Interim ECDC Risk Assessment - January 27th 2008

Emergence of seasonal influenza viruses type A/H1N1 with oseltamivir resistance in some European Countries at the start of the 2007-8 influenza season

1. Summary Statement

Ordinary seasonal influenza viruses with significant resistance to the antiviral oseltamivir (Tamiflu) have been detected within the earliest part of this winter's influenzas epidemics in Europe. The viruses are known as influenza A/H1N1 (H274Y), and they were fully sensitive to other influenza antivirals. These viruses have been detected by a European research and surveillance project known as VIRGIL (http://www.virgil-net.org/) (supported by an EU grant) which has been undertaking routine surveillance for resistance in circulating influenza strains since 2004-5.

H1N1 viruses are predominant in this winters epidemics worldwide, and these resistant viruses are a new phenomenon this winter. A limited amount of specialist testing has been undertaken for ten countries and a proportion of A/H1N1 viruses detected in four countries Denmark, France, Norway and the UK, have been found resistant to oseltamivir. Overall in Europe the proportion with oseltamivir resistant is around 13% but the proportions resistant are variable with Norway showing a markedly high proportion resistant (12 of 16). If the 16 Norwegian viruses are excluded the proportion with resistant would fall to around 5%. Data from Norway indicate that these viruses were transmitted in the country. To date there are no reports that they are making people any more ill than do other influenza A viruses.

Normally A/H1N1 viruses as a group cause milder disease than some other human influenza viruses. However it must be realised that all influenza A viruses are potentially lethal for vulnerable individuals (the old and the very young and those with chronic debilitating conditions). **It also needs to be remembered that antiviral resistant is a relative not absolute term. Patients ill with viruses that are deemed resistant in the laboratory often still seem to benefit when they receive antivirals.**

The source of these viruses is not known at present. It seems unlikely to have anything to do with antiviral use in Europe, since those drugs are very rarely used here. Specifically the resistance is not explained in the Norwegian patients by the source patients having taken any antivirals.

These data come from only some 150 viral isolates from very early in this winter epidemics in ten of the thirty European countries therefore the results are preliminary and it would be unwise to make any statement for Europe as a whole. However Norway is still seeing them in specimens collected this month. There are some indications that A/H1N1 viruses with the same resistance mutation are being seen in some other countries, notably North America. However, the data from Europe is showing the highest resistance levels at present.

A conclusive risk assessment cannot be attempted at present and there seems little reason to change clinical guidance except possibly where these new viruses predominate over all others. It could be that as the influenza season progresses these viruses will be overwhelmed by more fit and sensitive viruses (many influenza virus strains that are resistant to antivirals lack 'fitness'- i.e. they have a reduced ability to transmit or cause illness) Equally however the resistant viruses could come to spread and predominate. We simply do not know at present.

There is considerable risk of confusion of this development with the separate issues of avian influenza, pandemic threat and antivirals stockpile. However, **it is important to bear in mind that these new H1N1-H247Y viruses have limited pandemic potential as they are a variant of a widely circulating strain. This differs from a pandemic scenario, which is likely to be caused by a completely novel strain of influenza virus. Though guarantees of effectiveness against an unknown virus cannot be made there is no reason to believe that oseltamivir will be ineffective against novel strains. Equally it is important to appreciate that H1N1-H247Y is a human seasonal virus and must not be confused with avian influenza viruses notably the similarly named A/H5N1 which causes bird flu in poultry.**

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Comments and suggestions for improvement are especially welcome in this interim document. These should be sent to influenza@ecdc.europa.eu with the subject heading *Interim Risk Assessment – antiviral resistance*.

2. Specific Questions

What are the implications for human health from the emergence and detection of oseltamivir resistant influenza A/H1N1 seasonal influenza viruses in some European countries this winter?

What are the options for further actions that could be taken?

3. What has been detected - What is new?

Surveillance for antiviral resistance in influenza has been undertaken in the European Union (EU) and European Economic Area (EEA) since the winter of 2005-5. this is undertaken by the EU supported VIRGIL project. Following its agreed work-plan the central VIRGIL laboratories in London began testing specimens for the 2007-8 season in the week beginning January 23rd. These specimens came from people with proven human seasonal influenza in ten EU/EEA countries. They represented the earliest viruses detected in November and December 2007 before transmission started in earnest this month.
(EISS/ECDCPressRelease - http://www.ecdc.europa.eu/Press/press_releases/080124_pr.html), Arkema et al 2008). Preliminary data shows that overall 19 of the 148 (13%) influenza A(H1N1) virus isolates predominantly collected in November and December 2007 from 10 European countries, carry an amino-acid mutation histidine to tyrosine at position 274 (H274Y) in the neuraminidase protein, which in previous studies has been associated with high level of resistance to oseltamivir.

An analysis by country shows that 12 of 16 Norwegian isolates, 4 of 19 French isolates, 2 of 75 United Kingdom isolates, and 1 of 10 isolates from Denmark carry the H274Y mutation. No mutations were found in 3 specimens from Austria, 4 each from Germany and Latvia, 5 specimens from Slovakia, 1 from Slovenia and 11 specimens from Spain. The concentration in Norway is striking but without the specimens from there the prevalence of this type among all H1N1 isolates in Europe would be only around 5%. **This is a new observation.** Although early in the influenza season, the presence of oseltamivir-resistant viruses circulating in the community in at least 4 European countries is unusual if compared to the previous winter seasons (2004/2005, 2005/2006 and 2006/2007), when little or no evidence of oseltamivir resistance was detected in over 900 isolates tested from 24 countries [Monto et al, 2006; VIRGIL 2008, personal communication]. **However all the H1N1 viruses isolated and tested so far this year are sensitive to the other antineuraminidase drug zanamivir and to the anti-M2 drugs amantadine and rimantadine.** To date A/H1N1 have dominated the 2007-8 season in Europe and other parts of the Northern Hemisphere where surveillance is active (North America and Japan).(Arkema et al 2008)

Further testing and relevant investigations are now underway. This is involving WHO (European Region and Headquarters), EISS, ECDC and Member States. Investigations are well advanced in Norway and the information from the National Institute for Public Health (NIPH) is none of the 12 viruses were obtained from a person who has either been treated with oseltamivir, or knew they had been in close contact with another individual treated with oseltamivir. This makes it very unlikely that the finding of the emergence of the mutated strains represent the selection of resistant strains that sometimes can be seen in people who are receiving oseltamivir. Also none of the 12 cases report recent travel and there are no obvious epidemiological links between them. (NIPH personal communication) Therefore it can be assumed that viruses carrying the H274Y mutation represent community influenza transmissions in Europe (rather than importations or a focused outbreak). Certainly these viruses are to some extent *fit* and able to transmit and cause disease. The specimens tested by VIRGIL came from November and December

2007, The Norwegian National Influenza Centre has sequenced some specimens from this month (January 2008) and finds evidence that the resistant virus is persisting. To date there is no indication that the people with these infections are suffering from anything more than ordinary human influenza. While a few persons have been hospitalised that may be a *surveillance artefact* (in this case that people who are hospitalised are, in contrast to people who still at home, more likely to have a specimen taken and their influenza virus entering the surveillance system). However it must be realised that even ordinary seasonal influenza can be lethal, especially for those most vulnerable (older people, people with debilitating diseases and the very young). ECDC estimates that each year ordinary seasonal influenza is responsible for around 40,000 deaths in the EU/EEA. [ECDC Influenza Fact Sheets, 2007]

4. Scientific Background Information

4.1 Surveillance

A series of systems deliver surveillance for influenza viruses world-wide. These are choreographed by the World Health Organisation Global Influenza Surveillance Network (GISN). (http://www.who.int/csr/disease/influenza/influenzanetwork/en/index.html)
Central to it is the unrestricted sharing of information and viruses between countries. [ECDC Briefing 2007] In Europe there is the long-standing European Influenza Surveillance Scheme (EISS - http://www.eiss.org/index.cgi) which is currently integrating into other surveillance schemes with ECDC. In the Northern Hemisphere the season for intense clinical and virological influenza surveillance runs from Week 40 of one year in the Autumn to week 20 of the next year in the Spring. So seasons are referred to as Season 2004-5, Season 2006-7 etc. Background surveillance continues throughout the year. Monitoring of Influenza antiviral resistance in Europe is conducted as part of the European Surveillance Network for Vigilance against Viral Resistance (VIRGIL) (http://ec.europa.eu/research/health/influenza/proj13_en.html) in collaboration with the WHO Collaborating Centre at the MRC National Institute for Medical Research in London and the Community Network of Reference Laboratories for Human Influenza in Europe (CNRL) which is coordinated by EISS and is comprised of all the National Influenza Centres (NICs) in Europe [Meijer et al, 2005, 2006]. This central laboratory work on influenza for VIRGIL is undertaken at the Health Protection Agency Centre for Infection in London though this is supported by work across Europe and by the Neuraminidase Inhibitor Susceptibility Network (NISN) [Zambon M & Hayden FG, 2001]. Surveillance for antiviral resistance is carried out each winter influenza season and has been underway since 2004-5. The work is supported financially by the European Union though as it is based on a series of routine systems it also relies on considerable input and support from the countries concerned. Specimens are identified in EU member states and following initial work a subset of all virus isolates are sent centrally to the laboratories in London for full characterisation for the bi-annual WHO vaccine recommendations, and where they are tested for indicators of antiviral resistance [Gerbil 2003, Meijer A et al, 2007]. (http://www.eurosurveillance.org/em/v12n04/1204-222.asp)
At this time of year (January) surveillance work starts on the first specimens that have been gathered early in the season (November and December 2007). That is before proper

transmission begins. This year transmission started in earnest in January 2008 [Arkema JMS et al, 2008]. (http://www.eurosurveillance.org/edition/v13n04/080124_2.asp)

4.2 Seasonal Influenza Epidemics In temperate climates like Europe transmission of ordinary influenza (human seasonal influenza) takes place each year in the winter months with epidemics across Europe. In more tropical climates nearer the equator influenza transmission is thought to be more continuous through the year with over-laying epidemics. In the Southern Hemisphere the epidemics are the converse of those in Europe taking place in the period May to October. In recent years the European epidemics have rarely started in earnest before the New Year though early transmissions can always be detected in the late autumn. Human seasonal influenza is not caused by a single virus but by complex and variable mix of viruses the balance of which varies from year to year. Influenza viruses are inherently unstable and it is thought that small genetic changes are taking place in the viruses constantly (so called *antigenic drift*). This is the reason why there needs to be such careful surveillance of influenza and annual recommendations made by WHO as to the composition of the influenza vaccines produced by industry. [Gerdil 2003] (http://www.who.int/csr/disease/influenza/20078anorthreport.pdf)
In the 2007-8 season the dominant virus has been of type A/H1N1 which is usually associated with milder disease in humans than say the A/H3N2 [Arkema JMS et al 2008, Simonsen L, 2005]. The drivers for the changes are not fully understood though an important element is the instability of the RNA at the heart of the influenza viruses, their tendency to mutate spontaneously and to occasionally exchange genetic material (reassortment) when two viruses infect the same human or animal. Of late work known as *antigenic cartography* has suggested that many of the new variants of human influenza viruses that have emerged recently come from the Far East and South East Asia [Smith et al 2004 & Smith 2006]. It is thought that subtly different influenza viruses are emerging constantly but many do not transmit efficiently (often they are said to lack *fitness*) and these then die out as they do not compete well with fitter viruses.

4.3 Influenza Pandemics Very occasionally (four times since the late nineteenth century) (http://www.ecdc.europa.eu/Health_topics/Pandemic_Influenza/stats.html) larger changes led to the emergence of a new virus able to transmit efficiently from humans to humans and to which a large proportion of the population have no immunity. These spread as pandemics across the world. **It is important to bear in mind that when talking about these new H1N1-H247Y viruses there is no reason to imagine that they have any more pandemic potential than other influenza viruses. Equally it is important to appreciate that H1N1-H247Y is a human seasonal virus and must not be confused with avian influenza viruses notably the similarly named A/H5N1 which causes bird flu in poultry.**

4.4 Antiviral Resistance There are two main groups of antivirals used against influenza viruses. In order of discovery these are the M2 ion channel inhibitors (M2Is) adamantenes (amantadine and rimantadine), and the neuraminidase inhibitors (NIs) (zanamivir and oseltamivir). (Moscona 2005b) Amantadine and rimantadine were licensed in the US with antiviral indications in 1966 and 1993 respectively. Zanamivir

and oseltamivir became available in the late 1990s. Oseltamivir is often preferred over zanamivir for treatment purposes as it is available as tablets while zanamivir has to be inhaled. Oseltamivir has a Europe-wide license (EMEA), whilst adamantenes and zanamivir need to be licensed by country, and are not licensed in all countries.

Resistant Viruses in People on Treatment As influenza viruses continuously change through mutation and recombination, drug resistance viruses are occasionally detected briefly in infected individuals during treatment with antivirals. This is why information has to be gathered on what treatment if any people with resistant viruses have had, or whether they have been in contact with those on treatment. However these viruses usually lack *fitness* and do not transmit on. [Moscona 2005a]

Resistance to Neuraminidase inhibitors This has been reported as occurring at very low level (<1%) in immunocompetent individuals screened during seasonal influenza epidemics [Monto AS, 2006] though cross resistance between oseltamivir and zanamivir is not common. Oseltamivir resistance in influenza viruses is relative and despite its presence patients with oseltamivir-resistant viruses may still benefit from receiving oseltamivir.[Moscona 2005a] Often the clinical outcome for patients with resistant viruses and treated with antivirals is not any different from patients carrying fully sensitive strains [De Jong MD et al, 2005 & Hayden FG, 2006] However, higher levels of antiviral resistance has been reported developing during treatment of children and immunosuppressed individuals [Kiso M et al, 2004; Hayden FG, 1997; Gubareva LV, 1998].

Types of Resistance There are generally three levels of antiviral resistance according to the way that resistance can be detected or inferred:

a. **Genotypic Resistance**: detected through sequencing of the viral genome and identification of mutations previously associated with a certain level of drug resistance. Many national laboratories in Europe can undertake this testing

b. **Phenotypic Resistance**: resistance of the virus to drugs is tested *in vitro* (not in living systems) by measuring viral replication at different drug concentrations (IC50)

c. **Clinical Resistance**: based on animals (ferret or mice) and human patients and measuring or observing the actual response to treatment with antivirals.

Of course clinical resistance in humans is what is of most concern.[Moscona 2005b] Clinical resistance and the response to treatment with antivirals (the clinical response) remains the most important proof of antiviral effectiveness. A virus may have genetic markers associated with resistance (*genotypic resistance*) but still show satisfactory response either in the laboratory (*phenotypic resistance*) or when the antivirals are given to patients (*clinical resistance*).

Genotypic mutations associated with drug resistance against oseltamivir are often observed to have defects in their basic virus characteristics including transmissibility and ability to replicate. This makes them “unfit”. However the most commonly observed mutations conferring adamantane resistance did not seem to affect the virus fitness, and hence naturally resistant viruses are circulating at variable levels during seasonal epidemics.

Until these observations no rise in resistance to oseltamivir for ordinary transmitting human influenza viruses had been observed over time. This might be due to the fact that some of the NI resistance associated mutations are also associated with reduced influenza virus transmissibility and this might partially explain the low prevalence of primary NI resistance in viruses isolated from clinical and animal model settings.

The H274Y mutation has only been observed in viruses of neuraminidase type 1 (N1). This mutation has been associated with a 400-fold reduction in the sensitivity of the virus to oseltamivir in vitro. There is contradictory evidence on the role of such mutation in affecting the viral fitness with some studies showing reduced replicative capacity and transmissibility and other showing similar fitness than the wild type viruses [Hurt AC et al, 2006].

Resistance to the Adamantenes. Influenza viruses resistant to the adamantenes emerged as early as the late 1960s. The drivers for this emergence are not clear. In some areas of the world, like North America the proportion of influenza strains naturally resistant to this drug class has recently become unacceptably high to the point where they are not always the drug of choice for seasonal influenza [Fiore AE et al, 2007].

4.5 What is known about A/H1N1 viruses and specifically A/H1N1-H247Y?

In general A/H1N1 viruses are associated with milder illness than other influenza A viruses though like all influenza A viruses they can cause severe disease and death, especially in some vulnerable individuals. A/H1N1 viruses with the H274Y mutation have been seen before when they were detected in Japan in patients treated with oseltamivir. Those viruses were not subsequently detected to be circulating in the community except at very low levels so they may be a somewhat different phenomenon. However the earlier viruses were studied in animal models. This found that they could transmit from one animal to another but with lower efficiency than do ordinary A/H1N1 viruses. That said they are among the fitter of all antiviral resistant influenza viruses (F Hayden personal communication 2008).

4.6 What is being observed elsewhere?

WHO is now working intensively with its partners across the world to determine whether these first European observations are unique or occurring elsewhere. There are some indications that some of the same virus oseltamivir-resistant A/H1N1 viruses are being observed at low levels in the United States.. However detailed surveillance for the 2007-8 season is only just starting and more information will become available later.

4.7 Oseltamivir use in Europe It is important to bear in mind that apart from in Japan and to a lesser extent the United States oseltamivir is not much used for treatment of seasonal influenza. This is especially so in Europe where it seems that in most countries the numbers of prescriptions for treatment are negligible.(Communication from Roche 2007) There were many prescriptions in 2005 and 2006 in some countries but most reports suggest that these were driven by anxiety over the threat from bird flu and were stored in homes rather than used for treatment. This has two implications. Firstly it makes it even more unlikely that these viruses have emerged as a result of use of oseltamivir in Europe. Secondly it is not that the case that clinicians may be losing the utility of a widely used drug.

5. Risks of Misunderstanding – Risk Communication

Perhaps the greatest risk at present is that the information and risks are misunderstood, especially when WHO and ECDC lack the information to make definitive statements on risks. The possibility for confusion of these events with avian influenza (A/H5N1), pandemics and oseltamivir stockpiles is considerable. Therefore ECDC and WHO are preparing a series of Frequently Asked Questions and Answers (FAQs) and these will shortly be available on their web-sites. The ECDC FAQs are designed for adaptation and use by EU/EEA member states.

6. Formulation – What is likely to emerge?

These are early days in this new development and it really is too soon to undertake any proper risk assessment. There is only information for viruses detected early in the 2007-2008 (actually from people who become ill in November and December) from a limited number of isolates from ten European countries. Numbers tested for any individual country are low. Hence this interim assessment will be reviewed in the coming weeks and months and updated as more information rapidly becomes available from more testing, from other countries and especially as a result of work and consultations organised by WHO with which ECDC is working closely. It could be that as the season progresses these particular viruses are overwhelmed by other, fitter viruses. Equally however they could spread to other countries and become more prevalent as seasonal influenza transmission spreads across Europe.

7. Risk Statement

It is also too early to make any definitive risk statement and more information will be gathered for this as time goes along. However the most important points to be born in are:

- There is no indication as yet that these oseltamivir resistant viruses (A/H1N1 H274Y) are more virulent or are causing more severe disease than wild type influenza viruses. Though A/H1N1 usually causes less severe disease than say the A/H3N2 viruses it still can have severe outcomes even death in vulnerable people.

- This high level of resistance to oseltamivir observed in a relatively small number of samples tested might not be confirmed when more samples are tested and when there is widespread circulation of flu in the community.
- A/H1N1 viruses are well matched with the current seasonal influenza vaccine and therefore patients who received vaccination are already at lower risk of contracting the disease or developing severe complications than people who have not been immunised.
- Oseltamivir is not much used in Europe at present.
- While there are no international or EU guidelines for use of antivirals there are national guidelines in some EU countries which often recommend that antivirals are reserved mostly for prophylaxis or early treatment among patient with risk factors or those who are older (variously defined as being over 60 or 65 years).[National Institute for Health and Clinical Excellence (NICE) http://www.nice.org.uk/guidance/index.jsp?action=byID&o=11841 UK, National Institute for Public Health and the Environment (http://www.rivm.nl/cib/infectieziekten/Influenza/Influenza.jsp) RIVM, The Netherlands] Therefore the immediate implication of the reporting of oseltamivir resistance are limited and for those where antiviral prophylaxis or treatment is indicated alternative drugs can be used. However these national guidelines may need to be reviewed in the light of this development if the H1N1-H247Y viruses continue to appear and spread.

8. Options for Action in the EU

a. Further typing of specimens especially those detected after transmission started in earnest including specimens from the other EU-EEA countries with the objectives of seeing and tracking how H1N1-H247Y matures and evolves in Europe.
b. Proactive communication of further results and information for the public and professionals including 'rumour surveillance' looking for misunderstandings and responding to them
c. Descriptive investigations of the initial cases especially concerning their disease and response to any treatment with antivirals that they happen to be given.
d. Consideration of review of national guidelines clinical advice by those experienced in developing such guidance.

9. When will be reviewed - One month after publication date (may be updated Earlier if need be)

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