Review

Preliminary Success in the Characterization and Management of a Sudden Breakout of a Novel H7N9 Influenza A Virus

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Abstract

Influenza has always been one of the major threats to human health. The Spanish influenza in 1918, the pandemic influenza A/H1N1 in 2009, and the avian influenza A/H5N1 have brought about great disasters or losses to mankind. More recently, a novel avian influenza A/H7N9 broke out in China and until December 2, 2013, it had caused 139 cases of infection, including 45 deaths. Its risk and pandemic potential attract worldwide attention. In this article, we summarize epidemiology, virology characteristics, clinical symptoms, diagnosis methods, clinical treatment and preventive measures about the avian influenza A/H7N9 virus infection to provide a reference for a possible next wave of flu outbreak.

Key words: H7N9 virus; avian influenza A; infection; diagnosis; treatment; prevention.

Background

During February 19 through March 7, 2013, three patients with severe pneumonia and pulmonary dysfunction in Shanghai and Anhui of China were admitted to hospitals successively. All cases had typical influenza-like symptoms such as cough, fever in an early phase, and severe pneumonia and acute respiratory distress with lethal outcome several days after the onset of the initial symptoms. Before too long, many analogous cases were also found in Yangtze River Delta region of China. The following etiological studies revealed that the patients were infected with a novel reassortant avian influenza virus, A/H7N9 virus [1, 2]. On March 31, 2013, China National Health and Family Planning Commission informed that this new avian influenza A/H7N9 virus had caused human deaths in China [3]. It was the first report that the avian influenza A/H7N9 virus could infect human and that a low pathogenic avian influenza virus could cause human deaths [4, 5]. Thus, it has aroused global public health concerns about its risk and pandemic potential [5-7].

Epidemiology

Until October 25, 2013, the World Health Organization (WHO) has reported a total of 137 cases of human infection including 45 fatal cases from ten

provinces, two municipalities of China and Taiwan; Afterwards, other two cases was reported in China. Nearly 90% of the infection took place between March 19 and April 23 [8]. The infection was diffused from Yangtze River Delta region to the surrounding areas and inland (Fig. 1). So far, the case-mortality rate (33%) of the avian influenza A/H7N9 virus infection is significantly higher than that of previous influenza pandemic infection (range, 0.1%-2.5%) [9]. However, a study during March 4 to April 28, 2013 in China showed that only 6 of 20739 (0.03%) patients with influenza-like symptoms were confirmed as cases of infection with the A/H7N9 virus [10]. Accordingly, compared to previous assessment, this avian influenza A/H7N9 epidemic may cause high mortality rate, but it is unlikely to cause a large-scale outbreak [11].

Human infection with the A/H7N9 virus seems to occur through direct contact to infected poultry, most of cases have a history of exposure to live poultry [11, 12]. Zhang et al. investigated 10703 samples from different sources, and the survey report showed that all samples with the A/H7N9 virus were collected from live poultry markets except for one from a homing pigeon farm and another from a wild pigeon [13], suggesting that live poultry markets are the major origins of the A/H7N9 virus infection.

The A/H7N9 virus infection shows two typical epidemiological characteristics: Firstly, the elderly are more susceptible than the young (Fig. 2a) [11, 14, 15]. The average age of 125 infected cases was 62 [11, 16].

This may be explained by the facts that the aged spend more time in live poultry markets and therefore were more likely to be exposed to live poultry, and that they generally have weak immunity levels related to aging or comorbidities [11, 14, 17, 18]. Secondly, sex differences exist in the A/H7N9 virus infection (Fig. 2b), with the number of males being significantly greater than that of females [9, 11], whereas both of the avian influenza H5N1 and the pandemic H1N1 influenza virus infections exhibit no sex bias [15, 19]. A study conducted by Cowling group revealed that the preference for males occurs only in urban areas but not in countryside, implying that the current sex bias is attributed to different customs or habits which are associated with gender in different regions rather than some previous speculations about physiological differences between males and females [4, 15].

Although most of the patients appear to be sporadic and most of close contacts are negative for A/H7N9 virus [12], researchers found that human H7N9 virus isolates could efficiently spread among ferrets via direct contact [20] and respiratory droplets [13]. However, so far, human-to-human transmission remains very uncommon at this time. In fact, in a family cluster with two patients, the second patient developed symptoms after providing care for the index patient without protection, and genome sequences of the strains isolated from the two patients were almost identical, demonstrating that person-to-person transmission is feasible under certain conditions [21].
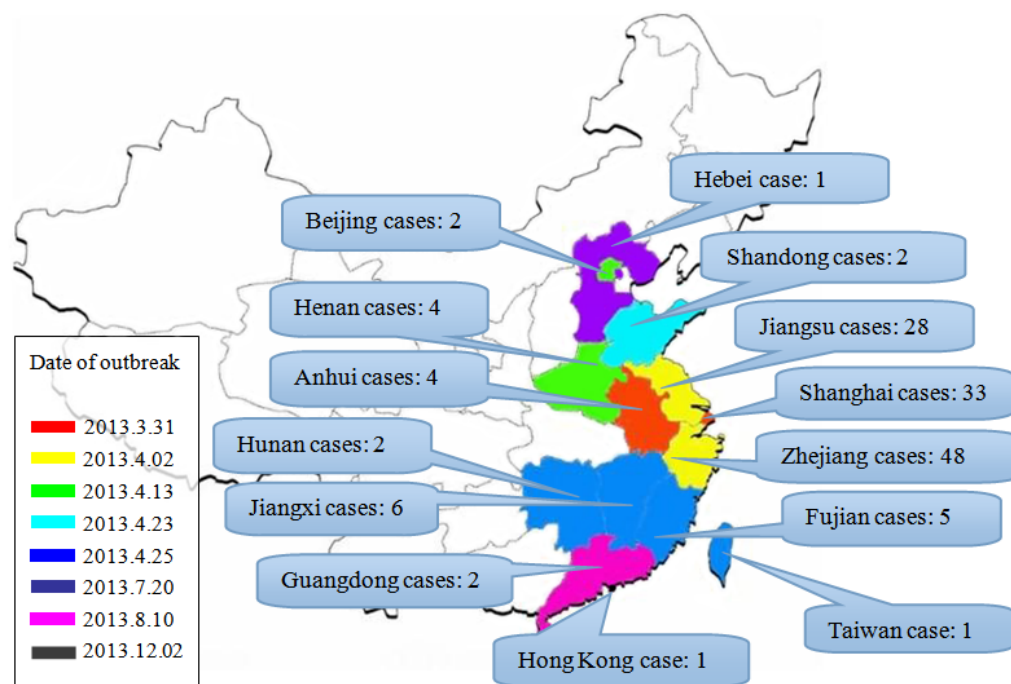

Figure 1. Geographical distribution of the A/H7N9 infection and the date when the first case of infection was reported in each region. Note: the statistical data are up until December 2, 2013.
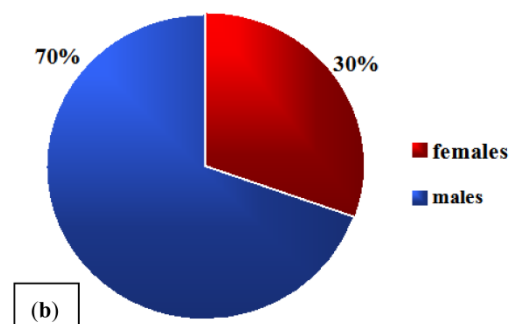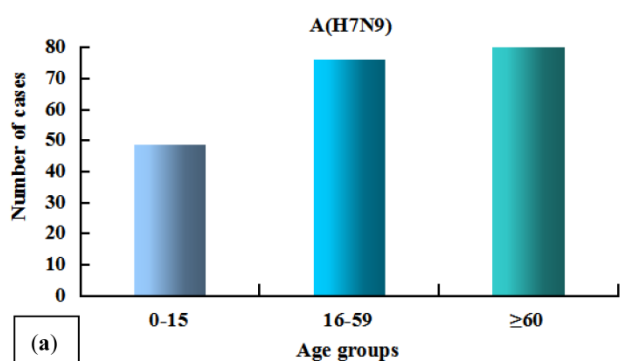
Figure 2. The age (a) and sex (b) distribution of 125 cases. The data are obtained from references [11] and [16].

Virology

Phylogenetic analysis. The new A/H7N9 virus is a reassortant of earlier H7N9, H7N3, and H9N2 viruses [22-24]. Its hemagglutinin (HA), neuraminidase (NA), nonstructural protein (NS) may come from A/duck/Zhejiang/12/2011-H7N3, A/wild bird/Korea/A14/2011-H7N9 and A/chicken/Dawang/1/2011-H9N2, respectively, whereas the other five internal genes matrix (M), nucleoprotein (NP), acidic polymerase (PA), basic polymerase 1 (PB1) and basic polymerase 2 (PB2) appear to be originated from more than one source [25]. For M, PA, PB1 and PB2, each of them has a cluster together with the A/brambling/Beijing/16/2012-H9N2 and the other H9N2 clusters previously found in chickens in Yangtze River Delta, indicating that they might stem from the A/brambling/Beijing/16/2012-H9N2, and have reassorted and evolved rapidly into several clusters [26, 27]. Based on these results, it is speculated that the reassortment path of the new A/H7N9 virus is as follows (Fig. 3): the earlier A/H7N9 virus in migratory birds reassorted with H9N2 virus in northern China, so the reassortant virus retained the NA of H7N9 virus and obtained five internal gene fragments (PB2, PB1, PA , NP and M) from H9N2; when birds migrated to Jiangsu, the reassortant virus HXN9 (x = 7/9) reasserted with H9N2 (A/chicken/Dawang/1/2011-H9N2) again and gained its NS segment; meanwhile, the A/duck/Zhejiang/12/2011-H7N3 virus reassorted with some H9N2 strains in Yangtze River Delta and formed H7NX (x = 2/3), which retained H7 of the H7N3 and obtained PB2, PB1, PA, NP and M from the H9N2 viruses; at last, HXN9 reassorted with H7NX, consequently generating this new human-pathogenic A/H7N9 virus.
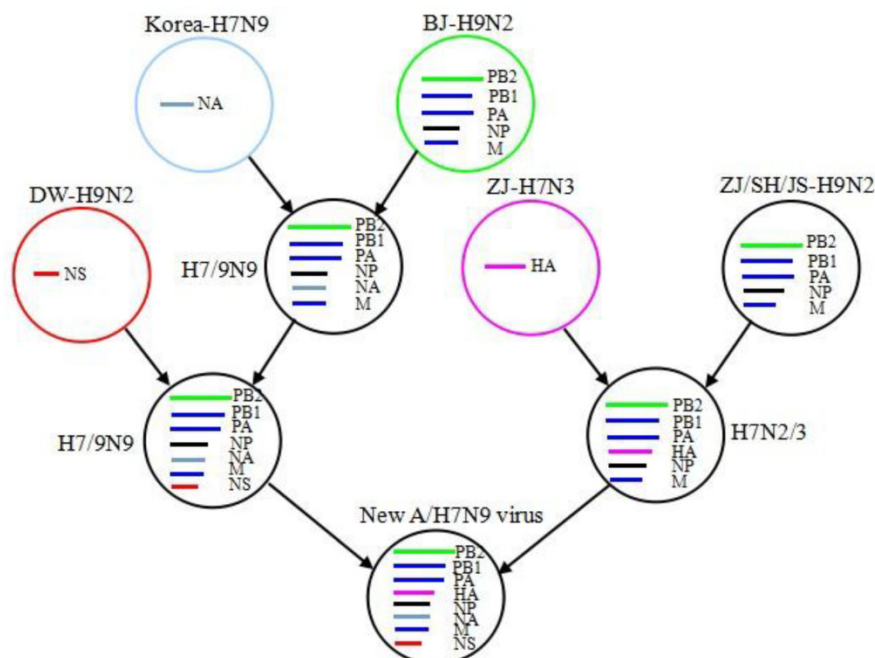

Figure 3. The reassortment path of a new A/H7N9 virus. BJ, Beijing; DW, Dawang; ZJ, Zhejiang; SH, Shanghai; JS, Jiangsu.

A series of reassortments enable the viruses to exchange their gene fragments very rapidly and facilitate a virus adaptation to human cells. Thus, it is necessary to care about ongoing reassortments and analyze the latest data timely, which will help us take prompt and effective measures to prevent potential infection outbreaks [28, 29].

Molecular signatures of new A/H7N9 viruses. Gene reassortments promote the formation of new virus strains while gene mutations facilitate the emergence of mammal adaptation and the increase in virulence. The new avian influenza A/H7N9 virus has possessed several signatures related to mammal adaptation and other characteristics so far (Table 1) [29-45]. The A/H7N9 virus could bind to both avian-type (α-2, 3 sialic acid) and human-type (α-2, 6 linked sialic acid) receptors. This property may attribute to several amino acid changes occurring in the receptor-binding site (RBS) of HA, such as Q226L (H3 numbering system) [23]. The single Q226L amino acid substitution could modulate the receptor-binding preference of viruses by generating a non-polar binding site for C-6 and the ring carbons of galactose-2 for the human receptor, thus providing a more hydrophobic binding site which favors combination with human receptor [46-50]. Watanabe et al. [51] reported that the A/H7N9 virus bearing L226 appears to be more efficient in infecting mammalian cells and releasing from infected cells. Further analysis revealed that the A/H7N9 virus bearing Q226 preferentially bound to the avian-type receptor; while the A/H7N9 virus bearing L226, exhibited an increased binding to both avian-type and human-type receptors [47, 52, 53]. The findings demonstrated that the viruses retained the preference for the avian receptor, which is not conducive to human-to-human transmission, at the same time, acquired other receptor-binding specificities characteristic of typical pandemic influenza viruses.

PB2 is another important determinant of the host range and virulence of influenza viruses. A critical mutation in PB2 of A/H7N9 virus, E627K, which was previously found in A/H5N1 [54] and A/H7N7 viruses [55], is considered contributing to the improvement of viral polymerase activity at human upper respiratory tract temperature (32-33°C) and thus facilitate the adaptation of the viruses to human. Notably, the E627K mutation can be detected in almost all the A/H7N9 isolates from human cases, but was not detected in any poultry or environmental isolates, suggesting that the E627K mutation took place after virus transmission from poultry to human [13, 29, 30, 51].

In addition, another interesting molecular feature of the A/H7N9 virus is the absence of multibasic amino acid motif in the HA cleavage site. The multibasic cleavage site is a sign of high pathogenicity for birds, but not for human, which may be the reason why the A/H7N9 virus infection causes human deaths while only mild symptoms in poultry [23, 50]. These important molecular signatures indicate that nascent A/H7N9 viruses may be readily transmitted to human, and they are, therefore, more difficult to deal with.

Clinical characteristics. The most common clinical symptom of patients infected with A/H7N9 viruses is severe pneumonia [56]. Most of cases manifested fever, cough with sputum production, wheeze, headache, myalgia and general malaise during early stage and later severe illnesses characterised by bilateral pneumonia, rapid progression to acute respiratory distress syndrome (ARDS), multiorgan dysfunction (impaired liver or renal function), septic shock, rhabdomyolysis and encephalopathy [29, 30, 57-61]. Bilateral ground-glass opacities, consolidation and pleural effusion are the typical radiologic findings [30, 56, 62]. Critically ill patients also generally exhibit lymphopenia and thrombocytopenia [49, 56], along with the increase of some serum cytokines or chemokines, including lactate dehydrogenase, creatinine kinase, aspartate aminotransferase, C-reactive protein, IP-10, MIG, MIP-1β, MCP-1, IL-6, IL-8 and IFN-α [46, 50, 62].

Table 1. Molecular signatures of the A/H7N9 virus.

Gene	Molecular signatures	Effect
HA	Q226L/I, G228S, T160A, A138S, G186V, G195V, D285N	Increased affinity to human-type receptors
	PEIPKGR*G in HA cleavage site	Low pathogenic to birds
NA	R292K, R152K	Resistance to neuraminidase inhibitors
	69-73 deletion	Adapted to terrestrial birds and increased virulence
PB2	L89V, E627K, N701D, A44S	Improved replication and virulence in mammals
PB1	H99Y, I368V	Related to H5 virus transmission among ferrets
M1	N30D, T215A	Increased virulence in mice
M2	S31N	Amantadine resistance
NS1	P42S	Increased virulence in mice
PA	V100A, K356R, S409N	Increased virulence to mammals

Diagnosis

The diagnosis of patients infected with the A/H7N9 virus should follow *the diagnosis and treatment scheme of human infection with H7N9 avian influenza (Version 2, 2013)* [63]. It is noteworthy that a sputum specimen is more likely to show A/H7N9 virus positive than that of throat swab [50, 64-66]. Infected cases can be confirmed by means of virus isolation, serologic testing, and real time reverse transcriptase polymerase chain reaction (RT-PCR) assays [67-69]. Among these methods, the RT-PCR assay is the most common method and the gold standard of influenza virus detection [12, 70].

Baas et al. examined the ability of six antigen detection-based rapid influenza point-of-care tests (POCTs) in detecting the A/H7N9 virus. The low Ct values suggested that these POCTs could not be employed in the detection for the A/H7N9 infection [71]. In resource-limited regions, a subtype-specific reverse transcription loop-mediated isothermal amplification assay (LAMP) could be used as a reliable method for the early diagnosis. The procedure of this reaction only contains one step, 63°C for 60 min in a single tube containing hydroxynaphthol blue dye, using a strand displacement DNA polymerase and 4 primers designed for six regions of target genes. Its detection limits are comparable to corresponding H7 and N9 RT-PCR assays [72, 73].

Treatment and prevention

Treatment recommendations. Due to the acute progresses and severe outcomes of the A/H7N9 virus infection, it is suggested that all the confirmed cases and suspected cases received antiviral treatment as soon as possible [74-75]. Mounting evidence indicated that the early treatment is probably the major decisive factor for survival in the A/H1N1 and A/H5N1 virus infection [76, 77]. Therefore, poor outcomes in some A/H7N9 virus-infected patients may be attributed to the delayed antiviral treatment [78]. As for severe cases with respiratory dysfunction, mechanical ventilation should be supported without delay.

Therapeutic use. Preliminary data suggest that the A/H7N9 virus is resistant to adamantane because of the S31N mutation in the virus matrix2 (M2) protein which has been found in all isolates [79, 80], however, it remains sensitive to neuraminidase inhibitors, such as oseltamivir and zanamivir [50, 78, 81].

It is worth noting that R292K in NA, a mutation in a seasonal H3N2 influenza virus, which is considered to confer resistance to oseltamivir [82], has emerged in avian influenza A/H7N9 viruses. The R292K mutation causes a significant expansion in the active site cavity and increases in its solvent accessible surface, thereby leading to a remarkable loss of total binding free energy for NA-oseltamivir and an unfavorable shift in van der Waals interactions which may contribute to drug resistance [83]. Even though patients, who are infected with the A/H7N9 virus carrying R292K mutation, are treated with neuraminidase inhibitors timely, some of them fail to respond to the treatment, exhibiting persistent or rebound virus titer and poor outcomes [78, 84]. Nevertheless, neuraminidase inhibitors are still the front-line therapeutic choices against the A/H7N9 virus infection.

In addition, a study on glucocorticoids used against the A/H7N9 virus infection was reported [30]. Results showed that although glucocorticoids can generally be used as anti-inflammatory agents and immunomodulators [85, 86], they have been proved no benefit to the A/H7N9 virus infection [30]. It is noteworthy that two isolates from patients gained R292K mutation after administration of corticosteroids [78]. It remains to be seen whether corticosteroids could accelerate the emergence of R292K mutation [87]. At least, corticosteroids should be avoided for the treatment of A/H7N9 virus infection at present [64, 88].

Advances in the development of anti-flu drugs. Gene mutations of the A/H7N9 virus associated with drug resistance emphasize the importance of developing new drugs with different action mechanisms [88]. Currently novel anti-flu agents that target viral key proteins, host factors have achieved varying degrees of progress [89-96]. Several novel anti-influenza virus drugs are currently in clinical trials, including favipiravir, laninamivir, DAS181, Vertex VX-787, AVI-7100, apomivir, ergoferon and nitazoxanide [97]. Some of the screening drugs studied will probably be effective to A/H7N9 influenza even though none of them have been specifically designed for the A/H7N9 influenza.

Vaccines for avian influenza A (H7N9). Vaccine is considered as the most important potential means for controlling the A/H7N9 influenza transmission [98]. Scientists around the world have been working on the development of vaccines specific to A/H7N9 virus in order to prevent a potential pandemic bird flu. Previous reports have demonstrated that H7 protein is poorly immunogenic in humans [5, 99-101] because of a very low T cell epitope content in the HA sequences and poor potential for cross-reactivity with the T cell epitope of currently circulating strains of influenza [101]. It, therefore, seems difficult to develop an effective vaccine for the A/H7N9 virus. Even so, at least four companies have succeeded in developing A/H7N9 candidate vaccines, including Greffex, Protein sciences, Medicago, and Novavax [102, 103].

All four companies adopted modern genetic engineering techniques to prepare key protein-containing vaccine for the A/H7N9 virus infection. Two virus-like particles (VLP) vaccines developed by Medicago and Novavax yielded promising results (pre-) clinical trials (Fig. 4) [103, 104].

The A/H7N9 VLP vaccine developed by Novavax consists of HA (A/Anhui/1/2013-H7N9), NA (A/Anhui/1/2013-H7N9) and M1 (A/Indonesia/05/ 2005-H5N1). This type of VLP not only elicits hemagglutination-inhibition (HAI) antibody titers of ≥1:64 and anti-neuraminidase (NA) antibody against the A/H7N9 virus, but also provides cross-protection for other H7 subtype virus, such as the H7N3 virus. In experimental mouse models, all animals receiving the A/H7N9 vaccine survived, while no survival was observed in control groups receiving an A/H5N1 vaccine or placebo [103]. These promising results may be ascribable to a fact that VLP can be presented not only by the MHC-II molecules for stimulation of CD4 helper T cells, but also by the MHC-I molecules for activation of cytotoxic T-lymphocytes (CTLs) [105]. Moreover, the path of VLP production is simple and time-saving. In fact, it needs only 26 days for the genetic construction of the vaccine for the production of an A/H7N9 VLP. The resulting VLP preparation does not contain genetic materials, which are essential for viral replication and infection. Therefore, compared with traditional inactivated or attenuated vaccines, the preparation of VLP is more convenient and safer when pandemic bird flu takes place suddenly. Other studies including phase I clinical trials in human are ongoing and anticipated be completed in August 2014 [97].

Prevention and control. Owing to profound lessons from SARS and experience from the A/H5N1 and A/H1N1 epidemics, responses of the Chinese Government to the A/H7N9 epidemic are swift and transparent [106-108]. Relevant departments quickly identified the cause of the disease and the source of the A/H7N9 virus, followed by a series of control measures, including: (1) closed live poultry markets, culled live poultry and prohibited the entry of adventive live poultry; (2) publicized preventive knowledge through various kinds of media; (3) strengthened surveillance on epidemic dynamics. These measures were proved effective as a prominent reduction occurs in the number of newly emerged cases since the closure of live poultry markets (Fig. 5) [111-113]. However, it remains a great challenge for the Chinese Government to control the epidemic. Because of the low pathogenicity of A/H7N9 virus to poultry and wild birds [4, 5], the virus may spread silently among poultry without being noticed and can infect human irregularly. Although measures currently taken by the government such as closing live poultry markets and culling live poultry have made great progress merely for disease control rather than prevention, these strategies can only control the transmission of the A/H7N9 epidemic but not block the formation of the epidemic. Therefore, searching for specific preventive measures to the A/H7N9 influenza is imperative.
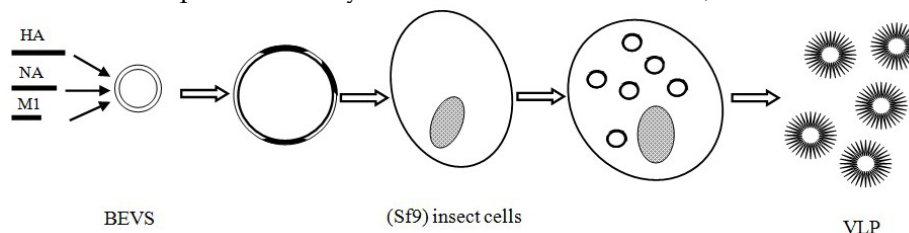

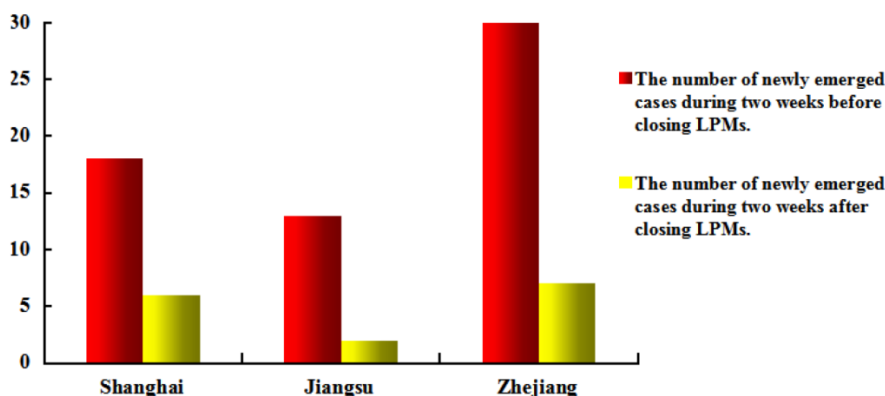**Figure 4.** The method of virus-like particles (VLP) vaccine production.

Figure 5. Impacts of closing live poultry markets (LPMs) on A/H7N9 epidemic.

Conclusion

The new reassortant A/H7N9 virus primarily infected human and has caused several deaths in China. Although there is no sufficient evidence to indicate sustained human-to-human transmission yet, this risk will increase as a result of frequent gene reassortants and mutations. Typical symptoms of the avian influenza A/H7N9 virus infection include fever, cough, even severe pneumonia, and multi-organ dysfunction syndrome. So far, real-time PCR is still the main diagnostic method, and neuraminidase inhibitors (oseltamivir, zanamivir, and peramivir) are used for clinical treatment but the M2 ion channel blockers (amantadine and rimantadine) are invalid; at the same time, the continuing resistance mutations have been hindering the treatment. It is thus urgent to explore new drugs and therapy strategies. Whereas closing live poultry markets, culling live poultry, and other control measures have prevented the transmission of the epidemic successfully, the pathologic characteristics of the A/H7N9 virus (low pathogenic to avian but high to human) may lead to insidious outbreak of deadly avian flu, and the molecular characteristics of the A/H7N9 virus make it difficult to develop effective vaccines. Along with a decrease in absolute humidity in autumn and winter, the survival and transmission abilities of influenza viruses will increase apparently [114]. If the A/H7N9 virus infection follows a similar epidemic pattern to that of the A/H5N1 or other pandemics, it will recur in autumn and winter. Thus, continually intensive surveillance and prepared measures for the prevention of major outbreaks remain quite important.

Acknowledgments

Competing Interests

The authors have declared that no competing interest exists.

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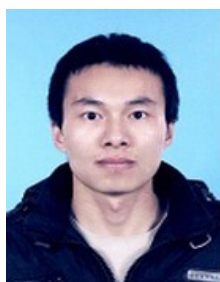
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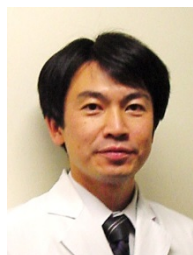
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