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Diversity, evolution and population dynamics of avian influenza viruses circulating in the live poultry markets in China

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ABSTRACT

Live poultry markets (LPMs) are an important source of novel avian influenza viruses (AIV). During 2015–2016 we surveyed AIV diversity in ten LPMs in Hubei, Zhejiang and Jiangxi provinces, China. A high diversity and prevalence of AIVs (totaling 12 subtypes) was observed in LPMs in these provinces. Strikingly, however, the subtypes discovered during 2015–2016 were markedly different to those reported by us in these same localities one year previously, suggesting a dynamic shift in viral genetic diversity over the course of a single year. Phylogenetic analyses revealed frequent reassortment, including between high and low pathogenic AIV subtypes and among those that circulate in domestic and wild birds. Notably, the novel H5N6 reassortant virus, which contains a set of H9N2-like internal genes, was prevalent in all three regions surveyed. Overall, these data highlight the profound changes in genetic diversity and in patterns of reassortment in those AIVs that circulate in LPMs.

1. Introduction

Avian influenza viruses (AIVs) are important pathogens of birds and can cause sporadic and non-sustained infections in human populations (Alexander, 2007; Kuiken et al., 2006). Of the diverse subtypes of AIV, H5 and H7 (also known as highly pathogenic avian influenza, HPAI) pose significant risks to both public and veterinary health (Guan et al., 2004; Kuiken et al., 2006; Lam et al., 2015). HPAI H5N1 virus was first recorded in humans in Hong Kong during 1997 (Claas et al., 1998), and there have been 855 laboratory confirmed cases with 452 deaths reported up to October 2016. Similarly, H7N9 virus was first reported to infect humans in mainland China in 2013, and since this time there have been 798 reported cases, of which 320 have died (Gao et al., 2013a, 2013b; Lam et al., 2013; WHO, 2016). More recently, novel AIV reassortants, such as H10N8 and H5N6, have emerged, causing sporadic infections in humans in China (Bi et al., 2016; Chen et al., 2014; Feng et al., 2016; Pan et al., 2016; Yang et al., 2015; Zhang et al., 2016). A better understanding of the diversity, evolution and population dynamics of AIVs in birds is clearly of considerable importance for understanding their potential to emerge in humans.

Live poultry markets (LPMs) contain a concentrated collection of birds of different species and from different geographic locations, making them a major source of AIV transmission, mixing, and reassortment (Choi et al., 2015). LPMs are also a hot bed for zoonotic infections by providing an efficient interface where humans come into

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close contact with infected birds. Indeed, exposure to LPMs has been reported in many patients infected by H5N1 or H7N9 viruses (Fournié et al., 2013; Pantin-Jackwood et al., 2014; Yu et al., 2014). Clearly, LPMs should be closely monitored for the emergence of novel AIVs. Furthermore, considering the complex patterns of AIV transmission within LPMs and the potential for influenza viruses to reassort, both high and low pathogenic AIVs should be monitored to reveal their evolution and population dynamics within LPMs (Pepin et al., 2013). Indeed, low pathogenic avian influenza viruses (LPAI) may be key contributors to the genesis of novel virus genotypes with the potential to cause human infection, although they are often ignored in surveillance schemes (Butt et al., 2005; Pepin et al., 2013; Pu et al., 2015; Yuan et al., 2013).

Following the appearance H5N1 and H7N9, a proliferation of novel H5Nx subtypes such as H5N2, H5N5, H5N6, and H5N8 have recently been reported in LPMs (Krauss et al., 2016; Su et al., 2015). This includes a wide array of novel H5N6 recombinant variants reported in 2014–2015 by both our (Chen et al., 2016) and other groups (Bi et al., 2015; Mok et al., 2015; Yuan et al., 2016). In this study we report the continued monitoring of AIV in various geographic regions in China. In particular, as we previously reported the presence of highly pathogenic H7N9 and H10N8 viruses in Zhejiang and Hubei provinces, we continued sampling these provinces with the addition of samples from the neighboring province of Jiangxi (Lam et al., 2015; Chen et al., 2014). The data generated provide the most up-to-date characterization of the diversity structure in LPMs and its implications for the evolution and population dynamics of AIV in general.

2. Results

2.1. AIVs in Zhejiang, Hubei and Jiangxi provinces

A total of 1189 (including 805 fresh fecal and 384 cloacal swabs) samples were collected from 10 wholesale markets in three Chinese provinces (Fig. 1). Among these, 566 (47.6%) were positive for influenza A virus by RT-PCR (Table 1), with positive rates of 49.9% and 42.7% for fresh fecal and cloacal swabs sample, respectively. The positive rates were high for LPMs within all three provinces: 58%, 41.9% and 36.5% for Hubei, Zhejiang and Jiangxi provinces, respectively. The comparisons among different bird species suggested that ducks had the highest positive rate (52%) while pigeon had the lowest (11.8%), which is consistent with our previous survey performed during 2014–2015 (Chen et al., 2016). Importantly, in addition to a high rate of positivity, the samples from LPM exhibited frequent co-infections, involving more than 30% of all positive samples. Collectively, these observations suggest a high intensity and complexity of AIVs circulating within the LPM.

2.2. Geographic distinctiveness and diversity shifts in AIVs circulating LPMs in Zhejiang, Hubei, and Jiangxi provinces

Sequencing of the HA and NA genes revealed a total 12 AIV subtypes, comprising combinations of 9 HA (H1, H2, H3, H4, H5, H6, H7, H9, and H11) and 5 NA (N1, N2, N6, N8, and N9) subtypes (Table 1). At least 7 subtypes were identified in each province. Although there were partial overlaps of the AIV subtypes among the three provinces, such as H5N6 and H9N2 which appeared in all three provinces and at a relatively high prevalence, the dominant subtypes differed between the provinces: these were H3N6 and H5N6 in Hubei, H1N2 and H2N8 in Zhejiang, and H6N6 and H9N2 in Jiangxi (Table 1). Notably, the highly pathogenic H5 subtypes were common in all three provinces, while H7 was only found in Zhejiang and Jiangxi. Finally, the new H5N6 reassortment strain (see below) was found in all three provinces, suggesting that it is spreading widely.

An important aspect of our study was the follow-up sampling of LPMs in Hubei and Zhejiang that we previously surveyed during November 2014 – January 2015 (Chen et al., 2016). Remarkably, the newly-identified subtypes were markedly different from those identified only one year previously. This is reflected in (i) the shift in the minor subtypes, from H5N6 to H3N6, and from H9N2 to H1N2, for Hubei, and Zhejiang, respectively; and (ii) the emergence of new subtypes, such as H2N8, H3N6, and H11N2. Clearly, the diversity of AIVs within LPMs can change rapidly. However, as no oropharyngeal (or tracheal) samples were collected in this study it is possible that we may have missed some AIVs).

2.3. Phylogenetic analyses of AIVs from LPMs

To better understand the evolutionary history of the AIVs currently circulating in the LPMs, we obtained the complete coding region sequence of 45 AIVs (Fig. 2). At least one virus of each subtype was sequenced to cover the entire coding regions, with the exception of the H11N2 virus for which we were only able to obtain a partial genome.

2.3.1. Highly pathogenic AIVs

The highly pathogenic AIV subtypes discovered in this study included the H5N6, H5N1, and H7N9. While the H5N1 and H7N9 viruses were similar to those reported previously (Chen et al., 2016), novel reassortment variants were observed for the H5N6 subtype. Among these, the dominant type was a reassortment between H5N6 and H9N2 (Figs. 2 and 3 and S1-2). The HA and NA gene segments of these viruses (labeled with two stars in the figures) fell into the previously defined H5N6 lineage A in both the HA and NA trees (Figs. 2 and 3 and S1-2), whereas the internal genes all grouped with H9N2-associated lineages (Figs. 2 and 4 and S3). Importantly, this reassortant did not form monophyletic cluster in the HA and NA trees (Fig. 3 and S1), suggesting that it was in fact derived from multiple reassortment events. Another H5N6 variant, which contained two viruses (A/Duck/Hubei/ZYSYG5/2015 and A/Duck/Hubei/ZYSYG18/2015) from Wuhan, had a complex reassortment history involving H5N2/8 (HA, PB2, PB1, NP, and MP), H6N6 (NA), and other poultry viruses (PA and NS) (Figs. 2–4 and S1-3; labeled with one star). Furthermore, we also discovered two H5N6 viruses from Jiangxi (A/Chicken/Ganzhou/GZ21/2015 and A/Chicken/Ganzhou/GZ27/2015) whose PB2 gene segment grouped with H7N7 viruses (Figs. 2–4 and S1-3).

2.3.2. Lowly pathogenic AIVs

2.3.2.1. H1N2. H1N2 virus was the dominant subtype in LPMs from Zhejiang province. To determine the evolutionary history of this virus we obtained whole genome sequences of both duck and chicken H1N2 viruses. Interestingly, the H1N2 viruses from chickens exhibited high sequence similarity to those viruses sampled from ducks. The HA of these viruses were closely related to the H1 from multiple subtypes, including H1N2, H1N8, and H1N4, circulating in LPMs within China, all of which belonged to the Eurasian AIV lineage (Fig. S4). Similarly, the NA gene of H1N2 viruses formed a cluster with the NA genes from H9N2 and H4N2 type viruses (Fig. S8). Although it was difficult to determine the evolutionary origin of the surface protein genes, the internal gene segments of all H1N2 viruses were related to H7N3 or H1N4 viruses circulating in China (Fig. S9).

2.3.2.2. H3N6. Viruses of the H3 subtype commonly circulate in LPMs in China and therefore have the potential to jump species boundaries to mammals, including humans (Cui et al., 2016). In our survey, H3N6 was the dominant subtype in Hubei. Whole genome sequences of H3N6 viruses were recovered from six duck fecal and cloacal swabs. Unfortunately, our attempts to recover some segments of H3N6 viruses from chickens were unsuccessful. While the HA gene