

Genetic characterisation of H9N2 influenza viruses in southern China

Y Guan *, GJD Smith

KEY MESSAGES

1. Two lineages of H9N2 influenza virus continue to be enzootic in terrestrial poultry. In China, one lineage is predominately found in quail (G1-like), and the other (Ck/Bei or Y280-like) in chickens.
2. Both lineages of H9N2 influenza viruses have undergone a series of reassortments with H5N1 and other influenza viruses in southern China.
3. Genetic characterisation of the H9N2 viruses suggests that all have the potential to infect

humans.

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Y Guan *, GJD Smith

State Key Laboratory of Emerging Infectious Diseases, Department of Microbiology, The University of Hong Kong

* Principal applicant and corresponding author: yguan@hkucc.hku.hk

Introduction

H9N2 influenza viruses have become enzootic in terrestrial poultry populations from Egypt and the Middle East, across South, South East and East Asia.¹ H9N2 viruses have sporadically infected humans and swine and have provided segments, through reassortment, to the Asian highly pathogenic H5Nx viruses.² Two main lineages are found in these viruses, the widely distributed G1 lineage and the Ck/Bei or Y280-like lineage found in East Asia, particularly China.^{1,3,4} Chickens are the primary hosts for these viruses, but they are also found in minor poultry, especially in the case of G1-like viruses in China.^{3,4} In this study we sought to characterise the H9N2 viruses found in poultry in southern China and the extent of their reassortment with other influenza viruses, particularly the highly pathogenic H5N1 viruses.

Results

This study was conducted from October 2006 to September 2008. H9N2 viruses were isolated from samples collected from chickens, ducks, and minor poultry from 2000 to 2005 in several provinces of southern China and from 2005 to 2007 in Hong Kong. The surveillance data provided 47 225 chicken samples, 49 150 duck samples, and 11 526 samples from quail and other minor poultry in provinces of southern China, as well as 30 770 chicken samples and 3422 samples from minor poultry in Hong Kong.

Prevalence of H9N2 viruses

From all the samples collected, 1189 (2.5%) of chicken samples, 89 (0.2%) of duck samples, and 938 (8.6%) of minor poultry samples from provinces

of southern China were positive for H9 influenza viruses.^{3,4} Among the minor poultry, quail had an unusually high prevalence of H9 influenza viruses, with 610 (13.3%) of 4601 quail samples positive.³ Of the Hong Kong samples, 845 (2.7%) from chicken and 22 (0.6%) from minor poultry were positive for H9 influenza viruses.⁵

Chicken isolates came primarily from oropharyngeal samples, with isolates from cloacal or faecal samples decreasing over time.⁴ In quail and other minor poultry, oropharyngeal samples accounted for over 90% of the isolates.^{3,4} In Hong Kong, the ability to take oropharyngeal samples was limited and most isolates were from drinking water or cloacal/faecal samples.⁵

While H9 isolates could be isolated year round, the isolation rate in the summer months was generally lower.^{3,4,5} In total, 73 quail, 84 other minor poultry, 139 chicken, and 15 duck H9N2 isolates were selected and subjected to full genome sequencing, with at least one isolate being taken from each positive sampling occasion.^{3,4,5}

Phylogenetic analysis

In the haemagglutinin (HA) gene phylogeny, approximately half of the quail viruses were part of the G1-like viruses, whereas the others were spread within two subgroups (1 and 2) of the Ck/Bei-like lineage.³ Subgroup 1 had A/Quail/Shantou/243/2000 (ST243) as an early virus and subgroup 2 was part of a much larger sub-clade containing A/duck/Hong Kong/Y280/1997 (Y280).³ Minor poultry viruses also fell into both these subgroups of the Ck/Bei lineage with subgroup 1 being almost exclusively from minor poultry viruses.^{3,4} Chicken viruses were mainly part of subgroup 2 of the Ck/Bei-like lineage

and two duck viruses were from the Korean Y439-like lineage.⁴ Viruses from both chickens and minor poultry in Hong Kong were part of the subgroup 2 (Y280-like) except for one chicken virus from the G1 lineage. The neuraminidase (NA) gene phylogeny showed that G1-like viruses remained predominantly within a consistent sub-clade and the NA of the Ck/Bei lineage poultry viruses from southern China also fell into two subgroups within that lineage, with one of them containing mostly minor poultry viruses.^{3,4} A smaller proportion of viruses were in sub-clades with virus from eastern and northern China. All but one of the Hong Kong viruses were part of the same Ck/Bei lineage subgroup.⁵

Antigenic analysis

Antigenic analysis was conducted using monoclonal antibodies raised against A/Quail/Hong Kong/G1/1997 (G1), A/duck/Hong Kong/Y280/1997 (Y280), and A/chicken/Hong Kong/G9/1997 (G9) in a haemagglutinin inhibition (HI) assay. A numerical analysis of the HI titres was conducted to provide a graphical overview of the patterns of antigenic changes. There were four different antigenic groups. Two corresponded to the G1-like viruses, primarily from quail, with one group associated with the prototype G1 virus and a smaller group showing an antigenic shift relative to the G1 virus. These viruses formed a sub-clade within the G1-like viruses.³ Of the Ck/Bei- or Y280-like viruses a distinct sub-clade of mostly minor poultry viruses formed one antigenic group, with the bulk of the chicken and duck viruses forming the other antigenic group.^{3,4}

Genotyping

Reassortment, and classification of genotypes, among the H9N2 viruses were examined by tracking the phylogenetic origins of the segments. G1-like viruses were designated to have A-series genotypes and those from the Ck/Bei-like lineage were designated to have B-series genotypes, while two duck viruses were from the Korean Y439-like genotype.^{3,4} The prototype G1 virus was designated to have genotype A0 and this persisted until 2002. Genotypes A1 and A2 had the NS segment and M (for A2) from the Ck-Bei lineage, indicating reassortment among the H9N2 major lineages. Genotype A3, which appeared in 2002, incorporated a PA segment of H5N1 origin and persisted and predominated during this study. A subset of viruses from this genotype showed antigenic drift. A transient genotype, A4, further incorporating a PB1 segment of unknown avian origin appeared in 2005.

The Ck-Bei-like lineage displayed a far greater variety of genotypes, whether or not the viruses came from minor poultry or chickens and ducks. This indicated extensive reassortment with other wild bird and poultry viruses, including the G1-like

viruses in quail and contemporary H5N1 viruses as well as with duck and aquatic bird viruses. Thirty B-series genotypes were reported in total, with only a few genotypes persisting for several years.^{3,4} Fewer genotypes were observed in viruses from ducks, which is consistent with the trend for the H9N2 viruses to be found predominately in chickens and minor poultry.

Molecular characterisation

The connecting peptides at the HA1/HA2 cleavage site (residues -1 to -4 of HA1) were predominately R-S-S-R.^{3,4} Korean lineage and early viruses did not have the R at -4 of HA1. A few viruses had amino acids other than Ser at positions -2 and -3 and R was occasionally replaced by K at both positions -1 and -4.⁴ One virus had an additional R at position -2, but this virus was not highly pathogenic in chickens.³ Residues in the receptor binding sites positions 226 and 228 (H3 numbering) that are characteristic of avian (2-3) or human (2-6) receptor binding were predominately L (more human-like) and G (avian-like), respectively.^{3,4} L226 predominated in the later viruses, which were infrequently isolated from ducks and more commonly isolated from oropharyngeal samples. Deletions of 2, 3, or 6 amino acids were found in the stalk regions of the NA proteins of several viruses.^{3,4}

Discussion

Viruses from the two major H9N2 lineages continue to circulate in southern China. The Ck/Bei-like lineage is predominant and is found primarily in chickens. In China, the G1-like lineage viruses were found mainly in quail, with few isolated from other minor poultry or chickens and ducks. This is in contrast with the situation in the Middle East and South Asia, where G1 viruses are prevalent and primarily isolated from chickens.¹ In southern China, the G1-like lineage viruses were relatively stable with respect to their genotypes, indicating limited reassortment with other viruses. This could be due to their more restricted prevalence in southern China and that quail, in particular, are more isolated from other birds in the poultry farming and marketing system. Evidence of antigenic drift in the quail G1-like viruses was found, which might be indicative of host selection pressure.

Ck/Bei-like viruses were more prevalent in southern China and predominately isolated from chickens. This lineage is not seen outside East Asia. A large number of genotypes were detected for viruses of this lineage indicating that they were readily able to reassort with viruses in the chicken or duck populations. However many of the genotypes were only transient. There were interchanges of segments with H5N1 viruses as well as viruses from

ducks and unknown birds. The interaction with H5N1 viruses raises the possibility of altering the way these highly pathogenic can be transmitted to humans. As with the G1-like viruses, two antigenic groups were detected. One group contained viruses mostly from quail and other minor poultry and was phylogenetically distinct from the other, predominately chicken, Ck/Bei-like viruses.

Overall, the H9N2 viruses from southern China and Hong Kong show patterns of both limited isolation and mixing within the poultry system. Quail and minor poultry are relatively more isolated in the farming and marketing system and were shown to host stable lineages of viruses. Ck/Bei-like viruses showed less stable lineages and had a greater level of exchange of segments with viruses from poultry and other birds. Therefore the poultry system in southern China allows H9N2 viruses to develop in multiple ways with mixing among species and the opportunity to transmit to humans.² To prevent these developments leading to public health problems like those caused by the highly pathogenic H5N1 viruses, better separation of birds within the poultry farming and marketing systems is advisable.

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References

1. Fusaro A, Monne I, Salviato A, et al. Phylogeography and evolutionary history of reassortant H9N2 viruses with potential human health implications. *J Virol* 2011;85:8413-21.
2. Lin YP, Shaw M, Gregory V, et al. Avian-to-human transmission of H9N2 subtype influenza A viruses: relationship between H9N2 and H5N1 human isolates. *Proc Natl Acad Sci U S A* 2000;97:9654-8.
3. Xu KM, Li KS, Smith GJ, et al. Evolution and molecular epidemiology of H9N2 influenza A viruses from quail in southern China, 2000 to 2005. *J Virol* 2007;81:2635-45.
4. Xu KM, Smith GJ, Bahl J, et al. The genesis and evolution of H9N2 influenza viruses in poultry from southern China, 2000 to 2005. *J Virol* 2007;81:10389-401.
5. Chu YC, Cheung CL, Hung Leung CY, et al. Continuing evolution of H9N2 influenza viruses endemic in poultry in southern China. *Influenza Other Respir Viruses* 2011;5(Suppl 1):68-71.