MEETING REVIEW

Bird flu at Oxford: A meeting review

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In September 2008, scientists from all across the globe gathered at Oxford to consider five related themes in combating avian influenza; epidemiology, vaccines, virulence and pathogenicity, immunology, and antivirals. Overarching this focus on the relationship of avian influenza to human health was the necessity of viewing veterinary and human medicine as an integrated whole. As Dr John McCauley (UK) pointed out, in his opening remarks to the conference, because there are so many unknowns, government pandemic plans can set out only a rough estimate of the human death rate. It appeared that no government pandemic plans were able to plan for a death rate as high as was being observed with the H5N1 virus currently recorded by the World Health Organization (WHO). The "panzootic" of the H5N1 virus was unusual for highly pathogenic avian influenza viruses, because usually such viruses were controlled within a limited area.

Focusing on the changing epidemiology of avian influenza, Dr Dennis Alexander (UK) explained the current theory that the mutation from low pathogenic avian influenza (LPHI) to high pathogenic avian influenza (HPAI) takes place *after* the introduction from wild birds to domestic poultry and domestic ducks, especially densely populated poultry and ducks in commercial farms (Alexander, 2006). The spread of numerous viruses can take place in many ways – bird migration, human and bird movement within and between farms, truck deliveries, the sale and slaughter of live birds, contaminated faeces and feathers, fighting cocks, smuggled birds. It is fairly certain that the viruses responsible for the most recent outbreaks of influenza A viruses were first detected at Lake Qinghai in Northern China in 2005 and spread in many directions (Alexander, 2007; Vijaykrishna et al, 2008). Whether that spread was by bird migration or human movement, especially as the spread seemed to follow the route of the Trans Siberian Railway, is not clear.

As the opening keynote speaker, Professor John Oxford (UK) suggested that pandemic plans should be viewed in the context of three walls of defence: An outer defence zone of anti-viral drugs, a stock of vaccines, and an inner defence zone based on self-empowering personal decisions about social distancing and hygiene. In his address outlining scientific, medical and social lessons from the Great Influenza Pandemic of 1918, he stressed the importance of World War I, "the teenage war," in gathering together so many soldiers in camps living under special strained circumstances. For each soldier who died fighting, ten died from disease. There were only 145 deaths in 1916, but some 50 million deaths by the end of 1920. In the current outbreak of H5N1 influenza, there had been only 158 deaths from 2003 to 2006, but the number in future years is, of course, not yet known. Recent demographic studies had highlighted how with each successive wave in 1916-1919 the number of children under 5 who died had increased significantly. Different cities had approached the pandemic with different methods of social distancing. Further study of how people reacted was necessary.

Professor Albert Osterhaus (The Netherlands) tackled the burning question of the availability of an effective vaccine at the next influenza pandemic. There were a number of significant medical tools for intervening in the face of a possible pandemic. The World Health Organization (WHO) surveillance system for human pandemic, as well as extensive surveillance systems for animals established by the World Organization for Animal Health (OIE), both focused on early warning and rapid response. Some 140 laboratories were involved, but there was a need to develop and manufacture an effective vaccine, and then arrange suitable repositories. A number of antivirals had already been developed, but there was increasing resistance to one antiviral, Oseltamivir/Tamiflu (Lackenby et al, 2008; Regoes and Bonhoeffer, 2006). Vaccination would remain the cornerstone of influenza prevention for both seasonal and pandemic influenza. However, there was a need to develop new production methods, because the current seasonal vaccine was being produced solely in

chicken eggs, with only one dose being produced from each egg, in a technology that was 60 years old. Furthermore, it was important to remember that the H5N1 virus attached itself to the *lower* respiratory tract in humans, which was difficult for the present virus to reach, explaining why the number of human deaths thus far had been limited (Van Riel et al, 2006).

The second keynote speaker, Professor Sir John Skehel (UK) discussed the influenza virus glycoproteins as drug targets. He stressed the importance of receptor binding strains, with major differences between Group I (H1, H3 and H7) and Group II (H9 and H13) haemagglutinins. The biological significance of the different structural formations is simply not known, although research is underway (Chandrasekaran et al, 2008). It may be that when molecules refold for fusion different groups may use different pathways, but there is no evidence yet for this. Perhaps there will be a site for small molecule binding for Group II; and these molecules will have no effect on Group I. Furthermore, the neuraminidases also form distinct groups, with N1, N4, N5 and N8 in Group I, and N2, N3, N6, N7 and N9 in Group II. It is the resistant mutation His-274 that is making Group I resistant to Tamiflu, not the wide-spread use of the drug. As greater understanding emerges of the underlying structure of the influenza A virus, combination therapy might be viable.

Continuing the search to understand the barriers to transmission of avian influenza viruses between humans, Professor Wendy Barclay (UK) spoke of a model of transmission between ferrets. She and her colleagues had also investigated the binding of H5 HA protein to different cell types in the human airway. Using a recombinant baculovirus expression system in insect cells, soluble proteins were used to probe slides of fixed human airway epithelium from cultures or *ex vivo* tracheal sections (Barclay et al, 2007). The resulting data showed that H5 HA was capable of adapting to acquire human virus-like bonding properties for the receptors displayed in the upper airways of people. However, the fact that such mutations have not yet been detected in naturally occurring viruses may explain why H5 has not yet acquired human transmissibility.

Professor Robert Webster (USA) in his keynote speech entitled, "Continuing Evolution of Avian Influenza Viruses: Is H5N1 beyond Control?" concluded: "The certainty is that an influenza pandemic will occur in humans at some future time; and preparation for H5N1 will serve global preparedness." He pointed out that H9N2, with its capacity to acquire human-like receptor binding properties poses a considerable danger and is already prevalent in "live markets" throughout Asia (Matrosovich et al, 2001 and 2007). The presence of H9N2 in a Mediterranean gull resulting from a gene segment re-assortment event between North American and European gull species has been reported elsewhere in this journal, setting a dangerous precedent for the evolution of pandemic spread of this virus (Lebarbenchon et al, 2008). There were many unresolved issues about the reservoirs and the carriers of influenza A viruses. Ducks have become a Trojan Horse, carrying the viruses without being harmed by them, but transferring viruses in a form that becomes pathogenic in other species (Gilbert et al, 2008). Professor Webster believed that there was only a low probability of the highly pathogenic Asian H5N1 virus being spread to the Americas by migratory birds, some probability from frozen meat, but a high probability of spread of the avian virus from smuggled birds (including fighting cocks). With some 500 million deaths of poultry since 1997, the present situation was disturbing.

Throughout the conference, there was much probing for new research possibilities. For example, in the context of human medicine, Dr Sarah Gilbert (UK) spoke of her experience with the clinical trials of a flu vaccine designed to induce cross-subtype immunity, which is being developed at the Jenner Institute at Oxford. This research built on the work of Lee et al (2008) on detectable T cell responses of 90% of the adult population to various influenza antigens. The aim was to create a new vaccine that would target the internal proteins of the flu virus in such a manner that the body would maintain a low-level of T cell response to flu from previous flu infections. The new vaccine would then boost immunity to levels high enough to protect against subsequent infection from flu viruses of any strain.

In an example of a research initiative linked to veterinary medicine, Dr Julia Chosy (USA) spoke on "Zoos as Disease Sentinels: Piloting an Avian Influenza Surveillance System in Zoological Institutions," explaining the launch of a Zoonotic Emerging Disease Surveillance Center in the United States. This extensive surveillance system on the presence of influenza A viruses and other diseases in zoo animals was significant, not only for future animal health, but because 60% of the emerging disease outbreaks world-wide among humans during the period from 1940 to 2004 have begun in animals, with the newly created pathogens then entering the human population for the first time (Jones et al, 2008). Furthermore, 72% of these zoonoses have originated with wildlife, as with Severe Acute Respiratory Syndrome (SARS) and Ebola Virus.

The international scope of the conference was indicated by a number of presenters from Asia, Australia and Europe; and in order to capture the international scope of the conference, this summary has left out many speakers, especially from the United Kingdom and the United States, as well as all of the poster presentations. (Abstracts of all presentations and posters are presented elsewhere in this issue of the journal).

Considering the strengthening of foothold of the avian influenza viruses in the African continent, Dr Nancy Gerloff (Luxembourg) presented her work linked with collaborators in Nigeria and Burkina Faso on "The Spread and Evolution of Highly Pathogenic Avian Influenza H5N1 in Africa." It was important to identify the various sublineages of H5N1 viruses, especially as poultry farming was the second largest industry in Nigeria after oil production. Scavenger birds in Africa that feed on many dead species may function as sentinels of H5N1 infection, in a manner similar to raptors or swans in Europe and cats in Indonesia.

Dr Giovanni Cattoli (Italy) presented an overview of molecular epidemiology and viral characteristics of avian H5N1 strains isolated in Africa and in the Middle East between 2006 and 2008, citing four distinct sublineages that have been introduced into Africa and the Middle East. Working with Ilaria Capua, he stressed that whole genome analysis was instrumental for rapid recognition of whether strains of the H5N1 virus were adapting to human hosts. Surveillance programmes were also essential in the midst of African farming practices and environmental conditions that were very different from Asia.

Investigating molecular mechanisms of the host-pathogen interactions, Dr Laura Sironi (Italy) spoke on the role of the Mx polymorphism coding for amino acid position 631 in chicken lines experimentally infected with a highly-pathogenic H7N1 avian influenza virus. This study suggested that there was genetically controlled variation in response to avian influenza virus challenge, and that analysis of genetic mechanisms controlling resistance to AI virus would be a valuable tool in reducing the threat of AI in poultry to human health.

Professor Hiroshi Kido (Japan) explained how the influenza A virus initially infected the airway epithelial cells in his presentation on proteolytic activation proteases of highly-pathogenic avian Influenza viruses, which cover wide strains, even for non-susceptible strains, by furin and PC5/6. This discussion was further supplemented by Professor Xiufan Liu's (China) presentation on molecular determinants of H5N1 avian influenza virus for high virulence to ducks. He noted the compatibility areas in eight genes that might be important factors in how HA genes exercised their function. The work of his laboratory demonstrated that the PB2, PB1, PA and HA genes of the H5N1 virus might all be involved in determining the viral virulence of ducks. Furthermore, L322 in combination with the deletion of position L329 in the HA cleavage motif was the major contributor to the high virulence in ducks.

Dr Jimmy Kwang (Singapore) set out an alternative method for the production of an influenza vaccine through expressing an influenza viral gene using baculovirus surface-displaying technique, in his presentation "Protective Immunity against Lethal H5N1 Viral Infection in Mice by Intranasal Co-administration of Baculovirus Surface-displayed Hemagglutinin Subunit and Recombinant CTB as an Adjuvant."

Later in the day, Dr Nazeer Kalboro (Germany) presented evaluation of a safe, single cycle vector vaccine in chickens that was protective against high does of HP AIV. At present, existing inactivated influenza virus vaccines did not prevent virus shedding; and there is no way to discriminate between infected and vaccinated animals.

While a large part of the conference was dedicated to discussing the avian influenza viruses and their impact on birds and humans, there was also some useful discussion of new diagnostic tools. Dr Susan Fereidouni (Germany) set out a new fast and reliable method of using restriction enzymes for pathotyping influenza viruses. This was critical for rapid diagnosis and accurate identification of H5N1 AIV in both animal and human influenza infection diagnosis. In the final conference presentation of research, Dr Astrid Gall (Germany) explained the limitations of existing diagnostic methods for detecting avian influenza viruses and presented their work on developing a diagnostic microarray approach for detection, haemagglutinin sub-typing and patho-typing of avian influenza viruses.

Closing the conference, Dr John McCauley, pointed out that in 1918 public health systems throughout the world were unable to cope, whereas with the much less extensive 1968 pandemic, public health systems coped. The political drama now was around the question of whether public health systems would be able to cope with the next influenza pandemic. The answer to that question would depend to a considerable extent on the effectiveness of research in both veterinary and human medicine to control the H5N1 virus in birds before it acquired the ability to sustain infection in human populations.

COMPETING INTERESTS

None declared.

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