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Reviews in Medical Virology

REVIEW

Avian influenza virus in pregnancy

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SUMMARY

The unprecedented epizootic of avian influenza viruses, such as H5N1, H5N6, H7N1 and H10N8, has continued to cause disease in humans in recent years. In 2013, another novel influenza A (H7N9) virus emerged in China, and 30% of those patients died. Pregnant women are particularly susceptible to avian influenza and are more likely to develop severe complications and to die, especially when infection occurs in the middle and late trimesters. Viremia is believed to occur infrequently, and thus vertical transmission induced by avian influenza appears to be rare. However, avian influenza increases the risk of adverse pregnancy outcomes, including spontaneous abortion, preterm birth and fatal distress. This review summarises 39 cases of pregnant women and their fetuses from different countries dating back to 1997, including 11, 15 and 13 infections with H7N9, H5N1 and the 2009 pandemic influenza (H1N1), respectively. We analysed the epidemic features, following the geographical, population and pregnancy trimester distributions; underlying diseases; exposure history; medical timelines; human-to-human transmission; pathogenicity and vertical transmission; antivirus treatments; maternal severity and mortality and pregnancy outcome. The common experiences reported in different countries and areas suggest that early identification and treatment are imperative. In the future, vigilant virologic and epidemiologic surveillance systems should be developed to monitor avian influenza viruses during pregnancy. Furthermore, extensive study on the immune mechanisms should be conducted, as this will guide safe, rational immunomodulatory treatment among this high-risk population. Most importantly, we should develop a universal avian influenza virus vaccine to prevent outbreaks of the different subtypes. Copyright © 2016 John Wiley & Sons, Ltd.

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Abbreviations
CFR, Case fatality rate; ECMO, Extracorporeal membrane oxygenation; HA, Hemagglutinin; HPAI, Highly pathogenic avian influenza; ICU, Intensive care unit; LPAI, Low pathogenic avian influenza; LPM, Live poultry market; LPS, Lipopolysaccharide; NA, Neuraminidase; PB2, Polymerase basic protein 2; PEEP, Positive end-expiratory pressure.

INTRODUCTION

Of the 16 haemagglutinin subtypes, extensive human epidemics have occurred with only three: H1, H2 and H3. Avian influenza refers to diseases caused by other influenza type A viruses, most often by the H5, H7 and H9 subtypes. Despite the fact that these viruses occur naturally among wild aquatic birds, they can also infect domestic birds and, to a lesser extent, other animal species and humans. Before 2000, only 72 human cases of avian influenza A viruses had ever been recorded, but the incidence has increased significantly to 1419 since 2000 [1]. In March of 2013, an unexpected influenza A (H7N9) virus emerged in China and caused

severe human illness; almost one-third of the individuals died. The fatal outcome was not only associated with virus replication but also with host factors, such as age, underlying diseases and pregnancy [2–4].

In past seasonal influenza A pandemics, pregnant women were approximately four to five times more likely to develop severe disease than non-pregnant women. Thus, the case fatality rate (CFR) in pregnant women increased to 10–25%, which is much higher than the overall population's risk of 0.3–1% [2]. Accordingly, these types of pandemics carry an increased risk of adverse outcomes in the fetuses, such as spontaneous abortion, preterm birth and fatal loss [5]. This was attributed to two major physiology changes during pregnancy. First, maternal respiratory system is significantly altered, and hyperventilation can create a maternal perception of dyspnoea [6–9]. Secondly, pregnancy also appears to shift the cell-mediated immunity toward humoral immunity [5]. These adaptations in pregnant patients represent a diminished ability to compensate for and resist the influenza/avian influenza virus [10,11].

Although a few studies have documented and recorded pregnant women infected with avian influenza virus [12,13], the current information is very fragmented. This systematic review attempts to compare the epidemiology in gravid patients caused by H7N9 (low pathogenic avian influenza [LPAI]) and H5N1 (highly pathogenic avian influenza [HPAI] and seasonal 2009 pandemic H1N1 [2009 pdm(H1N1)]). These data will be of benefit for the control of infection in pregnant women with avian influenza and therefore improve the outcome of both the mothers and their fetuses.

DISEASE DISTRIBUTION

Geographical distribution

For the H7N9 group, the first H7N9 case was reported in March of 2013; as of 31 January 2016, 721 laboratory-confirmed H7N9 cases have been reported worldwide, including 285 deaths [14], as follows: four cases were imported from Taiwan, 13 cases from Hong Kong, one case from Malaysia, two cases from Canada and 701 cases from mainland China. Most cases were distributed across one-third of the area of mainland China over three years, with major circulations in the south and east. Eleven cases of pregnancy were found in the 721 confirmed cases of the H7N9 virus in the world, accounting for 1.5% (11/721) of the total cases, as follows: 4 cases from Zhejiang Province (accounting for 1.9% [4/208] of the total cases), 3 cases from Guangdong Province (1.6% [3/188] of the total cases), two cases from Jiangsu Province (2.4% [2/85] of the total cases), and two cases from the Hunan Province (7.4% [2/27] of the total cases). See Figure 1A and Table 1.

For the H5N1 group, as of 26 January 2015, 718 avian influenza A (H5N1) cases, including 413 deaths, have been identified since the first case was diagnosed in Hong Kong in 1997. All confirmed cases were distributed across 16 countries/areas in the world. The most severe outbreaks were found in Southeast Asia and North Africa. In this review, we report a total of 15 H5N1 cases in pregnant patients: six from Indonesia (3%, 6/200), five from Egypt (2.2%, 5/227), two from Vietnam (1.6%, 2/125) and two from China (4.3%, 2/46). See Figure 1B and Table 1.

In conclusion, H7N9 was the major circulating type of avian influenza in east and south China, while H5N1 was predominantly identified in Southeast Asia and northern Africa. These areas are traditionally active sites for bird rearing, transporting and trading, which may explain their higher incidences of avian influenza. The pregnant patients that were infected with one of these two viruses were all found in the most severely affected areas/countries. However, the percentage of pregnant women with H7N9 (1.5% [11/721]) was lower than the number of H5N1 cases in pregnant patients (2.2% [15/718]) but still higher than the A (H1N1)pdm09 (0.6% [13/2078]) reported in China. In summary, pregnant women appeared more likely to be infected with the H5N1 virus.

Age and sex distribution

The sex characteristics of H7N9 cases are distinct from those of H5N1 and A (H1N1)pdm09 cases (21). The female cases accounted for only one-third of the total H7N9 cases (33.0% [238/721]), but there was a more equal sex distribution in H5N1 (female cases accounted for 54% [282/522]) and A (H1N1) pdm09 (female cases accounted for 46.2% [964/2087]) [7,15,16]. See Figure 2A and Table 1.

For the H7N9 group, the age characteristics of the H7N9 cases were very different from those of H5N1 and A (H1N1)pdm09. For H7N9, the average age was 61 years (4 months–91 years) in

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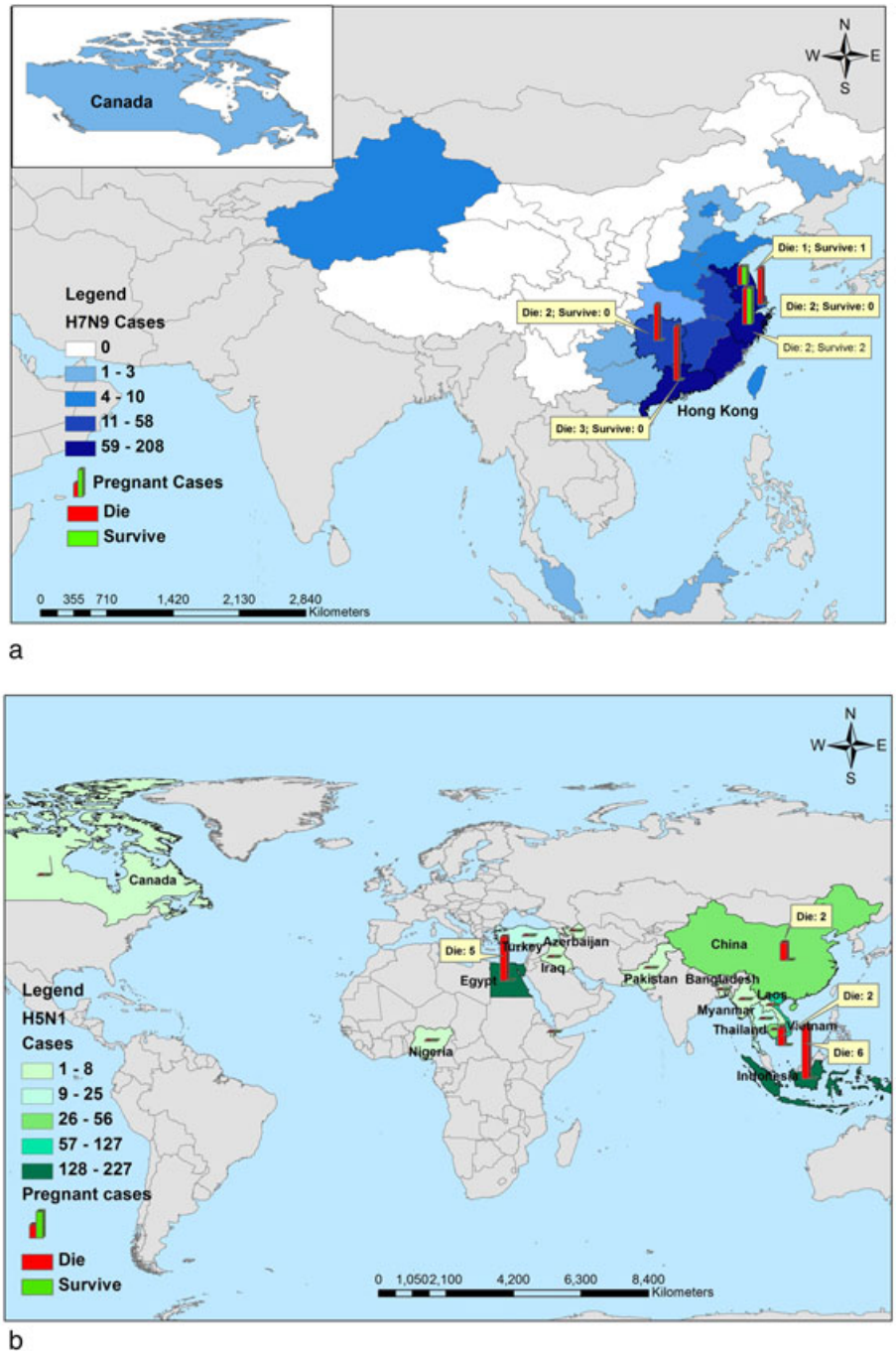

Figure 1. Geographic distribution of all laboratory-confirmed overall cases and pregnant cases infected with the H5N1 virus since 1997 and with the H7N9 virus since 2013 worldwide. [11]

all cases, while the average age was 50.1 years (4 months–85 years) in the female cases [1,14,17,18]. In addition, 24.4% (42/172) of the female cases were distributed in the 20-to 45-year reproductive age groups. The age findings have been consistent during the first, second and third waves. The predominance of an older male population is attributed to cultural practices in which older men may be more likely to shop at live poultry markets (LPMs) [1]. For the pregnant cases, the average age was

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Table 1. Epidemiological features of pregnant cases infected with influenza A/avian H7N9, H5N1 and 2009 pdm(H1N1) in the world as to 31 January 2016

	H7N9			H5N1	
Epidemiological features	**Pregnant cases $N = 11$**	**Total cases $N = 721$**	**Female cases $N = 238$**	**Pregnant cases $N = 15$**	**Total cases $N = 718$**
Percent of female cases (%)	-	33.0 (238/721)	-	-	54 (282/522)
Percent of pregnant cases (%)	-	1.5 (11/721)	-	-	2.2 (15/718)
CFR	72.7 (8/11)	39.5 (285/721)	-	100 (15/15)	57.5 (413/718)
Average age (Years old)	28 (20 ~ 35)	61 (4 months ~ 91)	50.1 (4 months ~ 85)	24.8 (20 ~ 35)	20 (8 month ~ 75)
Pregnancy Trimester					
First	27.2 (3/11)	-	-	6.7 (1/15)	-
Second	36.4 (4/11)	-	-	46.7 (7/15)	-
Third	27.2 (3/11)	-	-	13.3 (2/15)	-
Postpartum	9.1 (1/11)			0.0 (0/15)	
Unknown	0.0 (0/11)	-	-	33.3 (5/15)	-
Comorbidities (%)	9.1 (1/11)	73 (526/721)	37.9 (90/238)	No	Most patients with influenza A (H5N1) virus infection were previously healthy
Exposure to or sick or deadly poultry (%)	0.0	0.0	0.0	100	Handling of sick or dead poultry during the week before the onset of illness is the most commonly recognised risk factor
Exposure to LPM or contact with live poultry (%)	100	65 ~ 82	55.5	0.0	0.0
Incubation Period (Median Days)	4.0 (3 ~ 10)	3.3 (1.4 ~ 5.7)	-	3.0 (1.0 ~ 7.0)	2.0 ~ 5.0 (0 ~ 7.0)
No. of contacts infections	1	-	-	1	-
Cluster cases (%)	9.1 (1/11)	6.8 (49/721)	-	6.8 (1/15)	20 (144/718)
Cluster size	2	2 ~ 3	2 ~ 3	2	2 ~ 8

Notes: LPM, live poultry market; Pregnancy trimester: First referred <12 weeks; Second: referred between 13 weeks and 27 weeks; Third referred: 28–40 weeks; Postpartum period (≤2 weeks after giving birth)
CFR, Case fatality rate

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Table 1. (Continued)

Epidemiological features	H5N1	2009 pdm(H1N1)		
	Female cases $N = 316$	Pregnant cases $N = 13$	Total cases $N = 2078$	Female cases $N = 963$
Percent of female cases (%)	-	-	46.2 (964/2078)	-
Percent of pregnant cases (%)	-	-	0.6 (13/2078)	-
CFR	-	0.0 (0/13)	0.5 (10/2078)	-
Average age (Years old)	20.8 (14 months ~ 75)	28 (20 ~ 34)	22 (0 ~ 89)	23 (0 ~ 82)
Pregnancy Trimester				
First	-	7.7 (1/13)	-	-
Second	-	53.8 (7/13)	-	-
Third	-	15.4 (2/13)	-	-
Postpartum		0.0 (0/13)		
Unknown	-	23.1 (3/13)	-	-
Comorbidities (%)	-	38.5 (5/13)	50% of serious cases and 100% of deaths infected with A (H1N1)pdm09 had underlying diseases	-
Exposure to or sick or deadly poultry (%)	-	0.0	0.0	0.0
Exposure to LPM or contact with live poultry (%)	0.0	0.0	0.0	0.0
Incubation Period (Median Days)	-	3.0	3.0	-
No. of contacts infections	-	-	-	-
Cluster cases (%)	-	77 (10/13)		-
Cluster size	-	-	7 ~ 73	-

28 years and ranged from 20 to 35 years. Six cases were in their 20s, and five cases were in their 30s. See Figure 2B and Table 1.

For the H5N1 group, the average age was 20 years (8 months–75 years) for all H5N1 cases. Similarly, the average age was 20.8 years (14 months–75 years) in female cases, of which the reproductive age groups (20–45) accounted for 46.1% (130/282) of the female cases [17]. Additionally, the average age was 24.8 (20–35) years in the 15 pregnant women infected with the H5N1 virus. See Figure 2B and Table 1.

For the A (H1N1)pdm09 group, the average age was 22 (0–89) years and 23 (0–82) years in the general and female population infected with A (H1N1) pdm09, respectively [19]; 39% (379/964) of the

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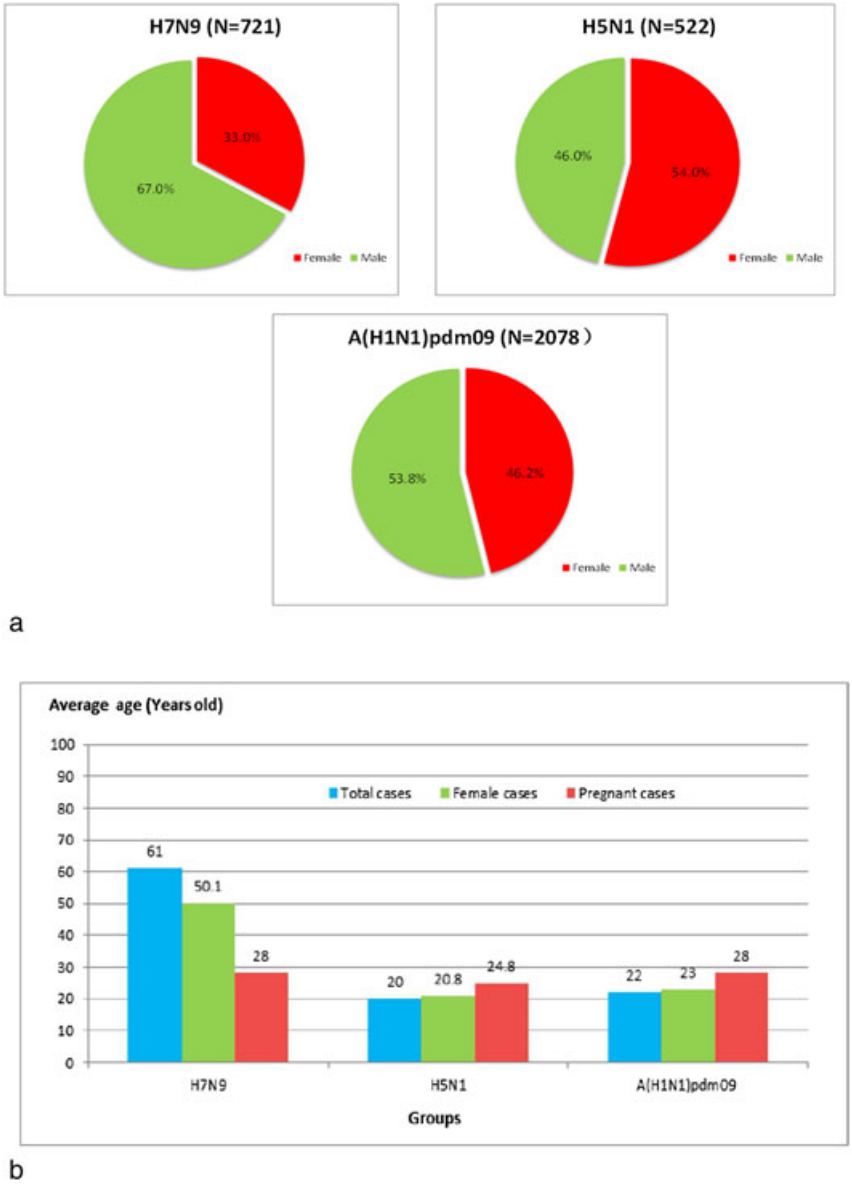

Figure 2. The age and gender distribution of total cases, female cases and pregnant cases infected with H5N1, H7N9 and A (H1N1)pdm09, respectively. [11]

female cases were found in the group of reproductive age, with a range from 20 to 45 years of age. The average age was 28 years old (20–34) among the pregnant women infected with A(H1N1) pdm09 [20]. See Figure 2B and Table 1.

In general, H7N9 was the major strain identified in the male population >60 years old, while H5N1 and A(H1N1)pdm09 were most often found in young adults, without a sex bias. Although the percentage of reproductive women was very different in the H7N9, H5N1 and A (H1N1)pdm09 cases, the average age was very similar in the pregnant cases infected with one of these three viruses.

TRIMESTER DISTRIBUTIONS

For the H7N9 group, from March 2013 to January 2016, only 11 pregnant women were reported to be infected with the H7N9 avian influenza. Of these, 27.2% (3/11) occurred in the first trimester (≤12 weeks), 36.4% (4/11) in the second trimester (13–27 weeks) and 27.2% (3/11) in the third trimester (28–40 weeks). A total of 9.1% of infection (1/11)

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was reported in the postpartum period (≤2 weeks after giving birth).

For the H5N1 group, 6.7% (1/15) of the pregnant cases occurred in the first trimester, 46.7% (7/15) in the second trimester, 13.3% (2/15) in the third trimester and 33.3% (5/15) were unknown.

For the A (H1N1)pdm09 group, 7.7% (1/13) of the pregnant cases occurred in the first trimester, 53.8% (7/13) in the second trimester, 15.4% (2/13) in the third trimester and 23.1% (3/13) were not clear.

In summary, H7N9, H5N1 and A(H1N1)pdm09 appeared to preferentially infect pregnant women in the middle to late gestational period [21]. This trend has been attributed to the higher susceptibility and higher chance of exposure to poultry and related environments among the middle to late stage cases. In contrast, pregnant women infected with A (H1N1)pdm09 were predominantly infected by human-to-human transmission [18,22,23]. The percentages by trimester are shown in Figure 3 and Table 1.

UNDERLYING DISEASES

Pre-existing concurrent health conditions were associated with the mother's outcome and also the pregnancy's outcome. Although 70–80% of the confirmed H7N9 cases were found to have chronic diseases in the total population, only 37.9% (90/238) of the female cases experienced the same conditions [15]. Even so, among the 11 H7N9 pregnant cases, 9.1% (1/11) of them had an underlying disease, and there were four cases with a pregnancy history. In contrast, most patients with the influenza A (H5N1) infection were previously healthy [16]. Similarly, all 15 H5N1 pregnant cases had no underlying diseases except one case with a pregnancy history before onset. Unlike the H7N9 and H5N1 cases, about 50% of the serious cases and 100% of the deaths of those infected with A (H1N1)pdm09 had underlying diseases in the overall population. Furthermore, the percentage with underlying diseases was 38.5% (5/13) among the pregnant cases infected with A (H1N1)pdm09. See Table 1.

POULTRY EXPOSURE HISTORY

For the H7N9 group, current research has indicated that LPMs appear to be critical in maintaining the transmission of H7N9 among poultry in urban settings [24]. However, H7N9 has not been found to any significant degree in wild birds, in waterfowl or on rural farms [25,26]. A series of studies indicated that 82% of the H7N9 cases had had recent animal exposure or had visited an LPM prior to illness onset [14,27]. The most common animal exposures were with chickens (82%), ducks (22%) and pigeons (12%). Similar to the general cases, all H7N9 pregnant cases reported an exposure history before their onset. Three of them had direct contact with poultry, while the other eight cases had visited an LPM. These data suggest that LPMs were an ongoing source of exposure for both birds and humans.

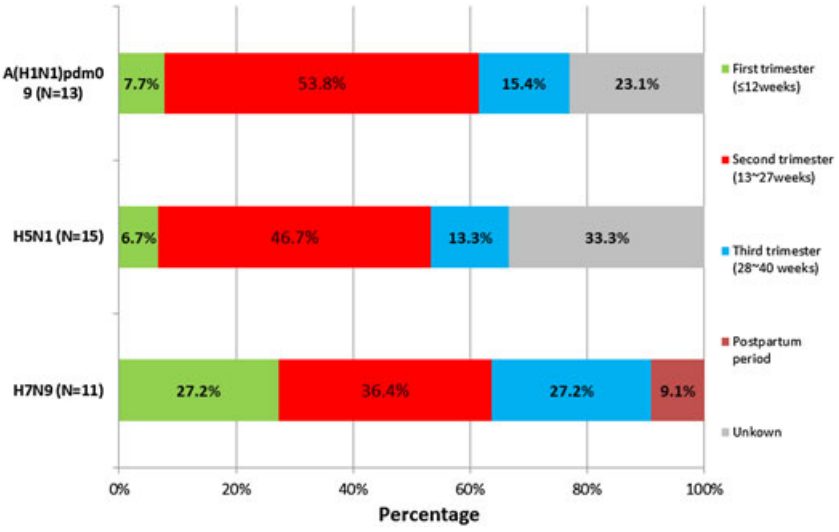

Figure 3. The trimester distribution was 15, 11 and 13 pregnant cases infected with H5N1, H7N9 and A (H1N1)pdm09 virus, respectively.

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For the H5N1 group, unlike H7N9, H5N1 circulates in wild birds and infects poultry in backyards and in small farms in rural areas [28,29]. Direct avian-to-human H5N1 virus transmission is the predominant means of human infection. Handling sick or dead poultry during the week before the onset of illness is the most commonly recognised risk factor for avian influenza [2,3,30]. All 15 of the pregnant cases in this study had been exposed to sick and dead poultry. Three cases involved the slaughtering and defeathering of chickens [13], and one case had had contact with contaminated fomites or with fertilizer that contained poultry faeces [16,31]. The birds most often implicated were chickens, turkeys and ducks in the backyard. See Table 1.

HUMAN-TO-HUMAN TRANSMISSION

For the H7N9 group overall, a total of 721 H7N9 cases have been identified, and at least 24 clusters of cases were reported. All H7N9 family clusters have involved two to three cases [32–34]. Family cluster cases accounted for only 6.8% (49/721) of all H7N9 cases.

The H5N1 group overall is similar to that of H7N9 in that at least 55 clusters of H5N1 cases have been reported in at least 10 countries. Family clusters, or two linked cases, represent approximately 20% (144/718) of all H5N1 cases [35,36]. Most clusters involved two or three family members, except only one cluster affected with eight cases. The cluster cases were produced by common-source exposures to poultry or person-to-person transmission that probably occurred during very close, unprotected contact with a severely ill patient [37]. Among the well-described clusters caused by the H5N1 and H7N9 viruses, there appears to be evidence of limited, nonsustained human-to-human transmission. The calculated transmission dynamics also do not support person-to-person transmission [1].

For the H7N9 pregnant group, family cluster cases accounted for 9.1% (1/11) of all H7N9 pregnant cases. Ten of the 11 pregnant cases with H7N9 were sporadic, except for one case at 25 weeks gestation found in Hunan Province, which was the index case of a family cluster, involved two confirmed cases. The patient's husband reported disease onset eight days after the patient became ill and developed severe symptoms, most likely due to human-to-human transmission from the index pregnant case. However, the secondary case did not transmit further to the other contacts. None of the close contacts of the remaining 10 pregnant women experienced any illness, and all proved negative by RT-PCR. These limited data suggested that the pregnant cases also produced no sustained human-to-human transmission.

For the H5N1 pregnant group, family cluster cases accounted for 6.8% (1/15) of all H5N1 pregnant cases. One out of the 15 H5N1 pregnant cases was identified in Egypt at 24 weeks gestation and was confirmed as the index case of the family cluster. This cluster involved two confirmed cases, and her young child was also verified as having the H5N1 infection. The mother and child developed symptoms on 26 November 2011; the mother died on 3 December even though she had been prescribed oseltamivir on 1 December 2011. The child was in stable condition after beginning the initial antivirus treatment on 2 December. Investigations into the source of the infection indicated that the mother and her child had both had exposure to sick and dead backyard poultry (chicken and turkeys). Most of the close contacts of the H5N1 pregnant cases have been monitored for symptoms of infection and were given oseltamivir prophylactically. No other contacts reported symptoms or tested positive. This report also supports previous research conclusions that the H5N1 virus has no sustained person-to-person transmission. See Table 1.

CLINICAL FEATURES

Incubation period

For the H7N9 group overall, using the available data, researchers have calculated the median incubation period to be 3.3 days (1.4–5.7 days) among the total H7N9 cases [17]. Among the 11 H7N9 cases diagnosed in pregnant women, the median incubation period was 4 days (3–10 days).

For the H5N1 infections overall, the median incubation period generally appears to be 7 days or less, while 2–5 days was found in those cases after exposure to sick and dead poultry. This median incubation period appears to be approximately 3–5 days in some family clusters [16].

For the three groups of pregnant women among the 15 pregnant cases infected with H5N1, the median incubation period was 3 days (1–7 days). The median incubation period was 3 days in the

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13 A (H1N1)pdm09 pregnant cases from China, similar to the overall cases with (H1N1)pdm09. See Table 1.

Clinical period

For the H7N9 cases overall, based on the report of 139 confirmed H7N9 cases and 47 fatal cases of Li *et al*., the median days from illness onset to the first consultation was 1.0 day (0–3.0), from illness onset to hospitalisation was 4.0 days (3.0–6.0), from illness onset to ICU admission was 7 days (5.0–9.0) and from illness onset to oseltamivir treatment was 6.0 days (5.0–9.0). The median days from onset to laboratory confirmation was 8.3 days (7.3–9.5), from illness onset to death was 21 days (12.5–36.0) and from illness onset to discharge was 34 days (4–138.0).

For the H5N1 cases overall, the onset-to-admission interval for H5N1 was different from that of the H7N9 virus. Based on the 308 patients infected with H5N1 that have been reported to the World Health Organisation (WHO), the median number of hospitalised days was estimated to be 5.0 days (0–25), while the median number of days from onset to confirmation was 8.0 days (1–29). The median number of days from illness onset to oseltamivir treatment was 5.0 days (0–25.0), the median number of days from illness onset to death was 9.8 days (2–30) and the median number of days that elapsed from the illness onset to discharge was 14 days (7–32) [17].

For the three groups of pregnant women, the median number of days in pregnant cases was very similar to that of the total cases. The median period from symptom onset to first consultation was 1, 2.5 and 2 days and 2 days in 11 H7N9, 15 H5N1 and 13 A (H1N1)pdm09 pregnant cases ($p < 0.05$), respectively. The median number of days from onset to hospital admission was 5, 4 and 2 days in the H7N9, H5N1 and A (H1N1)pdm09 groups, respectively. The median number of days from onset to ICU admission was 8, 5.5 and 10 days in the H7N9, H5N1 and A (H1N1)pdm09 groups, respectively. The median number of days from onset to confirmation was 8, 8.5 and 2 days in the H7N9, H5N1 and A (H1N1)pdm09 groups, respectively. The median number of days from onset to positive end-expiratory pressure (PEEP) was 10, 5.5 and 10 days in the H7N9, H5N1 and A (H1N1)pdm09 groups, respectively. The median number of days from onset to antivirus treatment was 7, 6 and 4.5 days in the H7N9, H5N1 and A (H1N1)pdm09 groups, respectively. The days that passed from onset to being discharged were 51 and 12 days in H7N9 and A (H1N1)pdm09 groups, respectively. The median from onset to death was 43 and 8 days in the H7N9 and H5N1 groups, respectively. Generally, the clinical timeline demonstrates that H5N1 involved a potentially rapid and aggressive course compared with H7N9 and seasonal A (H1N1) pdm09 in infected pregnant women. See Figure 4 and Figure 5A, 5B.

PATHOGENESIS AND VERTICAL TRANSMISSION

The viral and host factors that determine the transmission, symptoms, severity and clinical outcome

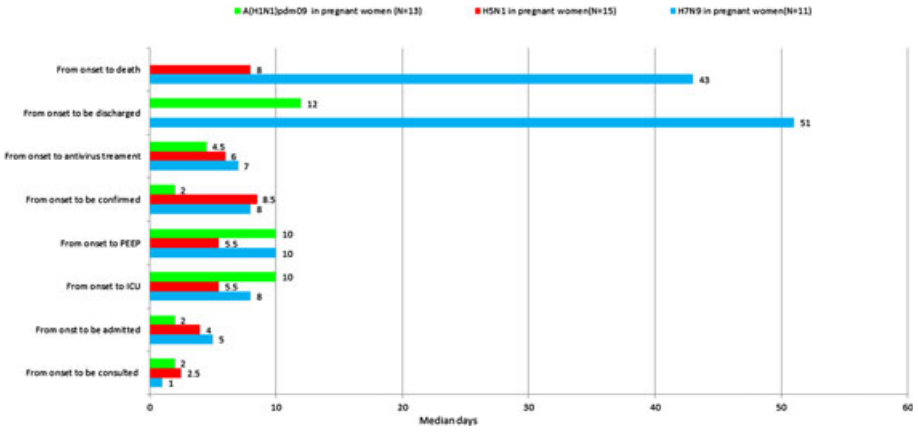

Figure 4. The median days for pregnant women infected with the avian influenza A H7N9 and H5N1 viruses and A (H1N1)pdm09, respectively.

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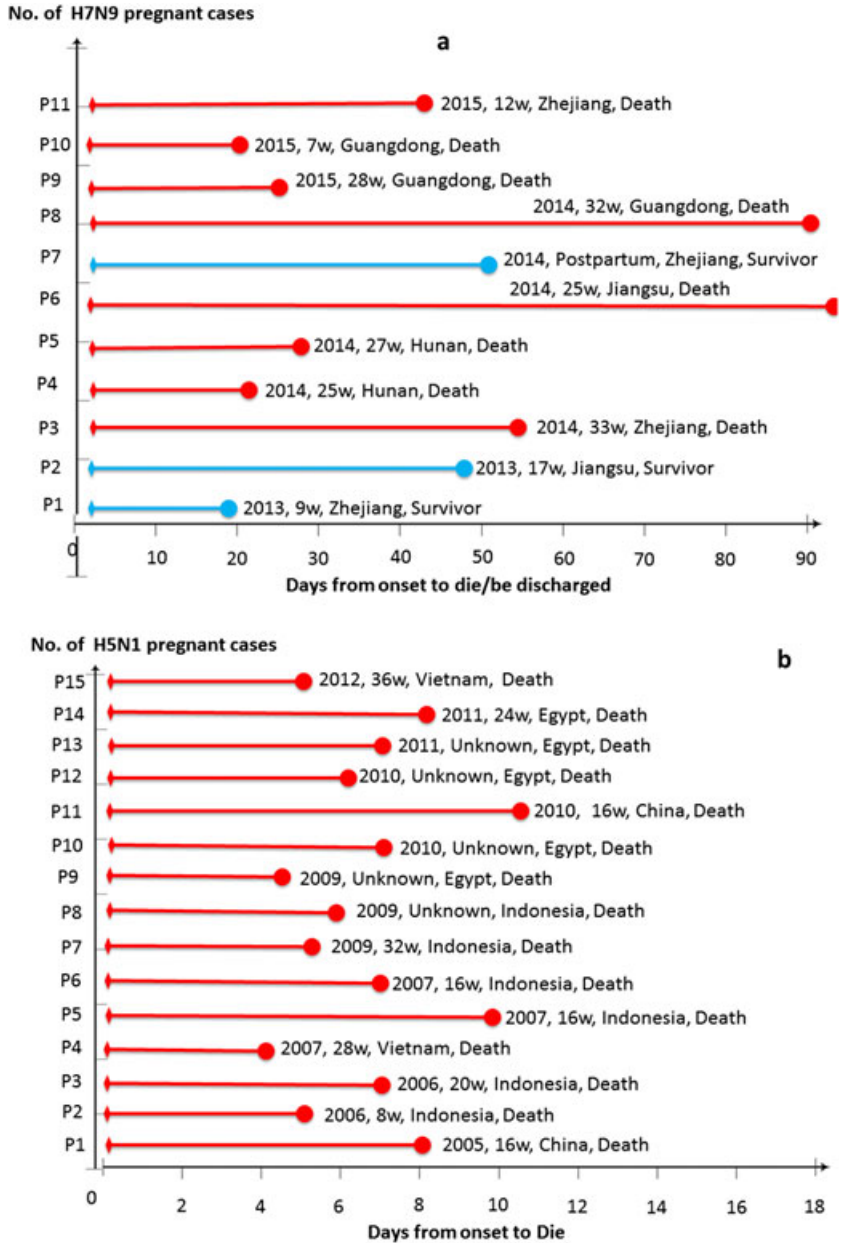

Figure 5. Median days from illness onset to death/discharge for pregnant women infected with avian the influenza A H7N9 and H5N1 viruses, respectively. 5A: For the H7N9 cases (11 pregnant women were infected, and 8 died). 5B: For the H5N1 cases (All 15 infected pregnant women died). [11]

have been only partially understood. First of all, viral features were found to be the key factors of avian virus infection, especially regarding human adaptability, mutation, pathogenicity and viral replication.

Viral infection

For the H5N1 group, H5N1 prefers to bind to α-2, 3-linked sialic acid receptors located predominantly in the human lower respiratory tract. This can prevent influenza A (H5N1) from readily infecting humans via poultry. Although some mutations that permit binding to both α-2,3- and α-2,6-linked sialic acid receptors have been found, these mutations appear to be insufficient for sustained human-to-human transmission [38–40]. Animal infection models have indicated that H5N1 viruses have no

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transmissibility or only poor transmissibility between ferrets and between swine [39].

For the H7N9 group, H7N9 viruses demonstrate an affinity to both α-2,3- and α-2,6-linked sialic acid receptors [41]. Furthermore, Q226L and G186V in hemagglutinin (HA) were identified in most human isolates, which have been associated with increased affinity for α-2,6-linked sialic acid receptors [1]. A further animal test proved that H7N9 viruses infect both upper and lower respiratory cells, causing significant disease in ferrets [42,43]. All the findings showed that H7N9 had an increased binding affinity to α-2,6 receptors compared with the H5N1 virus, which raises concerns about the potential for H7N9 to adapt further and cause a pandemic.

Avian influenza A viruses are designated as highly pathogenic avian influenza (HPAI) or low pathogenicity avian influenza (LPAI) based on the molecular characteristics of the virus and the ability of the virus to cause disease and mortality in chickens, but not humans, in a laboratory setting. Infection of poultry with low pathogenic avian influenza A (LPAI) viruses may cause no disease or mild illness (such as ruffled feathers and a drop in egg production) and may not be detected. Infection of poultry with highly pathogenic avian influenza A (HPAI) viruses can cause severe disease with high mortality (sourced from http://www.cdc.gov/flu/avianflu/influenza-a-virus-subtypes.htm). There are genetic and antigenic differences in cleavage sites (linked HA1 and HA2) between HPAI and LPAI. LPAI (H7N9) hemagglutinin outer-surface proteins do not have a multibasic cleavage site (only a single basic cleavage site "R": (RKRGLF)). Although the human disease is severe, the absence of the multibasic cleavage site in the HA indicates that H7N9 would be considered an LPAI virus. However, the multibasic cleavage site (RXXXXR/KRGLF) is seen in the HA protein of HPAI viruses (H5N1) and is associated with greater viral replication and systemic spread, manifesting as a much more severe disease in poultry and mammals [44,45].

The primary pathologic process in avian viruses that causes death in humans is fulminant viral pneumonia [16]. There are high levels of viral loads associated with the fatal outcome in both H7N9 and H5N1. For example, de Jong *et al*. reported that viral copies in the pharynx are higher and that viral RNA is more often detected in the plasma in fatal cases than in nonfatal cases [46]. H5N1 cases may carry detectable viral RNA in the respiratory tract for up to three weeks [46,47]. A fatal influenza A (H5N1) infection in one pregnant woman was associated with virus infiltration of the brain, placenta and fetus, which supported the possibility of vertical transmission [48]. A BALB/c mouse infection model also supported this opinion [49]. Similarly, a high viral load persisted in the throat throughout the first 10 days of the H7N9 illness in the absence of specific treatment. The medians of the viral loads in throat swabs taken at admission in the three groups of patients were 3.76 in the extracorporeal membrane oxygenation (ECMO) group, 2.26 in the mechanically ventilated group and 3.05 in the pneumonia group [50]. Another report on a pregnant case revealed that the H7N9 virus became undetectable in sputum 14 days after the onset of symptoms after effective antiviral therapy with oseltamivir and symptomatic/supporting treatments. Correspondingly, the reduction of the H7N9 viral load following antiviral treatment was correlated with an improved outcome. The emergence of neuraminidase (NA) R292K mutation in two patients, who had also received corticosteroid treatment, led to treatment failure and a poor clinical outcome [50]. Another report from Wang *et al*. showed that the Polymerase basic protein 2 (PB2) E627K mutation slightly increased the case fatality rate [51]. To date, there has been no mother-to-child transmission report in H7N9 infections.

Host responses

Other factors related to clinical outcome were host susceptibility and immune competence. The currently available evidence indicates that the general population is immunologically naive to the H7N9 and H5N1 viruses and therefore susceptible to both [1]. A recent article about sex disparities in influenza pathogenesis suggests that pregnancy is a risk factor for greater morbidity and mortality; females may generate higher proinflammatory cytokines and chemokine responses to avian influenza [52]. Another report *in vivo* also showed elevated levels of IL-10, IL-6 in H5N1-infected individuals — particularly in those deaths [53]. *In vitro* and animal experiments also implicate cytokine dysregulation in H5N1 pathogenesis, characterised by the evasion of the antiviral effects of interferons and tumour necrosis factor (TNF)-α6 in addition to the induction of proinflammatory cytokines and chemokines by

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H5N1 viruses. These responses are driven by the high-level viral replication in H5N1. However, in pregnancy, the inflammatory responses are increasingly attenuated. For example, in *in vitro* tests, the cytokine production in whole blood that was exposed to lipopolysaccharide (LPS) was significantly lower among 18 pregnant women during their third trimester of pregnancy as compared to those in the postpartum period, with three-fold lower IL-12 production and 40% lower TNF-α production [54]. In one pregnant case caused by the H7N9 virus, peripheral lymphocytes initially decreased in the early stage but the CD4+ T-cell percentage increased after 16 and 18 days since the onset of symptoms. The kinetic changes of proinflammatory and anti-inflammatory cytokines played a key role in the pathogenesis and clinical outcome [55].

These findings support the opinion that the tissue damage in avian influenza disease probably results from the combined effects of unrestrained viral infection and inflammatory responses induced by these viruses. More data and further research will be required to support this opinion and therefore guide safe, rational antiviral and immunomodulatory treatment in pregnant woman [16].

Antivirus treatments

The WHO recommends that pregnant women with suspected or confirmed avian influenza, regardless of the stage of pregnancy, should be treated with antiviral therapy. Early treatment with oseltamivir is recommended, and data from uncontrolled clinical trials suggest that the drug improves survival. The early administration of a standard dose of oseltamivir (75 mg twice daily for 5 days in adults) within 1–3 days after the onset of the illness is effective [15]. Mortality remains high despite the administration of oseltamivir due to late initiation, a short duration and a low dose of therapy. On the other hand, virus susceptibility to antiviral drugs is vitally important for treatment.

For the H5N1 group, CFR was 100% in pregnant women infected with the H5N1 virus, which is obviously much higher than that in the general population (60%). Late initiation of therapy (delayed for 6.0 days) appears to be a major factor, along with the physical changes that take place during pregnancy. Due to the high levels of replication of the H5N1 virus, a higher dose of oseltamivir (e.g. 150 mg twice daily in adults) combined with other antivirus treatments that prolong the treatment duration to 10 days has been recommended [16]. In a mouse model of H5N1 infection, the combination of oseltamivir and amantadine significantly increased the survival rates and inhibited viral replication in the internal organs [56]. Another contribution was that susceptibility to current antiviral agents varies among circulating strains of H5N1 viruses. Clade 1 isolates of H5N1 viruses appear to be 15–30 times more sensitive to oseltamivir than clade 2 isolates. During oseltamivir therapy, the emergence of highly resistant variants with an H274Y neuraminidase mutation may be associated with a fatal outcome [57]. H5N1 infection containing an N294S mutation could cause a reduction in oseltamivir susceptibility by a factor of 12–15 times and was reported in two fatal cases [58,59].

For the H7N9 group, CFR was 72.7% (8/11) in pregnant women infected with the H7N9 virus, which is also higher than that of the general population (39.5% [285/721]). Genomic sequences of H7N9 viruses infecting humans in China revealed that very few H7N9 viruses have a mutation in the neuraminidase gene (R292K), which confers significant resistance to oseltamivir and peramivir and offers reduced susceptibility to zanamivir [60–64]. The most likely contribution was the delay in confirmation (8.0 days) and also delayed antivirus treatments (7.5 days). However, it is necessary to conduct further in-depth research into the issues that these pregnancy delayed confirmed cases were required to give combined and high-dose antivirus treatments for H7N9.

Generally, pregnant women with suspected avian influenza should be treated early and aggressively with supportive care and antiviral therapy. The possible benefits of oseltamivir or any antiviral medication certainly outweigh the fatal risks given the high potential for maternal mortality, but prophylactic antibiotics are not recommended for H5N1 and H7N9 infection in pregnancy [8].

MATERNAL SEVERITY AND MORTALITY

For the A (H1N1)pdm09 group, in the general population, the CFR of seasonal A (H1N1)pdm09 is 0.1–2% across the world [19,65]. However, in pregnant women, A (H1N1)pdm09 causes increased cases of severe diseases and deaths. Liu *et al*. reported that 2,441 pregnant women infected with A (H1N1)pdm09 were found in seven countries; 157 patients died, and the case mortality was 6.4% (157/2441). An increased incidence and a greater

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severity of illness has also been observed during interpandemic periods [6]. Based on another report conducted among 13 pregnant women with A (H1N1)pdm09, mild cases accounted for 69.2% (9/13), while severe cases made up 30.8% (4/13); no deaths from (H1N1)pdm09 in pregnant patients were reported in the Zhejiang Province from 2009–2010 [19]. See Table 2.

For the H7N9 group, the CFR was 39.5% (285/721) in the total population infected with the avian H7N9 virus, which was strikingly different from that of the A (H1N1)pdm09 virus. Approximately 30% of the total deaths were in women. In 11 pregnant cases, 9.1% (1/11) in the first trimester of pregnancy had a mild case; two cases (18.2% [2/11]) were both severe, where one of the cases was in the second trimester, and the other case was in the postpartum period; and 72.7% (8/11) (two deaths in the first trimester, three deaths in second trimester and the third trimester, respectively) died. The mortality rate was 72.7% (8/11) in the pregnant women, which was much higher than that of the general population. Table 2**.**

For H5N1 group, although a few studies have reported asymptomatic and mild cases of H5N1 [30], most cases presented with severe and fatal symptoms. The CFR was 57.5% (413/718) in the overall population infected with the H5N1 virus. However, all pregnant patients died (CFR of 100%) in 15 cases, whether in the first, second or third stage of pregnancy. See Table 2.

In summary, these three viruses shared two features. Firstly, the clinical severity and fatality rates in pregnant cases were obviously higher than those

Table 2. Comparison of material severity and mortality and fetus outcome among the pregnant cases infected with influenza A/avian H7N9, H5N1 and 2009 pdm(H1N1) virus in the world as to January 31, 2016

Epidemiological features		H7N9 ($N=11$)	H5N1 ($N=15$)	2009 pdm(H1N1) ($N=13$)
Mother severity	Mild (%)	9.1 (1/11)	0.0 (0/15)	69.2 (9/13)
	Severe (%)	18.2 (2/11)	0.0 (0/15)	30.8 (4/13)
	Death (%)	72.7 (8/11)	100 (15/15)	0.0 (0/13)
Gestation weeks	Preterm delivery (<37 weeks) (%)	66.7 (2/3)	100 (1/1)	20.2 (108/534)
	Term delivery (≥37 weeks) (%)	33.3 (1/3)	0.0 (0/1)	79.8 (426/534)
Deliver ways	Abortion (%)	25 (1/4)	0.0 (0/1)	5.1 (19/372)
	Vaginal (%)	0.0 (0/4)	0.0 (0/1)	50.8 (189/372)
	Cesarean (%)	75 (3/4)	100 (1/1)	43.3 (161/372)
	Suction (%)	0.0 (0/4)	0.0 (0/1)	0.3 (1/372)
Fetus outcome	Survivor (%)	27.3 (3/11)	6.7 (1/15)	94.7 (396/418)
	Death (%)	72.7 (8/11)	93.3 (14/15)	5.3 (22/418)
Live birth rate		100 (3/3)	100 (1/1)	94.7 (396/418)
Fetus weight	Low (%)	66.7 (2/3)	100 (1/1)	-
	Normal (%)	33.3 (1/3)	0.0 (0/1)	-

Notes: 1. Definition of a mild case

An individual with confirmed infection who met the respiratory infection criteria was classified as a mild case, presenting with mild respiratory symptoms that did not have any complications throughout the clinical course, such as acute respiratory distress syndrome (ARDS), multi-organ failure, hypoxaemia, etc.

2. Definition of a severe case

An individual with confirmed infection who met any one of the following criteria was classified as a severe case: presenting with severe respiratory symptoms with any complications including ARDS, shock, multi-organ failure, hypoxemia, etc., requiring hospitalisation or intensive care unit admission or mechanical ventilation for medical reasons. The objective index was as follows: 1) X-ray showed lesions in multiple lobes or disease progression >50% within 48 h; 2) dyspnea with a respiratory rate >24 breaths per minute; 3) hypoxemia with oxygen saturation < 92% on oxygen at a flow rate of 3 to 5 liters/min; 4) Shock, ARDS or multiple organ dysfunction syndrome.

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of the general population infected with these three viruses. Secondly, most of the deaths occurred in the second and third trimesters. Increased abdominal pressure raising the diaphragm is another potential explanation for increased respiratory morbidity in the third trimester. In contrast, the maternal fatality rate for the H5N1 avian virus was statistically higher than that of the H7N9 virus, which was higher than that for A (H1N1)pdm09 influenza.

PREGNANCY OUTCOME

For the A (H1N1)pdm09 group, it is well known that adverse pregnancy outcomes, such as spontaneous abortion, preterm birth and fatal loss, are observed with pandemic influenza. For example, the rates of spontaneous abortion and preterm birth increased to 50% during the pandemics of 1918 (H1N1) and 1957 (H3N2). Similarly, during A (H1N1)pdm09, there were 1211 pregnant cases in the US, Japan, India, Austria and France; only 43.6% (528/1211) delivered living infants. Preterm births (<37 weeks) accounted for 20.2% (108/534) of these babies, while 79.8% (426/534) were term births (≥37 weeks). The proportion of deliveries by abortion, vaginal birth, caesarean section, premature birth and suction were 5.1% (19/372), 50.8% (189/372), 43.3% (161/372), 0.5% (2/372) and 0.3% (1/372), respectively. The live birth rate was 94.7% (396/418) [6]. See Table 2.

For the H7N9 group, in contrast with the A (H1N1)pdm09 scenario, only four pregnant woman gave birth among 11 H7N9 infections. Of these, 25% (1/4) of the pregnant cases were ended by artificial abortion during admission, and 75% (3/4) underwent an emergency caesarean surgery because of the severe situation as follows: one pregnant mother gave birth to a boy with a low birth weight (48 cm and 2.2 kg) at 33 weeks gestation. The baby needed to be admitted to the neonatal intensive care unit due to "septicemia and neonatal asphyxia," and he did not receive antiviral drugs. However, the mother died of multiple organ failure 53 days after onset and 47 days after delivery. Another pregnant case was confirmed as H7N9-positive in the 17 week of pregnancy but recovered completely 39 days after admission. She also received a caesarean section at 35 weeks gestation, 2 months after her discharge from the hospital. She gave birth to a healthy girl (48 cm and 3.3 kg) [22]. This mother survived the delivery. The third pregnant case delivered a healthy baby at 40 weeks gestation through caesarean surgery and survived. The three babies also proved to be survivors. The other eight babies (72.7% [8/11]) died with their mothers. See Table 2.

For the H5N1 group, among the 15 pregnant women infected with H5N1, only one underwent emergency surgery at 36 weeks gestation and gave birth to a boy with a low birth weight (2.3 kg) before she died. The boy exhibited respiratory symptoms, but he was proven to be H5N1-negative. The other 14 pregnant cases and their fetuses did not survive regardless of the pregnancy trimester.

These findings support the idea that pregnant women infected with one of the three avian influenza viruses had an increased risk of preterm birth and a low birth weight baby. All cases required emergency surgery due to the severe infection. However, the live birth rate ranged from 94% to 100% in deliveries by mothers infected with the three viruses. The effects of maternal influenza on the fetuses are not well understood. Viremia is believed to occur infrequently, and thus vertical transmission appears to be rare [66]. Highly pathogenic strains of the influenza virus, such as avian influenza A (H5N1), are more likely to be transmitted across the placenta [67]. In contrast, no evidence of vertical transmission was found in the seasonal H1N1 and LPAI (H7N9) viruses in other studies. Since a significant knowledge gap exists for the effects of this novel virus on the fetus, further studies will be beneficial. In conclusion, pregnant women with mild or controlled avian influenza do not require elective delivery. When a mother experiences a rapid deterioration, failure to maintain adequate oxygenation, multi-organ failure, fatal compromise and difficulty with ventilation secondary to a gravid uterus, early delivery should be suggested [8]. The decision should be made by the obstetrician after careful consideration of the mother's and fetus's situation and also after weighing the risks of both.

CONCLUSIONS

The current limited data from different countries/areas suggest that pregnant women infected with the H5N1 and H7N9 avian viruses are a high-risk population, especially in the second and third trimesters [14]. Generally, pregnant women acquired H7N9 infections from an LPM, live birds, and a general poultry environment (27)

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while H5N1 cases were caused by direct contact with ill and dead birds in backyards NN. There was no difference in the incubation periods between the two groups. Most infections developed their fatal outcome in 8 and 43 days in the H5N1 and H7N9 cases, respectively. The CFR was 72.7% vs. 100% vs. 6.4% in pregnant women infected with H7N9, H5N1 and A (H1N1) pdm09, respectively; these rates are significantly higher than those of the general population. In addition, there was limited human-to-human transmission in pregnant cases infected with H5N1 and H7N9.

The fatal outcome varied in seasonal A (H1N1) pdm09, H7N9 and H5N1 infections. The live birth rate was 100%, 100% and 94.7% in pregnant cases infected with H7N9, H5N1 and A (H1N1)pdm09, respectively [12]. Each fetus survived the emergency surgery performed due to the mother's infection with an avian virus. In contrast, only 43.3% of the pregnant cases with A (H1N1)pdm09 required a caesarean operation. Because of the preterm births, all pregnant cases gave birth to fetuses with low birth weights. No infants contracted avian virus infection, which supported that vertical transmission was not present in these limited cases.

Avian influenza viruses are constantly changing; a series of these novel viruses, such as H5N6, H5N3, H7N3 and H10N8, have been identified as infecting humans in recent years. Unlike seasonal influenza, no commercially available vaccine has been applied to prevent H5N1 and H7N9 infection to date [2]. Control of the infection source is the most efficient way to avoid infection among pregnant women. Given the higher mortality rates for both mothers and fetuses, pregnant women should be considered a population that requires special considerations for prevention and treatment of avian influenza. Therefore, in the future, the first step will be to build avian surveillance systems and emergency medical systems for pregnant women, especially those in highly circulating countries. It will also be beneficial to identify and diagnose pregnant women early and begin antivirus treatment as soon as possible. Second, further research into the immune mechanism is necessary, which will be helpful to guide safe, rational immunomodulatory treatment and to improve the outcome of both mother and baby. Third, we should develop a universal avian influenza vaccine in the near future for the next unexpected avian influenza threat.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding or financial holdings that might be perceived as affecting the objectivity of this review.

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