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Importance of rapid testing to combat the global threat of bird flu

'Through the application of advanced biotechnology diagnostic tools for early detection, it is hoped the mass culling of poultry and human losses can be minimized.'

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One of the important outcomes of the recently held meeting of the World Health Organization (WHO), the Food and Agriculture Organization (FAO) of the United Nations, the World Organisation for Animal Health (OIE) and the World Bank in Geneva November 7–9, 2005, on the threat of avian influenza A(H5N1), was that there is still a window of opportunity to minimize the threat to both the animal and human populations at the source (the birds) through rapid reduction of the viral burden of A(H5N1) influenza [101]. Through the application of advanced biotechnology diagnostic tools for early detection, it is hoped that mass culling of poultry and human losses can be minimized. It was stressed that surveillance by veterinary and human health services should be strengthened to allow early detection, timely notification and rapid mechanisms for an effective response.

Unrest in Europe & the rest of the world

In October 2005, 1 month earlier, Europe was startled by the occurrence of A(H5N1) bird flu cases among wild birds, including outbreaks among poultry in Turkey, Romania and Croatia [102]. The virus was likely introduced into Europe by migrating wild birds. The European Commission reacted promptly by placing bans on transport of birds and bird products, and giving the urgent advice to shelter free-range poultry from contact with wild birds and their droppings. The increased media attention, and the worry that this event was the prelude to the next influenza pandemic with reference to the casualties of the Spanish flu, has caused considerable disquiet

among Europeans. In some countries, there has been a rush on the regular human influenza vaccine and the flu inhibitor Tamiflu® (a neuraminidase inhibitor with the generic name oseltamivir). Vaccine shortage for the seasonal vaccination of risk groups was reported in the media, and bids of over €150 for a cure of Tamiflu were noted on the internet. The report of a resistant influenza A(H5N1) virus in a patient from Vietnam who received Tamiflu therapy added further to the unrest, as large amounts of already stockpiled Tamiflu were considered wasted money in the media [1].

Major causes of the unrest are the over- and misinterpretation of the available information by the media and the general public. The real situation is that:

- The seasonal flu vaccine does not protect against bird flu
- One resistant virus does not render the stockpiles of Tamiflu useless
- Most importantly, the risk of humans attracting bird flu is considered very low, as was also stated in a recent risk assessment by the European Centre for Disease prevention and Control [102]

Bird flu: a pandemic threat?

To better understand the unrest, some background information is necessary. Bird flu (or avian influenza) is caused by influenza A virus, and it can lead to epizootics with up to 100% mortality, especially among poultry [2,3]. Influenza A viruses are subdivided into 16 hemagglutinin subtypes (H1–16) and nine neuraminidase subtypes (N1–9). Any combination of

H and N subtypes can exist and all the H and N subtypes are endemic in wild waterfowl. The avian influenza A viruses are classified based on the amino acid composition of the hemagglutinin cleavage site and their pathogenicity index, as highly pathogenic avian influenza (HPAI) viruses, which cause severe disease with up to 100% mortality, and as low pathogenic avian influenza (LPAI) viruses, which generally do not cause severe disease [2]. Since the discovery in 1955 of avian influenza A viruses as the cause of fowl plague, HPAI A(H5) and A(H7) viruses have been reported to be associated with epizootics, with devastating effect among poultry [2].

Only the H1N1, H1N2, H2N2 and H3N2 subtypes cause, or have caused, the seasonal flu in humans since 1918. When a new virulent H and N subtype is introduced into the human population, by reassortment of genes of avian and human viruses or by direct transmission of avian viruses to humans, this can have an enormous impact, as the population is immunologically naive for such a virus [2,3]. The pandemic caused by the Spanish flu A(H1N1) in 1918 and 1919, which is considered exceptional, claimed an estimated 40–50 million deaths worldwide, the Asian flu A(H2N2) in 1957 approximately 1–4 million and the Hong Kong flu A(H3N2) in 1968 approximately 1–2 million. Evidence has been collected that important genes of these viruses (e.g., for the hemagglutinin) were avian in origin [2,3].

In 1997, 18 hospitalized persons, of which six died, were diagnosed with culture-confirmed influenza A(H5N1), which they contracted from poultry outbreaks on livestock markets in Hong Kong, demonstrating direct transmission of an avian influenza virus to humans for the first time [2,3]. The influenza A(H5N1) virus re-emerged in 2003 and, since then, has caused many outbreaks among poultry and wild birds in Asia and Europe. Over 200 million birds have been culled and by November 17, 2005, 130 people have been diagnosed with laboratory-confirmed influenza A(H5N1), of which 67 have died (for the current situation check [103] and [104], respectively).

HPAI A(H7N7) viruses are also a potential threat as, during the epizootic with this virus in The Netherlands in 2003, 89 people were found to be infected (laboratory confirmed), of which one died [4]. In addition, serologic analysis demonstrated the possibility of many more asymptomatic infections than expected, increasing the risk of the generation of a possible pandemic virus [5].

Until now, the last requirement for avian influenza viruses to cause a pandemic (i.e., efficient transmission between humans) has fortunately not been met. However, the devastating effect of the A(H5N1) bird flu on poultry holdings, and hence on economics and food supply, and the threat of the influenza A(H5N1) virus possibly becoming the next pandemic virus, are eminent. As the current window of opportunity to intervene is measured in days, rapid, simple and low-cost tests for diagnosis would be of great help in combating the global threat of bird flu.

Importance of rapid testing

Why is the current window of opportunity to intervene measured in days? The very first step to combat an infectious disease is the early detection of infection and, before that, the recognition of a suspect case. Recognition of suspect cases of bird flu in humans is quite difficult as the initial symptoms are not very specific for bird flu, in contrast to the symptoms of HPAI in a poultry flock [2,3].

Independent of avian or human bird flu, a rapid diagnosis is needed to commence measures to limit further spread of avian and human bird flu and to start therapy in case of human bird flu. Bird flu can decimate a poultry flock and spread to surrounding flocks in a few days. Veterinary A(H5) vaccines are available, but their use is limited due to trade regulations and, therefore, rapid culling is the primary measure to contain a poultry outbreak. Human therapy with Tamiflu decreases the severity of the symptoms and reduces shedding of the virus, thus limiting spread and possible generation of viruses capable of effectively transmitting from human to human. However, therapy is only effective when administered within 48 h after onset of symptoms. In addition, modelling showed that, in the absence of an effective human vaccine and under certain conditions, an emerging pandemic could be eliminated using antivirals if geographically targeted prophylaxis starts as quickly as possible, underlining the need for strengthened surveillance using rapid diagnostic tests [6,7].

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Classic virus isolation in cell culture or embryonated eggs (for diagnosis of human and avian flu, respectively) and subsequent H and N subtyping can take up to 14 days. Veterinary regulations require that the pathogenicity is determined in live chickens to denote a virus as HPAI, which takes another 10 days. Although in the case of an A(H5) or A(H7) influenza outbreak among poultry, interim measures (e.g., quarantine and preventive culling) are allowed as soon as the subtype has been determined, this reduces the analysis time to a maximum of only 14 days.

Therefore, it is obvious that there is a need for rapid testing, both in the veterinary and human field. However, rapid tests should never replace virus isolation fully, as the virus itself is still needed to study the characteristics and evolution of the virus and for selection of vaccine candidates.

Diagnosis by rapid tests

What options do we have in rapid tests for diagnosing bird flu? There are essentially three categories:

- Near-patient tests (NPTs) and field antigen detection tests
- Laboratory antigen detection tests
- Tests using nucleic acid amplification techniques (NATs)

Commercially available NPTs, such as the Directigen A[®] and A+B[®] (Becton Dickinson) and the QuickVue[®] (Quidel), are developed to diagnose human influenza and provide results within approximately 30 min [8,105]. The European Influenza Surveillance Scheme (EISS) task group on near-patient tests

recommended the use of NPT data as an adjunct to virus isolation for early warning of a change in influenza activity in humans [8]. It was only recommended as an adjunct because the sensitivity is limited to 70–75% compared with virus isolation and NAT [8,105]. In addition, the sensitivity for influenza A subtypes other than A(H1) and A(H3) is unknown. Application in diagnosis of culture-confirmed influenza A(H5N1)-positive patients in Vietnam and Thailand gave ambiguous results (nine and four positive patients in rapid tests of 11 and 11 virus culture-positive patients, respectively) [9,10]. Therefore, these NPTs are not recommended for bird flu diagnosis until they are thoroughly validated for this purpose. For veterinary purposes, at least one commercial field antigen detection test is available: the Anigen[®] H5 Avian Influenza Virus Antigen Rapid Test Kit (Animal Genetics, Inc., Korea). This kit had a sensitivity of 100% by farm and 82% by specimen compared with virus isolation, during the outbreaks of HPAI A(H5N1) in Korea in 2004 [106].

The second type of rapid tests are the antigen detection tests, which must be carried out in the laboratory (e.g., direct immunofluorescence on smears and enzyme-linked immunosorbent assay [ELISA]). Although these types of test can provide results within hours, their sensitivity is inferior to virus isolation (the gold standard) and, therefore, only recommended when the superior third type of tests is not available.

The third type of tests, NAT, is most sensitive as compared with virus isolation; these add 2–13% to the detection rate and results can be obtained within 4 h in real-time format [105]. A disadvantage of this type of test is the complexity of the test and the requirement of expensive equipment that, especially for laboratories in poor countries, are barriers to using NAT. Commercial kits for specific detection of the H5 gene are already available from at least four companies:

- HKDNA Ltd, Hong Kong SAR, China: nucleic acid sequence-based amplification (NASBA) and gel-based and real-time reverse transcriptase (RT) PCR
- EuroClone SpA, Pero (Milan), Italy: gel-based RT-PCR
- AJ Roboscreen, Leipzig, Germany: real-time RT-PCR
- Qiagen NV, Venlo, The Netherlands: real-time RT-PCR

The NASBA technique is of particular interest for poor countries as no expensive equipment is needed; NASBA is performed at one incubation temperature, and ELISA and a simple enzyme immunoassay reader can perform product detection easily. In addition, the Asia Biomedix group (Malaysia) provides a variant of the HKDNA Ltd NASBA, which allows discrimination of HPAI A(H5) viruses from LPAI A(H5) viruses, and this increases the possibility to intervene quickly in an HPAI A(H5) outbreak among poultry. In general, sequencing of the hemagglutinin cleavage site is used to discriminate between HPAI and LPAI on a molecular level.

If possible, NAT is recommended as the rapid test for bird flu diagnosis, but only by well-trained laboratories. Confirmation by an internationally recognized reference laboratory for

human A(H5) infection (WHO) or avian influenza (OIE) is required, but this should not delay taking extra preventative measures. Also, one should bear in mind that, for a stable quality of commercial or homemade NAT, continuous monitoring of the match of primers and probes with the circulating strains of the A(H5N1) virus is needed owing to the continuous drift of the virus. Related to this, one concern is the considerable delay in sharing of sequences of recent A(H5N1) viruses in the major nucleic acid databases (GenBank and The Influenza Sequence Database), and the WHO and the OIE together have a duty to fulfil, stimulate and realize rapid worldwide sharing of sequence information.

Laboratory preparedness

Worldwide, the WHO and OIE provide their networks of National Influenza Centres (NICs) and of veterinary reference laboratories with information on recommended tests and procedures for diagnosis of cases of human and avian A(H5N1) influenza, respectively. However, both organizations lack the resources to equip the laboratories with the necessary instruments and all reagents. In 2002, a survey performed by the WHO indicated that 70% of the NICs have a thermocycler and 32% a nucleic acid sequencer but that only 45% used NAT for influenza diagnosis [11]. Input of money is especially needed to

equip laboratories in poor countries with the right instruments and to teach the personnel how to use the sophisticated molecular techniques.

Collaborative efforts to strengthen the surveillance, like those by the EISS [107] for Europe, are encouraged and supported by WHO as they strengthen the existing network of NICs [12].

Over the last couple of years, EISS has established several mechanisms (reagents, protocols, controls and databases) to prepare for a pandemic with the A(H5N1) virus, and 26 of 32 European reference laboratories for human influenza and 22 of 25 European countries are now prepared for the detection of the A(H5N1) influenza virus (status at January 2005).

Concluding remarks

In conclusion, there is certainly a position for rapid tests, particularly the molecular tests, in combating the global threat of bird flu. In the current situation of very limited transmission of the virus from birds to humans, their usefulness is limited due to the time lag between the onset of illness and identification and testing of suspect cases, which, in turn, limit the effectiveness of treatment (as demonstrated in Vietnam) [3]. Improved human surveillance on clinical symptoms by strict triage using case definitions (e.g., those provided by the WHO) is urgently needed and will increase the effectiveness of using rapid test in combating bird flu. In the veterinary field, the rapid tests are particularly important for rapid investigation of the first occurrence or re-emergence of the HPAI A(H5N1) virus in a certain area, and to commence immediate

measures to limit further spread. However, the ultimate measure to combat the global threat of bird flu to the animal and human populations remains prevention by vaccination, and efforts for developing effective vaccines and vaccination strategies should be maximized.

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