

Burden of Influenza and Respiratory Syncytial Virus Infection in Pregnant Women and Infants under 6 months in Mongolia

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| 著者 | CHAW LI LING |
| 学位授与機関 | Tohoku University |
| 学位授与番号 | 11301甲第16850号 |
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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)

東北大学大学院医学系研究科医科学専攻

(病理病態学講座微生物学分野)

Chaw Liling

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1 2. SUMMARY

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

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

8 Design: Prospective and observational open cohort study.

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

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

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

19 Results: A total of 1260 unvaccinated pregnant women were enrolled, whereby 174 (13.8%)
20 ILI and 2 (0.2%) sARI cases were detected. From all tested ILI (94.8%) and sARI (100%) cases,
21 26 ILI and 1 sARI case tested positive for influenza A. The overall incidence rates for ILI, sARI

22 and influenza A were 11.8 [95% confidence interval (C.I): 11.2 – 12.4], 0.1 [95%C.I: 0.0 – 0.4],
23 and 1.7 [95% C.I: 1.5 – 1.9] per 1,000 person-days, respectively. Incidence rates and adjusted
24 hazard ratios for ILI and influenza A were lowest in the third trimester. Pregnant women
25 with co-morbidity were 1.4 times more likely to develop an ILI episode [Adj.HR: 1.4 (95%C.I:
26 1.1–1.9)].

27 A total of 1304 infants under 6 months were enrolled, whereby 246 (18.9%) ILI and 255
28 (19.6%) sARI cases were detected. The overall ILI and sARI incidence rates were 15.2 [95%C.I:
29 14.5 – 15.8] and 20.5 [95%C.I: 19.7 – 21.3] per 1,000 person-days, respectively. Among the
30 tested ILI (77.6%) and sARI (30.6%) cases, the overall positivity rates for influenza A,
31 influenza B and RSV were 6.3%, 1.1%, and 9.3%, respectively. Positivity rates of influenza A
32 and RSV tend to increase with age. sARI cases were 1.4 times more likely to be male [Adj.HR:
33 1.4 (95%C.I: 1.1–1.8)]. From all influenza A and RSV positive infant cases, 11.8% and 68.0%
34 were respectively identified among sARI hospitalized cases.

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

39 3. BACKGROUND

40 3.1. BASIC INTRODUCTION TO INFLUENZA VIRUS

41 Influenza viruses are enveloped, single-stranded, negative sense, segmented, ribonucleic
42 acid (RNA) viruses belonging to the *Orthomyxoviridae* family [1]. They are classified into
43 three genera or types (A, B, and C). Genus Influenza virus A has one species, influenza A. It
44 includes all avian and equine influenza viruses, and most influenza viruses of swine. This
45 virus can be classified into subtypes, based on the surface glycoproteins: haemagglutinin
46 (HA), and neuraminidase (NA). There are 16 HA (H1-16) and 9 NA (N1-9) recognized subtypes.
47 Genus Influenza virus B has one species, influenza B, which is less common than influenza A
48 and infects mainly humans. It can be divided into two antigenically and genetically distinct
49 lineages: B/Yamagata and B/Victoria. Influenza B mainly causes disease for school-aged
50 children [2]. Lastly, genus Influenza virus C has one species, influenza C, which is the least
51 common than the other types. It usually causes mild disease in children, and it can also
52 infect dogs and pigs. Out of the three types, Influenza A and B viruses are known to cause
53 influenza epidemics in humans, leading to significant morbidity and mortality. Giving the
54 rich source of host reservoirs mainly from birds and swine, influenza A viruses are able to
55 cause epidemics and pandemics through re-assortment and mutation processes. The five
56 known influenza pandemics occurred in 1918 A(H1N1) Spanish flu, 1957 A(H2N2) Asian flu,
57 1968 A(H3N2) Hong Kong flu, 1977 A(H1N1) Russian flu and 2009 A(H1N1) Swine flu.
58 Influenza B, on the other hand, has lesser chance of causing pandemics.

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

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

64 3.2. BASIC INTRODUCTION TO RESPIRATORY SYNCYTIAL VIRUS (RSV)

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

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

79 3.3. INFLUENZA AND RSV BURDEN FOR PREGNANT WOMEN

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

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

90 There were also reports on increased risk of influenza-associated hospitalization during the
91 inter-pandemic period, especially among pregnant women with co-morbidity [10] and
92 asthma [11, 12]. Women in the third trimester also have increased risk of influenza-
93 associated hospitalization, particularly if they have co-morbidities [13, 14]. When compared
94 to post-partum women, women in their third trimester of pregnancy were 3 – 4 times more
95 likely to be hospitalized for an acute cardiopulmonary illness during the influenza season
96 [14]. On the other hand, studies using databases from Kaiser Permanente Health Plan in
97 Oregon [15] and Northern California [16] reported low hospitalization rates for pregnant
98 women during the 1975-79 and 1997-2002 seasons, respectively. During the inter-pandemic
99 period, there is also one study that investigated the occurrence of influenza-like illness (ILI)
100 episodes at each pregnancy stage [17]. They found increasing risk of developing ILI with
101 increasing trimester stage, with highest risk during the post-partum period.

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

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

114 3.4. INFLUENZA AND RSV BURDEN FOR INFANTS UNDER 6 MONTHS

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

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

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

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

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

138 3.5. PHYSIOLOGICAL AND IMMUNOLOGICAL CHANGES DURING PREGNANCY

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

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

154 CD4+ and CD8+) decrease from the second half of the pregnancy period [42]. Reduced levels
155 of highly inflammatory Th1 cytokine release also occur during the later stages of pregnancy
156 [43]. This dampening of the pro-inflammatory response, particularly NK cells, results in viral
157 clearance delay of influenza infections, which leads to an increased risk of severe illness [43].

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

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

174 3.6. IMMUNE RESPONSES OF INFANTS UNDER 6 MONTHS

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

177 vaccinating infants under 6 months with the influenza vaccine is not allowed because their
178 adaptive immune system is not mature enough to develop adequate immune response.
179 Hence, one option to protect them against influenza is to acquire the influenza antibodies
180 from the mother *in utero*. Infants who had acquired maternal antibodies against influenza
181 [49, 50] and RSV [51] were reported to be protected from severe illness and had delayed
182 symptom onset.

183 3.7. MATERNAL VACCINATION FOR INFLUENZA AND RSV

184 Maternal influenza vaccination is one of the safe and cost-effective ways of protecting both
185 pregnant women as well as infants under 6 months [52, 53]. There is evidence that maternal
186 influenza vaccination can induce a strong immune response to protect both mother and the
187 unborn fetus. It was found to reduce influenza illness for both pregnant women [54-56] and
188 infants under 6 months [25, 56, 57], and also reduce hospitalizations for infants under 6
189 months [57-59]. In a randomized study in Bangladesh [56], maternal influenza vaccination
190 reduced 63% of laboratory-confirmed influenza illness in infants under 6 months. It also
191 reduced 29% and 36% of febrile respiratory illness in infants under 6 months and pregnant
192 women, respectively. Another study found an association between maternal influenza
193 vaccine use and reduced likelihood of premature and small for gestational age births [60].
194 No serious adverse effects have been reported for influenza vaccination during pregnancy
195 [61], though there were limited information available on the safety of vaccination during the
196 first trimester [62]. Also, vaccine effectiveness is similar between pregnant women and other
197 adults [54]. The WHO Strategic Advisory Group of Experts (SAGE) on immunization
198 recommended that pregnant women should be vaccinated against influenza at any
199 pregnancy stage [3].

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

211 Currently, the only available preventive measure against RSV infection among infants under
212 6 months is the monoclonal antibody, palivizumab [65]. However, it is expensive to
213 administer, has to be administered monthly throughout the RSV season, and does not
214 always prevent serious RSV disease [65, 68].

215 3.8. RESEARCH GAPS AND STUDY SIGNIFICANCE

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

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

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

235 In addition, the study results would also benefit Mongolia. There are a few reasons why
236 Mongolia is chosen as the study site. First, maternal and child health is currently one of the
237 important health issues in Mongolia due to its high birth rate and population growth.
238 Between 2005 and 2014, the number of live births increased at an average rate of 6.4% [69,
239 70]. In 2014, the estimated annual birth rate is 20.9 per 1,000 persons [71]. Second, it was
240 previously shown that children < 5 years old in Mongolia have the highest incidence of
241 influenza-associated illness [72]. Third, the burden among pregnant women has never been
242 assessed in the country, even though 24.1% of all influenza A(H1N1)pdm09-confirmed
243 deaths in Mongolia were pregnant [73]. During the study period, the country's national
244 influenza vaccination policy did not include pregnant women as one of the recommended
245 vaccine recipients [74]. Therefore, the study findings can thus help inform on any disease

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

248 4. STUDY OBJECTIVES

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

252 5. MATERIALS AND METHODS

253 5.1. STUDY SITE

254 Baganuur is a semi-urban district located at about 130 km away from the central part of
255 Ulaanbaatar, Mongolia's capital city (Fig. 1). In 2013, it had an estimated population of
256 27,440. Medical services are provided mainly by one district hospital and four primary
257 healthcare centers called Family General Practitioners (FGPs). The study was conducted in
258 this district for two consecutive influenza seasons (October 1st 2013 - April 30th 2014 and
259 October 1st 2014 - April 30th 2015).

260 5.2. DATA COLLECTION

261 In Mongolia, all pregnant women and children < 2 years old are required to register and
262 undergo medical check-ups at their FGPs, which are assigned based on their official
263 residential address. All four FGPs in the district were used as the study entry point. All
264 residents who registered at the FGPs and met the inclusion criteria (being pregnant or aged
265 < 24 weeks during the study period) were enrolled. To include those who had already
266 registered and those newly registered during the study period, enrolment was carried out
267 continuously throughout the whole study period. Follow-up ends if any of the following
268 occurred: a woman was no longer pregnant, a child reached 24 weeks of age, or any
269 participant moved away from Baganuur district.

270 At the time of enrolment, a baseline questionnaire was administered to all participants to
271 collect their demographic information. Prenatal information and household member
272 demographics were also collected from pregnant women for both seasons. For infants under
273 6 months, information on birthweight, gestational age at delivery and presence of birth

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

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

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

293 The primary outcome in this study was the occurrence of a case positive for either influenza
294 A, influenza B or RSV. The secondary outcome was the occurrence of an ILI or a sARI case. An
295 ILI case was defined as the sudden onset of fever of $\geq 38^{\circ}\text{C}$, 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 (QuickNaviTM – 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

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

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

324 5.3. ETHICAL APPROVAL

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

331 5.4. STATISTICAL ANALYSIS

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

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

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

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

359 **6. RESULTS**

360 **6.1. DISTRIBUTION OF ILI AND SARI CASES, AND CIRCULATING VIRUSES IN BOTH POPULATIONS**

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

366 Figure 4 shows the weekly number of virus positives detected in the two populations. In
367 general, positive cases were detected mainly from January to February, coinciding with the
368 high activities of both ILI and sARI. All three targeted viruses were detected during the
369 2013/14 season, while influenza B virus was not detected during the 2014/15 season. This
370 was quite consistent with data from the national ILI surveillance, except that RSV was not
371 detected during the 2013/14 season (Fig. 2). There were periods of limited point-of-care test
372 kit availability at the beginning of both seasons and early December in the 2014/15 season.
373 Hence, virus testing was compromised during those periods.

374 **6.2. INCIDENCE RATES OF ILI, SARI AND VIRUS POSITIVES FOR PREGNANT WOMEN**

375 We individually enrolled 1,260 pregnant women and they were followed for a total of
376 120,887 person-days. The population characteristics were quite different for the two
377 seasons (Table 1). Briefly, the 2014/15 season cohort tend to be more educated, employed,
378 overweight, enrolled at an earlier gestational age, and did not have co-morbidities. The
379 median age at enrolment was 27 years (range: 16 – 44) and 43.0% were enrolled during the

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

382 A total of 174 ILI episodes (13.8%) were detected from 160 pregnant women. There were 12
383 and 2 women with 2 and 3 ILI episodes respectively, during the same influenza season.
384 Among the ILI cases with recorded symptom resolution dates (85.6%), the mean interval to
385 resolution was 8.1 days (range: 3 - 20). The overall IR for ILI was 11.8 per 1,000 person-days
386 (95% C.I: 11.2 – 12.4) (Table 2a).

387 Among all tested ILI cases (n = 165), 26 (15.8%) tested positive for influenza A, 2 (1.2%) for
388 influenza B, and 4 (2.4%) for RSV. This gives an overall IR of 1.7 per 1,000 person-days (95%
389 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,
390 and 0.3 per 1,000 person-days (95% C.I: 0.2 – 0.4) for RSV (Table 2b). The majority of
391 samples (96.0%) were tested within 5 days after symptom onset. During the 2014/15 season,
392 2 women tested positive for both influenza A and RSV from separate ILI episodes.

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

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

403 adjusting for age and presence of prior pregnancy [Adjusted hazard ratio (Adj. HR): 0.22
404 (95% C.I: 0.07 – 0.73)]. Pregnant women were also 50% [Adj. HR: 0.50 (95% C.I: 0.34 – 0.75)]
405 and 63% [Adj. HR: 0.37 (95% C.I: 0.25 – 0.55)] less likely to develop an ILI episode in the
406 second and third trimesters, respectively, when compared to the first trimester. In addition,
407 pregnant women who have any co-morbidity were 43% more likely to develop an ILI episode
408 [Adj. HR: 1.43 (95% C.I: 1.06 – 1.94)].

409 6.3. ILI AND SARI INCIDENCE, AND VIRUS POSITIVITY RATES FOR INFANTS UNDER 6 MONTHS

410 We individually enrolled 1,304 infants under 6 months and they were followed for a total of
411 122,344 person-days. They were mainly healthy, whereby 88.4% were born full term, 2.9%
412 had low birthweight, and 3.5% were born with a certain birth defect. Their baseline
413 characteristics were similar for both seasons, except that there were significantly more full
414 term infants in the 2014/15 season (Table 4). The median age at enrolment was 12 days
415 (range: 0 – 167). During the 2013/14 season, 99.9% were breastfed for 6 months. Almost all
416 identified infants participated in this study (99.0%) and none was lost to follow-up.

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

426 A total of 255 sARI hospitalized cases (19.6%) were detected from 218 infants under 6
427 months, giving an overall sARI IR of 20.5 per 1,000 person-days (95% C.I: 19.7 – 21.3) (Table
428 5a). There were 30 and 8 infants who were hospitalized for sARI twice and thrice,
429 respectively, during the same influenza season. Also, 36 (14.1%) of them had prior visit to
430 the FGP for the same illness episode. The mean length of hospital stay was 8.9 days (range: 0
431 – 33). For all sARI cases, 74.3% were admitted within 5 days after symptom onset. A total of
432 23 (18.3%) and 55 (42.6%) cases were tested during the 2013/14 and 2014/15 seasons,
433 respectively. This gives an overall sARI testing rate of 30.6%. During the 2013/14 season, 4
434 (17.4%) tested positive for RSV and 2 (8.7%) for influenza A while 13 (23.6%) RSV and no
435 influenza A cases were detected during the 2014/15 season. No influenza B positive sARI
436 cases were detected from either season. No virus co-detection and deaths were also
437 observed during the study period.

438 Overall, a total of 17 (6.3%) influenza A, 3 (1.1%) influenza B, and 25 (9.3%) RSV cases were
439 detected among the tested ILI and sARI samples (n = 269) (Table 5b). Among the total
440 positive cases detected, 2 (11.8%) influenza A and 17 (68.0%) RSV cases were detected
441 among hospitalized cases with sARI. After stratification by age group, 16 – 24 weeks old
442 infants had the highest ILI IR [26.8 per 1,000 person-days (95% C.I: 25.2 – 28.5)] and sARI IR
443 [25.8 per 1,000 person-days (95% C.I: 24.2 – 27.4)] (Table 5a). The same trend was also
444 observed in the positivity rates of influenza A and RSV (Table 5b). The increase in the RSV
445 positivity rate started from the 8 – 15.9 weeks old group. Also, sARI cases were 1.4 times
446 more likely to be male, when compared to non-sARI [Adj. HR: 1.40 (95% C.I: 1.07 – 1.83)]
447 (Table 6).

448 7. DISCUSSION

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

461 One study in the United States [13] reported both ILI and sARI IR for pregnant women in all
462 three trimesters and they observed high IRs, especially for sARI. Both ILI and sARI IRs in the
463 study are also higher than that previously reported for the reproductive-age population (15
464 – 49 years) in Baganuur [82] and Mongolia [72], though it could possibly be due to active
465 case search in the study. Expanding this study to include non-pregnant women of
466 reproductive-age would allow us to see any impact of pregnancy in acquiring ILI and sARI.
467 These Mongolian studies also reported incidence rates for young children, though
468 aggregates of 0 – 11 months were used. Here, ILI IR in this study is higher than that reported
469 in Baganuur [82]. Relatively high infant ILI IRs were also previously reported from Bangladesh
470 [56] and the United States [25]. Nevertheless, caution should be taken for interpreting these

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

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

484 Both IR and adjusted HR of influenza A were lowest for pregnant women in the third
485 trimester, which could be due to differences in virus exposure at each trimester stage.
486 Studies in the United States have shown that women in the third trimester tend to engage in
487 lesser leisure activities [83] and spend more time at home [84]. Although it is uncertain
488 whether both tendencies are also observed among Mongolians, it can lead to less
489 interaction with others in the community, thus decreasing the probability of acquiring
490 respiratory infections. Notably, I also observed the same finding in the risk factor analysis for
491 ILI, after accounting for the presence of co-morbidity as well as possible transmission in the
492 household (presence of kindergarten-age child) and workplace (employment status). This
493 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.

540 Also, protection from maternal antibodies tends to wane after the first 2 - 3 months of life.
541 Previous studies reported the half-life of passively-acquired maternal antibodies to be 21 –
542 53 days against influenza [49, 50, 87, 88] and 26 – 78 days against RSV [89-91].

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

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

561 Infant sARI cases were 1.4 times more likely to be males, a trend that is consistent with
562 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

587 possibly affect the risk factor analysis results. Fifth, I did not detect enough virus-positive
588 cases from both seasons to assess their risk factors. This could merely reflect the low
589 influenza and RSV activities in this community and thus caution must be taken to extrapolate
590 these findings to other areas in Mongolia. Sixth, dates of follow-up and hospitalization were
591 used to determine the symptomatic period for ILI and sARI IR calculations, respectively.
592 These dates do not strictly reflect on each clinical course and may affect the calculated IRs.
593 Lastly, household demographic information was collected among infants only for the
594 2014/15 season. As a result, these data were excluded from the risk factor analyses for both
595 seasons.

596 8. CONCLUSION

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

611 9. REFERENCES

- 612 1. Kawaoka Y, editor. *Influenza Virology: Current Topics*: Caister Academic Press; 2006.
- 613 2. Frank AL, Taber LH, Glezen WP, et al. Influenza B virus infections in the community
614 and the family: The epidemics of 1976–1977 and 1979–1980 in Houston, Texas. *Am J*
615 *Epidemiol.* 1983;118(3):313-25.
- 616 3. WHO position paper on influenza vaccines - November 2012. *Wkly Epidemiol Rec.*
617 2012;87:461-76.
- 618 4. Borchers A, Chang C, Gershwin M, et al. Respiratory Syncytial Virus-A Comprehensive
619 Review. *Clin Rev Allergy Immunol.* 2013;45(3):331-79.
- 620 5. Groothuis J, Hoopes JM, Jessie VH. Prevention of serious respiratory syncytial virus-
621 related illness. I: Disease pathogenesis and early attempts at prevention. *Adv Ther.*
622 2011;28(2):91-109.
- 623 6. Harris JW. Influenza occurring in pregnant women: A statistical study of thirteen
624 hundred and fifty cases. *JAMA.* 1919;72(14):978-80.
- 625 7. Freeman DW. Deaths from Asian influenza associated with pregnancy. *Am J Obstet*
626 *Gynecol.* 1959;78(6):1172-5.
- 627 8. Van Kerkhove MD, Vandemaële KAH, Shinde V, et al. Risk Factors for Severe
628 Outcomes following 2009 Influenza A (H1N1) Infection: A Global Pooled Analysis. *PLoS Med.*
629 2011;8(7):e1001053.
- 630 9. Mosby LG, Rasmussen SA, Jamieson DJ. 2009 Pandemic Influenza A (H1N1) in
631 Pregnancy: A systematic review of the literature. *Am J Obstet Gynecol.* 2011;205(1):10-8.
- 632 10. Cox S, Posner S, McPheeters M, et al. Hospitalizations with respiratory illness among
633 pregnant women during influenza season. *Obstet Gynecol.* 2006;107(6):1315-22.
- 634 11. Schanzer DL, Langley JM, Tam TW. Influenza-Attributed Hospitalization Rates Among
635 Pregnant Women in Canada 1994–2000. *J Obstet Gynaecol Can.* 2007;29(8):622-9
- 636 12. Hartert TV, Neuzil KM, Shintani AK, et al. Maternal morbidity and perinatal outcomes
637 among pregnant women with respiratory hospitalizations during influenza season. *Am J*
638 *Obstet Gynecol.* 2003;189(6):1705-12.
- 639 13. Dodds L, McNeil SA, Fell DB, et al. Impact of influenza exposure on rates of hospital
640 admissions and physician visits because of respiratory illness among pregnant women. *Can*
641 *Med Assoc J.* 2007;176(4):463-8.
- 642 14. Neuzil KM, Reed GW, Mitchel EF, et al. Impact of Influenza on Acute
643 Cardiopulmonary Hospitalizations in Pregnant Women. *Am J Epidemiol.* 1998;148(11):1094-
644 102.
- 645 15. Mullooly JP, Barker WH, Nolan TF. Risk of acute respiratory-disease among pregnant-
646 women during influenza-A epidemics. *Public Health Rep.* 1986;101(2):205-11.
- 647 16. Black SB, Shinefield HR, France EK, et al. Effectiveness of Influenza Vaccine during
648 Pregnancy in Preventing Hospitalizations and Outpatient Visits for Respiratory Illness in
649 Pregnant Women and Their Infants. *Amer J Perinatol.* 2004;21(06):333-9.
- 650 17. Lindsay L, Jackson LA, Savitz DA, et al. Community Influenza Activity and Risk of Acute
651 Influenza-like Illness Episodes among Healthy Unvaccinated Pregnant and Postpartum
652 Women. *Am J Epidemiol.* 2006;163(9):838-48.
- 653 18. Martin A, Cox S, Jamieson D, et al. Respiratory Illness Hospitalizations Among
654 Pregnant Women During Influenza Season, 1998–2008. *Matern Child Health J.*
655 2013;17(7):1325-31.

- 656 19. McNeil SA, Dodds LA, Fell DB, et al. Effect of respiratory hospitalization during
657 pregnancy on infant outcomes. *Am J Obstet Gynecol.* 2011;204(6, Supplement):S54-S7.
- 658 20. Hall CB, Long CE, Schnabel KC. Respiratory Syncytial Virus Infections in Previously
659 Healthy Working Adults. *Clin Infect Dis.* 2001;33(6):792-6.
- 660 21. Nair H, Brooks WA, Katz M, et al. Global burden of respiratory infections due to
661 seasonal influenza in young children: a systematic review and meta-analysis. *The Lancet.*
662 2011;378(9807):1917-30.
- 663 22. Nair H, Nokes DJ, Gessner BD, et al. Global burden of acute lower respiratory
664 infections due to respiratory syncytial virus in young children: a systematic review and meta-
665 analysis. *The Lancet.* 2010;375(9725):1545-55.
- 666 23. Nair H, Simões EAF, Rudan I, et al. Global and regional burden of hospital admissions
667 for severe acute lower respiratory infections in young children in 2010: a systematic analysis.
668 *The Lancet.* 2013;381(9875):1380-90.
- 669 24. Henkle E, Steinhoff M, Omer S, et al. Incidence of influenza virus infection in early
670 infancy: a prospective study in South Asia. *Pediatr Infect Dis J.* 2011;30(2):170-3.
- 671 25. Eick AA, Uyeki TM, Klimov A, et al. Maternal influenza vaccination and effect on
672 influenza virus infection in young infants. *Arch Pediatr Adolesc Med.* 2011;165(2):104-11.
- 673 26. Nokes DJ, Okiro EA, Ngama M, et al. Respiratory Syncytial Virus Infection and Disease
674 in Infants and Young Children Observed from Birth in Kilifi District, Kenya. *Clin Infect Dis.*
675 2008;46(1):50-7.
- 676 27. Poehling KA, Edwards KM, Weinberg GA, et al. The Underrecognized Burden of
677 Influenza in Young Children. *N Engl J Med.* 2006;355(1):31-40.
- 678 28. Hall CB, Weinberg GA, Iwane MK, et al. The Burden of Respiratory Syncytial Virus
679 Infection in Young Children. *N Engl J Med.* 2009;360(6):588-98.
- 680 29. Izurieta H, Thompson W, Kramarz P, et al. Influenza and the rates of hospitalization
681 for respiratory disease among infants and young children. *N Engl J Med.* 2000;342(4):232-9.
- 682 30. Nolan T, Borja-Tabora C, Lopez P, et al. Prevalence and Incidence of Respiratory
683 Syncytial Virus and Other Respiratory Viral Infections in Children Aged 6 Months to 10 Years
684 With Influenza-like Illness Enrolled in a Randomized Trial. *Clin Infect Dis.* 2015;60(11):e80-e9.
- 685 31. Iwane MK, Edwards KM, Szilagyi PG, et al. Population-Based Surveillance for
686 Hospitalizations Associated With Respiratory Syncytial Virus, Influenza Virus, and
687 Parainfluenza Viruses Among Young Children. *Pediatrics.* 2004;113(6):1758-64.
- 688 32. Hasan R, Rhodes J, Thamthitiwat S, et al. Incidence and Etiology of Acute Lower
689 Respiratory Tract Infections in Hospitalized Children Younger Than 5 Years in Rural Thailand.
690 *Pediatr Infect Dis J.* 2014;33(2):E45-E52.
- 691 33. Simoes EAF. Environmental and demographic risk factors for respiratory syncytial
692 virus lower respiratory tract disease. *J Pediatr.* 2003;143(5, Supplement):118-26.
- 693 34. Jones M, Castile R, Davis S, et al. Forced Expiratory Flows and Volumes in Infants. *Am*
694 *J Respir Crit Care Med.* 2000;161(2):353-9.
- 695 35. Hoo AF, Dezateux C, Hanrahan J, et al. Sex-Specific Prediction Equations for V_{max}FRC
696 in Infancy. *Am J Respir Crit Care Med.* 2002;165(8):1084-92.
- 697 36. Klein SL. The effects of hormones on sex differences in infection: from genes to
698 behavior. *Neurosci Biobehav Rev.* 2000;24(6):627-38.
- 699 37. Friedrich L, Stein RT, Pitrez PMC, et al. Reduced Lung Function in Healthy Preterm
700 Infants in the First Months of Life. *Am J Respir Crit Care Med.* 2006;173(4):442-7.
- 701 38. Kourtis AP, Read JS, Jamieson DJ. Pregnancy and Infection. *N Engl J Med.*
702 2014;370(23):2211-8.

- 703 39. Gabriel G, Arck PC. Sex, Immunity and Influenza. *J Infect Dis.* 2014;209(suppl 3):S93-9.
- 704 40. Sheffield J, Cunningham F. Community-Acquired Pneumonia in Pregnancy. *Obstet*
705 *Gynecol.* 2009;114(4):915-22.
- 706 41. Mor G, Cardenas I. The Immune System in Pregnancy: A Unique Complexity. *Am J*
707 *Reprod Immunol.* 2010;63(6):425-33.
- 708 42. Kraus T, Engel S, Sperling R, et al. Characterizing the Pregnancy Immune Phenotype:
709 Results of the Viral Immunity and Pregnancy (VIP) Study. *J Clin Immunol.* 2012;32(2):300-11.
- 710 43. Pazos M, Sperling R, Moran T, et al. The influence of pregnancy on systemic immunity.
711 *Immunol Res.* 2012;54(1-3):254-61.
- 712 44. Jost S, Quillay H, Reardon J, et al. Changes in Cytokine Levels and NK Cell Activation
713 Associated with Influenza. *PLoS One.* 2011;6(9):e25060.
- 714 45. Robinson DP, Klein SL. Pregnancy and pregnancy-associated hormones alter immune
715 responses and disease pathogenesis. *Horm Behav.* 2012;62(3):263-71.
- 716 46. Straub RH. The Complex Role of Estrogens in Inflammation. *Endocr Rev.*
717 2007;28(5):521-74.
- 718 47. Haggerty CL, Ness RB, Kelsey S, et al. The impact of estrogen and progesterone on
719 asthma. 2003;90(3):284-91.
- 720 48. PrabhuDas M, Adkins B, Gans H, et al. Challenges in infant immunity: implications for
721 responses to infection and vaccines. 2011;12(3):189-94.
- 722 49. Reuman PD, Ayoub EM, Small PA. Effect of passive maternal antibody on influenza
723 illness in children: a prospective study of influenza A in mother-infant pairs. *Pediatr Infect Dis*
724 *J.* 1987;6(4):398-403.
- 725 50. Puck J, Glezen W, Frank A, et al. Protection of Infants from Infection with Influenza A
726 virus by Transplacentally Acquired Antibody. *J Infect Dis.* 1980;142(6):844-9.
- 727 51. Eick A, Karron R, Shaw J, et al. The role of neutralizing antibodies in protection of
728 American Indian infants against respiratory syncytial virus disease. *Pediatr Infect Dis J.*
729 2008;27(3):207-12.
- 730 52. Rasmussen SA, Jamieson DJ, Uyeki TM. Effects of influenza on pregnant women and
731 infants. *Am J Obstet Gynecol.* 2012;207(3, Supplement):S3-S8.
- 732 53. Steinhoff MC, MacDonald N, Pfeifer D, et al. Influenza vaccine in pregnancy: policy
733 and research strategies. *The Lancet.* 2014;383(9929):1611-3.
- 734 54. Thompson MG, Li D-K, Shifflett P, et al. Effectiveness of Seasonal Trivalent Influenza
735 Vaccine for Preventing Influenza Virus Illness Among Pregnant Women: A Population-Based
736 Case-Control Study During the 2010–2011 and 2011–2012 Influenza Seasons. *Clin Infect Dis.*
737 2014;58(4):449-57.
- 738 55. Madhi SA, Cutland CL, Kuwanda L, et al. Influenza Vaccination of Pregnant Women
739 and Protection of Their Infants. *N Engl J Med.* 2014;371(10):918-31.
- 740 56. Zaman K, Roy E, Arifeen SE, et al. Effectiveness of Maternal Influenza Immunization in
741 Mothers and Infants. *N Engl J Med.* 2008;359(15):1555-64.
- 742 57. Dabrera G, Zhao H, Andrews N, et al. Effectiveness of seasonal influenza vaccination
743 during pregnancy in preventing influenza infection in infants, England, 2013/14.
744 *Eurosurveillance.* 2014;19(45):2-5.
- 745 58. Benowitz I, Esposito DB, Gracey KD, et al. Influenza Vaccine Given to Pregnant
746 Women Reduces Hospitalization Due to Influenza in Their Infants. *Clin Infect Dis.*
747 2010;51(12):1355-61.
- 748 59. Poehling KA, Szilagyi PG, Staat MA, et al. Impact of maternal immunization on
749 influenza hospitalizations in infants. *Am J Obstet Gynecol.* 2011;204(6, Supplement):S141-S8.

- 750 60. Omer SB, Goodman D, Steinhoff MC, et al. Maternal Influenza Immunization and
751 Reduced Likelihood of Prematurity and Small for Gestational Age Births: A Retrospective
752 Cohort Study. *PLoS Med.* 2011;8(5):e1000441.
- 753 61. Munoz FM. Safety of influenza vaccines in pregnant women. *Am J Obstet Gynecol.*
754 2012;207(3, Supplement):S33-S7.
- 755 62. Mak TK, Mangtani P, Leese J, et al. Influenza vaccination in pregnancy: current
756 evidence and selected national policies. *The Lancet Infectious Diseases.* 2008;8(1):44-52.
- 757 63. Anderson LJ, Dormitzer PR, Nokes DJ, et al. Strategic priorities for respiratory
758 syncytial virus (RSV) vaccine development. *Vaccine.* 2013;31, Supplement 2 (Decade of
759 Vaccines):B209-B15.
- 760 64. Chu HY, Englund JA. Maternal Immunization. *Clin Infect Dis.* 2014;59(4):560-8.
- 761 65. Meng J, Stobart CC, Hotard AL, et al. An Overview of Respiratory Syncytial Virus. *PLoS*
762 *Pathog.* 2014;10(4):e1004016.
- 763 66. Munywoki PK, Koech DC, Agoti CN, et al. The Source of Respiratory Syncytial Virus
764 Infection In Infants: A Household Cohort Study In Rural Kenya. *J Infect Dis.*
765 2014;209(11):1685-92.
- 766 67. Poletti P, Merler S, Ajelli M, et al. Evaluating vaccination strategies for reducing infant
767 respiratory syncytial virus infection in low-income settings. *BMC Med.* 2015;13.
- 768 68. Groothuis J, Hoopes JM, Hemming V. Prevention of serious respiratory syncytial
769 virus-related illness. II: Immunoprophylaxis. *Adv Ther.* 2011;28(2):110-25.
- 770 69. Social and economic situation of Mongolia (As of the preliminary result of
771 2013) National Statistical Office of Mongolia; 2014 [cited 2014 May 20]. Available from:
772 <http://en.nso.mn/uploads/users/6/files/web201401en.pdf>.
- 773 70. Social and economic situation of Mongolia (As of the preliminary result of
774 2014) National Statistical Office of Mongolia; 2015 [cited 2015 May 15]. Available from:
775 <http://en.nso.mn/content/127>.
- 776 71. Central Intelligence Agency (CIA). The World Factbook: Mongolia [cited 2014 Nov 18].
777 Available from: <https://www.cia.gov/library/publications/the-world-factbook/geos/mg.html>.
- 778 72. Burmaa A, Kamigaki T, Darmaa B, et al. Epidemiology and impact of influenza in
779 Mongolia, 2007-2012. *Influenza Other Respi Viruses.* 2014;8(5):530-7.
- 780 73. Baigalmaa J, Tuul T, Darmaa B, et al. Analysis of fatal outcomes from influenza
781 A(H1N1)pdm09 in Mongolia. *Western Pac Surveill Response J.* 2012;3(3):43-8.
- 782 74. Dwyer D, Barr I, Hurt A, et al. Seasonal influenza vaccine policies, recommendations
783 and use in the World Health Organization's Western Pacific Region. *Western Pac Surveill*
784 *Response J.* 2013;4(3):51-9.
- 785 75. Harper SA, Fukuda K, Uyeki TM, et al. Prevention and control of influenza.
786 Recommendations of the Advisory Committee on Immunization Practices (ACIP). *MMWR*
787 *Recomm Rep.* 2005;54(RR-8):1-40.
- 788 76. Spong CY. Defining "term" pregnancy: Recommendations from the defining "term"
789 pregnancy workgroup. *JAMA.* 2013;309(23):2445-6.
- 790 77. American College of Obstetricians and Gynecologists. Definition of term pregnancy.
791 Committee Opinion No. 579.: *Obstet Gynecol*; 2013. p. 1139-40.
- 792 78. Ministry of Health Mongolia, National Centre of Communicable Diseases, National
793 Influenza Centre 2014 [cited 2014 May 9]. Available from: <http://flu.mn/eng/>.
- 794 79. Macfarlane JT, Macfarlane RM, Rose DH, et al. Prospective study of aetiology and
795 outcome of adult lower-respiratory-tract infections in the community. *The Lancet.*
796 1993;341(8844):511-4.

- 797 80. Jedrychowski W, Galas A, Pac A, et al. Prenatal Ambient Air Exposure to Polycyclic
798 Aromatic Hydrocarbons and the Occurrence of Respiratory Symptoms over the First Year of
799 Life. *Eur J Epidemiol*. 2005;20(9):775-82.
- 800 81. R Core Team. R: A language and environment for statistical computing. R Foundation
801 for Statistical Computing, Vienna, Austria 2014. Available from: <http://www.R-project.org/>.
- 802 82. Nukiwa N, Burmaa A, Kamigaki T, et al. Evaluating influenza disease burden during
803 the 2008-2009 and 2009-2010 influenza seasons in Mongolia. *Western Pac Surveill Response*
804 *J*. 2011;1(1):16-22.
- 805 83. Evenson KR, Wen F. National trends in self-reported physical activity and sedentary
806 behaviors among pregnant women: NHANES 1999–2006. *Prev Med*. 2010;50(3):123-8.
- 807 84. Nethery E, Brauer M, Janssen P. Time-activity patterns of pregnant women and
808 changes during the course of pregnancy. *J Expo Sci Environ Epidemiol*. 2008;19(3):317-24.
- 809 85. World Health Organization. WHO recommendations on the composition of influenza
810 virus vaccines 2015 [cited 2015 September 8]. Available from:
811 <http://www.who.int/influenza/vaccines/virus/recommendations/en/>.
- 812 86. Sande CJ, Cane PA, Nokes DJ. The association between age and the development of
813 respiratory syncytial virus neutralising antibody responses following natural infection in
814 infants. *Vaccine*. 2014;32(37):4726-9.
- 815 87. Steinhoff MC, Omer SB, Roy E, et al. Influenza Immunization in Pregnancy —
816 Antibody Responses in Mothers and Infants. *N Engl J Med*. 2010;362(17):1644-6.
- 817 88. Englund JA, Mbawuike IN, Hammill H, et al. Maternal immunization with influenza or
818 tetanus toxoid vaccine for passive antibody protection in young infants. *J Infect Dis*.
819 1993;168(3):647-56.
- 820 89. Brandenburg AH, Groen J, Steensel-Moll HAv, et al. Respiratory syncytial virus specific
821 serum antibodies in infants under six months of age: Limited serological response upon
822 infection. *J Med Virol*. 1997;52(1):97-104.
- 823 90. Chu HY, Steinhoff MC, Magaret A, et al. Respiratory Syncytial Virus Transplacental
824 Antibody Transfer and Kinetics in Mother-Infant Pairs in Bangladesh. *J Infect Dis*.
825 2014;210(10):1582-9.
- 826 91. Ochola R, Sande C, Fegan G, et al. The Level and Duration of RSV-Specific Maternal
827 IgG in Infants in Kilifi Kenya. *PLoS One*. 2009;4(12):e8088.
- 828 92. Model IMCI handbook: Integrated management of childhood illness: World Health
829 Organisation; 2005. Available from:
830 http://www.who.int/maternal_child_adolescent/documents/9241546441/en/.
- 831 93. Chartrand C, Leeflang MMG, Minion J, et al. Accuracy of Rapid Influenza Diagnostic
832 Tests: A Meta-analysis. *Ann Intern Med*. 2012;156(7):500-11.
- 833 94. Falsey AR, Walsh EE. Respiratory Syncytial Virus Infection in Adults. *Clin Microbiol Rev*.
834 2000;13(3):371-84.

835

836

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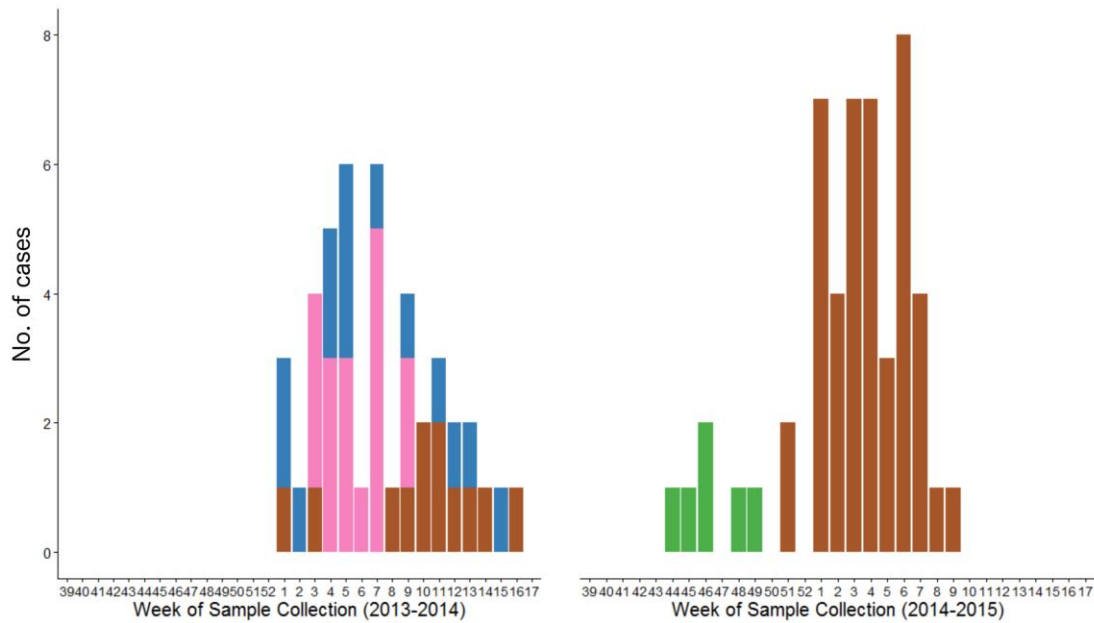
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855 11. FIGURES



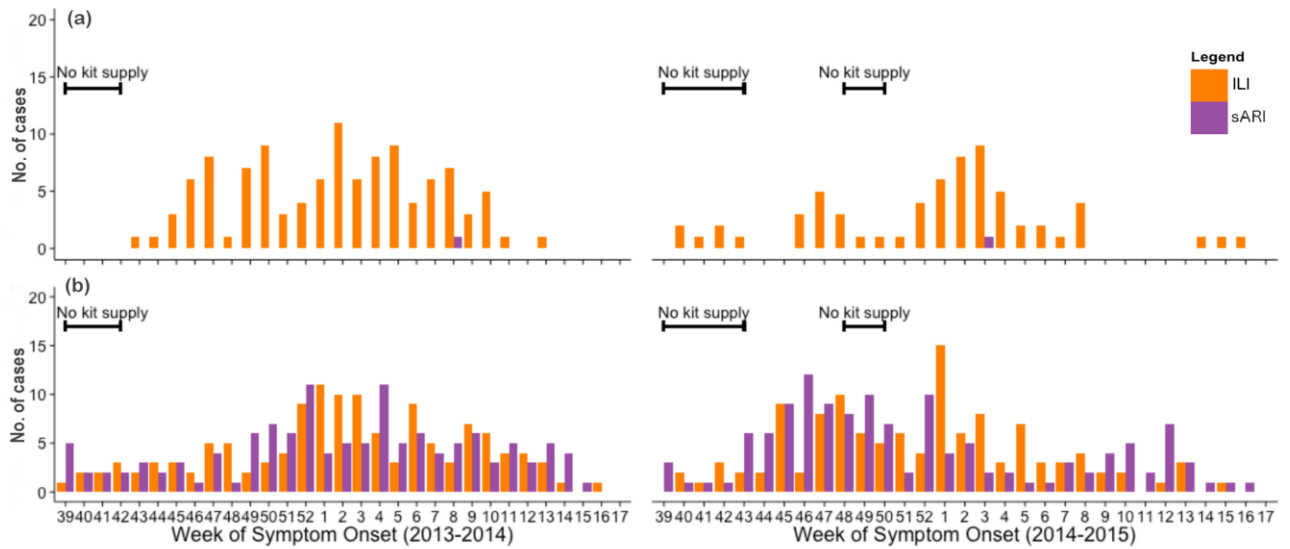
856

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



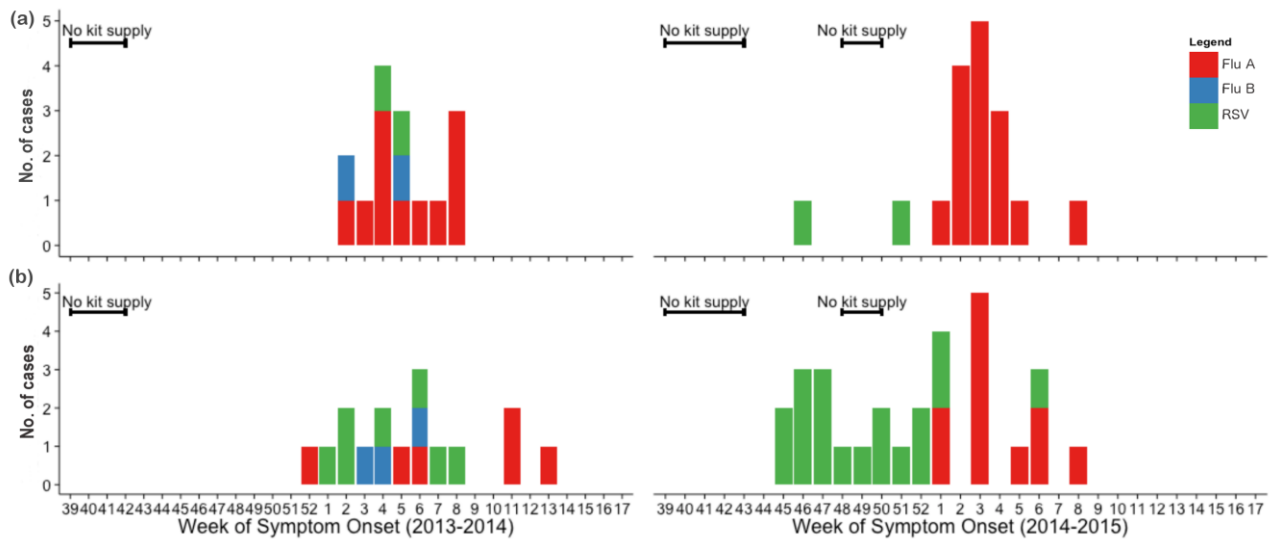
858

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.



864

865 **Fig 3.** The weekly number of influenza-like illness (ILI) [orange] and severe acute respiratory
 866 infections (sARI) [purple] cases during the 2013/14 (left) and 2014/15 (right) seasons, for
 867 pregnant women (a) and infants under 6 months (b). The black lines indicate periods of
 868 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

878 **12. TABLES**

879 **Table 1.** Baseline characteristics of the pregnant women cohort and its comparison between
 880 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

881

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

884 (a)

885

| 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) |

886

887 (b)

888

| 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) |

889

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

894 **(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

895

896 **(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) |

897

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 \pm sd | 12.0 \pm 50.9 | 13.0 \pm 51.7 | 9.0 \pm 49.9 | 0.06 |
| | Range | 0 - 167 | 0 - 167 | 0 - 167 | |
| <i>Newborn characteristics</i> | | | | | |
| 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 (\geq 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

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

906 **(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) |

907
 908 **(b)**
 909

| 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) |

910

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 \pm sd | 29 \pm 6.0 | 27 \pm 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 (\geq 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 (\geq 27 weeks) | 14 (8.1) | 274 (24.9) | |
| Gestational age at enrolment | Median \pm sd | 12.4 \pm 7.7 | 16.6 \pm 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

917 **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 \pm sd | 21 \pm 34.3 | 11 \pm 53.1 | <0.001** | 8 \pm 39.5 | 12 \pm 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) | 0.49 | 5 (2.0) | 4 (0.4) | 0.03** |
| | Early term (37-38 weeks) | 25 (10.2) | 120 (10.9) | | 10 (3.9) | 41 (3.8) | |
| | Full term (\geq 39 weeks) | 221 (89.8) | 974 (88.3) | | 240 (94.1) | 1043 (95.8) | |
| FGP consulted | A | 50 (20.3) | 278 (25.2) | 0.002** | 63 (24.7) | 268 (24.6) | 0.77 |
| | B | 88 (35.8) | 291 (26.4) | | 64 (25.1) | 302 (27.8) | |
| | 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) | <0.001** | 77 (59.7) | 206 (40.6) | <0.001** |
| | Ger | 71 (60.7) | 178 (34.2) | | 38 (29.4) | 198 (39.0) | |
| | Private house | 16 (13.7) | 102 (19.6) | | 14 (10.9) | 104 (20.5) | |
| Household size | 1 - 2 | 3 (2.6) | 18 (3.4) | 0.78 | 7 (5.4) | 15 (3.0) | 0.20 |
| | 3 - 4 | 55 (47.0) | 225 (43.3) | | 62 (48.1) | 221 (43.5) | |
| | 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

918 **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) |

