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Vaccination of poultry successfully eliminated human infection with H7N9 virus in China

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The H7N9 viruses that emerged in China in 2013 were nonpathogenic in chickens but mutated to a highly pathogenic form in early 2017 and caused severe disease outbreaks in chickens. The H7N9 influenza viruses have caused five waves of human infection, with almost half of the total number of human cases (766 of 1,567) being reported in the fifth wave, raising concerns that even more human infections could occur in the sixth wave. In September 2017, an H5/H7 bivalent inactivated vaccine for chickens was introduced, and the H7N9 virus isolation rate in poultry dropped by 93.3% after vaccination. More importantly, only three H7N9 human cases were reported between October 1, 2017 and September 30, 2018, indicating that vaccination of poultry successfully eliminated human infection with H7N9 virus. These facts emphasize that active control of animal disease is extremely important for zoonosis control and human health protection.

H7N9 influenza virus, evolution, vaccination, human infection, elimination

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Influenza A viruses are important zoonotic pathogens that continually challenge animal and human health. The genome of influenza A virus comprises eight gene segments: basic polymerase 2 (PB2), basic polymerase 1 (PB1), acidic polymerase (PA), hemagglutinin (HA), nucleoprotein (NP), neuraminidase (NA), matrix (M), and nonstructural protein (NS). Each of these segments encodes one to three proteins (Vasin et al., 2014). On the basis of the antigenicity of the HA and NA proteins, influenza viruses are divided into different subtypes. H1N1, H2N2, and H3N2 viruses have caused four influenza pandemics since 1918, and H1N1 and H3N2 viruses continue to co-circulate in humans globally (Kilbourne, 2006; Li and Chen, 2014). The highly pathogenic H5 and H7 viruses bearing different NA subtypes have caused numerous disease outbreaks in domestic poultry and wild birds around the world since 1959 (Alexander and Brown, 2009; Chen, 2009b; Swayne, 2012).

The H7N9 influenza virus caused severe human infections as soon as it emerged in China in 2013 (Gao et al., 2013), and it is considered one of the viruses most likely to cause a human influenza pandemic (Watanabe et al., 2013; Zhang et al., 2013a; Zhu et al., 2013). To monitor H7N9 viruses in poultry, we performed active surveillance by collecting over 177,000 samples from poultry markets, farms, wild bird habitats, and slaughterhouses all over China between March 2013 and January 2017, and isolated 666 H7N9 viruses and two H7N2 viruses; the two H7N2 viruses were respectively isolated from a chicken in 2014 and from a duck in 2018 (Shi et al., 2017; Shi et al., 2018; Shi et al., 2014; Zhang et al., 2013a). Detailed analysis of these strains provided a complete evolutionary picture of H7N9 viruses in nature. Importantly, the detection of H7N9 highly pathogenic virus in early 2017 and studies in animal models that revealed the high pandemic potential of the H7N9 viruses and their in-

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creased threat to human health (Shi et al., 2017) have provided very important information for the active control of H7N9 influenza in China. In this review, we briefly summarize our current understanding of the properties and evolution of the H7N9 influenza viruses, discuss the effectiveness of the poultry H7N9 vaccine, and describe the advantages and challenges of vaccination as a control measure for zoonotic H7N9 influenza.

GENETIC PROPERTIES AND EVOLUTION OF THE H7N9 INFLUENZA VIRUSES

The H7N9 influenza viruses were initially detected in humans in Shanghai and Anhui provinces who suffered rapidly progressing lower respiratory tract infections (Gao et al., 2013). To investigate the possible origins of the H7N9 viruses that caused these human infections, samples from poultry markets, poultry farms, wild bird habitats, and poultry and swine slaughterhouses were collected and analyzed. H7N9 viruses were detected in the samples that were collected from live poultry markets, but not in other locations (Shi et al., 2013; Zhang et al., 2013a), indicating that the live poultry markets were the source of the H7N9 viruses that caused these human infections.

Genetic analysis of the 2013 H7N9 viruses indicated that the viruses were reassortants that derived their six internal genes from the local H9N2 avian influenza viruses and their HA and NA genes from unknown H7Nx and HxN9 viruses (the x represents the unknown subtype) (Shi et al., 2013;

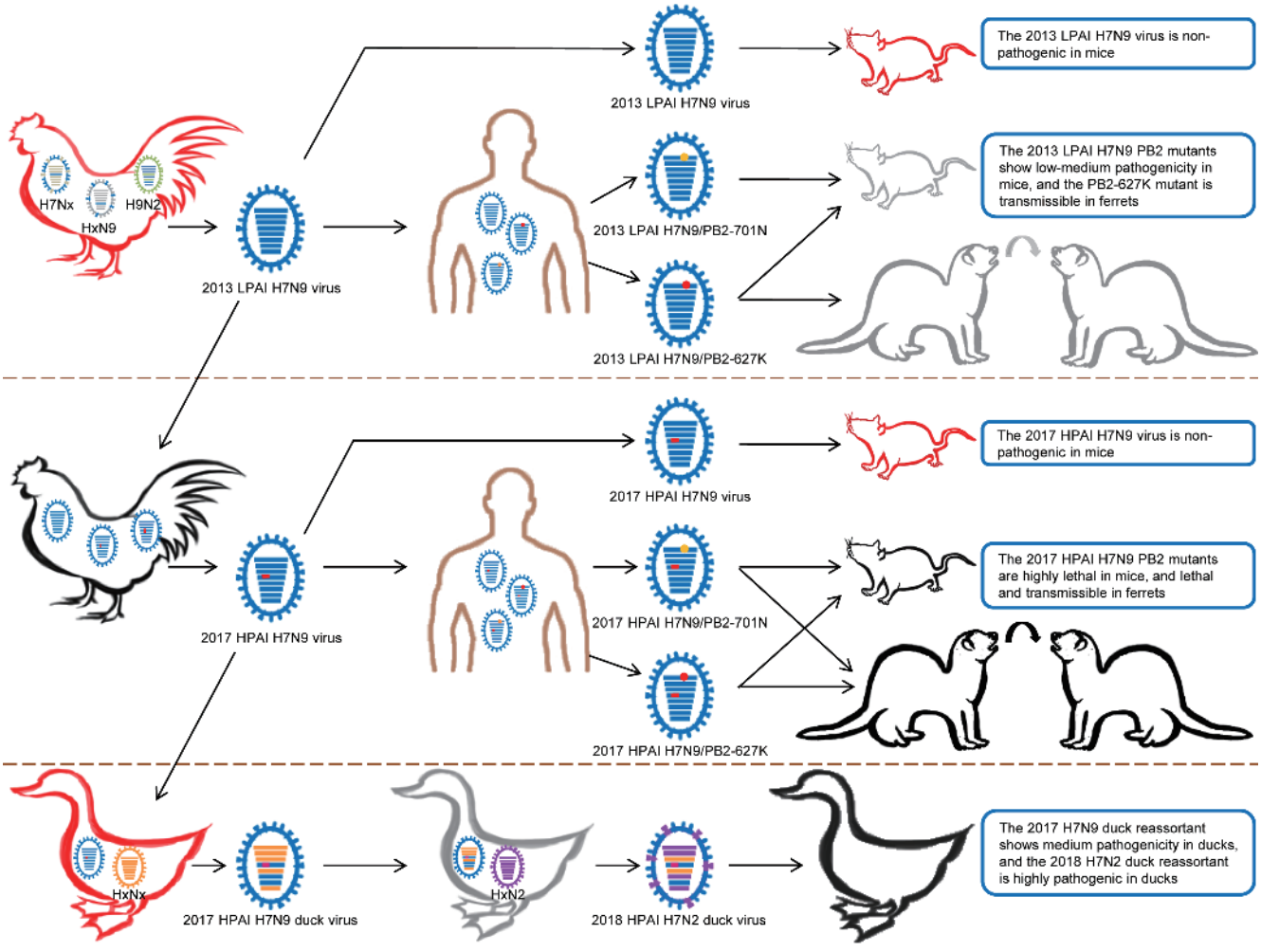

Figure 1 Genetic and biologic evolution of H7N9 influenza viruses. The 2013 low pathogenic avian influenza H7N9 virus (2013 LPAI virus) mutated to a highly pathogenic form in 2017 (2017 HPAI virus) by obtaining an insertion of four amino acids (indicated by the short red bar in the blue virus sketch) in its hemagglutinin (HA) cleavage site. The 2017 HPAI virus further reassorted with different duck influenza viruses and formed new H7N9 and H7N2 reassortants (genes from other duck viruses are shown in dark yellow and purple). Both 2013 LPAI H7N9 virus and 2017 HPAI virus could obtain the PB2 627K or PB2 701N mutation (indicated by the red dot and yellow dot, respectively, in the blue virus sketch) during their replication in humans. The pathotypes of the viruses in different birds or mammals are indicated by the color of the animal sketches: red, non-lethal; grey, low-medium pathogenicity; black, highly pathogenic.

Zhang et al., 2013a) (Figure 1). The cleavage site of their HA genes contains a single basic amino acid, a characteristic of low pathogenic influenza virus in chickens. In this review, we refer to these viruses as 2013 low pathogenic avian influenza H7N9 virus (2013 LPAI H7N9 virus). After four years of circulation in nature, a few strains isolated from chickens in Guangdong province in 2017 were found to have acquired 12 extra nucleotides (-aaacggactgcg-) encoding four amino acids (-KGKRTAR/G-) in the cleavage site of HA (Shi et al., 2017), a motif for increased virulence of H5 and H7 viruses in chickens (Neumann and Kawaoka, 2006). In subsequent surveillance, four more different motifs were detected in the HA cleavage sites of H7N9 strains: -K*R*KRAAR/G-, -K*R*KRTAR/G-, -KGKRIAR/G-, and -K*R*RRTAR/G- (the four amino acids underlined were insertions, whereas the amino acid R shown in italics was a mutation of the amino acid G at that position), and viruses bearing the motif "-K*R*KRTAR/G-" were widely detected among viruses isolated from birds and humans (Shi et al., 2018; Yang et al., 2017). We refer to the virus with the HA insertion as 2017 highly pathogenic avian influenza H7N9 virus (2017 HPAI H7N9 virus) in this review.

The internal genes of the H7N9 viruses are more diverse than their HA and NA genes, which suggests that the viruses underwent frequent reassortment and rapid evolution. It is important to note that the internal genes of the 2013 LPAI H7N9 viruses detected so far are all derived from the H9N2 viruses (Shi et al., 2013). H9N2 subtype avian influenza viruses (AIVs) have widely circulated throughout the world since their first detection in turkeys in Wisconsin in 1966 (Homme and Easterday, 1970). In China, H9N2 AIVs were first isolated from chickens in Guangdong province in 1994 (Chen et al., 1994); the viruses then spread to poultry in other provinces (Li et al., 2005a). Li et al. performed extensive analysis of the H9N2 viruses circulating in poultry in China and found that these viruses preferentially bind to the human-type receptor, and that some of them can cause disease and transmit between ferrets by respiratory droplets (Li et al., 2014b). All of the transmissible H9N2 viruses have a similar internal gene constellation, which is also present in the H7N9 and H10N8 viruses (Chen et al., 2014; Deng et al., 2015; Li et al., 2014b; Shi et al., 2013; Zhang et al., 2013a). Li's study also suggested that the stable internal-gene-constellation of H9N2 viruses may favor the rapid adaptation of the H7N9 virus in chickens.

In May 2017, some viruses isolated from ducks in Fujian province were proven to be novel H7N9 reassortants bearing the HA, NA, and M genes of a 2017 HPAI H7N9 virus and the PB2, PB1, PA, NP, and NS genes from an unknown duck virus (Shi et al., 2018). In January 2018, the duck H7N9 virus further reassorted with an unknown HxN2 virus and generated an H7N2 virus, which bears the HA and M genes of a 2017 HPAI H7N9 virus and its other six genes from unknown duck viruses (Shi et al., 2018) (Figure 1).

The receptor-binding property of influenza virus is determined by the HA protein. The HA of human infective influenza subtypes preferentially recognizes α-2,6-linked sialic acids (SAs) (human-type receptors), whereas the HA of avian-infective influenza subtypes preferentially recognizes α-2,3-linked SAs (avian-type receptors) (Herfst et al., 2012; Rogers and Paulson, 1983). The H7N9 viruses bind to human-type receptors with high affinity, but they also maintain the ability to bind avian-type receptors, with affinities that vary according to the strain (Belser et al., 2013; Watanabe et al., 2013; Xiong et al., 2013; Zhang et al., 2013a; Zhou et al., 2013). Two amino acids—valine (V) at position 186 and leucine (L) at position 226—in the HA protein (H3 numbering) were reported to have a significant role in H7N9 virus binding to the human-type receptor (Xiong et al., 2013). It is important to note that over 90% of H7N9 viruses detected from both avian species and humans bear these two amino acids (Shi et al., 2017), which explains why the H7N9 avian influenza viruses more readily infect humans compared with other avian influenza viruses circulating in poultry.

Two key amino acid mutations, glutamic acid (E) to lysine (K) at position 627 (E627K) and aspartic acid (D) to asparagine (N) at position 701 (D701N), in the PB2 protein have been widely reported to increase the replication, virulence, and transmission of avian influenza virus in mammalian hosts (Gabriel et al., 2005; Gao et al., 2009; Hatta et al., 2001; Hatta et al., 2007; Li et al., 2005b; Steel et al., 2009; Subbarao et al., 1993). Detailed comparisons of avian and human isolates indicated that all of the H7N9 viruses isolated from avian species have 627E and 701D in their PB2 gene, but over 80% of the H7N9 viruses isolated from humans have 627K or 701N in their PB2 gene (Shi et al., 2017), indicating that the H7N9 viruses can easily obtain such mutations during their replication in humans (Figure 1).

INSIGHTS FROM ANIMAL STUDIES

Various species have been used to evaluate the replication and virulence of H7N9 influenza viruses. The 2013 LPAI H7N9 viruses replicated efficiently in chickens and were shed for up to 7 days. These viruses also transmitted efficiently from infected chickens to chickens that came into contact with them, but they did not cause any disease in chickens (Zhang et al., 2013a). The 2013 LPAI virus also replicated in quails, and these birds shed high titer viruses but did not show disease (Pantin-Jackwood et al., 2014). Ducks inoculated with the 2013 LPAI H7N9 viruses do not shed virus, and rarely seroconvert (Pantin-Jackwood et al., 2014; Zhang et al., 2013a). These studies indicate that the 2013 LPAI H7N9 virus can silently replicate in chickens and

quails, suggesting that these birds may play an important role in 2013 LPAI H7N9 virus spread.

The 2017 HPAI H7N9 viruses replicated systemically in chickens, killed chickens within 24 hours of intravenous inoculation, and yielded an intravenous pathogenicity index value of up to 3 (0=least pathogenic; 3=most pathogenic) (Qi et al., 2018; Shi et al., 2017). The early chicken isolates had limited replicative ability in ducks and were only detected in the pharynx of inoculated ducks. However, several viruses isolated from ducks have exhibited increased replicative ability in ducks; in addition of the pharynx, these viruses could also be detected in other organs of ducks (Shi et al., 2018). The H7N9 and H7N2 viruses that bearing certain genes from other duck influenza viruses replicated systemically in ducks and showed medium-to-high pathogenicity in ducks (Shi et al., 2018) (Figure 1).

Surveillance data and studies performed in chickens and ducks indicate that, during circulation in chickens, the 2013 LPAI H7N9 viruses obtained certain amino acids in their HA cleavage site and then became highly pathogenic in chickens; the replicative ability of 2017 HPAI H7N9 viruses enables them to reassort with other duck viruses and generate novel lethal H7N9 and H7N2 viruses in ducks (Shi et al., 2018) (Figure 1). These facts indicate that AIVs could obtain their increased virulence through different mechanisms in different hosts.

Mice have been widely used to evaluate the replication and virulence of AIVs in mammals (Feng et al., 2016; Jiao et al., 2008; Li et al., 2005b), and studies indicate that the virulence of H5N1 virus in mice correlates with its virulence in humans (Hatta et al., 2001; Li et al., 2010; Lu et al., 1999). Zhang et al. compared the replication and virulence of 2013 LPAI H7N9 viruses isolated from avian species and humans and found that all three avian isolates tested replicated in the nasal turbinates and lungs of mice but did not cause any disease or death in mice. The three human isolates tested—A/Anhui/1/2013 (AH/1), A/Shanghai/1/2013 (SH/1), and A/Shanghai/2/2013 (SH/2)—caused body weight loss and killed mice in the high-dose (10^5 or 10^6 EID_{50})-inoculated groups (Zhang et al., 2013a). The AH/1 virus was also tested in mice by two other laboratories (Belser et al., 2013; Watanabe et al., 2013), and it seems that their AH/1 stocks were more lethal in mice than the stock in our laboratory.

Ferrets and guinea pigs have been widely used to evaluate the transmissibility of influenza viruses (Gao et al., 2009; Herfst et al., 2012; Imai et al., 2012; Lowen et al., 2006; Wang et al., 2017; Yang et al., 2016; Zhang et al., 2012; Zhang et al., 2013b). Respiratory droplet transmission is commonly used to evaluate the airborne transmission potential of influenza viruses. Generally, a novel virus that is highly transmissible in these animal models is thought to have a higher pandemic potential than one that does not transmit in these models. Usually three to six pairs of animals are used to test for transmission of a virus. The donor animals are inoculated intranasally with the test virus and then housed separately in specially designed cages within an isolator. Twenty-four hours later, naïve animals are placed in adjacent cages. Each pair of animals is separated by a double-layered net divider. Nasal washes are collected every 2 days from all of the animals beginning 2 days post-inoculation or 1 day post-exposure for the detection of virus shedding. Sera are collected from all animals on day 14 or 21 post-inoculation to look for seroconversion. Respiratory droplet transmission is confirmed when virus is isolated or seroconversion is detected in the naïve exposed animal.

Upon report of H7N9 virus infection in humans (Gao et al., 2013), at least five research groups tested the transmissibility of the 2013 LPAI H7N9 human isolates in ferrets (Belser et al., 2013; Richard et al., 2013; Watanabe et al., 2013; Zhang et al., 2013a; Zhu et al., 2013). Our lab tested two avian isolates and all three of the human isolates reported by Gao et al. (Zhang et al., 2013a), whereas other labs tested one or two of the three human isolates (Belser et al., 2013; Richard et al., 2013; Watanabe et al., 2013; Zhu et al., 2013) (Table 1). We found that one of the avian isolates did not transmit in ferrets, the other avian isolate (PG/S1421) and two of the human H7N9 isolates (SH/1 and SH/2) transmitted in one of three ferret pairs, but the AH/1 virus transmitted in all three pairs (Zhang et al., 2013a) (Table 1). Belser et al. found that the AH/1 and SH/1 viruses transmitted in two of six pairs and one of three pairs, respectively (Belser et al., 2013); Richard et al. found that AH/1 transmitted in three of four pairs (Richard et al., 2013); Watanabe et al. found that AH/1 transmitted in one of three pairs (Watanabe et al., 2013); and Zhu et al. found that SH/2 transmit in two of three pairs (Zhu et al., 2013) (Table 1). The AH/1 virus was also highly transmissible in guinea pigs (Kong et al., 2015). Even though the transmission efficacy varies among different strains, and the transmission efficacy of the same strain varies in the hands of different labs, all of these studies confirmed that the H7N9 human isolates are airborne transmissible in mammals, posing a higher pandemic potential than the H5N1 viruses, which transmit in these animal models only after they have obtained additional mutations in HA and PB2 or have reassorted with other human influenza viruses (Chen et al., 2012; Herfst et al., 2012; Imai et al., 2012; Zhang et al., 2013b).

The virulence and transmissibility of 2017 HPAI H7N9 viruses have also been evaluated extensively (Shi et al., 2017). The index virus, A/chicken/Guangdong/SD008/2017 (CK/SD008), is not lethal in mice; however, when the virus obtained the PB2 627K or PB2 701N mutation, its virulence in mice increased over 10,000-fold (Shi et al., 2017). Recent studies have shown that after circulation in poultry for a few weeks, some viruses acquired the ability to kill mice, with virulence increasing over 1,000-fold compared to the CK/

Table 1 Summary of transmission studies in ferrets of the 2013 H7N9 low pathogenic influenza viruses isolated from humans

Study leader	Animal pairs per experiment	Respiratory droplet transmission (Number of positive pairs/Total number of pairs)[a)]			
		A/Anhui/1/2013 (HA 186V/226L and PB2 627K)	A/Shanghai/1/2013 (HA 186V/226Q and PB2 627K)	A/Shanghai/2/2013 (HA 186V/226L and PB2 627K)	Reference
Hualan Chen	3	3/3	1/3	1/3	Zhang et al., 2013a
Terrence M. Tumpey	3 or 6	2/6	1/3	Not done	Belser et al., 2013
Ron A. M. Fouchier	4	3/4	Not done	Not done	Richard et al., 2013
Yoshihiro Kawaoka	3	1/3	Not done	Not done	Watanabe et al., 2013
Yi Guan and Yuelong Shu	3	Not done	Not done	2/3	Zhu et al., 2013

a) Pair were deemed positive if virus was isolated or seroconversion was detected in the naïve exposed animal.

SD008 virus, even though these viruses did not have the PB2 627K or PB2 701N mutation (Shi et al., 2018).

Shi et al. and Yang et al. tested the transmissibility of 2017 HPAI H7N9 viruses isolated from chickens in ferrets and guinea pigs, and found that these chicken isolates only transmitted in one of three pairs of animals tested (Shi et al., 2017; Yang et al., 2018). As stated above, the H7N9 influenza viruses readily obtain the PB2 627K or 701N mutation during their replication in humans. Shi et al. investigated the transmission potential of 2017 HPAI H7N9 viruses that obtained the PB2 mutations after replication in ferrets (CK/SD008-PB2/627K and CK/SD008-PB2/701N) and found that these viruses were not only highly transmissible in ferrets, but also caused severe disease, killing one of the three ferrets inoculated with each virus (Shi et al., 2017).

Together, these studies using animal models revealed that, after obtaining key mutations in its PB2 protein during replication in humans, both the 2013 LPAI H7N9 virus and the 2017 HPAI H7N9 virus could become highly transmissible in ferrets. The difference is that the 2017 HPAI H7N9 virus could also be highly lethal in mice and ferrets, suggesting an increased threat to humans, which soon proved to be the case when 50% of humans infected with the 2017 HPAI H7N9 virus died (Yang et al., 2017).

EFFECTIVENESS OF H7N9 VACCINATION OF POULTRY

The H7N9 influenza viruses that emerged in February 2013 have caused 1,567 human infections, with a mortality rate of greater than 30%. Between October 1, 2016 and September 30, 2017, there were 766 human cases (48.9% of the total) reported (Figure 2), which raised a concern that an even large number of human infections may occur in the sixth wave. During each human H7N9 infection wave, tons of uninfected poultry and poultry products were destroyed because people were afraid to consume them, which caused considerable damage to the poultry industry in China. The 2017 H7N9 HPAI viruses spread from Guangdong to several other provinces and caused vast outbreaks; millions of chickens were killed in efforts to control the disease (World Organisation for Animal Health, 2017). Given the damage the H7N9 lethal virus has and will cause in poultry and the high risk it poses to human health, control and eradication of both the low and highly pathogenic H7N9 viruses became the highest priority for animal disease control authorities in China in 2017.

China has been a leader in H5 avian influenza vaccine development and application. A series of inactivated vaccines (with seed viruses generated by plasmid-based reverse genetics) have been widely used to control H5 influenza in poultry in China and several other Asian and African countries (Li et al., 2014a; Tian et al., 2005). A novel Newcastle virus-vectored H5 avian influenza bivalent live vaccine has been used in chickens in China since 2006 (Chen, 2009a; Ge et al., 2007), and the first H5 DNA vaccine was recently approved (Jiang et al., 2007; MARA of China, 2018). A duck enteritis virus-vectored bivalent live vaccine has been constructed and found to provide fast and complete protection in ducks against H5N1 avian influenza virus and highly lethal duck enteritis virus (Liu et al., 2011).

An H7N9 vaccine seed virus was generated by reverse genetics. This seed virus (H7N9-Re1) bears the HA and NA genes of a low pathogenic H7N9 virus and the six internal genes of the high-growth A/Puerto Rico/8/1934(H1N1) (PR8) virus (Shi et al., 2018). An H5 inactivated vaccine (Re-8 strain) has been developed and used in poultry in China since 2015 (Zeng et al., 2016). To increase the efficiency of poultry vaccine inoculation, an H5/H7 bivalent inactivated vaccine was developed by using the H7N9-Re1 and H5 Re-8 viruses as seed viruses. The vaccine was extensively evaluated for safety and efficacy against challenge with different H5 and H7 viruses in the laboratory setting. The vaccine provided solid protection against the H7N9 low pathogenic virus, and different H7N9 highly pathogenic viruses in chickens (Shi et al., 2018). The safety and immunogenicity of the vaccine were also evaluated in farmed poultry, including 105,600 chickens, 11,800 ducks, and 6,600 geese, in Guangdong, Guangxi, and Heilongjiang provinces. Although the vaccine was highly immunogenic in

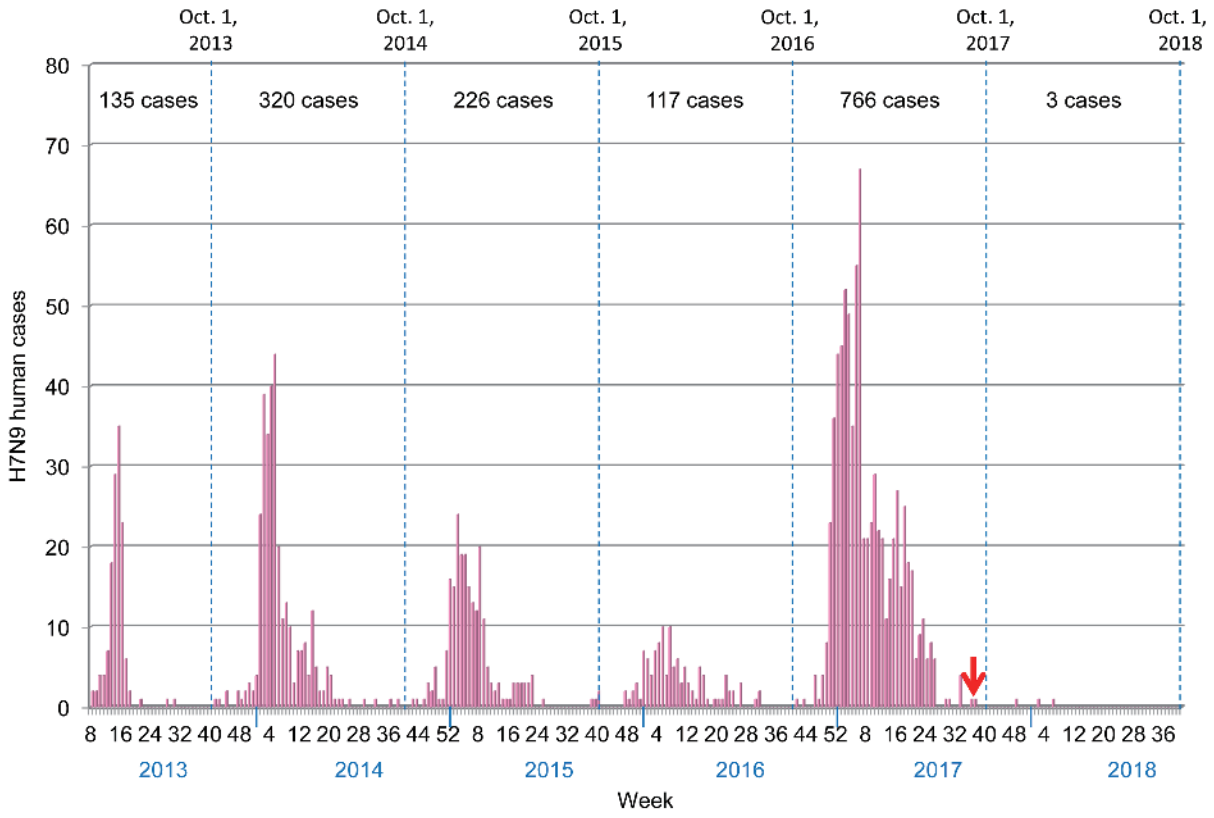

Figure 2 Human infection with H7N9 viruses. The red arrow indicates when H5/H7 vaccine administration to poultry was initiated in China.

chickens, ducks, and geese, H7N9 viruses isolated from chickens did not replicate efficiently and caused disease in ducks and geese; therefore, the vaccine was limited to use only in chickens by the vaccine evaluation expert panel of the China Institute of Veterinary Drug Control.

Eight vaccine companies in China were assigned by the Ministry of Agricultural and Rural Affairs to produce the H5/H7 bivalent inactivated vaccine, the application of which was initiated in September 2017 (Shi et al., 2018). Post-vaccination serological surveillance from October 2017 until January 2018 shown that 73% of farms with layer chickens and slow-growing meat chickens were vaccinated with the H5/H7 vaccine; 20% of farms with layer chickens and slow-growing meat chickens were vaccinated with the H5 single vaccine. The vaccination coverage in farms of fast-growing broilers (meat chickens that are usually slaughtered at 40 days of age) was very low, with only one of the 21 farms investigated being vaccinated with the bivalent vaccine (Table 2) (Shi et al., 2018).

Large scale virus surveillance performed before and after the H5/H7 bivalent vaccine application indicated that the vaccination dramatically reduced the prevalence of H7N9 virus in poultry. The first round of surveillance was performed before the vaccination, from February 2017 to May 2017, and 306 H7N9 viruses were isolated from 30,201 samples collected (isolation rate: 1.013%). The second round of surveillance was performed after the vaccination, from October 2017 to January 2018, and only 16 H7 viruses (15 H7N9 and one H7N2) were isolated from 23,683 samples (isolation rate 0.068%) (Shi et al., 2018); in other words, the H7N9 virus isolation rate was reduced by 93.3% after chickens were inoculated with the H5/H7 vaccine.

More importantly, the vaccination of chickens successfully prevented or eliminated the next human infection wave with H7N9 virus. As shown in Figure 2, the H7N9 influenza virus caused five waves of human infection since 2013, and the second through the fifth waves started in around October each year. There were 766 human cases (49.0% of the total cases across the five waves) detected between October 1, 2016 and September 30, 2017, which had raised concern that an even large number of human infections with H7N9 virus would occur in the "sixth" wave. However, after the introduction of H5/H7 vaccine in chickens, there have been only three H7N9 human cases reported since October 1, 2017 (World Health Organization, 2018) (Figure 2).

CHALLENGES OF THE VACCINATION STRATEGY

The vaccination of poultry with the H5/H7 vaccine successfully reduced the H7N9 virus prevalence in chickens and

Table 2 Post-vaccination serological surveillance in chicken farms from October 2017 to January 2018 in China

Chicken species	Number of farms visited	Vaccinated farm[a), b)]			
		H5	% vaccinated	H5/H7	% vaccinated
Broiler[c)]	21	2	14.3	1	4.8
Layer and slow-growing meat chickens[d)]	230	47	20.4	168	73.1
Total	251	49	19.5	169	67.3

a) Serum samples were collected from 10 chickens in each farm; the chicken farm was considered vaccinated when seven or more chickens had hemagglutinin inhibition (HI) antibody titers and the mean titer was ≥4 log2. b) Chickens in some farms were only vaccinated with the H5 single vaccine and only had H5 HI antibodies. c) Fast-growing meat chickens that are usually slaughtered at 40 days of age. d) Slow-growing meat chickens that are usually taken to markets at 90–120 days of age.

dramatically reduced the incidence of human H7N9 virus infection; however, this vaccination strategy still faces several challenges before the H7N9 virus is completely eradicated.

First, the 2017 HPAI H7N9 virus has adapted to ducks, and high-level vaccination coverage in ducks is difficult to achieve. In China, up to 4 billion ducks are reared annually, often in open fields with no biosecurity measures, and they are mainly traded through the live poultry market system. Many influenza viruses replicate efficiently in ducks but usually do not cause disease or death in ducks, especially adult ducks. Therefore, farmers are reluctant to use influenza vaccines in ducks. Our serological surveillance in recent years showed that H5 vaccination coverage in ducks is always less than 30%, which means that more than 70% of the ducks in China are unvaccinated. This explains why even though the H5 vaccine has been used in poultry in China for over ten years, H5 virus can still be detected in the live poultry markets, mainly from ducks (Shi et al., 2018). Given the high risk the H7N9 virus poses to humans, coupled with the fact that the current H5/H7 vaccine is highly immunogenic and provides sound protection in ducks, duck vaccination with the H7N9 vaccine should be mandated.

Second, an H7N9 antigenic variant may emerge in unvaccinated naïve birds. The 2017 HPAI H7N9 viruses replicate more efficiently than the 2013 LPAI H7N9 viruses, and therefore may mutate more rapidly in nature, especially in unvaccinated naïve birds. Mutations in the HA protein may alter the antigenicity of the viruses, and the currently used vaccine may not be able to provide solid protection. Although the prevalence in chickens and human infections of H7N9 viruses have been successfully controlled to date, the H7N9 viruses have not yet been eradicated. Active surveillance still needs to be enforced and any newly detected viruses must be carefully evaluated. Moreover, if the current vaccine does not protect against a new virus, the vaccine strain must be updated in a timely manner.

In summary, the H7N9 influenza viruses are typical zoonotic pathogens: they emerged as low pathogenic viruses in chickens in 2013, and then mutated to a highly pathogenic form in 2017. Both low pathogenic and highly pathogenic viruses easily infect humans. Importantly, they have a high probability of becoming highly lethal, highly transmissible viruses and causing a human influenza pandemic. Introduction of an H5/H7 vaccine in chickens reduced the H7N9 virus prevalence in poultry and prevented human infection with H7N9 virus. Although the vaccination strategy still faces some challenges, the success of H7N9 influenza control in China reflects the effectiveness of the avian influenza vaccine and demonstrates the importance of active control of animal disease for zoonosis control and human health protection.

Compliance and ethics *The author(s) declare that they have no conflict of interest.*

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