TECHNICAL REPORT

of the Scientific Panel on Influenza in reply to eight questions concerning avian flu Stockholm, 5 June 2006

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1. INTRODUCTION

The main task of the Scientific Advice Unit (SAU) of the European Centre for Diseases Control (ECDC) is to provide sound and independent technical and scientific advice. This is accomplished through the collaboration of a strong scientific core within the Centre with leading European scientists in the relevant disciplines.

According to ECDC founding regulation[1], the Unit can be supported in its scientific work by *ad hoc* Scientific Panels, consisting of 11 members, selected following a well defined procedure, among those who have expressed their interest to work with the ECDC by applying in response to the ECDC call for scientists across the Member States.

Panels are expected to provide scientific opinions on questions put to the ECDC, to promote the scientific agenda of the Centre and to aid the Centre in keeping abreast of scientific developments. They are also expected to: (i) take part in the development of guidelines to harmonize the response to communicable diseases in Europe; (ii) advise the ECDC on toolkits to be proposed to Member States; and (iii) identify gaps in scientific research.

The current report has been produced by the ECDC with the help of an *ad hoc* Panel established to advise on replies to eight questions, regarding H5N1 avian influenza, requested by the European Commission and Member States. A standard format – consisting of background, conclusions, future research priorities and references – has been developed to reply to each question.

The members of this Panel, the first since ECDC was established, have been selected, amongst highly reputed scientists fulfilling the above-mentioned requirements, with the aim of covering all the needed expertise.

The first Panel meeting was held in Stockholm on 28th February 2006. After two further formal meetings and regular e-mail exchanges of information, the Report of the ECDC Scientific Panel on avian influenza has been finalized by a written procedure.

The ECDC is now working to develop a solid system for rapid consultation and updates for future work.

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[1] Article 6 of Regulation (EC) No 851/2004 of the European Parliament and of the Council of 21 April 2004 establishing a European Centre for disease prevention and control.

2. QUESTIONS AND REPLIES

Question n. 1

Development of resistance to neuraminidase inhibitor drugs: what evidence in relation to their use in prevention and treatment of avian influenza?

Reply to question n. 1

Background

Neuraminidase Inhibitor (NI) Drugs (Zanamivir and Oseltamivir) work by inhibiting the enzyme activity of the influenza viral neuraminidase (NA) protein, as they are analogues of the natural ligand sialic acid. The activity of the enzyme is required to release newly formed viral particles from infected cells and may also play a role in preventing entry of virus particles into cells. The structure and active site of the NA enzyme is highly conserved across all influenza A subtypes and also in influenza B, thus making possible the development of a drug which can inhibit all influenza A and B virus strains.

The initial drug design and development used the N9 influenza subtype crystal structure for modelling and drug design work. Resistance to NI drugs is mediated *in vivo* by mutations in the viral NA, primarily around the active site. Viruses bearing mutations in NA conferring resistance to drug action are usually compromised in their enzyme activity, or stability and appear to have reduced transmissibility in animal models (1).

In more recent years further structural models of other influenza subtypes have become available. Although it is recognized that there is strong conservation of the architecture of the enzyme active site, there are some subtle differences in the detailed amino acid configurations at the active site between different influenza A subtypes and influenza B. These may influence exact binding of drug at the active site. Different mutations may be expected to give rise to resistance in different subtypes.

Use in human and zoonotic influenza A infections

Clinical use of NI drugs has been most extensive in treatment of human H3N2, H1N1 and B infection. Drugs have been licensed worldwide since 1999, and clinical trials prior to this involved the treatment or prophylaxis of approximately 7,000 individuals infected/exposed with these subtypes. Prophylaxis efficiency in the family and institutional settings is well demonstrated for both NI drugs, with efficacy of 80-90% in prevention of household influenza cases (2).

Since 2003, there has been treatment of less than 100 cases of H5N1 and even fewer records of prophylaxis. There is at present no centralized clinical database which can be used for formal evaluation of prophylaxis efficiency against H5N1 in humans as these data, if any will be held in the various different countries where H5N1 has occurred. Mouse model work also suggests efficient NI drug prophylaxis against H5 strains (3).

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Moreover, there is no documented evidence of emergence of resistance during prophylaxis for exposure to H7 influenza which was undertaken during the outbreak of H7N7 in the Netherlands in 2003 (4).

Surveillance of circulating Influenza strains

Surveillance for resistance amongst influenza A human isolates since 1999, indicates a low level of detection of resistance (<1%) (5). The highest global per capita usage of NI inhibitors has taken place in Japan, and analysis of approximately 2000 Japanese isolates from 2003/2004 indicates a similar level of resistance (6). This type of surveillance provides a snapshot of prevalence of resistance in circulating human viruses, including H1N1 viruses, but is not linked to knowledge of whether the individuals shedding virus have been drug treated. It is possible that resistant viruses have been detected in individuals who have not been drug treated, in which case this may be considered as evidence of a low level of transmission of resistant viruses.

There is at present only limited NI drug susceptibility data from circulating H5N1 strains (7). Among 97 A/H5N1 human and poultry isolates tested, only a single sample from a treated patient described below (A/Vietnam/30408/2004) has shown partial resistance to oseltamivir (8).

Analysis of influenza strains from treated individuals

Intensive investigations involving analysis of sequential samples taken post treatment in an infected individual will give rise to different sorts of information, in particular the detection of resistant variants during treatment. This sort of data addresses the question of whether resistant viruses can arise in a treated individual, but not whether these resistant viruses are transmitted from the individual carrying them. Depending on the virus type and assay method used, oseltamivir resistant variants emerge during therapy of <1% of adults infected with human influenza viruses, and between 5 and 18% of children. Infection in children is generally considered to lead to higher viral replication than in adults on account of limited pre-existing immunity. This situation favours the emergence of resistance and may account for the observed higher rates of resistance in treated children. The highest rate of resistance observed in Japanese treated children (18%) (9) was derived from a study where sensitive molecular techniques were used to dissect different populations of virus present in individuals after treatment, without a suggestion that there was transmission of resistant viruses. Japanese children were treated on a dose per kilogram basis, rather than as a unitary dose as elsewhere in the world. Suboptimal drug concentrations may create conditions which favour emergence of resistant variants in a situation of high rates of viral replication. The emergence of resistance in human subtypes in the immunocompetent host does not correlate with clinical failure of treatment, although children, immunocompromised adults and individuals infected with H5N1 may represent a different situation.

Analysis of H5N1 influenza strains from treated individuals

Limited data from treatment of human cases of H5N1 has indicated that resistant variants can be detected in treated individuals. In one case report, a respiratory sample collected on the fourth day of oseltamivir treatment was shown to contain an A/H5N1 virus mixture

(A/Vietnam/30408/2004) that was partially resistant to oseltamivir by neuraminidase (NA) inhibition assay and contained both drug-susceptible and drug-resistant virus clones (10). The majority of the resistant clones had the His274Tyr NA mutation, which is known to confer high-level oseltamivir resistance in N1 NAs; some had an Asn294Ser mutation, which was associated with moderate oseltamivir resistance. This sample was collected from a teenager who had received 3 days of oseltamivir prophylaxis with once-daily dosing but who, in retrospect, was symptomatic and most probably already infected before starting the drug. Increased fever and respiratory complaints on prophylaxis prompted an increase to a standard twice-daily therapeutic dose of oseltamivir, after which no further samples were positive for virus and recovery ensued.

The two other patients, in whom emergence of oseltamivir-resistant A/H5N1 was observed during or after therapy, both had fatal outcomes (11). One was a 13 year old girl who presented with fever and focal pneumonia and received conventional therapeutic doses of oseltamivir starting 1 day after illness onset and continuing for a total of 5 days. She developed increasing pneumonia and respiratory distress on the fourth treatment day in association with detection of A/H5N1 virus harbouring the His274Tyr mutation in her pharynx and died 3 days later (2 days after cessation of therapy) with continued pharyngeal detection of resistant virus. An 18 year old patient had resistant virus with His274Tyr mutation isolated 3 days after completion of a 5-day oseltamivir treatment that had been started on the sixth day of illness; she died on the 20th day, of respiratory failure. No autopsies were performed and no other viral data are available. However, including these two patients in this case series, there was only one survivor among nine who presented 2–7 days (median, 6 days) after illness onset and had detectable virus at the end of therapy (n=3) or who did not have serial samples collected (n=6). In comparison, all 4 patients who started treatment 4–8 days after illness onset and who had undetectable pharyngeal RNA levels at the end of 5 days' therapy survived. This indicates that very high viral replication rates associated with the H5N1 virus may have a particularly unfavourable outcome if therapy is initiated late after illness onset.

Frequency of oseltamivir resistance in H5N1

The detection of resistant A/H5N1 variants during oseltamivir therapy is not unexpected, given the experience with oseltamivir treatment of human influenza in children, particularly if suboptimal dosing regimens are used. A study of 43 A/H1N1-infected Japanese children found that 16% shed resistant variants with the His274Tyr resistance mutation during or after oseltamivir treatment (12). A similar frequency of detection of phenotypically resistant variants (18%) was observed in hospitalized children infected with A/H3N2 virus during or after oseltamivir treatment (9). In these reports, some of the patients may have received suboptimal dosing of the drug, which may not suppress viral replication adequately and so provide appropriate conditions for the emergence of resistant mutants. The frequency of resistance emergence during oseltamivir treatment of A/H5N1 paediatric patients is uncertain, because little sequential viral sampling has been performed, but it is likely to be no less than that observed in A/H1N1-infected children. Whenever possible, sequential respiratory samples should be collected from A/H5N1-infected patients receiving antivirals, for subsequent analysis in qualified laboratories.

Oseltamivir treatment regimens

At present, it is uncertain whether higher doses or longer oseltamivir treatment regimens may reduce the likelihood of emergence of resistance variants and/or provide greater clinical benefit in severe infection particularly due to A/H5N1. In one murine model study using a human isolate A/H5N1 from 2004 higher oseltamivir doses (10mg/kg.d compared with 1 mg/kg.d) and more prolonged administration (8 days instead of 5 days) were required to inhibit viral replication compared to a human A/H5N1 isolate from 1997 (13). This was related to the higher levels of replication of the former virus and drug-resistance variants were not detected. If dose regimens requiring more oseltamivir or combination of oseltamivir with other antiviral drugs would help in reducing resistance emergence, it would have important implications for clinical management and stockpiling decisions. Rigorous data are needed to determine whether differing course length regimens are as clinically effective as standard ones, including the possible consequences with respect to the emergence of viral resistance. Until such data become available, it would be sensible to advise use of the approved therapeutic 5-day regimen of oseltamivir for cases of avian influenza, with consideration of longer treatment and higher dose if clinical deterioration or inadequate response is observed.

Conclusions

Based on currently available data there is no reason to suspect that prophylaxis efficiency against H5N1 in adults should be reduced compared with efficiency against H1N1, although the NA proteins do differ between the avian H5N1 and human H1N1. There is a very low level (<1%) of N1 drug resistance detectable in circulating human influenza viruses. This is a surrogate measure for transmissibility of resistant viruses. These data suggest that the likelihood that oseltamivir-resistant variants of human influenza strains will transmit and circulate at the community level at current drug dosages appears to be much lower than the previously documented experience with amantadine (14). Detailed analysis of viruses isolated from infected individuals indicates that the emergence of resistant variants does occur during the course of treatment and at a higher rate in children. This is not necessarily associated with treatment failure and so far has not been directly associated with transmission of resistant variants. The exact implications of these data for treatment of H5N1 infections remain uncertain.

Future Research Priorities

- Global surveillance for drug resistance linked to knowledge of drug use on a country by country base.
- Investigation of the emergence of drug resistance and its relationship to the rate of viral replication.
- Clinical/Model investigation of effects of higher dose and prolonged duration of oseltamivir/zanamivir in H5N1 infections.
- Investigation of viral fitness and drug susceptibility

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Question n. 2

Oseltamivir post-exposure prophylaxis in avian influenza: how long after exposure would it be useful to start its administration?

Reply to question n. 2

Background

The decision about when to begin prophylaxis following exposure to H5N1 should relate to the clinical course of incubation for H5N1. Summary data from WHO on cases of H5N1 presents known information about the natural history of H5N1. Incubation times for H5N1 in humans may be up to 8 days (generally 3–5 days) and highly pathogenic H5N1 is associated with visceral dissemination and virus shedding, including viraemia, for up to 9 days after the onset of illness. These are the data which should be used to determine when to begin prophylaxis (1).

Conclusions

Best practice suggests that prophylaxis should be started as soon as possible following exposure, and starting a prophylaxis course can still be useful until 8 days after exposure to an infected source.

Future Research Priorities

–   Human to human transmission potential of H5N1 viruses

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Question n. 3

Many countries are considering stockpiling zanamivir, especially in the light of some reports of emerging resistance to oseltamivir. As this drug has a local focus of action, what is the evidence to support its use in treating or preventing the systemic complications of avian influenza?

Reply to question n. 3

Background

Zanamivir has not been used in treatment of avian influenza (H5 or H7 subtypes). It is a topically delivered antiviral drug, targeted to the respiratory tract. Analysis of body fluids from models of H5N1 indicates that the highest viral loads of H5N1 in the respiratory tract are to be found in the lower respiratory tract, with relatively little replication in the upper airways (1, 2). Zanamivir does penetrate throughout the respiratory tract (3). Zanamivir can and should therefore be considered as treatment for H5N1, where over 90% of cases have clear evidence of respiratory system disease at the point of presentation. Two cases of H5N1 have been described with an atypical presentation involving the gastrointestinal tract, although virus has also been found in respiratory secretions, indicating it remains suitable for use even if there is an atypical presentation (4). Disseminated infection would be optimally treated with a systemically available drug, but this does not appear to occur in the majority of cases of avian influenza, nor does disseminated disease mean that reducing lung virus replication would not be helpful in reducing severity of disease. Also it should be noted that the mutation associated with oseltamivir resistance (His274Tyr) remains sensitive to zanamivir.

Conclusions

Zanamivir is a useful drug for treatment of avian influenza. The major mutations associated with oseltamivir resistance in treatment of H5N1 remain sensitive to zanamivir.

Future Research Priorities

– New antiviral therapies
– Mechanisms of resistance generation

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Question n. 4

It is generally considered that the high attack rate of A/H5N1 in children and young adults reflects higher exposure of this age group. Is there any evidence that prior exposure to the N1 component of A/H1N1 is providing some immunity to older age groups?

Reply to question n. 4

Background

Data on protection in humans against infection with HPAI viruses of the H5N1 subtype, on the basis of antibodies to the N1 component based on prior exposure to human H1N1 viruses are currently not available. However, on the basis of the genetic and antigenic distances between the N1 components of both variant groups of viruses, it may not be expected that cross neutralizing antibodies between these virus groups would exist. Preliminary unpublished studies in animals confirm this notion (Rimmelzwaan et al., personal communication)

Cross-protective immunity between influenza A virus subtypes based on T cell mediated immunity may, however, exist. Data from animal experiments have clearly demonstrated that pre-exposure to a virus from an influenza A virus subtype may reduce morbidity and mortality due to infection with another influenza A virus subtype.

A study carried out by Jameson et al (1) addressed the question whether influenza A specific human CD8+ and CD4+ T lymphocytes would recognize epitopes on influenza A virus strains derived from swine or avian species, including the 1997 H5N1 Hong Kong virus strains. The results demonstrate that adults living in an urban area of the USA possess influenza A-cross-serotype reactive CD8+ and CD4+ CTL that recognize multiple epitopes on influenza A viruses of other species. Cytotoxicity was observed in vitro in peripheral blood lymphocytes against avian and human influenza A viruses. ELISPOT assays detected precursor CTL specific for both human CTL epitopes and the corresponding A/HK/97 viral sequences. The authors hypothesized that such cross-reactive CTL might provide partial protection to humans against novel influenza A virus strains introduced to humans from other species.

If a future influenza virus strain would result from a re-assortment event between a human influenza A virus and a HPAI virus H5N1, a certain degree of cross-protective immunity based on shared T cell epitopes between the pandemic virus on one hand and both human and avian viruses from which the pandemic strain originated on the other hand, may exist.

Conclusions

Given the limited number of cases and limited data on possible exposure, it is currently not possible to correlate the observed higher attack rates of H5N1 in children and young adults with any particular parameter of previous exposure to influenza.

There is some evidence, however, that older individuals who have been exposed to more human influenza A viruses in their lifetime than younger individuals, may have a higher degree of cross-protective immunity to newly emerging animal or pandemic influenza A

viruses. The basis of this cross protective is at least in part due to the presence of shared T cell epitopes between the respective viruses.

It is unlikely that pre-existing antibodies in the human population to N1 of circulating H1N1 viruses will provide significant cross neutralization (and therefore cross protection) to H5N1 on account of substantial differences in genetic and antigenic properties of these N1 proteins.

Future Research Priorities

– Correlates of Protection to Influenza
– Age-related susceptibility to non-human influenza subtypes
– Nature of cross protective cell mediated immunity to influenza
– Characterization of cross-reactive epitopes among diverse influenza A virus strains
– Identification of invariant viral determinants to be used in future vaccines to induce cross-protective immunity

References

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Question n. 5

Transmission of HPAI viruses to non-avian animal species: what is its role in the spread of infection and risk for further transmission to humans?

Reply to question n. 5

Background

HPAI viruses of the H5N1 subtype are avian viruses that may be transmitted to humans and other mammalian species:

Birds

Apart from poultry, in which infection with these viruses causes severe disease with high mortality ("fowl plague"), numerous (virtually all) domestic and non-domestic bird species, including migratory birds, can be infected with these viruses. This includes cage and aviary birds, although their possible exposure to these viruses is often more limited (for review see 1).

In a review paper by Olsen et al (2) an overview of the current knowledge on the ecology, epidemiology, genetics and evolution of the avian influenza viruses in relation to the ecology of their avian hosts is presented. Especially the knowledge on global patterns of influenza virus infections in wild birds, in relation the host ecology and bird's behaviour are addressed.

The transmission of HPAI viruses of the H5N1 subtype from birds to humans has so far been related to contacts with infected domestic poultry, their excreta or infected poultry products. No proven cases of human infections through contacts with wild birds have been identified so far. This is probably related to the relatively low transmissibility from birds to humans (see below).

Infected domestic poultry develop a disseminated infection, with high concentrations of the virus in virtually all their tissues and excreta. In other birds, the pathogenesis and distribution of the virus throughout the body and therefore the presence of virus in their excreta, differs largely per species. Some bird species develop a fatal infection similar to that in poultry, whereas others only develop sub-clinical infection with limited excretion of the virus. Pre-exposure to low pathogenic viruses of the same and possibly also other virus subtypes, may influence the pathogenesis and severity of the infection. Depending on the bird species, the age of the birds and their history of previous influenza virus infections, a whole range of susceptibilities is likely to exist. Therefore, some species (e.g. swans) may act as "sentinel" animals in a certain area, due to their high susceptibility, indicating the presence of the virus in the environment and thus providing an early warning signal. Finally it should be realised that the recently emerged influenza A-viruses of the H5N1-subtype originate from a complex and dynamic process of genetic reassortment (3) and mutation, which has resulted currently in at least two major lineages of H5N1-viruses with many variants. This dynamic process may result in different pathogenicities of the resulting viruses in different bird species.

Humans

To date, in about 200 people symptomatic infection with HPAI viruses of the H5N1 subtypes has been identified in Asia, Turkey and the Middle East since 2003 (4). More than half of them have died from the infection. Given the large areas in which the viruses of this subtype have spread and the high endemicity of the viruses in poultry and wild birds in these areas, the frequency of bird to human transmission is low. Intensive contact with including ingestion of infected poultry, their excreta or inadequately cooked infected poultry products is apparently necessary for this interspecies transmission to humans. Recent papers by Van Riel et al (5) and Shinya et al (6) have indicated that the receptors for these avian viruses are predominantly found in the lower respiratory tract of humans. This may explain, at least in part, why bird-to-human transmission as well as human-to-human transmission with these viruses does not occur efficiently or is virtually absent, respectively.

Carnivore mammalian animal species

There are increasing numbers of reports from Asia and Europe of domestic cats dying from infection with HPAI viruses of the H5N1 subtype. The available evidence, albeit incomplete, suggests that cats may unexpectedly play a role in epidemiology of these viruses (7). This was unexpected since cats have only rarely been shown to be infected with influenza A-viruses, generally without developing clinical signs. It has become evident that fatal infections with H5N1-virus among cats are common in countries such as Indonesia, Thailand and Iraq, where the virus seems to be endemic in domestic poultry. Dead or moribund cats were found to be infected with H5N1 virus soon after the virus was detected in wild birds in Germany. This suggests that H5N1 virus can be transmitted from wild birds to cats, but also that unusual mortality in cats may act as an early warning signal for the presence of the virus. There are three publications (5,7,8) so far reporting experimental studies in which cats were infected with H5N1 virus. Experimental infection by the respiratory tract, by feeding infected chicks or through close contact between infected and non-infected cats, resulted in excretion of the virus from the pharynx, nose and rectum. The amount of excreted virus was much lower than the levels excreted by infected chickens. Signs of severe respiratory and disseminated infection that may cause the death of animals were noted. Similarly, in H5N1 outbreaks in tigers and leopards fed with infected chicken carcasses, disseminated infection and animal-to-animal transmission of the infection were observed.

The H5N1 viruses have the ability to infect an unprecedented range of hosts, including carnivores (for review see 1). In addition to felids, fatal infection has been described in a stone marten, a palm civet and a mink. Dogs may also be infected with certain influenza A-viruses and in some cases even develop severe disease. Recently infection with an influenza A-virus (H3N8) has caused serious problems in dogs (9). A preliminary report was made concerning H5N1 infection of a dog; however, the evidence for clinical infection is not strong and warrants further investigation (10). We can expect other domestic and wild carnivores such as foxes, mustellids and seals to be vulnerable to this infection. Moreover, fatal experimental infection of laboratory ferrets has been demonstrated (1,11).

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Non-carnivore mammalian animal species (for review see 1)

Experimental infections of cynomolgus macaques with H5N1 virus resulted in severe infection of the respiratory tract. (12)

Experimental and natural infection of pigs with H5N1 has been demonstrated, but no wide spread infection in areas where the virus in endemic in birds has been noted.

Experimental infection of laboratory rabbits and rats with H5N1 virus has resulted in non-fatal diseases, whereas fatal infections in mice have been described.

Conclusions

To date, only domestic poultry are known to have played a major role in the transmission cycle of the H5N1 virus from animals to humans. However, given the potential contribution of especially carnivore hosts to both virus transmission and adaptation to mammals, the time for increased surveillance, vigilance and precaution concerning the possible role of these species is here.

Future Research Priorities

– There is clearly an urgent need for further research in the area of H5N1-virus infections of carnivores (cats, dogs, foxes….) and pet animals
– Characteristics governing transmission potential of influenza viruses
– Host range and tissue tropism of influenza viruses
– Adaptation of avian influenza virus to mammalian hosts
– Intrinsic and innate host defence mechanisms need to be better explored

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Question n. 6

Presence of HPAI viruses in the environment resulting from wild bird droppings: what is potential for transmission to humans (susceptibility of the virus to desiccation, its survival in water with different salt degree, pH, etc.).

Reply to question n. 6

Background

Only limited data exist on environmental persistence of avian flu viruses under different conditions; moreover, available data concern different strains and subtypes and are difficult to generalize.

Viral persistence in bird faeces

Infectivity of wet bird faeces containing the highly pathogenic H5N1 virus declined over time, but remained detectable for at least 35 days at 4°C and for six days at 37°C (1). Virus titres in wet faeces remained detectable for 7 days at 25°C. A very high titre of H5N1 viruses in fresh duck faeces became undetectable after the faeces were dried overnight at room temperature.

Data available on infectivity persistence in faeces of H5N2 (2-4) and H7N2 (5) viruses have been recently reviewed by the EFSA (6). Field studies on H5N2 virus indicated infectivity still detectable after 105 days at room temperature (unknown), after 44 days (unknown temperature), 35 days at 4°C and only 2 days at 25°C. Experimental studies with a high infectious dose of H7N2 indicated that: (i) at 4°C infectivity was still detected after 23 days(last day tested); (ii) at ambient temperature (15-20°C) after 19, but not after 23 days; (iii) at 35°C after 14 but not 16 days. When the test was carried out with "field chicken" manure held between 15 and 20°C infectivity was detected after 4 (but not 6) days and at 37°C infectivity was detected after 12 (but not 36) hours (see also reference n. 6).

Viral persistence in fresh and salt water

No data are available on the persistence in water of the H5N1 virus.

Linear response models applied to persistence data obtained with high doses of five avian influenza viruses (i.e. H3N8, H4N6, H6N2, H12N5 and H10N7) per milliliter of distilled water at different temperatures, indicated that the time needed to reduce the virus infectivity titre by 90% varied from 21 to 34.5 days at 17°C according to the virus strain and from 5 to 17 days at 28°C (7). In a similar test carried out with lake water on H3N6 virus the time needed to reduce the virus infectivity titre by 90% was found to be 8.4 days at 0°C (Webster et al., 1978). The combined effects of water temperature, salinity and pH on persistence of three avian influenza viruses (i.e. H6N2, H4N2 and H10N7) were evaluated in a model distilled water system using isolates sampled from ducks by Stallknecht et al (9); estimated persistence of infectivity of a high infectivity dose was longest at 17°C/no added salt/pH 8.2 (100 days) and shortest at 28°C/20ppt added salt/pH 8.2 (9 days). It is unclear to what extent these data, obtained under laboratory conditions, may be actually representative on

environmental conditions under which viruses shed into the water may follow different dispersion pathways.

Ito et al (10) and Okazaki et al (11) provided evidence that in the paleartic regions, avian influenza viruses are preserved in frozen lake water during the winter in the absence of their migrating natural hosts; upon returning for breeding purposes during the subsequent season, birds or their offspring have been re-infected with viruses released from melting water.

Viral persistence in carcasses

The majority of human infection cases with H5N1 is associated with close contact with infected poultry thus confirming viral infectivity survival in carcasses that may also play a role in local spread of infection among wild birds and scavenging species.

Viral persistence in the presence of specific inactivating agents or processes

When exposed to 70% ethanol for 10 min, one out of four field isolated of H7N2 virus lost 100% infectivity and the other three lost 75% of infectivity. When each of the isolates was exposed to 70% ethanol for 15 min, each one lost all infectivity (7). The four isolates remained infectious when mixed with a commercial disinfectant (i.e. DC & R containing formaldehyde 2.3%; alkyl dimethyl benzyl ammonium 3.1%; and a derivative of propanediol 19.2%) for 5 min. Exposure to the disinfectant for 10 min, however, inactivated all the isolates. When exposed to pH 2 for 5 min, all the four above mentioned isolates lost 100% of their infectivity, whereas exposure to pH 5, 10 and 12 for 15 min had no effect on infectivity of the four isolates.

Based on the structure, surface charge, similar size and inactivation rates of avian influenza viruses and enteroviruses, it is assumed that avian influenza viruses are reduced by these treatments at least as much as enteroviruses.

On such a basis it is possible to estimate the survival of avian influenza viruses as follows:

- **Extremely unlikely** in presence of several disinfectants including 2-3% sodium or calcium hypochlorite, 4% quaternary ammonium salts, 2-3% calcium or sodium hydroxide, 2% cresolic acid or synthetic phenols or 2% glutaraldehyde (12-13);
- **Very unlikely** after the steps normally used in drinking water treatment plants (e.g. a properly carried out chlorination with an adequate residual free chlorine concentration under controlled turbidity or ozonization treatment is estimated to result in a $4\log_{10}$ reduction, whereas an adequate physical treatment is likely to result in a $2\log_{10}$ reduction) (14-15);
- **Likely** in waste treatment plants unless reliable viricide systems would be included in the sewers and water treatment plants. This assessment takes into account the low efficacy of disinfectant treatments as applied on effluents, if any. Furthermore, virus concentrations may be enriched in certain treated or separated waste fractions (such as waste solids) by sedimentation and solid-liquid separation processes (16).

Conclusion

Once shed into the environment, avian influenza viruses have a remarkable capability to retain their infectivity. Persistence of viral infectivity depends on several factors, including the virus subtype, temperature, acidity and other environmental factors and conditions. Available data indicate that viruses in faecal deposits on land may be more rapidly inactivated than in water. Residual infectivity tests carried out on several avian influenza viruses in fresh and salt waters, indicated that viral inactivation takes longer time at low temperatures in fresh water with a pH close to neutrality and that, under optimal environmental conditions, complete viral inactivation may even take months. Only very limited data concerning persistence of avian influenza viral infectivity in the presence of specific inactivating agents or treatments are available.

Future Research Priorities

−   Survival and inactivation of H5N1 and other avian influence virus strains in different environmental compartments as well as in bird and poultry carcasses.
−   Development of practical measurements methodologies of the viral load in different environmental compartments would also be very helpful.
−   Dose of influenza required to establish infection of avian influenza in adults and children

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Question n. 7

Drinking/swimming water contaminated by HPAI viruses: is there evidence to reassure the public on the safety of such waters for human consumption/bathing?

Reply to question n. 7

Background

Bathing in contaminated water

Considering the known ability of HP influenza viruses shed into the environment by wild infected birds or farm animals to remain viable for considerable periods of time especially in water (see the reply to Question n. 6), the infectivity of water resources depends on a variety of factors such as: (i) the accessibility of water to faeces from wild birds or from animal farms (that is higher for superficial waters including water reservoirs and rooftop waters than for underground water); (ii) the infectious load discharged as compared to the size of the water body; (iii) the temperature, pH and salinity of the water; (iv) the presence of other substances in the water; (v) the time elapsed since the shedding. Examples exist of detection of significant concentrations of avian influenza viruses, without any preliminary concentration step, in water samples from six lakes in Canada where ducks gathered and deposited large amounts of faeces and from lakes in the USA (1-3) as well as from concentrated pond water samples from Hong Kong (4).

Potential routes of transmission of avian influenza virus to humans during recreational use of waters contaminated with faeces from wild birds of farm animals include oral ingestion or aspiration of water as well as direct intranasal or conjunctival inoculation. Water bodies used for recreational purposes are almost always not treated and treatment may not be feasible.

Assessment of the risk associated with swimming in water contaminated with the H5N1 virus is made difficult by:

– the lack of data on the infectivity for humans of H5N1 virus, although it is generally accepted that this virus is not efficiently transmitted to human beings from birds (see also the reply to Question n. 5); and
– the many factors that influence the viral load to which human beings may be exposed (see also the reply to Question n. 6).

On the other hand, three cases out of 194 total cases of human avian influenza cases have been associated so far with recreational exposure to water containing H5N1 viruses, although in none of these cases could other possible sources of infection categorically be outruled (see the Annex for a more detailed analysis of these data). Therefore, although the risk for humans of being infected with H5N1 virus from swimming in contaminated water is much lower than that from direct contact with infected poultry/birds, the swimming risk cannot be dismissed altogether, particularly in case of heavily contaminated waters with the H5N1 virus.

Risks associated with human exposure to avian flu viruses through recreational use of open water bodies receiving large numbers of wild birds should be assessed, on a case by case

basis, by a multi-disciplinary group with all the relevant expertise including agriculture, environmental sciences, public health, veterinary and virology, taking into account all the relevant factors as well as available information on unusual bird mortality data or avian flu outbreaks among wild birds. Monitoring data concerning the presence of the virus in the considered water body, if any, are also useful for the risk assessment, as long as it is understood that the detection of the virus in the water does not necessarily imply an infectivity risk to human beings from swimming.

Risks associated with swimming in highly contaminated waters can be minimized by advising the public of the risk and restricting the human access to potentially hazardous sites.

In relation to the possible contamination of water bodies with faeces from farmed animals, risk assessment and control may be carried out by exploiting measures foreseen in Directive 2005/94/EC that provides guidance on how to establish restricted zones in case of HPAI outbreaks for the control of avian influenza.

Safety of drinking water

The risk of being exposed to avian flu viruses through drinking water is negligible as long as raw water is submitted to all the treatment steps generally applied, according to good practice, to control enteroviruses in drinking water treatment plants (see also the reply to Question n. 6). This evaluation relies on the assumption, based on the structural features, surface charge, similar size and inactivation rates of avian influenza viruses and enteroviruses, that avian influenza viruses, are reduced by denaturing agents and treatments at least as much as the enteroviruses. Moreover, it also relies on the assumption that a careful control is applied throughout all the chain of operations needed to ensure safety and quality of drinking water.

On the other hand, case by case risk assessments may be appropriate if:

(i)   not all the needed treatments can be applied in the production of drinking water;
(ii)   a very high human infectivity emerges for specific viral strains;
(iii) specific areas are affected by avian influenza outbreaks among wild birds or farmed animals; and
(iv)   monitoring of raw water sources indicates very high viral contamination levels associated with excretions of wild birds and/or farmed animals.

Risks deemed to be unacceptable can be controlled by:

(i)   excluding the water source from use;
(ii)   boiling water before consumption; or
(iii) water chlorination resulting in a concentration of free chlorine of at least 0.5 mg/ml after at least 30 min contact time pH<8.0.

Additional risk reduction measures consist of:

(iv)   inclusion of reliable viricide systems in all the sewers and water treatment plants;
(v)   strengthening the sampling and testing of surface waters considered more susceptible to viral contamination.

Conclusions

Based on the structural features, surface charge, similar size and inactivation rates of avian influenza viruses and enteroviruses, it can be assumed that survival of avian influenza viruses is reduced by denaturing agents and treatments at least as much as that of enteroviruses. Therefore, the risk of being exposed to avian flu viruses through drinking water is negligible as long as raw water is submitted to all the treatment steps generally applied, according to good practice, to control enteroviruses in drinking water treatment plants. Although the risk for humans of being infected with H5N1 virus from bathing in contaminated water is much lower than that from direct contact with infected poultry/birds, the bathing risk in case of contaminated waters cannot be dismissed all together and should be assessed on a case by case basis.

Future Research Priorities

Direct comparison of environmental persistence of the H5N1 virus with known enteroviruses under the conditions occurring in drinking water and wastewater treatment plants would be particularly valuable. Further investigations are also needed to better define the infectivity for humans of H5N1 virus and the relative importance of viral transmission routes and mechanisms in the environment, including aerosols.

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Annex to Question n. 7

What is the evidence that human cases of H5N1 avian influenza have been caused by exposure to water containing H5N1 viruses?

There is a paucity of data on this subject.

A report from the Cooperative Research Centre for Water Quality and Treatment (Australia) published in Health Stream in December 2005 states (1):

"A total of 138 confirmed cases of human infection with H5N1 have been reported between 26 December 2003 and 7 December 2005. Cases have occurred in Cambodia, China, Indonesia, Thailand, and Vietnam. There have been 71 deaths among infected people, a 51% mortality rate. The majority of human infections have been attributed to close contact with infected poultry or poultry faeces. However, the possibility of water-related transmission has been suggested in two of the cases. A thirty-five year old woman and an unrelated nine year old boy in Vietnam were reported to have developed the disease after swimming in water bodies used for disposal of dead poultry. In Vietnam and other countries in the region, dead birds are often dumped into lakes and rivers to feed fish, or simply as a means of disposing of carcasses. Chicken manure and litter from cages are also used as a cheap protein source for feeding fish. However if the manure comes from birds infected with avian influenza, this practice may introduce a high virus load to lakes and rivers that also serve as drinking water sources."

The above-mentioned report is anonymous and unreferenced. However, these data are quoted again in the WHO 'Review of latest available evidence on risks to human health through potential transmission of avian influenza (H5N1) through water and sewage' (last updated 24th March 2006) (2). In addition, the WHO report notes:

"…Another potential case occurred in Cambodia where an 8-year old female may have been exposed to the virus through recreational contact with contaminated water, where asymptomatic ducks may have shed virus into the pond. However, again there is no evidence to confirm this hypothesis (Megge Miller, WHO: personal communication)". The case of the 9-year old child is also referred to in a case series (n=2) of cases presenting with non-respiratory symptoms (3).

Thus the total available evidence appears to be related to 3 cases of 194 so far reported since late 2003 (1.5%) in which there is an association (not necessarily causal) between recreational contact with H5N1 contaminated water and the onset of confirmed human H5N1 disease.

However, in all 3 cases it was not possible to rule out another form of non-water exposure, nor to conclude whether (if water was the source of the infection) the route of transmission was by ingestion, inhalation, or by direct nasal or conjunctival inoculation.

But likewise, no data are available regarding the extent to which the other 191 confirmed cases referred to above, were ever exposed to recreational water. In other words, although extremely unlikely, it is possible that 3 human cases have occurred as a result of far fewer than 194 exposures to recreational water (in other words the true denominator is far smaller)

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and that the risks associated with exposure to recreational water are therefore underestimated by the summary data available from WHO.

A general brief review of environment-human transmission has been published in the New England Journal of Medicine (4), but it does not add any new data.

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Question n. 8

How significantly could euro banknotes contribute to the spreading of the avian flu? Should the spreading of avian flu through banknotes be a risk, what recommended measures are there for banks?

Reply to question n. 8

Background

A banknote is a piece of paper and as such could serve as a surface onto which influenza virus particles could be deposited, but no better and no worse than any other piece of paper used in everyday life and handled by more than one person in rapid succession, e.g. train tickets, papers transferred from person-to-person during business transactions, letters and envelopes, and stray discarded copies of newspapers which others often pick up on the tube, trains or buses for a 'quick read'. The one advantage of the banknote over some of these other items is perhaps that it is generally carried concealed on the person (in a pocket or wallet) and is not therefore in the direct 'firing line' of most coughs and sneezes. We know that viruses survive for hardly any length of time in viable numbers on soft surfaces anyway (15 minutes) and so the risk would be negligible, unless someone had just sneezed hard onto a banknote before handing it on to someone else. With regard to coins, these items are classified as hard surfaces, where it is possible that the virus may survive in transmissible quantities for periods of up to 24 hours. However the risk from handling coins is not likely to be appreciably different (and should not be taken out of context) from the risks posed by communal hard surfaces such as hand rails, escalator hand belts, door handles, etc. encountered in everyday living.

Conclusions

For the general public:

The answer is that during a pandemic, handling banknotes and coins is not practically avoidable and will confer no discernible increased risk compared with handling almost any other communal object used in daily life; and compared with exposure to respiratory droplets (coughs and sneezes) and communal hard surfaces and fittings (hand rails, escalator hand belts, door handles, etc.) the ability of money to transmit influenza will pretty much pale into insignificance compared with what else is going on in society at the time. The overwhelmingly important issue will be compliance with frequent hand-washing and 'no-touch-face' advice.

For occupational groups likely to handle money (and other communal objects e.g. tickets/documents) on a regular basis:

The answer is that during a pandemic, handling large quantities of banknotes or coins on a repeated daily basis might confer an increased risk, if the notes have already been handled by others within a short period (15 minutes) and if the coins have been handled by others within a longer period, perhaps up to 24 hours.

However, two further observations are highly pertinent:

1   The risks to workers who have to handle notes and coins can be almost completely mitigated by avoiding the practice of licking fingers before counting notes, by avoiding touching the face during note and coin counting activities, and practicing immediate hand hygiene at the end of note and coin counting activities; in practice this might mean increasing staff access to alcohol based hand rubs/gels at their work stations.

2   The risks to staff in the financial sector in particular would apply no more and no less to other occupations (e.g. cashiers, ticket inspectors/fare collectors) whose daily work involves the handling of banknotes, coins, or other communal items such as travel tickets, stationery, etc. Again these risks would be significantly reduced across a wide range of occupations by compliance with hand hygiene regimens and 'no-touch-face' advice.

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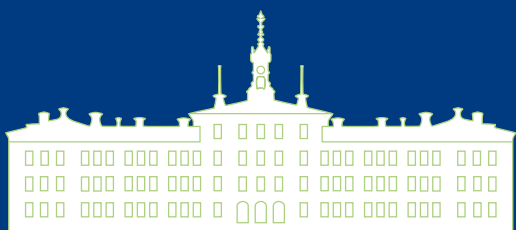ECDC
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