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Interspecies transmission of influenza viruses: H5N1 virus and a Hong Kong SAR perspective

Kennedy F. Shortridge[a,*], Peng Gao[b], Yi Guan[c], Toshihiro Ito[d], Yoshihiro Kawaoka[b], Deborah Markwell[a], Ayato Takada[e], Robert G. Webster[c]

[a]*Department of Microbiology, The University of Hong Kong, University Pathology Building, Queen Mary Hospital, Hong Kong SAR, China*
[b]*School of Veterinary Medicine, University of Wisconsin-Madison, Madison, WI 53706, USA*
[c]*Department of Virology and Molecular Biology, St. Jude Children's Research Hospital, Memphis, TN 38105, USA*
[d]*Faculty of Agriculture, Tottori University, Tottori 680-0945, Japan*
[e]*Graduate School of Veterinary Medicine, Hokkaido University, Sapporo 060-0818, Japan*

Abstract

This account takes stock of events and involvements, particularly on the avian side of the influenza H5N1 'bird flu' incident in Hong Kong SAR in 1997. It highlights the role of the chicken in the many live poultry markets as the source of the virus for humans. The slaughter of chicken and other poultry across the SAR seemingly averted an influenza pandemic. This perspective from Hong Kong SAR marks the coming-of-age of acceptance of the role of avian hosts as a source of pandemic human influenza viruses and offers the prospect of providing a good baseline for influenza pandemic preparedness in the future. Improved surveillance is the key. This is illustrated through the H9N2 virus which appears to have provided the 'replicating' genes for the H5N1 virus and which has since been isolated in the SAR from poultry, pigs and humans highlighting its propensity for interspecies transmission.

Keywords: Influenza; Birds; Interspecies transmission; Hong Kong; Poultry markets; Surveillance

1. First 'wave'

In May 1997, a young boy died of viral pneumonia and multiorgan failure on the heels of an outbreak of highly pathogenic H5N1 avian influenza in chicken on farms in Hong

* Corresponding author. Tel.: +852-2855-4345; fax: +852-2855-1241.
E-mail address: microgen@hkucc.hku.hk (K.F. Shortridge)

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Kong's New Territories (de Jong et al., 1997; Claas et al., 1998; Subbarao et al., 1998; Yuen et al., 1998). When it was announced in August 1997, that influenza H5N1 virus had been isolated from him, emotions were mixed.

Were we to witness a new pandemic? After all, it had been 29 years since the H3N2 pandemic virus was first isolated in Hong Kong. And the region was subsequently recognized as a hypothetical epicentre for the emergence of pandemic influenza viruses (Shortridge and Stuart-Harris, 1982). Was the H5N1 virus smouldering in the region as may have been the case with the catastrophic 1918 H1N1-like virus (Shortridge, 1999a), or was it confined to Hong Kong? Confusingly, the boy had been nowhere near the chicken farms (although it was later learnt that chicks and ducklings had been on display at his kindergarten prior to his being infected). Whatever the situation, this was the first time that an avian influenza virus had been isolated from a human with respiratory infection, fuelling the hypothesis that a pandemic human influenza virus was derived wholly or partly from an influenza virus resident in animals (Webster and Laver, 1975).

Interestingly, the recognition of the index case of the so-called 'bird flu' in early summer was at the time of the year hypothesized to be opportune for the interspecies transmission of avian influenza viruses to humans in the region. This is because of their greater abundance in domestic poultry particularly ducks (Shortridge, 1992). However, there were no further human H5N1 cases in the mid-summer months of June, July and August, when there is often much human influenza activity in Hong Kong (Richeldelfer et al., 1989, Peiris, unpublished data), suggesting that the May case may have been a one-off event. Nonetheless a state of alert was maintained.

In the meantime, evidence was emerging that all eight genes of the human H5N1 virus were of avian origin, the eight RNA segments of both viruses showing >99% sequence homology (Claas et al., 1998; Swayne et al., 1998). This indicated that the human virus had not undergone genetic reassortment with a prevailing human influenza A virus in a mammal, such as pig, the host or 'mixing vessel' in which it was thought this would most likely occur (Scholtissek et al., 1985). Such a virus is unlikely to produce a full-blooded pandemic at the outset. In fact, in 4 years of regular surveillance in Hong Kong of pigs from China, the H5N1 virus was not isolated. It did not exclude the possibility that this reassortment could occur later in a human or that the virus could possibly 'adapt' to humans through repeated contact by, say, nasal mucosal infection as probably occurs in rural southern China (Shortridge, 1992).

2. Second 'wave'

And so the situation lay until November 1997 when the floodgates of concern of a possible pandemic were opened with a second 'wave' of human infection through to the end of December. There were a further 17 cases, five of which were fatal with an overall case fatality rate of 33% (Yuen et al., 1998). All genes of the human isolates were still avian. During November, it was becoming apparent through the haphazard distribution of cases that there was an association with having visited a poultry market or having been in the vicinity of one. This suggested that the virus was established in the markets and that infection was the result of direct avian-to-human transmission. It was felt at the time that

the SAR was in the throes of an incipient pandemic. The critical question that needed to be addressed was the source of the virus in the live poultry markets. Chicken were the predominant poultry in both wholesale and retail markets. They were often mixed with aquatic ducks and geese along with a variety of aquatic and non-aquatic minor poultry. To this end, an ad hoc 'international rapid reaction team' — so named after an earlier proposal in connection with pandemic preparedness (Hannoun et al., 1997) — was formed, comprising those who co-author this report.

Initial surveillance of the markets based on faecal and cloacal sampling indicated that the chicken was the principal source of the virus. Ultimately, it was shown that around 20% were infected (Shortridge, 1999b). In previous surveillance of poultry markets, isolations of influenza viruses from chicken were uncommon, the H5 subtype not one of them. Only ducks and geese, the other members of the triad of major poultry, were infected at around 2.5%. The H5N1 virus was not isolated from any of the other birds examined, namely, five types of wild duck, domestic and wild pigeon, quail, francolin, chukka, pheasant, ornamental birds, feral birds in the markets and miscellaneous birds in parks or gardens. Nor was it isolated from mammals such as dogs and cats, or from rats examined in the wholesale market (although haemagglutination inhibiting activity was detected in some rat sera). This last finding needs to be explored further given that experimental studies show that both mice and rats may be infected by the H5N1 virus (Shortridge et al., 1998).

These findings indicated that the H5N1 virus must have gained access to the wholesale and retail markets in the SAR at a time unknown and had amplified greatly in them, spilling over to the local population and imposing an unprecedented H5N1 'virus load' upon it.

A voluntary ban on the import of poultry into the SAR was put into effect on 24 December and the slaughter of around 1.2 million chicken and 0.3 million other poultry that had been in contact with, or in the vicinity of, chicken commenced on 29 December. This was a complex operation extending to the almost 1000 live poultry retail markets across the SAR, two large wholesale markets and numerous small farms tucked away in the villages and scrubland of the New Territories.

The SAR went through a difficult time over the 2 months of the 'second wave' perhaps no more so than in the slaughter that ended the incident. This was vindicated by the fact that the last human case was recognized the day before the slaughter commenced. It immediately cut off the source of infection for humans. It also had the theoretical benefit of eliminating the possibility of reassortment with the Sydney variant of the H3N2 virus that was troublesome in early 1998 as well as possibly preventing direct zoonotic 'adaptation' to humans through repeated contact. In the case of the latter, recent information suggests that such a situation may have prevailed with the H1N1-like virus that caused the catastrophic 1918 pandemic. Adaptation of a smouldering avian progenitor (Shortridge, 1999a) may have been enhanced through, say, sequestration of plasminogen by the viral neuraminidase leading to unprecedented virulence and pandemicity (Goto and Kawaoka, 1998).

As it turned out, the H5N1 virus was subsequently shown to have been undergoing rapid evolution during the incident, acquiring a number of amino acids in its 'internal' proteins that correlate with replication in the human host (Zhou et al., 1999): scientific

vindication of the slaughter. It is probably fair to say that a pandemic had been averted. Untoward respiratory infection had been recognized in humans and, before that, infection (by the same virus) in animals. These were the two ideals for getting ahead of a pandemic virus espoused by Prof. Sir Charles Stuart-Harris in 1971, in the aftermath of the H3N2 pandemic first recognized in Hong Kong in 1968.

3. Poultry markets

Prior to the slaughter, all types of poultry could be found in the SAR's wholesale and retail markets providing the opportunity for the spread of influenza viruses which, in the case of H5N1 virus, was able to amplify in them.

To reduce the risk, aquatic and non-aquatic poultry are now segregated and come from approved farms in the Shenzhen area across the border. Ducks and geese go to one wholesale market where they are killed and prepared for sale. No other aquatic birds are allowed into the SAR. Non-aquatic birds after various checks either side of the border, including serological examination for evidence of H5N1 infection — are released into the other wholesale market some distance away and then onto the many retail markets.

4. Better pandemic preparedness?

Two major concerns during the incident were the preparation of adequate supplies of reagents for the laboratory diagnosis of H5N1 infection and the difficulty in preparing a vaccine because of the pathogenicity of the virus for cell cultures and the chick embryo. Awareness of such problems is but one of the lessons to be learnt for pandemic preparedness. Notwithstanding the capriciousness of influenza A viruses, perparedness might be improved through a better knowledge of the likelihood of one H subtype of virus over another in giving rise to a pandemic. Improved surveillance of animals and humans is the key.

For example, during the incident, H9N2 viruses were isolated from around 4.7% of chicken as well as from a smaller percentage of ducks, geese, domestic and wild pigeon, quail and from environmental swabs in a market (Shortridge, 1999b). At the time of writing, they may still be found in market poultry. In genetic studies comparing H5N1 and H9N2 viruses, the 'replicating' genes of the H5N1 virus were found to be most closely related to those of an H9N2 virus isolated from quail, suggesting that the H5N1 virus in the incident was the product of reassortment between two avian influenza viruses (Guan et al., 1999). Was the H9N2 virus the genetic driving force behind the H5N1 virus and, is it in itself, a virus with the potential for pandemicity?

Concern about the H9N2 virus increased in April 1998 when it was isolated in the SAR from two pigs originating from China, a concern that increased later when it was shown that contemporary human H3N2 variants were also present in pigs around the time (Markwell and Shortridge, unpublished data), potentially paving the way for avian-human influenza virus reassortment in the hypothetical porcine 'mixing vessel'. This was the first time that an H subtype of a virus other than H1 or H3 had been isolated from

pigs. The World Health Organization was notified of the isolation of these H9N2 viruses from pigs and influenza collaborating laboratories were accordingly advised. The viruses were made available to these laboratories for characterization studies and preparation of diagnostic reagents. Then in March 1999, the virus was isolated in the SAR from two young children with mild influenza-like illness (Weekly Epidemiological Record, 1999). Interestingly, the virus had been isolated in July and August 1998 from five cases of influenza-like illness in patients aged one to 75 years in Guangdong Province adjacent to Hong Kong SAR (Guo et al., 1999). The isolation of H9N2 virus from poultry, pigs and humans is the result of enhanced surveillance.

This suggests that the H9N2 virus or a reassortant of it, HxNy, has the potential for pandemicity. Viruses of the H6 subtype have recently been isolated from poultry at the same time as H9N2 raising the possibility of these two viruses reassorting. The fact that the H9N2 virus can be spread by the respiratory route in addition to the faecal route, the route of spread for the H5N1 virus (Shortridge et al., 1998), could account for its wider occurrence amongst different types of poultry (Shortridge, unpublished data). Moreover, the virus seems to have spread from chicken in eastern Asia in the early 1990s (Shortridge, 1995) to chicken in the Middle East, Europe and Africa today (Alexander, 2000). Could a pandemic virus derived from H9N2 arise outside the hypothetical Asian epicentre?

This fall-out of the 1997 incident has created the potential for even quicker reaction to an incipient pandemic in the future, and to influenza pandemic preparedness generally. It may be a turning point toward better influenza pandemic preparedness for the beginning of the new millennium (Shortridge, 1995).

5. Conclusion

The H5N1 incident was a time of great pressure on the fledgling SAR as the eyes of the world refocused on it following its ceremony of reunification with China on 1 July. It was grappling with an influenza incident with implications for the rest of the world that had never been possible to recognize before. It was as if the weight of the world was pressing down upon the SAR — the 'bird flu' must not 'escape' its confines. Local and international media attention exemplified this particularly in the final phase, the slaughter probably being the most difficult and controversial (Fig. 1). The responsibility for all concerned was enormous, neither overreacting nor underreacting.

The Hong Kong SAR did not go-it-alone and brought in representatives of international agencies, such as the World Health Organization and the Centers for Disease Control and Prevention as well as taking on board the support and advice of many beyond the SAR. It was a time when everybody pulled together for the good of all, an important message for dealing with a future influenza situation that must inevitably occur since influenza is a non-eradicable disease. Pleasingly, the incident fostered a new spirit of camaraderie amongst influenza investigators.

But first and foremost, the nuts-and-bolts of the incident were put together by many in the SAR who gave unstintingly in their effort. The brunt of this was borne by the SAR's Department of Agriculture and Fisheries, Department of Health and Hospital Authority.

Fig. 1. One of many signs posted by stallholders at a wholesale market expressing concern about their livelihood during the mass slaughter of poultry across the SAR. Translation: 'Who cares about H5N1, our jobs are in jeopardy'.

The SAR public are well informed about influenza through in-depth media coverage, particularly in mass circulation newspapers and magazines, often incorporating detailed information on the genetics of influenza viruses and how they reassort. The human influenza surveillance system is probably the most compact and wide ranging of any. The isolation of H9N2 viruses from two children in the SAR would probably have been missed were it not for this system and the awareness of influenza and its import by the medical community.

The Hong Kong SAR is, in effect, a sentinel post for influenza events taking place in the region.

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