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The changing epidemiology of avian influenza

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Only viruses of the *Influenzavirus A* genus have been isolated from birds and termed avian influenza [AI] viruses, but viruses with all 16 haemagglutinin [H1-H16] and all 9 neuraminidase [N1-N9] influenza A subtypes in the majority of possible combinations have been isolated from avian species.

Influenza A viruses can be divided into two groups on the basis of their ability to cause disease and death in poultry. The very virulent viruses cause highly pathogenic avian influenza [HPAI], with flock mortality as high as 100% in chickens and other susceptible poultry. These viruses have been restricted to subtypes H5 and H7, although not all H5 and H7 viruses cause HPAI. All other viruses cause a milder, primarily respiratory, disease [LPAI], unless exacerbated.

Until recently HPAI viruses were rarely isolated from wild birds, but for LPAI viruses extremely high isolation rates have been recorded in surveillance studies, with overall figures of about 11% for ducks and geese and around 2% for all other species. Influenza viruses may infect all types of domestic or captive birds in all areas of the world, the frequency with which primary infections occur in any type of bird usually depending on the degree of contact there is with feral birds. Secondary spread of HPAI viruses has been associated with human involvement, usually by bird or bird product movement or by transferring infective faeces from infected to susceptible birds. Potentially wild birds could be involved in spread, but until the emergence of the Asian H5N1 HPAI virus there was no evidence that they had.

Since the mid-1990s AI infections due to two subtypes have been widespread in poultry across a large area of the World. LPAI H9N2 appears to have spread across the whole of Asia in that time and has become endemic in poultry in many of the affected countries.

However, all other outbreaks of AI in recent years have been overshadowed by infections caused by the H5N1 HPAI virus, initially isolated in China, which has now spread in poultry and/or wild birds throughout Asia and into Europe and Africa, resulting in the death or culling of hundreds of millions of poultry and posing a significant zoonosis threat. This represents an entirely new situation, where the HPAI H5N1 virus is endemic in sectors of the poultry industries in a number of Asian countries and continues to perpetuate despite attempts at control. Additionally the HPAI H5N1 has caused several epizootics in wild birds.

Novel proteolytic activation proteases of highly-pathogenic avian influenza viruses which cover wide strains, even for non-susceptible strains by furin and PC5/6

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The post-translational proteolytic cleavage of precursor of the membrane fusion glycoprotein, hemagglutinin (HA), of influenza A virus is a prerequisite for viral membrane fusion activity and viral entry into cells. As is the case for highly pathogenic avian influenza (HPAI) viruses, endoproteolytic processing of HA takes place by means of ubiquitous cellular processing proteases. There are two types of the multi-basic consensus cleavage motifs of HA in HPAI viruses. One is the motif Arg-X-Lys/Arg-Arg, which is cleaved by Furin and PC5/6 and the other is the motif Lys-X-Lys/Arg-Arg, which is not cleaved by Furin. Cellular processing protease(s) for the latter HA motif of HPAI viruses has not been reported so far.

In the present study, we reported type II transmembrane serine proteases, MSPL (mosaic serine protease large–form) and TMPRSS13, which have strict cleavage specificities for the paired basic residues, unique enzymes in the family of type II transmembrane proteases. These proteases are distributed predominantly in the plasma membrane and partly distributed in the intracellular compartments. Both genes are ubiquitously expressed in various organs of human, mice and chickens and predominantly expressed in the enteric ducts, blood vessels, lungs, placentas and

prostates. These proteases selectively cleaved both HA motifs of HPAI viruses, such as Lys-Lys-Lys-Arg motif of A/chicken/Pennsylvania/ 1/1983(H5N2) and Arg-Lys-Lys-Arg motif of A/chicken/ Scotland/59 (H5N1) and induced membrane fusion activities. Pathogenicity of these viruses and effects of protease inhibitors of these enzymes are currently under investigation.

References
Kido H et al. 2007. Curr Pharm Des, 13, 405-414.
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Keywords
Processing protease, pathogenecity, haemagglutinin, membrane fusion activity, furin

Molecular determinants of H5N1 avian influenza virus for high virulence to ducks

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Two strains of H5N1 virus, A/Mallard/Huadong /S/05(S) and A/Mallard/Huadong/Y/03(Y) with distinct virulence to ducks were characterized in a surveillance program of domestic waterfowl. Both strains were highly pathogenic for chickens with IVPI values of 3.00 and 2.88 respectively. Strain S was highly pathogenic for ducks with IVPI of 2.65 whereas strain Y was nonpathogenic for ducks with IVPI of 0. The S virus replicated to significantly higher titer in various visceral organs and in tracheal and intestinal mucosa than did the Y virus. When we compared the entire genome sequences of the two viruses we found that both were distinct reassortants of genotype Z and there were 72 variations in amino acid sequences, distributing in seven genes except M. However, the two viruses shared all the amino acid positions known to be critical to virulence and host specificity, except those in HA cleavage site. Interestingly, the two viruses carried different amino acid in positions 322 and 329 (numbered according to H3) of the HA cleavage site in which the S virus had the sequence PLRERRRK-R//G, same as that of FJ-like strain, whereas the Y virus had the sequence PQRERRRKKR//G, same as that of strain Gs/Gd/1/96.

To investigate the molecular mechanism of the virulence difference of the two viruses, we constructed a series of recombinant viruses with the two backbone viruses by reverse genetics and measured their virulence (IVPI) in domestic mallards. Recombinant S viruses with PB2, PB1 and PA genes or with HA gene of Y virus significantly reduced the virulence while the recombinant with M and NS genes of Y virus only slightly reduced the virulence when compared with the S virus. On the other hand, recombinant Y viruses with PB2, PB1 and PA genes or with HA gene of S virus significantly increased the virulence while the recombinant with M and NS genes of S virus did not increase the virulence when compared with the Y virus. To further study the influence that the difference in sequence of HA cleavage site might exert, we constructed a panel of recombinant viruses with mutations or chimerical sequences in HA gene and measured their virulence in domestic mallards. Recombinant S viruses with mutations in HA gene that resulted in L 322 Q or -329K or both became nonpathogenic to mallards. Meanwhile, recombinant Y viruses with mutations in HA gene that led to Q322L or K329- or both significantly increased the virulence to mallards whereas recombinant viruses with chimerical sequences in HA gene that did not lead to the change of amino acid in position 322 or 329 did not change the virulence. The present study indicated that the PB2, PB1, PA and HA genes of H5N1 virus may involve in determining the viral virulence to ducks and the L322 in combination with deletion of position 329 in HA cleavage motif is the major contributor to the high virulence in ducks.

Keywords
H5N1 virus, high virulence to ducks, molecular determinants

Presentation of an infection control algorithm for use in healthcare facilities, ambulatory services and other emergency services in the event of pandemic influenza

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The increasing threat of an influenza pandemic has urged immediate review of pharmaceutical and non-pharmaceutical infection control measures for healthcare professionals and for those in the front line community services. Infection control practices include the appropriate use of personal protective equipment, social distancing measures, vaccination and antibiotic prophylaxis to prevent occupational acquisition of pandemic influenzae. We have undertaken an evidence based assessment of the literature for the infection control of SARS, avian influenza and pandemic influenza. Using guidelines set by the National Health and Medical Research Committee (Australia), our review analysed publications for levels of evidence given the inherent study design biases and confounding factors and the generalisability of the findings to the different healthcare professional groups. Consideration has also been given to the practicality and feasibility of implementing identified infection control practices as well as the compliance issues associated with each practice. The primary outcome of this assessment is our generation of an infection control algorithm based on evidence while considering the practical and compliance issues. This algorithm will be employed in healthcare facilities and other emergency and community front line services. The resultant evidence-based infection control protocol also has potential for application in the wider community setting.

Keywords
Infection control, pandemic planning, evidence based, healthcare

Zoos as disease sentinels: Piloting an avian influenza surveillance system in zoological institutions

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The avian influenza virus could serve as a model for the majority of infectious disease events that have occurred in humans over the last 60 years. Namely, it is a disease that originated in non-human animals but has since demonstrated a capacity for zoonotic infection. Relying on historical evidence and projected population trends, future emerging infectious diseases are likely to be zoonotic in nature. Monitoring the emergence and spread of these infectious agents with complex natural histories might be best approached with novel surveillance strategies.

One such strategy involves the use of zoological institutions as zoonotic disease sentinels. Captive wildlife systems, such as those housed at zoological institutions, possess all the characteristics of an ideal sentinel for the detection of zoonotic disease. Specifically, zoos host a broad range of species, each with its own susceptibility to a particular disease. Second, zoo animals are regularly observed and monitored by veterinary staff and often have extensive documented medical histories. Third, zoos house a relatively stationary population, allowing for sequential evaluation of individuals and groups on a regular basis and over time. Fourth, the zoological community provides a wide geographical distribution of sentinel sites with over 200 institutions in 47 states. These facilities span urban, suburban, and rural environments, and overlap with all four of the major North American migratory bird flyways. Fifth, zoos represent a potential interface between wildlife and humans, serving as a possible source for zoonotic disease transmission.

On behalf of the American Association of Zoos and Aquariums (AZA) and with the support of the United States Department of Agriculture (USDA), Lincoln Park Zoo (LPZ) is launching a Zoonotic Emerging Disease Surveillance Center that will use zoological institutions across the country as disease sentinels to monitor for avian influenza virus. The system includes free sample diagnostics for participating institutions and access to real-time test results from a centralized, confidential database. This presentation will describe the surveillance system and its importance as a novel form of surveillance. Additionally, data obtained from the pilot test of the system will be presented along with a progress report on the status of the full launch.

Continuing Evolution of avian influenza viruses: Is H5N1 beyond control?

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Pandemic influenza is a zoonotic disease caused by the transfer of influenza A viruses or virus gene segments from aquatic bird reservoirs to humans and domestic animals. In wild aquatic birds – the natural hosts of influenza viruses – these viruses exist in harmony with their hosts and are non-pathogenic. After transfer to other species influenza viruses evolve rapidly. It is now a decade since the H5N1 virus emerged in Asia and the virus continues to spread and to increase its host range, and genetic and antigenic diversity. The probability is that we are witnessing in real-time the evolution of a pandemic strain and the virus is continuing to evolve.

The remarkable genetic heterogeneity of the H5N1 highly pathogenic influenza virus may have occurred because this virus has moved successfully between multiple species of birds and mammals. The original virus which emerged from an unknown source in the natural wild bird reservoir became highly pathogenic and established in the domestic waterfowl of Asia. From 1997 to 2004 the highly pathogenic H5N1 was largely confined to Southeast Asian countries with high lethality in domestic poultry with rare transmissions to humans. In humans over 60% of those infected die. In May 2005 the highly pathogenic H5N1 infected Bar-headed geese and other waterfowl on Qinghai Lake, China killing a high percentage of infected birds. Subsequently the H5N1 spread rapidly to the Indian, African and European regions of Eurasia.

The role of migrating birds in the spread of H5N1 and exchange of viruses between domestic and wild birds is a continuing concern. The unanswered question concerning H5N1 influenza is whether the highly pathogenic H5N1 viruses are being perpetuated in the wild migratory birds of the world. What are the ultimate reservoirs of highly pathogenic H5N1 virus? If wild migratory birds are the ultimate reservoirs of highly pathogenic H5N1 this would be a paradigm shift that means, that like low pathogenic subtypes the highly pathogenic H5N1 is not eradicable and that the frequency of outbreaks of highly pathogenic H5N1 in gallinaceous poultry will increase. If highly pathogenic H5N1 is established in migratory birds why have the highly pathogenic viruses not spread to the Americas and to Australia? What are the prospects for the H5N1/07 virus to become consistently transmitted from human to human and cause a global catastrophe? These are some of the questions we will address as the virus continues to evolve.

Currently there are multiple genetically distinguishable clades and subclades of H5N1 that necessitate the preparation of multiple different vaccines and continued evaluation of anti-influenza drugs. The certainty is that an influenza pandemic will occur in humans at some future time and preparation for H5N1 will serve global preparedness.

The spread and evolution of highly-pathogenic avian influenza (HPAI) H5N1 in Africa

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In Africa HPAI H5N1 virus was first detected in Northern Nigeria and since then in 9 other African countries: Niger, Egypt, Cameroon, Burkina Faso, Côte d'Ivoire, Ghana, Togo, The Sudan and Djibouti. Phylogenetic analysis and substitution rates of complete genome sequences of Nigerian strains from the South-West and the North showed that three sublineages were present in Nigeria as early as February 2006 and that three independent introductions of H5N1 into the country are likely. (MF Ducatez et al. Avian flu: Multiple introductions of H5N1 in Nigeria. Nature 442, 37, 2006). These three sublineages include all African strains and by now have a distinct geographic distribution: sublineage A (initially Southwest Nigeria, Lagos, Niger), B (initially Southwest Nigeria, Lagos, Egypt, Djibouti) and C (initially Northern Nigeria, Burkina Faso, Sudan, Côte d'Ivoire) within Africa. Probable non-African ancestors within the West-Asian/Russian/European lineage distinct from the Southeast Asian lineages were identified for each sublineage. Evidence of reassortments between these sublineages is currently emerging. In 2006 all reported human cases in Africa (Egypt, Djibouti) were caused by sublineage B. A serological study in poultry workers and vendors at life-bird markets in Nasarawa State, North-Central Nigeria, provided no evidence of past H5N1 infections despite sustained large scale infections of the poultry industry in this State until end of 2006. We have also characterized the first avian influenza strains from Burkina Faso and the first strains from African wild birds (sublineage C). Between February and June 2006, 48 hooded vultures (*Necrosyrtes monachus*) were found dead or sick throughout Ouagadougou. We present here the first sequences from African wild birds and compare them to strains found in Burkina poultry. The sequences clustered with strains found in Côte d'Ivoire, initially Northern Nigeria and The Sudan (sublineage C). We showed that the infection of scavenger birds in Africa is likely to cause spill-backs from poultry to wild birds, which is rarely seen in other countries and has important consequences for surveillance in Africa and beyond. As they scavenge on many dead species, they may also function as conspicuous sentinels in the African continent, similar to raptors or swans in Europe or cats in Indonesia. Learning from the experience in implementing molecular and surveillance in techniques under appropriate biosafety condition in resource poor countries is critical to further remove bottlenecks in world-wide HPAI H5N1 surveillance.

Keywords
Highly pathogenic avian influenza H5N1, Nigeria, poultry, wild birds reassortment, sublineages

The pig as a mammalian model of resistance to H5N1 infections: Evaluation of host receptor distribution and innate resistance

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Influenza A viruses have a wide host range for birds and mammals, posing a major threat to animal health as well as a zoonotic threat to humans. Swine influenza, in particular, is a major respiratory disease, and along with other respiratory pathogens, such as the Porcine Reproductive and Respiratory Syndrome (PRRS) virus, can cause severe production losses to the pig industry. However, the clinical manifestation of uncomplicated influenza infection in pigs is usually mild and non-fatal, with full recovery within a week after the onset of clinical signs (such as dyspnoea or coughing). Indeed, in experimental infections, complete virus clearance can be demonstrated within a week of virus exposure.

The respiratory tract of pigs is permissive to the replication of avian, swine and human influenza viruses, and, as such, pigs have the potential to act as hosts for the generation of new pandemic influenza viruses of humans, by reassortment. One distinguishing feature of highly pathogenic H5N1 infections is the severity of the human condition with high mortality (>50%) in contrast to the relatively mild clinical signs observed in pigs. Pigs are seemingly more resistant, showing reduced clinical severity and more rapid recovery, following infection with highly pathogenic H5N1 avian influenza viruses than chickens and humans.

In order to evaluate the pig as a mammalian model of resistance to highly pathogenic avian influenza viruses, in particular H5N1 subtypes, we need to establish the relative susceptibility and pathogenicity of the pig to avian and mammalian influenza virus infections. To this end, we undertook extensive investigations into the organ distribution of virus receptors in the pig. By lectin immuno-histochemistry, using confocal microscopy, we profiled the anatomical distribution of SAα2,3 Gal and SAα2,6 Gal receptors in key organs of the pig. Additionally, we are conducting ex vivo organ and in vitro primary tracheal epithelial cultures to establish virus infectivity, virus replication and cellular pathogenicity for avian (H2N3) and swine (H1N1) influenza viruses. One interesting finding to date is that the avian H2N3 virus appears much more pathogenic than swine H1N1 virus on porcine tracheal epithelial cells. Supporting evidence from *ex vivo* and *in vitro* infection cultures, suggests that this high cell death to avian virus infection may paradoxically be a host protective mechanism to resist virus replication subsequent to virus entry.

Keywords
Host receptors, resistance, susceptibility, pig

Clinical trials of a flu vaccine designed to induce cross-subtype immunity

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Recent infections of avian influenza (H5N1) in humans could lead to a new pandemic if the virus acquires the ability to transmit between humans, with potentially devastating effects across the world. Current widely-used vaccines for seasonal influenza A act by stimulating production of antibodies to the highly polymorphic HA and NA, and induce little or no cross-subtype protection. Annual revaccination with the current H1N1/H3N2/B combination is required to maintain immunity. The current high rate of diversification of H5N1 strains suggests that vaccines made now may differ so much in their H5 sequence from any pandemic strain that emerges that these vaccines would have little or no efficacy. Seasonal influenza infection results in a cross-subtype T cell response to the virus which can protect against subsequent infection. Recent studies in Oxford (Laurel Lee and Tao Dong, WIMM) have shown that 90% of the adult population have detectable T cell responses to one or more influenza antigens, including the highly conserved internal proteins. However over the course of a few years these responses decline below protective levels. The new vaccine being tested in this Phase I open-label observational study is designed to boost these cross-reactive T cell responses back to protective levels. If satisfactory safety and immunogenicity results are obtained, a Phase IIa influenza challenge study will follow.

A single-cycle vesicular stomatitis virus (VSV) vector vaccine protects chicken from highly pathogenic avian influenza virus (H7N1)

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Fowl plague is a fatal disease of poultry caused by highly pathogenic avian influenza A viruses (HPAIV) of subtypes H5 and H7. These pathogens pose a permanent threat not only to domestic poultry but also as potential zoonotic agents to humans and other mammalian species. So far, general vaccination of domestic poultry is prohibited in Europe. The common inactivated influenza virus vaccines do not prevent virus shedding, and there are no established means to discriminate between infected and vaccinated animals (DIVA).

We have generated a novel marker vaccine that is based on a replication-incompetent vesicular stomatitis virus (VSV) lacking the VSV glycoprotein G gene. The vector can be propagated to high titers (1 x 10^8 infectious units/ml) on a trans-complementing helper cell line. The complemented vector was used to express the HA and NP antigens of influenza A/FPV/Rostock/34 (H7N1) in avian cells at high levels. Because no progeny virus was produced after infection, the vector was classified as a biosafety level 1 organism ("safe").

The efficacy of this novel vaccine was evaluated in chicken. Three-week-old SPF chicken were inoculated intramuscularly with either vehicle, vector expressing the control antigen GFP, vector expressing HA, or two separate vectors expressing HA and NP, respectively (2 x 10^7 infectious units/bird). Because infected cells did not express the VSV glycoprotein, no neutralizing antibodies directed against the vector were produced. Three weeks after primary immunization the animals were boostered with the same vaccines using the same route and dose. Two weeks after booster vaccination neutralizing serum antibodies were detected, and the animals were challenged oculo-nasally with $10^{7.8}$ EID_{50}/bird of A/chicken/Italy/445/99 (H7N1). SPF chicken vaccinated with vector expressing HA or HA+NP kept well and survived the challenge infection. In contrast, all control birds showed severe signs of illness and died on day 3 or 4 post challenge. Challenge virus to be detected by RT-PCR in oropharyngeal and cloacal swabs collected from vaccinated animals is currently being investigated. Seroconversion of the vaccinated animals was observed 3 weeks after challenge using an ELISA for detection of either NA or NP antibodies.

In summary, we have generated a single cycle vector vaccine that is safe, fits the DIVA principle and is protective even against high doses of heterologous HPAIV. These novel vector vaccines may help to control avian influenza virus epidemics in future. Improved vectors that will further reduce virus shedding are in progress.

Comparison of sensitivity of three methods for detection of type A influenza virus

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Laboratory diagnostic of avian influenza (AI) is based on the isolation and identification of the virus or determination of the viral proteins or genes. Although the diagnostic procedures for AI viruses (AIV) are described in details, there are several questions arising, concerning the specifity and sensitivity of the used methods. Two molecular methods, a one-step reverse transcriptase-polymerase chain reaction (RT-PCR) and a real time RT-PCR (RRT-PCR), both targeting gene M and gene H5 of AIV and detection of the virus by isolation in SPF fowl eggs were compared to determine the sensitivity of different methods. The allantoic fluid containing $10^{6,4}$ ELD of HPAI isolate of H5N1 subtype (A/mute swan/Slovenia/649/06), with hemagglutination titre 1:128

was diluted in ten-fold dilutions up to 10^{-10}. The serial dilutions were directly tested for the presence of the virus by hemagglutination test, RT-PCR and RRT-PCR. Same dilutions were also inoculated in the SPF fowl eggs for virus isolation. Allantoic fluids, harvested from dead embryos or on 5th day post inoculation, were again tested for the presence of AIV by all three methods. The results indicate that 10^{-1} and 10^{-2} dilutions of virus could be detected by hemagglutination test. One step RT-PCR was able to detect virus up to 10^{-4} or 10^{-5} dilutions, depending on the target gene, while RRT-PCR amplified virus from up to 10^{-6} dilutions. Inoculations of up to 10^{-6} dilutions of the virus into SPF embrionated eggs yielded positive result by hemagglutination test, while RT-PCR targeting gene M detected virus after inoculations of up to 10^{-7} dilutions. RT-PCR targeting gene H5 and RRT- PCR targeting M gene and H5 gene amplified virus after inoculations of up to 10^{-9} dilutions. The results of the study suggested a 1000 to 10000-fold higher sensitivity of PCR- based methods compared to detection of the virus by hemaglutination test. The results also indicated that low concentrations of virus that could not be detected by hemaglutination test or by molecular methods gave positive results by RT-PCR and RRT-PCR after one passage in SPF chicken embryos. The PCR-based methods have higher sensitivity compared to conventional methods, like virus isolation and detection of the virus by its hemagglutination activity. The sensitivity of molecular methods could be upgraded by using combination of at least one passage of the virus in the SPF embryonated chicken eggs coupled with molecular method for the detection of virus in allantoic fluid.

Keywords
Avian influenza viruses, virus isolation, RT-PCR, RRT-PCR, sensitivity

Economics and livelihood impacts of HPAI shock and stress in Nigeria

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Less attention has been paid to the specific nature of HPAI implications for livelihood of the smallholders in developing countries. This paper reviews 75 studies conducted by both national and international researchers with the aim of identifying key issues associated with macroeconomic and livelihood impacts of HPAI at national, community and household levels using Nigeria as a case study. In 2004, the percentage annual growth in the poultry sub-sector in Nigeria declined from 9.17% to -0.77% due to the ban on poultry importation. This policy change and emergence of HPAI might have contributed to the sharp decrease in poultry trading activities in 2006. The study reveals that every category of household members disaggregated by age and gender groups participates in extensive poultry production. However, women are more involved in the south and men in the north. We found that poultry significantly helps poor households in achieving sustainable livelihood outcomes. Up to 80% of households in the north may keep poultry mainly as a source of protein. Women especially depend on poultry income to provide household needs. At the onset of HPAI outbreak in the country, about 80% of households stopped purchase and consumption of poultry in 2006. However, very few studies have investigated such livelihood implications of HPAI. Generally, we found that a considerable level of research effort is required in the areas of intra-household dynamics of importance of poultry. Also, an analysis of disease risk, spread pattern, and contribution of compensation policy in household recovery will assist in understanding the ex-ante/ex-post coping/risk management behaviour of households in Nigeria.

Keywords
Livelihood, poultry, HPAI, risk and food security, Nigeria

Inactivation of airborne avian influenza a H5N1 virus with positive and negative ions

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The virucidal efficacy of cluster ions against airborne viruses was evaluated. Highly pathogenic H5N1 avian influenza virus (NIBRG14 Vietnam/1194/2004) was used in these experiments. Avian Influenza A H5N1 virus at a concentration of >107TCID50 per ml was aerosolized into a one cubic meter enclosure, and exposed to circulating ions (average density: 7000 pairs/cm3). The virus was then extracted via a vacuum pump into a pair of impingers. Vacuum pressure was applied for 10 minutes to collect 100 litres of air containing viruses into the two impingers which contained cell infection media. Collected samples were supplemented with protein (0.3%BSA) and stored in small aliquots at -80 degrees Celsius. The samples were then inoculated onto monolayers of MDCK cells and titrated to determine the viral titre of the residual virus. The efficacy of the positive and negative cluster ions was assessed by comparing the virus titre with the ionizer on and the ionizer off. 5 to 15 minutes after spraying virus, the virus titre without ions was 104.29TCID50 per ml, while the virus titre with ions was 102.33TCID50 per ml. It was observed that the cluster ions have the ability to significantly reduce the viral titre of Avian Influenza A H5N1 virus. Exposure of H5N1 virus to the positive and negative cluster ions for 5 to 15 minutes can result in reduction of up to 98.9%. In previous experiments: 1) H+(H2O)m and O2-(H2O)n ions were detected by TOF (Time of flight) mass spectrometer. 2) The inactivation effect was not obtained by using only negative ions. 3) OH radicals were detected by observing reaction with H2O2+WST-1*. *WST-1: 2-(4-Iodophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium, monosodium salt. In view of these results, we believe that the mechanism of inactivation is as below. Step1) Positive and negative ions surround the floating viruses. Step2) Active OH radicals are generated by the ionic reaction on the

surface of viruses. Step3) OH radicals combine with hydrogen from the surface proteins of the influenza viruses, destroying their ability to bind to and infect cells.

Keywords
Avian influenza, H5N1, ion, inactivation, Airborne, Positive, Negative

Innovative initiatives to mitigate pandemic influenza: An interdisciplinary review

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The science is clear: There is going to be an influenza pandemic; however, no one knows when such a pandemic will begin nor how lethal or contagious the virus will be. Some 95 per cent of the deaths will be in the developing world (See McKibbon at: http://www.lowyinstitute.org/Publication.asp?pid+345) certain countries, such Indonesia, are rightly worried about whether they will obtain vaccines which have been produced from samples of the virus that they have sent to labs approved by the World Health Organization (WHO). It is essential to share H5N1 viruses to stop a global influenza pandemic and create strategically-located stockpiles of anti-viral drugs (Garrett & Fidler, PLOS Med, Nov 2007).

This presentation offers a meta-analysis of selected articles, books and web pages about pandemic influenza from scientific, medical and health sources. The relevance of the SARS (Severe Acute Respiratory Syndrome) experience is linked to the importance of identifying and strengthening key networks (as set out in the March 2008 Editorial in PLOS Medicine) and to the One Health Initiative to unite human and veterinary medicine (www.infectiousdiseasenews.com/200802/veterinary.aspp) . This review focuses on three different perspectives: the animal community, the human community and the H5N1 virus itself.

Within the animal community, the spread of the H5N1 virus is linked to an abundance of domestic ducks (www.pnas.org/cgi/doi/10.1073/pnas.071058 11055), live poultry markets and commercial poultry farming (www.birdflubook.com). Topics covered include which species are susceptible to the virus, the role of animal vaccination policies and animal-human interaction.

For the human community, attention is directed to the feasibility of mitigating a pandemic once WHO Phase 4 (small clusters of cases with limited person-to-person transmission) has been reached. This is of considerable importance because of mounting evidence of probable limited person-to-person transmission of the virus in Thailand, Indonesia and China (See The Lancet, 8 April 2008). Significant attempts to contain H5N1 have been launched by www.google.org, InSTEDD (www.instedd.org), Global Health and Security Initiative (www.ghsi.org) and PATH (Program for Appropriate Technology in Health) (www.path.org/news/pr080114_flu_research.php), as well as WHO.

The H5N1 virus itself is elusive. Identifying and containing it will require linking networks of disease surveillance and laboratory analysis with anti-viral and vaccine research, production and distribution. Although there are hopes for the effectiveness of a pre-pandemic vaccine, development of a fully effective vaccine will be possible only after the mutated H5N1 virus has been identified. Much work remains to be done.

Keywords
Epidemiology, pandemic, H5N1, vaccine, anti-virals, Indonesia, China

Molecular quantitation of the H9N2 avian influenza virus in various organs of broiler chickens using Taq-Man Real time PCR

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During the past decade, H9N2 low pathogenic avian influenza virus (LPAI), has caused considerable economic losses due to decreased production, increased mortality and cost for vaccination in Iranian poultry industry. Because of the widespread occurrence of this disease and the virus potential to mutate to highly pathogenic (HP) form and transmission to humans, it is essential to investigate the virus pathogenecity. It was shown that real time PCR is a rapid, sensitive and specific method for detection and quantitation of influenza viruses. In this study, a two step Taq-Man real time PCR assay was performed for the quantitation of A/chicken/Iran/772/1998(H9N2) virus in various organs of broiler chickens at different days post inoculation (DPI).

Forty 5-week-old commercial broiler chickens were inoculated intranasallly with 10 6.5 EID50/100 µl of the virus. Five chickens were randomly selected on days 1, 3, 6 and 9 PI. They were sacrificed and gross lesions were recorded. The trachea, lung, spleen, kidney, pancreas, blood and feces were collected for virus detection. On days 2 and 6 PI two inoculated chickens died.

PCR was performed with specific primers of H9 protein gene of influenza A. The positive samples were used for quantitative real time PCR assay. Recombinant plasmids cloned with matrix protein gene were used as a standard in the real time PCR. The primers and probe used for real time PCR assay were selected from M1 protein gene of influenza A.

The result of RT-PCR assay showed the presence of the virus in trachea (40%, 33%), lung (20%, 66.6%) and spleen (20%, 50%) of infected chickens on days 3 and 6 PI respectively. The virus was also detected in kidneys of inoculated chickens on 3 (40%), 6 (60%) and 9 (100%) DPI. In fecal samples the virus was only detected on day 6 PI (83.3%).

The molecular quantitation of AIV showed that the AIV titer in the trachea, lung and spleen of chickens at 3 DPI is lower than the AIV titer at 6 DPI in these organs. Although the most frequency of virus detection was seen in the kidneys, the lowest titer was observed in this organ. There were no significant differences between virus titer in the kidneys on days 3, 6 and 9 PI. The highest titer was observed in the feces. The AIV titer in all organs of the birds died at 6 DPI were higher than those of the same organs in the other experimental birds.

Keywords
Avian Influenza, H9N2 , Real time PCR

Mortality by avian influenza (H9N2) in Iran

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Avian influenza (AI) is one of the major causes of mortality among chickens. Iran is an established endemic region for AI. There are many reports about its different subtypes, outbreak and distribution from all around the world; however data on its prevalence and epidemiology are scarce from Iran. In this cross sectional questionnaire survey, we evaluated the prevalence of mortality related to AI in different provinces of Iran for a 15- month period between September 2004 and November 2005. All broilers covered by the country's national insurance program were included in the analysis. From the 30118 flocks (total chickens=439,188,406) covered under this program, 11751 flocks (188,680,459 chickens) developed some kind of disease and consequently were reported to the insurance organization. Analysis of the questionnaires showed that 1484 flocks (24,959,890 chickens) were encountered with AI. The incidencece of AI mortality was 25% and 1.4% among AI encountered and total chickens respectively. The number of flocks which had mortality in relation with AI significantly decreased in the spring of 2005 compared to the fall and winter of 2004. The mortality rate slightly increased in the fall and winter of 2005. Close analysis of the data also showed that about 88% of AI-related mortalities occurred in chickens of 4-6 weeks old. Results of this study show that AI is a disease with heavy burden on chicken industry in Iran and implementation of mechanisms to control the spread of this disease is necessary.

Keywords
Mortality, avian influenza, broiler, H9N2, Iran

The pig as a mixing vessel for influenza A viruses

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Influenza A viruses are enveloped, single-stranded RNA viruses belonging to the family *Orthomyx-oviridae* and are highly infectious respiratory pathogens in their respective natural hosts, which includes birds, lower mammals and humans. All 16 HA and 9 NA subtypes of influenza A viruses have been isolated from wild waterfowl and seabirds. Therefore, birds have been considered the reservoir for influenza A viruses from which novel viruses can emerge to infect mammalian species. The ecology of influenza A viruses is dynamic and very complicated involving multiple host species and viral genes. Occasionally, influenza A viruses are transmitted to mammals from avian species which can lead to the development of human pandemic strains. Because swine are susceptible to infection with both avian and human influenza viruses, genetic reassortment between these viruses and/or swine influenza viruses can occur. The potential to generate novel influenza viruses in pigs has led to the "mixing vessel" theory. Although there is no direct evidence that the reassortment events leading to the 1918, 1957 or 1968 pandemic viruses occurred in pigs, genetic reassortment among avian, human and/or swine influenza viruses has occurred in pigs and some novel reassortant viruses have transmitted from pigs to humans. For example, the double reassortant (avian/human) H3N2 and the triple reassortant (avian/human/swine) H1N1 swine influenza viruses have infected humans in Europe and in the US, respectively. Recently, triple reassortant (avian/human/swine) H2N3 influenza viruses were isolated from US pigs, which contained leucine at position 226 and glycine at position 228 of HA, similar to the early isolates of the H2N2 human pandemic (Ma et al, 2007, *PNAS*, 104, 20949). These novel H2N3 swine viruses were able to cause disease in swine and mice and were infectious and highly transmissible in swine and ferrets without prior adaptation. These viruses pose a substantial risk to humans because H2 influenza viruses have been absent from humans since 1968. This demonstrates that pigs can transmit novel viruses from an avian reservoir to other mammalian species. Although swine can generate novel influenza viruses capable of infecting man, at present it is difficult to predict which virus, if any, will cause the next human pandemic. Based on history, influenza is and will continue to be a serious threat to human health.

Keyswords
Swine, influenza A virus, "mixing vessel" theory