	643 (51.0)	617 (49.0)	
Age at enrolment (years)	Median ± sd	27.4 ± 6.1	27.0 ± 6.1	28.0 ± 6.0	0.07
	Range	16 - 44	16 - 43	16 - 44	
Educated at high-school level or lower		278 (22.1)	212 (33.0)	66 (10.7)	<0.001*
Employment status	Employed [#]	825 (65.5)	381 (59.3)	444 (72.0)	<0.001*
	Unemployed	326 (25.9)	193 (30.0)	133 (21.5)	
	Student	109 (8.6)	69 (10.7)	40 (6.5)	
BMI category ^	Underweight (< 18.5)	51 (4.1)	22 (3.4)	29 (4.7)	<0.001*
	Normal (18.5-24.9)	845 (67.1)	471 (73.4)	374 (60.6)	
	Overweight (25-29.9)	296 (23.5)	126 (19.6)	170 (27.6)	
	Obese (≥ 30)	67 (5.3)	23 (3.6)	44 (7.1)	
Household characteristics					
Type of household structure ^	Apartment	544 (43.2)	297 (46.3)	247 (40.1)	<0.001*
	Ger	549 (43.6)	236 (36.7)	313 (50.7)	
	Private house	166 (13.2)	109 (17.0)	57 (9.2)	
Household size	1 - 2	173 (13.7)	82 (12.8)	91 (14.7)	0.5
	3 - 4	693 (55.0)	353 (54.9)	340 (55.1)	
	5 and above	394 (31.3)	208 (32.3)	186 (30.2)	
Young child present (< 2yrs)		135 (10.7)	61 (9.5)	74 (12.0)	0.18
Kindergarten-age child present (2 - 5yrs)		491 (39.0)	244 (37.9)	247 (40.0)	0.48
School-age child present (6 - 17yrs)		638 (50.6)	325 (50.5)	313 (50.7)	0.99
Obstetrics characteristics					
FGP consulted	A	296 (23.5)	159 (24.7)	137 (22.2)	0.51
	B	309 (24.5)	157 (24.4)	152 (24.6)	
	C	404 (32.1)	195 (30.3)	209 (33.9)	
	D	251 (19.9)	132 (20.6)	119 (19.3)	
Gestational age at enrolment	Median ± sd	16.1 ± 9.6	19.4 ± 10.0	13.0 ± 8.4	<0.001*
	Range	1.7 - 41.0	1.7 - 41	2.6 - 38.7	
Has prior pregnancy		879 (69.8)	435 (67.7)	444 (72.0)	0.11
Has any co-morbidity ^^		440 (34.9)	323 (50.2)	117 (19.0)	<0.001*
Classified as high risk pregnancy		601 (47.7)	264 (41.1)	337 (54.6)	<0.001*

[#] Includes employment in power stations, coal mining, agriculture, offices, schools and healthcare
^ Missing value for one participant
^^ Missing value for two participants
* *p*-value of < 0.05 is considered to be significant

Table 2. Incidence rates (IRs) of (a) ILI and sARI, and (b) influenza A, influenza B and RSV detected for pregnant women, stratified by trimester stage and for the overall cohort.

(a)

Trimester stage	ILI		sARI	
	No. of cases	IR per 1,000 person-days (95% CI)	No. of cases	IR per 1,000 person-days (95% CI)
1st	35	29.0 (25.9 – 32.5)	1	0.9 (0.2 - 5.2)
2nd	83	11.7 (10.9 – 12.6)	1	0.1 (0.03 - 0.8)
3rd	56	8.70 (7.96 – 9.51)	0	0 (0.0 - 0.4)
Overall	174	11.8 (11.2 - 12.4)	2	0.1 (0.04 - 0.4)

(b)

Trimester stage	Influenza A		Influenza B		RSV	
	No. of cases	IR per 1,000 person-days (95% CI)	No. of cases	IR per 1,000 person-days (95% CI)	No. of cases	IR per 1,000 person-days (95% CI)
1st	6	4.4 (3.3 – 5.9)	1	0.9 (0.5 – 1.6)	1	0.8 (0.4 – 1.5)
2nd	15	2.1 (1.7 – 2.5)	0	0	1	0.1 (0.1 – 0.3)
3rd	5	0.7 (0.5 – 1.0)	1	0.1 (0.1 - 0.3)	2	0.3 (0.2 - 0.5)
Overall	26	1.7 (1.5 – 1.9)	2	0.1 (0.1 - 0.2)	4	0.3 (0.2 - 0.4)

Table 3. Risk factor analysis results when comparing (a) ILI cases and non-ILI, and (b) influenza A positive cases and non-ILI among pregnant women. Covariates were chosen based on initial univariate analyses (Table 7). Estimates in bold indicate statistically significant covariates.

(a)

Pregnant women and ILI episodes		Hazard ratio (95% C.I)	
		Un-adjusted	**Adjusted**
Trimester of symptom onset	1st	1 (ref)	1 (ref)
	2nd	**0.52 (0.35 - 0.78)**	**0.50 (0.34 - 0.75)**
	3rd	**0.38 (0.25 - 0.56)**	**0.37 (0.25 - 0.55)**
Highest education attained	College or higher	1 (ref)	1 (ref)
	High school or lower	0.69 (0.45 - 1.04)	0.92 (0.54 - 1.56)
Employment status	Employed [#]	1 (ref)	1 (ref)
	Unemployed	0.86 (0.59 - 1.26)	1.00 (0.67 - 1.50)
	Student	0.54 (0.27 - 1.08)	0.75 (0.31 - 1.81)
FGP	A	1 (ref)	1 (ref)
	B	1.46 (0.94 - 2.26)	1.44 (0.91 - 2.28)
	C	1.34 (0.87 - 2.06)	1.30 (0.85 – 1.99)
	D	1.06 (0.65 - 1.74)	1.13 (0.69 - 1.83)
Age at enrolment		1.02 (1.00 – 1.05)	1.00 (0.97 – 1.03)
Has any co-morbidity ^^		**1.41 (1.04 – 1.91)**	**1.43 (1.06 – 1.94)**
Classified as high risk pregnancy		1.28 (0.94 – 1.73)	1.15 (0.76 – 1.80)
Has prior pregnancy		1.32 (0.92 - 1.89)	1.17 (0.76 - 1.80)
Has kindergarten-age child (2 - 5yrs) in household		1.19 (0.88 - 1.60)	1.11 (0.81 - 1.51)

[#] Includes employment in power stations, coal mining, agriculture, offices, schools and healthcare
^^ Missing value for two participants

(b)

Pregnant women and influenza A cases		Hazard ratio (95% C.I)	
		Un-adjusted	**Adjusted**
Trimester of symptom onset	1st	1 (ref)	1 (ref)
	2nd	0.62 (0.24 - 1.59)	0.63 (0.24 - 1.62)
	3rd	**0.22 (0.07 - 0.72)**	**0.22 (0.07 - 0.73)**
Age at enrolment		1.02 (0.96 - 1.09)	0.98 (0.90 - 1.07)
Has prior pregnancy		3.09 (0.93 - 10.3)	3.45 (0.94 - 12.70)

898 **Table 4.** Baseline characteristics of the infants under 6 months cohort and its comparison

899 between two seasons.

Population characteristics		Total cohort (%)	2013/14 season (%)	2014/15 season (%)	p-value
No. of infants < 6 months enrolled		1304	692 (53.0)	612 (47.0)	
Male gender		673 (51.6)	364 (52.6)	309 (50.5)	0.48
Age at enrolment (days)	Median ± sd	12.0 ± 50.9	13.0 ± 51.7	9.0 ± 49.9	0.06
	Range	0 - 167	0 - 167	0 - 167	
Newborn characteristics					
Birth defect present ^		46 (3.5)	31 (4.5)	15 (2.5)	0.051
Had low birthweight		38 (2.9)	25 (3.6)	13 (2.1)	0.14
Term of pregnancy	Preterm (< 37 wks)	9 (0.7)	6 (0.9)	3 (0.5)	<0.001*
	Early term (37-38 wks)	142 (10.9)	111 (16.0)	31 (5.1)	
	Full term (≥ 39 wks)	1153 (88.4)	575 (83.1)	578 (94.4)	
FGP consulted	A	318 (24.4)	177(25.6)	141 (23.1)	0.28
	B	360 (27.6)	199 (28.8)	161 (26.3)	
	C	371 (28.4)	192 (27.7)	179 (29.2)	
	D	255 (19.6)	124 (17.9)	131 (21.4)	

900 ^ Missing value for one participant

901 * *p*-value of < 0.05 is considered to be significant

Table 5. (a) Incidence rates (IRs) of ILI and sARI cases for infant under 6 months, stratified by age groups and for the overall cohort. (b) Positive case numbers of influenza A, influenza B and RSV in the study and for sARI cases only, stratified by age groups and for the overall cohort.

(a)

Age group (weeks)	ILI		sARI	
	No. of cases	IR per 1,000 person-days (95% CI)	No. of cases	IR per 1,000 person-days (95% CI)
0 - 7.9	46	9.10 (8.21 – 10.1)	80	21.3 (20.0 – 22.8)
8 - 15.9	69	10.9 (10.0 – 11.9)	72	15.7 (14.7 – 16.9)
16 - 24	131	26.8 (25.2 - 28.5)	103	25.8 (24.2 – 27.4)
Overall	246	15.2 (14.5 - 15.8)	255	20.5 (19.7 – 21.3)

(b)

Age group (weeks)	Total samples tested	Influenza A		Influenza B		RSV	
		Total cases (%)	sARI cases only (%)	Total cases (%)	sARI cases only (%)	Total cases (%)	sARI cases only (%)
0 - 7.9	54	2 (3.7)	0 (0.0)	1 (1.9)	0	3 (5.6)	3 (5.6)
8 - 15.9	79	2 (2.5)	1 (1.3)	0 (0.0)	0	8 (10.1)	7 (8.9)
16 - 24	136	13 (9.6)	1 (0.7)	2 (1.5)	0	14 (10.3)	7 (5.1)
Overall	269	17 (6.3)	2 (0.7)	3 (1.1)	0	25 (9.3)	17 (6.3)

911 **Table 6.** Risk factor analysis results when comparing sARI cases and non-sARI among infants
912 under 6 months. Covariates were chosen based on initial univariate analyses (Table 8).
913 Estimates in bold indicate statistically significant covariates.

Infant < 6 months and sARI cases		**Hazard ratio (95% C.I)**	
		Un-adjusted	**Adjusted**
Age group (weeks)	0 - 7.9	1.05 (0.78 - 1.42)	1.03 (0.76 - 1.39)
	8 - 15.9	0.76 (0.55 - 1.04)	0.75 (0.55 - 1.04)
	16 - 24	1 (ref)	1 (ref)
Gender	Female	1 (ref)	1 (ref)
	Male	**1.42 (1.09 - 1.85)**	**1.40 (1.07 - 1.83)**
Has birth defect^	No	1 (ref)	1 (ref)
	Yes	1.40 (0.86 - 2.28)	1.09 (0.57 - 2.11)
Gestational age at delivery	Full term	1 (ref)	1 (ref)
	Preterm	2.64 (1.28 - 5.44)	2.26 (0.83 - 6.16)
	Early term	1.10 (0.73 - 1.66)	1.10 (0.71 - 1.70)

914 ^ Missing value for one participant

915 **Table 7.** Characteristics of the ILI cases and non-ILI for the pregnant women cohort.

Population characteristics		ILI (%)	non-ILI (%)	p-value
No. of pregnant women enrolled	Total	174 (13.8)	1100 (87.3)	
	2013/14 season	110 (63.2)	542 (49.3)	<0.001*
	2014/15 season	64 (36.8)	558 (50.7)	
Age at enrolment (years)	Median ± sd	29 ± 6.0	27 ± 6.0	0.01**
	Range	17 - 44	16 - 44	
Educated at high-school level or lower		26 (14.9)	253 (23.0)	0.02**
Employment status	Employed [#]	127 (73.0)	707 (64.3)	0.04**
	Unemployed	39 (22.4)	292 (26.5)	
	Student	8 (4.6)	101 (9.2)	
BMI category ^	Underweight (< 18.5)	8 (4.6)	44 (4.0)	0.54
	Normal (18.5-24.9)	122 (70.5)	731 (66.5)	
	Overweight (25-29.9)	37 (21.4)	263 (23.9)	
	Obese (≥ 30)	6 (3.5)	62 (5.6)	
Household characteristics				
Type of household structure ^	Apartment	83 (47.7)	468 (42.6)	0.2
	Ger	75 (43.1)	481 (43.8)	
	Private house	16 (9.2)	150 (13.6)	
Household size	1 - 2	21 (12.1)	153 (13.9)	0.78
	3 - 4	99 (56.9)	603 (54.8)	
	5 and above	54 (31.0)	344 (31.3)	
Young child present (< 2yrs)		14 (8.0)	122 (11.1)	0.29
Kindergarten-age child present (2 - 5yrs)		78 (44.8)	417 (37.9)	0.09**
School-age child present (6 - 17yrs)		85 (48.9)	560 (50.9)	0.67
Obstetrics characteristics				
Trimester at enrolment	1st (0 - 13 weeks)	101 (58.0)	452 (41.1)	<0.001**
	2nd (14 - 26 weeks)	59 (33.9)	374 (34.0)	
	3rd (≥ 27 weeks)	14 (8.1)	274 (24.9)	
Gestational age at enrolment	Median ± sd	12.4 ± 7.7	16.6 ± 9.7	<0.001**
	Range	2.0 - 34.0	1.7 - 41.0	
FGP consulted	A	31 (17.8)	266 (24.2)	0.07**
	B	52 (29.9)	263 (23.9)	
	C	63 (36.2)	348 (31.6)	
	D	28 (16.1)	223 (20.3)	
Has prior pregnancy		134 (77.0)	757 (68.8)	0.04**
Has any co-morbidity ^^		77 (44.3)	371 (33.7)	0.006**
Classified as high risk pregnancy		98 (56.3)	511 (46.5)	0.02**

[#] Includes employment in power stations, coal mining, agriculture, offices, schools and healthcare

^ Missing value for one participant

^^ Missing value for two participants

* *p*-value of < 0.05 is considered to be significant

** Variable was included in Cox PH model

916

Table 8. Characteristics of the (a) ILI cases and non-ILI, and (b) sARI cases and non-sARI for the infants under 6 months cohort.

Population characteristics		ILI cases (%)	Non-ILI (%)	p-value	sARI cases (%)	Non-sARI (%)	p-value
No. of infants < 6 months enrolled	Total	246 (18.9)	1103 (84.6)		255 (19.6)	1088 (83.4)	
	2013/14 season	129 (52.4)	583 (52.9)	0.96	126 (49.4)	580 (53.3)	0.35
	2014/15 season	117 (47.6)	520 (47.1)		129 (50.6)	508 (46.7)	
Male gender		125 (50.8)	571 (51.8)	0.84	149 (58.4)	551 (50.6)	0.03**
Age at enrolment (days)	Median ± sd	21 ± 34.3	11 ± 53.1	<0.001**	8 ± 39.5	12 ± 52.6	0.01**
	Range	1 - 144	0 - 167		0 - 155	0 - 167	
Newborn characteristics							
Birth defect present ^		10 (4.0)	39 (3.5)	0.83	14 (5.5)	32 (2.9)	0.06**
Had low birthweight		6 (2.4)	33 (3.0)	0.83	11 (4.3)	27 (2.5)	0.17
Term of pregnancy	Preterm (< 37 weeks)	0 (0.0)	9 (0.8)		5 (2.0)	4 (0.4)	
	Early term (37-38 weeks)	25 (10.2)	120 (10.9)	0.49	10 (3.9)	41 (3.8)	0.03**
	Full term (≥ 39 weeks)	221 (89.8)	974 (88.3)		240 (94.1)	1043 (95.8)	
FGP consulted	A	50 (20.3)	278 (25.2)		63 (24.7)	268 (24.6)	
	B	88 (35.8)	291 (26.4)	0.002**	64 (25.1)	302 (27.8)	0.77
	C	76 (30.9)	308 (27.9)		73 (28.6)	309 (28.4)	
	D	32 (13.0)	226 (20.5)		55 (21.6)	209 (19.2)	
Household characteristics (For 2014/15 season only)							
Type of household structure	Apartment	30 (25.6)	240 (46.2)		77 (59.7)	206 (40.6)	
	Ger	71 (60.7)	178 (34.2)	<0.001**	38 (29.4)	198 (39.0)	<0.001**
	Private house	16 (13.7)	102 (19.6)		14 (10.9)	104 (20.5)	
Household size	1 - 2	3 (2.6)	18 (3.4)		7 (5.4)	15 (3.0)	
	3 - 4	55 (47.0)	225 (43.3)	0.78	62 (48.1)	221 (43.5)	0.20
	5 and more	59 (50.4)	277 (53.3)		60 (46.5)	272 (53.5)	
Young child present (< 2yrs)		12 (10.3)	43 (8.3)	0.61	18 (14.0)	36 (7.1)	0.02**
Kindergarten-age child present (2 - 5yrs)		58 (49.6)	213 (41.0)	0.11	58 (45.0)	206 (40.6)	0.42
School-age child present (6 - 17yrs)		47 (40.2)	246 (47.3)	0.19	51 (39.5)	242 (47.6)	0.12

** Variable was included in Cox PH model

^ Missing value for one participant

Table 9. Risk factor analysis results when comparing ILI and non-ILI cases for pregnant women, in the (a) 2013/14 and (b) 2014/15 seasons.

Pregnant women and ILI		(a) 2013/14 season		(b) 2014/15 season	
		Hazard ratio (95% C.I)		Hazard ratio (95% C.I)	
		Un-adjusted	Adjusted	Un-adjusted	Adjusted
Trimester at infection	1st	1 (ref)	1 (ref)	1 (ref)	1 (ref)
	2nd	**0.55 (0.32 - 0.94)**	**0.57 (0.33 - 0.99)**	**0.44 (0.25 - 0.79)**	**0.42 (0.23 - 0.77)**
	3rd	**0.39 (0.23 - 0.65)**	**0.44 (0.26 - 0.74)**	**0.32 (0.17 - 0.59)**	**0.33 (0.17 - 0.62)**
Highest education attained	College or higher	1 (ref)	1 (ref)	1 (ref)	1 (ref)
	High school or lower	0.56 (0.35 - 0.89)	0.70 (0.40 - 1.22)	0.60 (0.22 - 1.60)	1.28 (0.31 - 5.36)
Employment status	Employed	1 (ref)	1 (ref)	1 (ref)	1 (ref)
	Unemployed	1.00 (0.64 - 1.55)	1.20 (0.74 - 1.94)	0.43 (0.20 - 0.94)	0.49 (0.21 - 1.17)
	Student	0.67 (0.27 - 1.19)	1.10 (0.46 - 2.60)	0.25 (0.03 - 1.90)	0.19 (0.01 - 4.09)
FGP	A	1 (ref)	1 (ref)	1 (ref)	1 (ref)
	B	1.33 (0.74 - 2.39)	1.37 (0.74 - 2.53)	1.68 (0.86 - 3.27)	1.48 (0.76 - 2.89)
	C	1.86 (1.09 - 3.15)	1.78 (1.04 - 3.05)	0.86 (0.42 - 1.80)	0.81 (0.41 - 1.59)
	D	1.47 (0.81 - 2.68)	1.57 (0.86 - 2.86)	0.67 (0.28 - 1.57)	0.69 (0.29 - 1.62)
Age at enrolment		1.04 (1.01 - 1.07)	1.01 (0.97 - 1.06)	1.00 (0.96 - 1.04)	0.97 (0.91 - 1.03)
Has any co-morbidity		0.96 (0.66 - 1.39)	0.88 (0.62 - 1.27)	**1.98 (1.17 - 3.36)**	**2.05 (1.09 - 3.83)**
Classified as high risk pregnancy		1.46 (1.01 - 2.13)	1.27 (0.84 - 1.92)	1.24 (0.74 - 2.10)	1.08 (0.59 - 2.00)
Has prior pregnancy		1.62 (1.02 - 2.58)	1.24 (0.72 - 2.14)	1.01 (0.57 - 1.78)	1.09 (0.55 - 2.15)
Had kindergarten-age child (2-5 yrs) in household		1.34 (0.92 - 1.93)	1.27 (0.88 - 1.84)	1.00 (0.61 - 1.66)	0.91 (0.54 - 1.54)

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