



RISK ASSESSMENT

Laboratory-created A(H5N1) viruses transmissible between ferrets

29 February 2012

Executive summary

The results of two, as yet unpublished, investigations of laboratory-induced genetic changes in avian influenza A(H5N1) viruses have been reported to have found that a surprisingly few number of changes make the viruses transmissible between ferrets, the most commonly used model for the way influenza behaves in humans. The possibility that this could have resulted in the development in laboratories of A(H5N1) influenza viruses transmissible between humans has caused concern for public safety and generated unusually high levels of debate, especially in the United States. The American authorities that are funding the work have requested that part of the scientific papers (carried out independently in the USA and the Netherlands) be restricted because of a fear that this could represent *dual-use* research and development, i.e. research that could be used for malign as well as good purposes. An almost unprecedented voluntary moratorium on the research has been declared by the groups capable of undertaking such research, including six European groups.

These developments have raised a complicated series of interlocking issues: Public concern that laboratory-modified H5N1 viruses could accidentally cause an influenza pandemic; the need for assessment of the balance of public health risk and benefit in research; appropriate laboratory biosafety requirements of research on the evolution of pathogens to become more virulent or transmissible; the need and ability to revise risk assessments concerning A(H5N1) viruses; a potential threat to the new pandemic influenza preparedness framework for influenza virus and benefit sharing; and academic freedom to publish.

To resolve some of these issues WHO held a successful first meeting of the principals involved in the research and its publication (16–17 February 2012, Geneva). That meeting achieved a considerable degree of consensus, including the view that progress in this research area was vital to global health security, that the suggested mechanism of restricting publication was undesirable and impractical in the short term and that the moratorium on research should continue. It was agreed that wider meetings were now needed and that there should be a further review of laboratory biosafety both in the laboratories concerned and generally for this kind of research. Finally it was recommended that considerable public communication work was needed to explain the importance of the research work that is now on hold.

This risk assessment report summarises and explains the complex public health and scientific issues around these developments including the positive and negative aspects of some of the responses that have been proposed internationally. These proposed measures include withholding the research methodologies and genetic sequences from publication, destruction of the created viruses, raising their biosafety classification, handling them in maximum biosafety and security facilities and conditions, and restricting the numbers of institutions and groups working in the field. This document's starting point is that without sight of data and analyses it is very difficult to undertake risk assessments. It is not even clear at present how pathogenic these viruses are in animal models. The document also puts forward the ECDC position on some of these issues according to ECDC's limited mandate, recognising the value of the research but also the potential risks. ECDC stresses the need to consider mechanisms for a robust biorisk-management approach along the lines of international standards and EU-wide guidance on

laboratory biosafety/biosecurity for any future emerging threats. ECDC indicates that it would advocate open publication of the findings and emphasises the importance of sustaining and enacting the pandemic influenza preparedness framework with its underpinning global virological surveillance and sharing of information and benefits in order to enhance global health security. It is ECDC's intention to support the European Commission and Member States, to monitor these developments closely and with its stakeholders and collaborators to revisit its risk assessment for A(H5N1) viruses as the research findings emerge.

Enquiries concerning this document should be directed to the ECDC Chief Scientist Johan Giesecke, johan.giesecke@ecdc.europa.eu

Background

In September 2011, Prof. Ron Fouchier of the Erasmus Medical Centre (Rotterdam, Netherlands) reported at a public scientific meeting the results of genetically modifying influenza A(H5N1) viruses in animal research. The broad objective of this work is to determine whether these avian influenza viruses have the potential to adapt to humans and result in a pandemic [1,2]. Professor Fouchier announced that his team had produced viruses that were transmissible through the air between ferrets, a species often used in influenza research as a model for humans, and produced infections that were sometimes fatal for the animals. Similar research has been underway at the University of Wisconsin at Madison, USA under Dr Yoshihiro Kawaoka where the results have been similar except that the transmissible viruses are reportedly not lethal to ferrets [3]. No pathogenicity data are available as yet for the viruses in the Netherlands [1,2]. These studies are an extension of animal research experiments undertaken by several groups of scientists over a number of years exploring the structure and functioning of A(H5N1) and other animal influenza viruses. All the research can be seen as contributing to virological risk assessments* [4–7].

Manuscripts from the researchers in the Netherlands and the USA were submitted to the journals *Science* and *Nature* respectively and were accepted for publication after peer review and revision. Both sets of studies had been funded by the US National Institutes of Health (NIH) and their results therefore came under review by the [U.S. National Science Advisory Board for Biosecurity \(NSABB\)](#) prior to publication† [4]. The NSABB asked that parts of both papers be 'redacted' and the United States Government later endorsed this request on the grounds that the information might allow researchers with malign intent to potentially create a biological weapon [4]. This meant withholding parts of the paper that described exactly how the viruses were made more transmissible and which mutations were responsible for the change in biological properties. It was suggested that this information and the genetic code could be made available to a few researchers on a 'need to know' basis; the articles could not be published unless such a mechanism was in place or another solution was found. In response, the journals and the two groups of authors announced that they would respect the NSABB advice and assist in identifying a mechanism through which details of the studies can be shared with individuals and organisations with a 'need to know' [4].

The debate and concern in the media continued, especially in the United States, with surprise being expressed by some that researchers were seeming to try to create dangerous viruses [9–11]. Though that was not the prime objective of their work, on 20 January 2012, the Erasmus and Madison research groups, the US CDC and more than thirty other publicly funded researchers (including seven from four European countries) announced that they had voluntarily decided to impose a 60-day moratorium on this research [4]. These were many of the groups who had been undertaking work on animal influenza structure and functioning, including transmissibility studies, for some years with a strong research output which has increased understanding of influenza virus structure and the way the viruses work. This has made it possible to perform scientific risk assessments for influenza [5–7,12–17]. The researchers gave the reassurance that they would continue to assess the transmissibility of A(H5N1) and other influenza viruses that emerge in nature and pose a threat to health [4]. Such a moratorium has a precedent; something similar was done in 1975 when popular concern was expressed over genetic engineering and researchers successfully applied a voluntary pause in research of short duration while understanding increased and agreement on safeguards was achieved [18].

The A(H5N1) research in question is important for delivering global health security and so cannot be abandoned lightly [11,19,20]. Therefore, the World Health Organization (WHO) agreed to try to solve the impasse. WHO, Member States and Agencies like ECDC were also motivated by the recognition of a threat to the new pandemic influenza preparedness agreement made by World Health Assembly in May 2011 [21]. This improves global health security but it is based on the safe but unfettered exchange of viruses and open dissemination of research findings and benefits [21,22].

As a first step, WHO convened a limited meeting on 16–17 February of the principals involved in the research, experts representing the scientific journals, virological and public health specialists (including the heads of the six [WHO Influenza Collaborating Centres](#)) and representatives from the countries both where the viruses originated and where the research was carried out [23]. That meeting was successful in achieving consensus on a series of items though the issue of publication remains unresolved and the research moratorium is to continue for an unknown period. WHO anticipates a period of further work and larger meetings [23,24].

The objectives of this ECDC risk assessment are to summarise and explain the complex public health and scientific issues around these developments, commenting on some of the responses that have been proposed (Table 1). The ECDC position on some of the issues is put forward within the limits of its mandate as a non-regulatory body, recognising the value of the research but also the potential dangers and the rationale for review of appropriate laboratory biosafety precautions. It is recognised that some of the most important risks cannot be assessed without publication of the Erasmus and Madison work, nor without further research (Table 2) [11].

* See Annex for definition.

† 'Dual-use research and development' refers to work that potentially leads to creation of dual-use agents, i.e. an agent that can be used for good or ill purpose. It is potentially subject to EU legislation [8].

Acknowledgements and contributions

Drafts of this paper were reviewed and commented upon by ECDC's Advisory Forum and selected individuals. The paper was also received by the Health Security Committee, the European Food Safety Authority and European Commission (and through this the EU animal influenza reference laboratory) and comments provided. Details of the biosafety aspects in the Netherlands were kindly supplied by Professor Fouchier. Specific advice on biosafety aspects came from Ingegerd Kallings and Åsa Björndal (Swedish Institute for Infectious Disease Control) and Allan Bennett (UK Health Protection Agency) through their work and involvement in an ECDC-funded project on biorisk issues (2009–2011)*. However, final responsibility for the paper's content rests with ECDC which is an independent Agency of the European Union. The ECDC staff primarily involved in drafting this report are Angus Nicoll, Marc Struelens (Chief Microbiologist), Amanda Ozin, Eeva Broberg and Cornelius Bartels. Drafting and final approval was overseen by the Chief Scientist, Professor Johan Giesecke.

History of the threat from A(H5N1)

Highly pathogenic avian influenza A(H5N1) viruses[†] of the type that has caused concern among veterinary and public health communities were first detected in southern China in 1996[‡] and then found in poultry in Hong Kong in 1997 where drastic veterinary measures were taken because of the implications for animal health, and the apparent pandemic potential [25]. The viruses spread from the Far East and became entrenched in domestic poultry in a few lower resource countries where all attempts at control and eradication have been unsuccessful [26–27]. Good biosafety practices on farms, eradication and surveillance programmes implemented in EU Member States and co-financed by the European Union [28] have prevented the viruses becoming established in European poultry despite occasional introductions.

Despite the measures taken in Hong Kong in 1997, there were still occasional human A(H5N1) infections due to exposure to sick poultry or contaminated environments. These human infections often had a lethal outcome and the case-fatality rate recorded is uniquely high for influenza viruses [29]. Some of the infections were the result of human-to-human transmission. Such transmissions are difficult to distinguish from those due to contact with poultry or fomites, but they have certainly taken place [29]. However, to date, the A(H5N1) viruses have not adapted well to humans; the transmissibility and ability to infect humans has been inefficient [29,30]. That is to say there has not been: efficient virus attachment to human upper respiratory tract tissues, replication to high titres in those tissues, and then release and aerosolisation of virus particles [12].

Nevertheless, public health concern remains high for two reasons: as well as the extraordinarily high case-fatality ratio for an influenza (around 60% on average with a national range from 30% to 80%) the viruses persist in poultry and continue to evolve genetically by mutation, raising the possibility that they might eventually adapt to humans [29,30]. Concern among policymakers over what came to be known as 'bird flu' waxed with initial investment in antiviral stockpiles and so-called 'pre-pandemic' A(H5N1) vaccines, but was followed by a long period of low H5N1 activity. This, together with the experience of a mild pandemic in 2009, albeit from a very different animal influenza, resulted in waning interest and investment [31]. However, the scientific reasons for concern and public health risk have persisted and identifying the viral determinants of the host range and transmissibility became a WHO priority for research [32]. Though a formal risk assessment cannot yet be undertaken for the Erasmus and Madison viruses, these new developments are considered by WHO, ECDC and others to have heightened those concerns [24].

What exactly has been done in the laboratories, and what has been found?

Reports from these experiments have not been published. However, according to the information available in the public domain the researchers worked with ferrets as a model system for humans and carefully genetically modified the viruses in high security laboratories (level BSL3+[§]) with the objective of seeing whether any modifications would make them transmissible among mammals [1–4,12–15]. This form of *virological risk assessment* is recommended in the WHO Public Health Research Agenda for Influenza [32]. Such work could never

* See http://ecdc.europa.eu/en/activities/microbiology/biosafety/Documents/101117_Bioriskproject_Description.pdf

[†] The term *highly pathogenic* is a veterinary term referring to the effect of avian influenza on domestic poultry, not necessarily to any pathogenic effect in humans. Avian influenza A(H5N1) was first recorded in 1958 but the genetic type that emerged, spread and evolved from 1996 onwards has been of a distinct genetic type.

[‡] For more detail on the spread of H5N1, see the WHO time-line http://www.who.int/influenza/human_animal_interface/H5N1_avian_influenza_update.pdf

[§] See Annex for definition.

be done with human subjects and so ferrets are often used as surrogates because they represent one of the better, though still imperfect, models of how influenza viruses behave in humans [15]. In the Netherlands the viruses were also passed sequentially from one ferret to another to allow further adaptation. All due care and attention to ethics and safety were undertaken: a research permit was granted in advance by the Ministry of Infrastructure and the Environment of the Netherlands; on this matter, the Ministry was advised by the Dutch Commission on Genetic Modification, who also advised that a BSL3+ biosafety level* should apply to the experiments. Biosafety rules compatible with EU legislation and biosecurity measures compliant with a national code of conduct were followed. Similar precautions were followed by the researchers in the USA in accordance with its national regulations for working with pathogens on the 'select agents' list of the USA [33,34]. There were external inspections by CDC biosafety and biosecurity specialists in both countries in agreement with these US regulations, as the USA was the source of the main funding [4,33,34]. In December 2011 the European Commission contacted the Dutch authorities and ascertained that the virus samples are safely and securely stored and that the research project is bound by the Code of Conduct on Biosecurity set by the Royal Dutch Academy of Science. It has been clarified that the Dutch legislation on laboratory biosafety aligns with the European legislation on worker protection and work with genetically modified organisms. The necessary permits had been granted prior to commencing the research and the projects conform to the USA's biosafety and biosecurity requirements.

Similar work has been undertaken for some years by a small number of academic and other publicly funded groups, but these are the first two experiments resulting in a seemingly transmissible virus [1,4,12–17]. It is reported that surprisingly few mutations were necessary to make the A(H5N1) viruses transmissible among ferrets and that all of these individual mutations have already occurred naturally, though never in this combination [1]. It is also suggested that the resulting viruses in Erasmus are somewhat pathogenic to the ferret host, but that is not the case with the viruses in Wisconsin [3]. However, little is known beyond that about the results and a number of important research questions essential for a thorough risk assessment remain.

A need to revise A(H5N1) risk assessments?

If the reported results are confirmed then the risks of an emerging influenza pandemic arising from natural evolution and mammal/human adaptation of avian A(H5N1) viruses will need to be reconsidered by ECDC and others for A(H5N1) viruses in general and specifically for the transmissible viruses. The main question is whether this new work makes the natural emergence of an A(H5N1) pandemic any more likely. The changes in the nucleotide sequence that confers transmissibility being few and the 'natural' element of passaging the virus through an animal host may make it seem that the researchers have demonstrated that this is more of a natural evolutionary possibility than previously thought. Certainly that is WHO's view and ECDC agrees that there is a case to be answered [19,23,24]. The information that is needed for any reconsideration of the risk assessment is only available from the unpublished papers and further research will be required on the dose needed, the degree of transmissibility, the mechanisms of transmission, etc (Table 2). Hence it is impossible to undertake the scientific virological risk assessments which are needed to inform difficult decisions about reinvesting in preparation for an A(H5)-based pandemic and how to prioritise investment in diagnostic and vaccine development for A(H5) versus A(H7), A(H3N2)v, A(H9N2), etc [5,32].

Is there a threat to pandemic preparedness, virus- and benefit-sharing?

The implications for pandemic influenza preparedness and global health security are giving WHO and ECDC particular concern [20–22]. In the Spring of 2011, after a number of years, WHO and national stakeholders concluded a complex and difficult set of negotiations about access to influenza viruses and subsequent benefit-sharing [21]. This was endorsed by the World Health Assembly in May 2011 and implementation of the agreement has started [35]. One of the pivotal points of this is trust between suppliers of original viruses (often in moderate-resource or resource-poor countries), industry and researchers and public health institutions (often in better resourced countries). Countries where these viruses occur naturally should make them available for public health purposes while countries, organisations and companies receiving the viruses should share the resultant benefits, notably diagnostics and vaccines. Equally important is the free sharing of research findings needed to protect global health [21,22]. If as an outcome of the current restrictions the Dutch scientists could not share the results of their experiments with colleagues and collaborators that originally supplied the viruses or benefits from developing diagnostics, or A(H5)-based vaccines were not available to poorer countries at prices they could afford, that would implicitly threaten the agreement and international health security [20,21]. Another important issue is whether a country where the research results in a novel transmissible virus should make a notification under Article 6 of the International Health Regulations (2005) [36]. This is presently required of a country discovering a natural variant virus according to Annex 2 of the Regulations. In ECDC's view there is a case for this, as well as (if the country is based in Europe) making a notification through the European [Early Warning and Response System](#). There is also a case for tracking the transfer of modified viruses through [WHO's Influenza Virus Traceability Mechanism](#).

What are the laboratory biosafety risks?

Laboratory biosafety and biosecurity issues are taken very seriously in Europe and there is EU legislation in place designed to protect workers (individual risk)* and broader public/animal health (community/population risk)† in the context of research on the A(H5N1) influenza viruses. Further, there is also legislation regulating more general infectious disease research involving genetically modified organisms‡ It is a Member State's responsibility to implement and enforce these in their national laws. At international level there are various WHO guidance documents and manuals for laboratory biosafety and biosecurity, CEN standards for biorisk management, and the World Animal Health Organisation (OIE) has published guidance and recommendations on laboratory containment of animal pathogens (see Annex for list of these resources).

In the current situation, there has been an assurance from both the research groups and regulators that the experiments in question were fully compliant with all regulations and necessary safety measures [4]. This was elaborated in bilateral discussions between the European Commission and the Dutch authorities (as described above). There are always laboratory biosafety issues and biorisk management considerations surrounding this type of research. The first is to question whether this type of research is justifiable in the first place. In the view of WHO and ECDC the answer is a qualified 'yes', while remaining concerned as to how to minimise the risks from such work [24]. A further question is whether the demonstration that certain mutations result in a laboratory-created strain with potential to be transmissible and have severe outcome in ferrets (and by extension perhaps in humans) justifies a re-consideration of the safety categorisation of the viruses and procedures for handling them. As ECDC is not a regulatory agency, such decisions are not part of its mandate. There are also practical aspects of containment level, handling procedures, and other considerations including laboratory biosecurity issues [23]. For example, what is the risk from laboratory accidents with these engineered influenza viruses and what mechanisms are in place for initial detection (i.e. routine mechanism for medical 'syndromic surveillance' of laboratory staff irrespective of suspected exposure or laboratory accident).

It has been suggested by some that the viruses and any work that might result from them should only be conducted at the highest containment level, BSL4, rather than BSL3+§[9]. Such a solution would be premature as we do not know the threat posed by these viruses (see Table 2). Also there are discrepancies in terminology within Europe as to what constitutes BSL3+ and BSL4. The consequences, practicalities and costs of enacting BSL4 could be inhibitory, and it should be realised – as past examples have shown – that a universal requirement for a higher containment level could be interpreted as also applying to routine diagnostics or even clinical work for humans and animals [37, 38, 39]. It would thus be essential to distinguish between the level of control applied for the experiments like those undertaken by Fouchier and Kawaoka's group and diagnostic and clinical care work should the viruses escape or appear naturally in animals or humans [39]. An additional important consideration is the need to ensure adequate protection for laboratory workers by, for instance, offering vaccination [37,38,40].

The letter from the A(H5N1) researchers gave reassurances that all mandated biosafety and biosecurity precautions are being taken in accordance with the relevant regulations [4]. However, regulations are only as good as those that implement them in the laboratories and those acting as regulators. Further, the likelihood of accidents increases with the number of laboratories involved, particularly when handling procedures are concerned that require specialised training and facilities. The letter from the researchers [4] can be misunderstood as indicating that they are the only researchers working with A(H5N1) viruses. Though they are probably the only ones undertaking this experimental work they are by no means the only people working with unmodified non-transmitting A(H5N1) viruses; these have been widely shared in the academic and commercial laboratory community to the point where it is impossible to know where they all are, even in Europe. There is therefore a case for restricting the numbers of laboratories undertaking the most dangerous laboratory work and ensuring strong biosafety practices at those laboratories, even at a BSL3+ level. One of the welcome developments under the 2011 World Health Assembly agreement was that the movements of new emergent influenza viruses can be tracked through the [Influenza Virus Traceability Mechanism](#)[41]. However the weak points are that as yet not all academic groups are aware of this and it is not clear what happens when viruses move out of the [WHO Global Influenza Surveillance and Response System](#), for example for use in vaccine development and the private research setting [42].

These viruses potentially represent dual-use technology [8]. Although this is an important consideration, it distracts from the primary question of the balance between public health benefit and risk of conducting such research

* Directive 2000/54/EC of the European Parliament and of the Council of 18 September 2000 on the protection of workers from risks related to exposure to biological agents at work. OJ L 262, 17.10.2000, p. 21–45.

† Council Directive 2005/94/EC of 20 December 2005 on Community measures for the control of avian influenza and repealing Directive 92/40/EEC. OJ L 10, 14.1.2006, p. 16–65; and 2006/437/EC: Commission Decision of 4 August 2006 approving a Diagnostic Manual for avian influenza as provided for in Council Directive 2005/94/EC. OJ L 237, 31.8.2006, p. 1–27.

‡ Directive 2009/41/EC of the European Parliament and of the Council of 6 May 2009 on the contained use of genetically modified micro-organisms. OJ L 125, 21.5.2009, p. 75–97.

§ see Annex for definitions.

[11,43]. It is reassuring that the researchers have taken a self-governance approach with their voluntary moratorium. Together with a regulatory approach, this is the type of engagement between scientists and policymakers needed to tackle dual-use issues and to develop robust mechanisms to move forward on research topics with clear public health benefit that outweighs any inherent risks to the individual workers and the global community at large [42]. While the risks of dual use are difficult to assess, the risks of laboratory accidents are real and documented. Repeated case studies of laboratory-acquired infections and accidental dissemination (for example for the coronaviruses that caused SARS on a number of occasions, and once for A(H5N1) influenza in Europe) demonstrate that while these events are always avoidable by strict adherence to fundamental biosafety principles, the risks cannot be totally eliminated [43].) Hence the cumulative risk increases as more institutions undertake such work and more viruses are moved through transportation laboratories. For the current A(H5N1) research, the potential consequences of such an event could be an influenza pandemic severe by any standards. Therefore, the risks inherent in laboratory containment suggest the need to review mechanisms for the authorisation of research which has the potential to develop such dangerous agents.

Is redaction of research ever justified or workable?

The decision to request redaction of parts of the manuscripts is inconsistent with earlier approaches in very similar circumstances. When the genetic code for the 1918 pandemic A(H1N1) virus was published in 2005 and the live virus recreated, the same Board that is now urging redaction then judged that no restriction should be placed on the academic process. Considerable research benefit followed and no adverse events emerged [16–17]. A very active approach to evaluating all papers for potential dual-use topics would create huge difficulty in judging which papers to consider, which to hold back and which parts of a potential publication are actually dangerous. Perversely, the current process has ensured wider awareness of the work than the normal open publication process would have done. While it is possible to imagine some research work that should never be published, a restriction that stopped publication of any work that might result in a product that could be used maliciously would have an impact on a lot of research projects. It would be highly discouraging for researchers and make some important fields of study unattractive [10]. Regarding the question of who would oversee this task, a suggestion that any one national body should do this for the world would be unlikely to be acceptable and WHO has already indicated that it could not take on this role [40]. It would certainly be an enormous task at a European level. In the light of the above it is not surprising that the WHO meeting consensus was at variance with the position taken previously, perhaps because of slightly different judgement of the content of the papers and of the impact and practicalities of a redaction process.(24) One possible medium-term approach would be for all editors of biomedical science, microbiology and infectious disease journals to develop and adopt an international consensus statement about specific criteria for handling publication of information with potential biosecurity sensitivities.

What may be the way forward?

Considering these complex issues of safety and security, a number of short-term technical solutions have been suggested (Table 1). These are not mutually exclusive options. While these short-term solutions are worth considering, an international debate is needed for full evaluation and for global engagement of responsible scientists and other public health authorities. Moreover, there needs to be wider discussion of the longer term needs, practical solutions, and how to implement them [23,24]. WHO argues that particular emphasis should be placed on an education process to explain the importance of this kind of work and how it can strengthen global health security [20,23,24]. ECDC welcomes that such a process has now started and will contribute to it within its mandate. For all solutions, the bottom line will be the balance of the benefits of the potential research outcomes for public health protection and the risks inherent in the research from an evidence-based standpoint of effective containment and mitigation strategies in the event of a laboratory accident or other escape of the virus. This is not only applicable to influenza research, but will be a welcome debate on an overall approach to research on dangerous pathogens in countries of varying resource [39].

Table 1. Some non-mutually exclusive options that have been suggested, with ECDC comments

Option	ECDC comment
Stopping all research in this area	Would mean the cessation of research that is considered essential for global health security
Withholding the research methodologies and genetic sequences from publication	Would threaten the Pandemic Influenza Preparedness Framework. Not clear it would stop the information becoming available by other means. It is difficult to imagine a system for determining which material to restrict that would be practical or acceptable at an international level.
Destruction of the laboratory-modified H5N1 viruses	Would greatly inhibit research that is considered essential for global health security.
Raising the handling of the laboratory-modified H5N1 viruses or undertaking of all research that might result in their creation to the highest category of biosafety classification (BSL4)	There are definition problems here because different and sometimes conflicting terminology is used for levels of laboratory containment. Would result in restriction of the centres that could undertake this work. May be impractical and costly but will not overcome the general problem of laboratory accidents which can happen at any level of containment [39].
Review of the biosafety precautions required for handling these agents in specific research work	ECDC would agree with the suggestion from biorisk experts that once more information is available, in the short term a risk-based approach is taken and restriction be maintained on research projects on A(H5N1) which potentially increases the inherent dangers through increasing transmissibility of the viruses that are pathogenic to mammals.[4] This might for example be by heightening the level of their handling or restricting the number of centres undertaking the work, or both.
Restricting the numbers of institutions and groups working in the more dangerous fields	Would reduce the overall risk of accidental release of these and similar viruses through biosafety breaches

Table 2. Research and public health questions concerning these transmissible A(H5N1) viruses that need resolution

Question	Comments on how to resolve
How dangerous are these viruses for humans through direct infection?	Cannot be determined directly but animal and <i>in vitro</i> laboratory work will inform the risk assessment, e.g. where are they replicating in the ferrets, how are they transmitting and entering the receiving animals, what is their tissue tropism (receptor binding), etc.
How dangerous are these viruses for humans through any pandemic potential and does this change views on the pandemic potential of A(H5N1)?	The preliminary reports on findings in the two independent experiments seem to give weight to the pandemic potential of H5N1 but a virological risk assessment is needed based on the full data from the current and future research.
How likely are these described mutations in laboratory-modified H5N1 viruses to take place in nature in this combination?	Cannot be calculated but it is important to note that all these mutations have been observed naturally, though not in this combination.
How likely is it that these viruses might escape from the laboratory?	This is not within ECDC's mandate – but a high level of safety and security in the current laboratories has been noted.[4] The risk of an accidental biosafety breach will inevitably increase if many laboratories perform research and development work on these viruses.
How should these laboratory-modified H5N1 viruses be classified in terms of biosafety level or handled for research purposes?	Determining classification is not within ECDC's mandate. It is not clear how such decisions are made in Europe. It is important to distinguish between classification or research authorisation conditions for this kind of research and classification for diagnostic procedures and clinical care should these viruses occur in nature [38].
Should it be checked whether current diagnostic tests will detect these viruses and the likely effectiveness of current A(H5N1) vaccines and antivirals against them and then these products developed if the tests, vaccines and antivirals are considered sub-optimal?	Needs a virological risk assessment* using these current and future research findings.

*Note that undertaking a virological risk assessment will require access to the data and analyses from the Fouchier and Kawaoka groups.

Annex. Resources

EU legislation

Directive 2000/54/EC of the European Parliament and of the Council of 18 September 2000 on the protection of workers from risks related to exposure to biological agents at work. OJ L 262, 17.10.2000, p. 21–45.

Council Directive 2005/94/EC of 20 December 2005 on Community measures for the control of avian influenza and repealing Directive 92/40/EEC. OJ L 10, 14.1.2006, p. 16–65

Directive 2009/41/EC of the European Parliament and of the Council of 6 May 2009 on the contained use of genetically modified micro-organisms. OJ L 125, 21.5.2009, p. 75–97.

2006/437/EC: Commission Decision of 4 August 2006 approving a Diagnostic Manual for avian influenza as provided for in Council Directive 2005/94/EC. OJ L 237, 31.8.2006, p. 1–27.

WHO Biosafety and Laboratory Biosecurity programme

WHO Laboratory biosafety manual (3rd ed)

First published in 1983, the document provides practical guidance on biosafety techniques for use in laboratories at all levels. It comprises risk assessment and safe use of recombinant DNA technology guidelines for the commissioning and certification of laboratories, laboratory biosecurity concepts and the latest regulations for the transport of infectious substances. WHO. Laboratory biosafety manual. Geneva; World Health Organization; 2004. Available at: http://www.who.int/csr/delibepidemics/WHO_CDS_CSR_LYO_2004_11/en/

WHO Biorisk management - Laboratory biosecurity guidance

The guidance aims to expand the laboratory biosecurity concepts introduced in the manual (see above). It is intended for the use of national regulatory authorities, laboratory directors (laboratory managers) and laboratory workers, all of whom play key roles in the field of biosciences and in public health in general. WHO. Biorisk management - Laboratory biosecurity guidance. Geneva; World Health Organization; 2006. Available at: http://www.who.int/csr/resources/publications/biosafety/WHO_CDS_EPR_2006_6.pdf

CEN workshop agreements

CEN workshop agreements

CEN Workshop Agreements were introduced by the Comité Européen de Normalisation, or the European Committee for Standardisation, to satisfy market demands for a more flexible and timely alternative to the traditional European Standard (EN). Several workshops have been hosted by CEN in the area of biorisk. They are available at: <http://www.cen.eu/cen/Sectors/Sectors/ISSS/CEN%20Workshop%20Agreements/Pages/default.aspx>

CWA 15793 Biorisk Management Standard

The Laboratory Biorisk Management standard was developed through a joint action between European Biosafety Association, the American Biosafety Association and Det Norske Veritas with funding from the European Commission and is intended to safeguard life, property and the environment from biological risks through the development and adoption of recognised standards in the area of management of biological organisms and their products within laboratory environments.

CWA 16335:2011 Biosafety professional competence

CEN Workshop 53 Biosafety Professional Competence aims to describe competences of a biosafety professional. It provides models for the role profile and tasks of a biosafety professional in an organisation. It also provides model training specifications that help define individual competence.

World Organisation for Animal Health

International transfer and laboratory containment of animal pathogens. In: OIE. Terrestrial Animal Health Code (20th ed). 2011. Available at: http://www.oie.int/index.php?id=169&L=0&htmfile=chapitre_1.5.8.htm

Biosafety and biosecurity in the veterinary microbiology laboratory and animal facilities. In: OIE. Terrestrial Manual (6th ed). 2008. Available at:

http://www.oie.int/fileadmin/Home/eng/Health_standards/tahm/1.1.02_BIOSAFETY.pdf

Joint OIE/FAO worldwide scientific network for the control of animal influenzas: <http://www.oie.int/en/our-scientific-expertise/offlu-the-animal-influenzas-network/>

Biological threat reduction: <http://www.oie.int/en/our-scientific-expertise/biological-threat-reduction/>

Factsheet on bioterrorism:

http://www.oie.int/fileadmin/Home/eng/Current_Scientific_Issues/docs/pdf/BIOTE_EN_FS%20.pdf

Key definitions

Biosafety: The containment principles, technologies and practices that are implemented to prevent the unintentional exposure to biological agents and toxins or their accidental release.

Biosecurity: The protection, control and accountability for biological materials within laboratories in order to prevent their unauthorised access, loss, theft, misuse, diversion or intentional release.

Biorisk management: Encompasses hazard identification, risk assessment and risk control both in the fields of biosafety and laboratory biosecurity.

BSL (biosafety level): There are four accepted levels in the field of human health (containment levels 1, 2, 3 and 4). Community classification is available in Annex III of Directive 2000/54/EC (on the protection of workers from risks related to exposure to biological agents at work) and as well applying to containment and other protective measures as indicated in Annex IV of Directive 2009/41/EC (on the contained use of genetically modified organisms).

BSL3+: Biosafety level 3. The '+' refers to additional procedures and methods of handling in place (i.e. handling methods of materials for example for decontamination and storage, type of personnel protective equipment used, etc) and any other relevant considerations for the type of work being conducted within a BSL containment area.

Virological risk assessment: Process undertaken in order to identify novel influenza viruses that should go forward for development of diagnostic tests and vaccines. In the autumn of 2011 international researchers and agencies attended a meeting in Washington DC organised by CDC at which a tentative formalised virological risk assessment procedure was put forward and discussed. It was proposed to have an internationally standardised approach, based on ten or so parameters from virological, animal and human studies [5].

References

1. Mackenzie D. Editorial: Five easy mutations to make bird flu a lethal pandemic. *New Scientist*. 2011 Sept 26. Available at: <http://www.newscientist.com/article/mg21128314.600-five-easy-mutations-to-make-bird-flu-a-lethal-pandemic.html>
2. Herfst S, Schrauwen E, Chutinimitkul S, de Wit E, Munster E, Linster M et al. ESWI 2011 abstract B2020 September 2011 (from Abstract book)
3. Kawaoka H. H5N1: Flu transmission work is urgent [comment]. *Nature*. 25 January 2012. DOI:10.1038/nature10884. Available at: <http://www.nature.com/nature/journal/vaop/ncurrent/full/nature10884.html>.
4. Fouchier R, García-Sastre A, Kawaoka Y, Barclay W, Bouvier N, Brown I. et al. Pause on avian flu transmission research [letter]. *Science*. 20 January 2012. DOI 10.1126/science.1219412. Available at: <http://www.sciencemag.org/content/335/6067/400.full> (same letter published simultaneously in *Nature*).
5. European Centre for Disease Prevention and Control. The public health risk from highly pathogenic avian influenza viruses emerging in Europe with specific reference to influenza type A/H5N1. Stockholm:ECDC; 2006. Available at: http://ecdc.europa.eu/en/publications/Publications/0606_TER_The_Public_Health_Risk_from_Highly_Pathogenic_Avian_Influenza_Viruses_Emerging_in_Europe.pdf
6. European Centre for Disease Prevention and Control. Swine-origin triple reassortant influenza A(H3N2) viruses in North America. Stockholm:ECDC; 2006. Available at: http://ecdc.europa.eu/en/publications/Publications/i111129_TER_swine_origin_triple_reassortant_influenza_%20A_H3N2_viruses%20in%20North%20America.pdf
7. ECDC Scientific Advance [Internet]. [Application of Virological Risk Assessments - US Department of Health and Human Services \(HHS\) sponsors clinical trials of a vaccine targeted to a novel A\(H3N2\)v influenza strain](http://www.ecdc.europa.eu/en/scientific-advance/2012/01/17/application-of-virological-risk-assessments-us-department-of-health-and-human-services-hhs-sponsors-clinical-trials-of-a-vaccine-targeted-to-a-novel-a-h3n2-v-influenza-strain). 17 January 2012.
8. European Commission. Green Paper. The dual-use export control system of the European Union: ensuring security and competitiveness in a changing world. Brussels:European Commission; 30.6.2011 COM(2011) 393 final.
9. Osterholm M, Henderson RA. Life Sciences at a Crossroads: Respiratory Transmissible H5N1 [science express policy forum]. *Science*. 17 February 2012 <http://www.sciencemag.org/content/early/2012/01/18/science.1218612.full.pdf>
10. Palese P. Don't censor life-saving science. *Nature*. 2012;481:115. doi:10.1038/481115a <http://www.nature.com/news/don-t-censor-life-saving-science-1.9777>
11. Perez DR. H5N1 Debates: Hung Up on the Wrong Questions [science express policy forum]. *Science*. 19 January 2011. <http://www.sciencemag.org/content/early/2012/01/18/science.1219066.full.pdf>
12. Sorrell EM, Schrauwen EJA, Linster M, De Graaf M, Herfst S and Fouchier RAM. Predicting 'airborne' influenza viruses: (trans-) mission impossible? *Current opinion in virology* 2011; 1: 635-642. <http://www.sciencedirect.com/science/article/pii/S187962571100054X>
13. Maines TR, Chen LM, Van Hoeven N, Tumpey TM, Blixt O, Belser JA, et al. Effect of receptor binding domain mutations on receptor binding and transmissibility of avian influenza H5N1 viruses. *Virology*. 2011;413:139-47. <http://www.sciencedirect.com/science/article/pii/S0042682211000766>
14. Chen L, Blixt O, Stevens J, Lipatov A, Davis C, Collins B. In vitro evolution of H5N1 avian influenza virus toward human-type receptor specificity, *Virology*, Volume 422, Issue 1, 5 January 2012, pp 105-113, 10.1016/j.virol.2011.10.006.
15. Belser JA, Szretter KJ, Katz JM, Tumpey TM. Use of animal models to understand the pandemic potential of highly pathogenic avian influenza viruses. *Adv Virus Res*. 2009;73:55-97.
16. Tumpey TM, Basler CF, Aguilar PV, Zeng H, Solórzano A, Swayne DE, Cox NJ, Katz JM, Taubenberger JK, Palese P, García-Sastre A. Characterization of the reconstructed 1918 Spanish influenza pandemic virus. *Science* 2005 Oct 7;310(5745):77-80.
17. Garcia-Sastre A, Whitley RJ. Lessons learned from reconstructing the 1918 influenza pandemic. *J Infect Dis*. 2006;194 Suppl 2:S127-32.
18. Baringa M. Asilomar Revisited: Lessons for Today? *Science* 2000, 287 (5458) 1584-5.

19. WHO. WHO Comment on the importance of global monitoring of variant influenza viruses. December 2011. Available at: http://www.who.int/influenza/human_animal_interface/avian_influenza/h5n1-2011_12_19/en/index.html
20. WHO. WHO concerned that new H5N1 influenza research could undermine the 2011 Pandemic Influenza Preparedness Framework. 30 December 2011. Available at: http://www.who.int/mediacentre/news/statements/2011/pip_framework_20111229/en/index.html
21. Fidler DP, Gostin LO. The WHO Pandemic Influenza Preparedness Framework: A Milestone in Global Governance for Health. *JAMA*. 2011;306(2):200-201.doi:10.1001/jama.2011.960
22. ECDC [Internet]. Global influenza surveillance and virus sharing. Available at http://ecdc.europa.eu/en/healthtopics/seasonal_influenza/epidemiological_data/Pages/global_influenza_surveillance.aspx
23. WHO. Public health, influenza experts agree H5N1 research critical, but extend delay. News Release 17 February 2012. Available at: http://www.who.int/mediacentre/news/releases/2012/h5n1_research_20120217/en/index.html
24. WHO. Technical consultation on H5N1 research issues - consensus points. 16–17 February 2011. Available at: http://www.who.int/influenza/human_animal_interface/consensus_points/en/index.html
25. Li KS, Guan Y, Wang J, Smith GJ, Xu KM, Duan L, et al. Genesis of a highly pathogenic and potentially pandemic H5N1 influenza virus in eastern Asia. *Nature* 2004;430(6996):209–13.
26. Yee KS, Carpenter TE, Cardona M. Epidemiology of H5N1 avian influenza. *Comp Imm Micro & Inf Dis* 2009; 32: 325-340
27. Food and Agricultural Organization of the United Nations (FAO). Stamping out H5N1 avian influenza could take decades (FAO, Rome, 2011). Available at: www.fao.org/news/story/en/item/66118.
28. European Commission Directorate General for Health & Consumers [Internet]. Avian Influenza. Available at:http://ec.europa.eu/dgs/health_consumer/dyna/influenza/index.cfm
29. Writing Committee of the Second World Health Organization Consultation on Clinical Aspects of Human Infection with Avian Influenza A (H5N1) Virus Update on Avian Influenza A (H5N1) Virus Infection in Humans. *NEJM* 2008; 358:261-273
30. WHO. Summary of human infection with highly pathogenic avian influenza A (H5N1) virus reported to WHO, January 2003–March 2009: cluster-associated cases. *WER* 15 January 2010, vol. 85, 3: 13–20. Available at: <http://www.who.int/wer/2010/wer8503/en/index.html>
31. Nicoll A, McKee M. Moderate pandemic, not many dead. Learning the right lessons in Europe from the 2009 pandemic. *EJPH* October 2010 vol. 20 no. 5 486-488 doi:10.1093/eurpub/ckq114
32. WHO. Global Public Health Influenza Research Agenda WHO 2009. Available at: http://www.who.int/influenza/resources/research/2010_11_report_of_the_first_global_consultation_november_2009.pdf
33. United States Centers for Disease Control and Prevention [Internet]. Office of Public Health Awareness and Response. Regulation of Select Agents and Toxins. CDC <http://www.cdc.gov/phpr/dsat.htm>
34. United States Animal and Plant Health Inspection Service (APHIS) and Centers for Disease Control and Prevention [Internet]. National Select Agent Registry. <http://www.selectagents.gov/>
35. WHO. The Pandemic Influenza Preparedness (PIP) Framework [Internet]. WHO 2011 <http://www.who.int/influenza/pip/en/index.html>
36. WHO. International Health Regulations (2005). 2nd ed. Geneva; World Health Organization; 2008.
37. Le Duc JW, Franz DW. Genetically Engineered Transmissible Influenza A/H5N1: A Call for Laboratory Safety and Security and Biosecurity and Bioterrorism: Biodefense Strategy, Practice, and Science. <http://online.liebertpub.com/doi/pdf/10.1089/bsp.2012.0006>
38. Alison K. Hottes A, Benjamin Rusek B & Sharples F, Rapporteurs; Committee on Anticipating Biosecurity Challenges of the Global Expansion of High-Containment Biological Laboratories; National Academy of Sciences and National Research Council. Biosecurity Challenges of the Global Expansion of High Containment Biological Laboratories. Pre-publication copy National Academies Press 2011.

39. Public Health Agency of Canada Biosafety advisory: Efficiently transmissible engineered influenza A(H5N1) viruses. 1 February 1 2012. Available at: <http://www.phac-aspc.gc.ca/lab-bio/res/advi-avis/sbn-asb-2012-01-31-eng.php>
40. WHO. Responsible life sciences research for global health security – a guidance document. WHO:Geneva; 2010. Available at: http://whqlibdoc.who.int/hq/2010/WHO_HSE_GAR_BDP_2010.2_eng.pdf
41. WHO Influenza Virus Traceability Mechanism (IVTM). Available at: <http://www.who.int/influenza/pip/ivtm/en/>
42. Parvia AT. Laboratory Creation of a Highly Transmissible H5N1 Influenza Virus: Balancing Substantial Risks and Real Benefits <http://www.annals.org/content/early/2012/01/25/0003-4819-156-6-201203200-00386.full>
43. Lim W, Ng King-Cheung, Tsang D. Laboratory containment of SARS virus (Review) Ann Acad Med Singapore 2006; 35: 354-60 <http://www.annals.edu.sg/pdf/35VolNo5200606/V35N5p354.pdf>