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Epidemiology, Evolution, and Recent Outbreaks of Avian Influenza Virus in China

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Novel reassortants of H7N9, H10N8, and H5N6 avian influenza viruses (AIVs) are currently circulating in China's poultry flocks, occasionally infecting humans and other mammals. Combined with the sometimes enzootic H5N1 and H9N2 strains, this cauldron of genetically diverse AIVs pose significant risks to public health. Here, we review the epidemiology, evolution, and recent outbreaks of AIVs in China, discuss reasons behind the recent increase in the emergence of novel AIVs, and identify warning signs which may point to the emergence of a potentially virulent and highly transmissible AIV to humans. This review will be useful to authorities who consider options for the detection and control of AIV transmission in animals and humans, with the goal of preventing future epidemics and pandemics.

Avian influenza viruses (AIVs) are generally considered species specific, with infections chiefly limited to birds. Considering their widespread geographical prevalence, it is remarkable that AIVs relatively rarely cross the species barrier to infect humans and other mammals. However, the segmented genome of influenza A viruses affords considerable opportunities for rapid and substantial viral changes through both antigenic shift and drift, in which the increased diversity of available AIVs leads to a greater chance for the emergence of novel viruses.

There have been four such instances of influenza-driven pandemics in the 20th century (H2N2 in 1957, H3N2 in 1968, and H1N1 in 1918 and 2009) in which millions of people perished. It was once thought that AIVs require the infection of an intermediate host, such as a pig, to generate the necessary viral changes to cross the species barrier and directly infect humans. However, the H5N1 AIV outbreak in 1997 caused 18 human infections and resulted in 6 deaths, in which virus transmission was first recognized to have occurred directly from poultry to humans (1). As of March 2015, H5N1 strains have become enzootic in a number of avian species across wide geographical areas, with at least 784 laboratory-confirmed human infections with H5N1 AIVs and 429 deaths across 16 countries, amounting to a case fatality rate (CFR) of 55%.

China is recognized as a geographical area with suitable conditions for the emergence of novel influenza viruses (2). New strains of AIVs currently widely circulate in China, occasionally resulting in human infections. These include at least six subtypes of AIV (H5N1, H6N1, H7N9, H9N2, H10N8, and H5N6) (3). With an overall goal to reduce the threat of future human infections with novel or enzootic AIV subtypes, there is an urgent need to examine the factors which contribute to the emergence of novel AIVs.

EPIDEMIOLOGY AND PAST OUTBREAKS OF AIVs IN CHINA

The first H9N2 low-pathogenicity avian influenza virus (LPAIV) and H5N1 high-pathogenicity avian influenza virus (HPAIV) in Asia were isolated from Guangdong Province, China, in 1994 and 1996, respectively (2, 4). Currently, H9 and H5 are the most prevalent AIV subtypes (5) and can be found throughout most of the areas in China (Fig. 1A). Interestingly, the H9N2 and H5N1 AIVs are prevalent in both unvaccinated and vaccinated poultry farms, often causing sporadic outbreaks (2, 4).

Along with the high prevalence of H9 and H5 AIVs in poultry, novel AIVs are also emerging in China. In March 2013, a new H7N9 LPAIV emerged in the Yangtze River Delta region and quickly spread to more than 18 provinces and municipalities (Fig. 1A). As of April 2015, at least 630 laboratory-confirmed human H7N9 infections (CFR > 30%) have been documented in China. During December 2013, a novel H10N8 LPAIV emerged from Jiangxi Province, China, resulting in 3 human infections and 2 deaths. In contrast to H5N1 strains, both H7N9 and H10N8 strains are classified as LPAIV. Therefore, poultry show few symptoms of infection with H7N9 and H10N8, but these viruses retain the ability to cause severe disease in humans. A high prevalence of H9 and H5 AIVs can be found in poultry species regardless of location, but H7 and H10 viruses are rarely detected on poultry farms and seem to be amplified mainly among birds in live-poultry markets (LPMs) (6, 7). Novel H5N1, H5N2, H5N6, and H5N8 HPAIVs strains have been found in China since 2000, and the overall prevalence of H5 viruses appears to be increasing since 2010. H5N6 and H5N8 AIVs have been most prevalent among China's domestic waterfowl during previous years; however,

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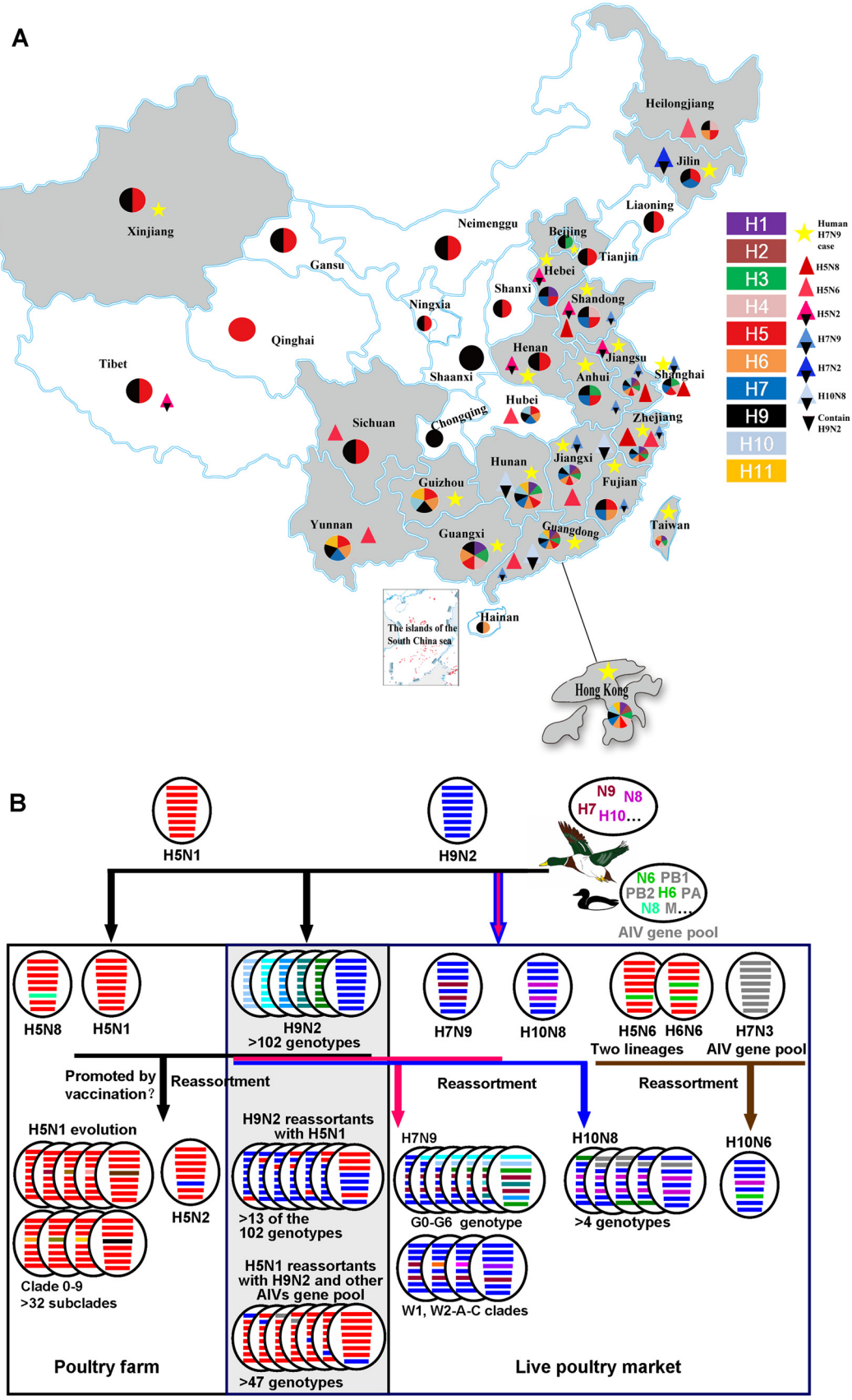

FIG 1 Emergence and distribution of avian influenza viruses in China. (A) Distribution of AIVs found in China (from the NCBI database [http://www.ncbi.nlm.nih.gov/genomes/FLU/FLU.html] and various news reports). Various colors depict different HA subtypes. Yellow stars indicate provinces with confirmed H7N9 human infections. The shaded regions represent the emergence of AIV cases in humans. (B) Schematic for the emergence of novel AIVs in China. The eight bars, from top to bottom, represent the PB2, PB1, PA, HA, NP, NA, M, and NS gene segments. Different colors represent different genetic origins.

H5N6 AIVs caused several outbreaks in chickens in China, Vietnam, and Laos during 2014 and 2015. H5N8 AIVs have caused outbreaks in poultry across several nations in Asia, Europe, and North America. H5N1 and H5N2 variants have also been isolated from chickens and waterfowl, and H5N2 AIV was the cause of one outbreak among chickens in Hebei Province, China, during December 2013, and it is currently a serious problem in the United States (OIE data).

In summary, H9N2 and H5Ny AIVs are the prevalent subtypes in Chinese poultry farms and LPMs, whereas H7N9 and H10N8 viruses are currently circulating mostly in LPMs only. Therefore, novel H7N9 and H10N8 viruses should be targeted for early detection and containment at LPMs before these viruses are able to spread to the much larger poultry farms.

GENETIC EVOLUTION OF AIVs IN CHINA

After the isolation of the first strain in 1994, H9N2 AIVs have rapidly differentiated such that more than 102 genotypic variants have now been recognized based on the nomenclature system (8), and 69 genotypes have been recognized by a new nomenclature method (4). Notably, H9N2 viruses belonging to genotype 57 (G57) appear to have a selective advantage in chickens, with increased infectivity and the ability to escape selective pressure via antigenic shift. G57 AIVs have been the cause of widespread H9N2 outbreaks among chickens during 2010 to 2013 in China (4).

Similarly, since the first identification of H5N1 HPAIV (clade 0), these viruses have evolved into at least 32 clades, in which clades 2.3.2.1, 2.3.4.2, and 7.2 are currently the most prevalent in China. Recently, H5 viruses from clade 2.3.4.6 (recently redefined as clade 2.3.4.4) appear to gradually be replacing viruses from clade 2.3.4.2, especially in waterfowl. It seems troubling that novel H5N5, H5N8, H5N2, and H5N6 reassortants bearing the genetic backbone of clade 2.3.4.4 H5N1 variants have been undergoing reassortment with H3, H5, H6, H7, H9, and H11 AIVs, and these novel viruses are becoming increasingly prevalent in domestic birds (9, 10) (Fig. 1). Interestingly, H5N2 viruses expressing the hemagglutinin (HA) gene from clade 2.3.2, 2.3.4, or 7.2 have been isolated with internal genes from diverse origins (10). The recent outbreak of H5N2 in the Hebei, Shandong, and Henan Provinces of China are mainly reassortant viruses between clade 7.2 H5N1 and H9N2 AIVs (10). There are now more than 47 genotypes of H5 AIVs; of these genotypes, many are of viruses with reassortments between H5N1 and H9N2 or occasionally other AIV subtypes (2, 11).

The recently emerged and currently prevalent H7N9 and H10N8 AIVs are also reassortant viruses, containing HA and NA genes that originated from wild birds but bearing the internal gene backbone of H9N2 AIVs (12). Importantly, H7N9 and H10N8 viruses have been undergoing reassortment with H9N2 as well as other AIVs (6, 13) (Fig. 1B).

The recently emerged reassortant AIVs, such as the H7N9, H10N8, and H5N2 subtypes found in domestic poultry, appear to be related to the widespread H9N2 viruses (6, 10, 12). To our knowledge, AIVs originating from wild birds are not always adapted to infect domestic birds (12). However, wild-bird AIVs may gain the ability to infect domestic poultry and humans more effectively after reassortment with an AIV that is already well adapted to domestic birds, such as H9N2. For instance, the H5N1 strain responsible for the 1997 human outbreak in Hong Kong was found to contain internal genes derived from H9N2 (14). Conversely, the incorporation of H5N1-derived internal gene segments into H9N2 increases its virulence in mice (8), and increasing numbers of H9N2 strains have been discovered to bind to human-type receptors (15), which potentially increases the risk of human infections.

VACCINATION AND THE EVOLUTION OF AIVs

Vaccinations have been carried out in poultry in an attempt to control HPAIV outbreaks by reducing the opportunity for inter- and intraspecies virus transmission (16). In China, large-scale immunizations with an inactivated, reassortant H5N1 virus containing the A/Puerto Rico/8/34(H1N1) internal genes have become the primary method to prevent and manage H5N1 virus infections among poultry since 2004 (2); however, AIV transmission has been documented from infected animals that have been vaccinated. Immune escape and antigenic drift, as a result of the selective pressure induced by immunization, may be one of the critical reasons behind vaccine failure (4, 17). Inactivated H9N2 vaccines have been used for reducing the impact of H9N2 infection in chickens since 1998. The vaccine was initially successful in containing H9N2, but the virus eventually spread throughout most of China. To date, H9N2 virus continues to circulate in vaccinated chicken flocks (4).

These results suggest that vaccination may actually play a role in driving the evolution of AIVs (4, 17). Since current vaccines against H5N1 and H9N2 cannot completely eliminate AIV infections in poultry (4, 16, 17), both viruses are still able to replicate in immunized domestic birds without apparent clinical signs or mortality. The vaccinated animals may then act as silent carriers for AIVs, spreading the virus to naive animals through poultry transports or LPMs (1, 2, 4, 9, 11). It should be noted that both the H9N2 and H5N1 AIVs gradually evolved into distinct clades, sublineages, antigenic groups, and genotypes after the large-scale vaccination of poultry (Fig. 1B) (2, 8).

DETERMINANTS OF TRANSMISSIBILITY FOR AIVs

Past studies have provided insight into specific mutations in AIVs that lead to enhanced infection of mammals. The Qinghai-like H5N1 virus (clade 2.2), which possesses the amino acid K627 in PB2, exhibits high transmissibility from Qinghai, China, into other countries located in Asia, Africa, and Europe (18). This virus demonstrates the highest infectivity to mammals and humans out of all known H5N1 viruses and has caused 292 human infections in Egypt, with 99 deaths (CFR = 34%) (WHO data; http://www.who.int/influenza/human_animal_interface/EN_GIP_20150623cumulativeNumberH5N1cases.pdf?ua=1).

H5N1 AIV has not been confirmed to transmit efficiently between humans. However, only four mutations in the HA antigen (H110Y, T160A, Q226L, and G228S in A/Indonesia/5/2005 virus or N158D, N224K, Q226L, and T318I in A/Vietnam/1203/2004 virus) is required to increase the preference of AIVs for human-type receptors (sialic acid [SA] α2,6-Gal) and result in a mutant H5N1 virus that is readily transmissible in ferrets (19). The T160A mutation has already been identified in some natural H5 isolates (9, 20). The presence of amino acids N701 and A160 in PB2 and HA, respectively, also enhances the efficient transmission of H5 AIVs in mammals (20).

The ability of AIVs to bind human-type receptors will increase their infectivity and transmissibility among humans. H7N9 AIVs are able to bind human receptors, and the S138A, G186V, T221P,

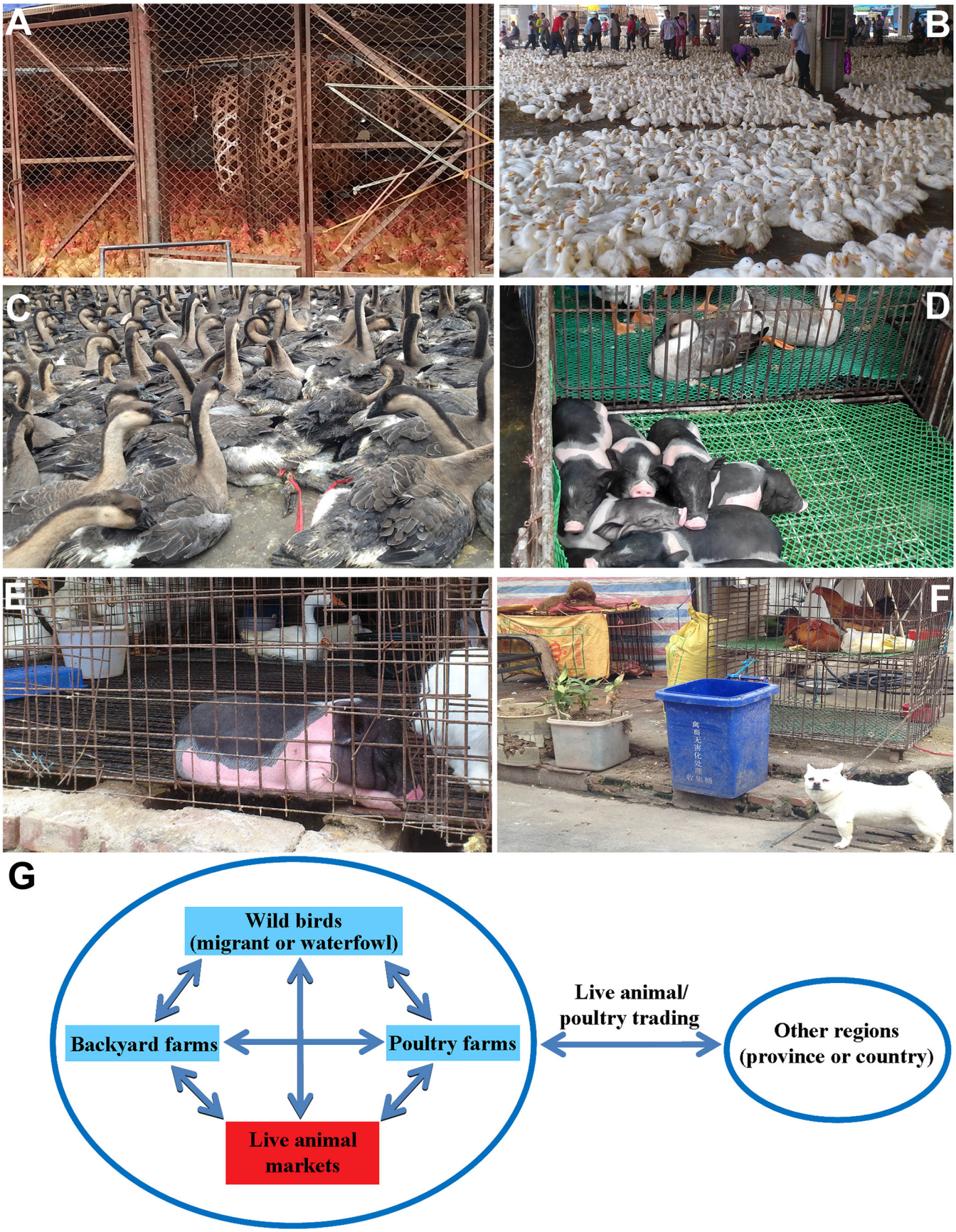

FIG 2 Live-poultry markets and live-animal markets in China. (A to C) Photographs taken from a typical LPM in China; (D to F) photographs representative of a live-animal market in Guangdong Province; (G) proposed ecosystem model for the production and cross-species transmission of novel reassortant AIV in China. The cycles represent different regions in China.

and Q226L mutations in the HA antigen have been shown to enhance this interaction (19). H10N8 viruses preferentially bind to avian receptors (21); however, documented human infections with H10N8 virus suggests that the virus may also be able to bind human receptors (6). The increasingly numerous H9N2 AIVs with the Q226L mutation in HA also appear to be able to bind to human-type receptors (15). The E190V, G228S, and P186L substitutions in the H6 protein are important for H6N1 viruses to acquire the capacity to bind human receptors (22).

POTENTIAL REASONS BEHIND THE EMERGENCE OF DIVERGENT AIV REASSORTANTS

Due to conditions which promote AIV reassortment, southern China is considered an ideal breeding ground for the emergence of novel AIVs. The practice of backyard farming and live-animal markets (LAMs) contributes to this, due to the constant close proximity of humans to poultry (chicken, ducks, geese, and numerous other species) and other animals (pigs, cats, dogs) (Fig. 2) (23). Pigs are considered the most likely mixing vessels in many backyard farms, producing numerous H1 and H3 reassortant viruses. The practice of backyard farming is gradually disappearing and being replaced by regular poultry farms, in which chickens are raised in closed henhouses. However, a slowdown in the emergence of novel AIV reassortants in China has not yet been observed, as evidenced by the presence of novel H7N9, H10N8, and H5N6 AIVs in LPMs and H5N6 in poultry farms (6, 9, 13). LAMs or LPMs are able to provide ample opportunities for wild aquatic birds, domestic poultry, mammals, and humans to closely interact and potentially share influenza pathogens, resulting in the emergence of novel viruses (Fig. 1B and 2).

Waterfowl, especially ducks, play an important role in the transmission of AIV. Wild waterfowl was regarded as a natural reservoir for AIVs and contribute to the geographical spread of AIVs via long-distance migration (18, 24). In shared wetlands or LPMs, the wild-bird-derived viruses or their genes might be conserved in domestic poultry by reassortment with a poultry-adapted AIV, such as novel H7N9 and H10N8 viruses. Several duck species have also been found to be carriers of H5N1 HPAIV. These ducks can shed and transmit LPAIVs and even HPAIVs from both the respiratory and intestinal tracts, with few or no clinical signs (1, 24), whereas H5N1 HPAIV infection in chickens is generally lethal. H5N1 HPAIVs have been isolated previously in apparently healthy chickens from LPMs, but these viruses are highly pathogenic for specific-pathogen-free (SPF) chickens (1, 11). These observations suggest that apparently healthy birds in LPMs or LAMs may also act as mixing vessels for AIV reassortment and shed virus without any clinical signs of the disease (25). The novel H7N9, H10N8, and H5N6 viruses may have first emerged from the complex influenza ecosystem in LPMs, which act as a gene pool for the generation of new reassortant AIVs and a source of human infections (Fig. 2) (6, 9). Furthermore, LPMs are now considered a pivotal site for the spread of the H7N9 subtype in China.

CONCLUSIONS

There are at least five subtypes of AIV (H5, H6, H7, H9, and H10) which cause sporadic, epidemic, or panzootic influenza diseases in poultry farms and LPMs in China. These viruses are continually evolving due to viral mutation, recombination, and reassortment as well as selection pressure from vaccinations in poultry. The migration of wild birds, increased interaction of large concentrations of birds with other animal species, exposure to human influenza viruses, poor sanitary practices on farms, and transport of animals for commercial purposes are the main factors leading to the emergence of novel AIVs (Fig. 2G). Novel and existing AIVs possessing H5N1-derived internal genes and other AIVs possessing specific mammal-derived mutations may exhibit enhanced virulence and transmissibility in humans, respectively. Therefore, AIVs with these properties warrant further investigation in order to preemptively control and prevent future influenza epidemics and pandemics.

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