SCIENTIFIC OPINION OF EFSA, ECDC AND EMA

Scientific opinion on the possible risks posed by the influenza A (H3N2v) virus for animal health and its potential spread and implications for animal and human health[1]

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ABSTRACT

Swine are an important host in influenza virus ecology since they are susceptible to infections with both avian and human influenza A viruses. In 2011 and 2012, clusters of human infection with a swine-origin influenza A(H3N2) variant virus (H3N2v) containing the matrix (M) gene from the 2009 H1N1 pandemic virus were reported in the United States (US). The likelihood of introduction of H3N2v virus into the EU, and subsequent exposure and infection of EU pig herds was assessed. The overall likelihood of a pig holding in the EU being infected by exposure to H3N2v virus through either imported infectious pigs or humans coming from the US was estimated to be low. Efficient separation of imported pigs for 30 days would reduce the likelihood of exposure to a negligible level. The likelihood that H3N2v would spread to other pig holdings was judged to be high, assuming frequent movements of pigs between holdings. Currently, applied real time RT-PCRs can detect all swine influenza A viruses and, combined with gene sequencing, would identify the emergence of H3N2v virus. However, sequencing is not done on a routine basis in EU. Experimental studies in pigs show that the

infection is purely of respiratory nature and follows a relatively mild course with fever, coughing and inappetence, similar to that of the endemic swine influenza viruses. Immunity resulting from vaccination with European vaccines may provide some cross-protection against infection with H3N2v virus whereas vaccines based on US swine H3N2 strains would offer superior protection. It is not possible to predict which changes within H3N2v virus might enable it to develop pandemic properties. Hence, it is not possible at present to set up a specific system to monitor such a risk. Nevertheless, it is recommended to reinforce the monitoring of influenza strains circulating in pigs in EU.

KEY WORDS

SUMMARY

Following a request from the European Commission, the Panel on Animal Health and Welfare (AHAW) was asked to deliver a scientific opinion on the possible risks posed by the Influenza A(H3N2)v virus for animal health and its potential spread and implications for animal and human health.

In 2011, the United States of America reported a cluster of cases of human infection with a swine-origin influenza A(H3N2) variant virus H3N2v containing the matrix (M) gene from the 2009 H1N1 pandemic virus (A(H1N1)pdm09). In 2012, 309 influenza H3N2v virus infections in humans were identified in the US and 12 cases in 2013. Most of the human infections occurred in persons that had contact with live pigs at county fairs, especially children.

Swine are an important host in influenza virus ecology since they are susceptible to infections with both avian and human influenza A viruses and can play a role in interspecies transmission. This can lead to co-infection and genetic reassortment of viruses of swine, human or avian origin. Today, influenza is a common infection of pigs worldwide, sometimes causing severe respiratory disease in non-immune animals. Infection is maintained in endemic cycles without clear seasonality. Currently, H1N1, H1N2 and H3N2 are the predominant subtypes of swine influenza viruses (SIVs) worldwide, but other virus subtypes have also been isolated occasionally from pigs in some parts of the world, e.g. H9N2 and H5N1.

Following the detection of human cases of influenza H3N2v virus, this virus has subsequently been detected in pigs in several US states designated H3N2pM virus. **With respect to significance for the health of pigs of the occurrence of H3N2v virus**, if the pig population is completely naïve, it can be assumed that the significance of infection with H3N2pM virus will be comparable to infection of a naïve population with other swine influenza viruses (SIV), as happened in the past in Europe or US.

In field infections with H3N2pM in pigs in the USA (agricultural fairs) a subclinical course was very common, and when clinical signs were observed (coughing, fever), they were generally mild, with low morbidity and no mortality.

Pathogenicity studies in naive pigs experimentally inoculated with H3N2v show that the infection is purely of respiratory nature and shows a relatively mild course with fever, coughing and inappetence similar to that of other SIVs currently circulating in the swine population. Thus the impact of H3N2pM, if introduced, on the health of the European pigs is not expected to differ significantly from the impact of already circulating, endemic SIVs.

With respect to the risk of introduction of H3N2v in EU, the likelihood of a possible introduction of H3N2v virus into EU pig holdings by movement of live pigs according to EU animal health import legislations was assessed qualitatively and considered to be low. Moreover, in particular, the likelihood of pig holdings in the EU being exposed to H3N2v virus by persons working in the pig sector or regularly visiting pig fairs in the USA was judged to be low, whereas the likelihood was considered negligible for other persons travelling from the USA. Efficient separation of imported pigs for 30 days upon the farmers' decision would reduce the likelihood of exposure of EU pigs to a negligible level.

However, given that the a first holding has become infected, the likelihood of spread of H3N2pM from pigs of that holding to pigs in a second holding located in the same Member State was expected to be high, assuming frequent movement of pigs between holdings and a high likelihood for pigs in a second holding to be susceptible.

With respect to the diagnostic capabilities to early detect H3N2v incursion in EU, early detection of H3N2pM/H3N2v in the EU is not likely due to the limited current surveillance effort in

combination with routine use of diagnostic approaches which are not able to specifically identify this new strain.

Currently applied real time RT-PCRs based on the matrix (M) or the nucleoprotein (NP) gene are capable of detecting all of the influenza A viruses known to be endemic in European pigs plus emergent strains such as rH3N2p from North America. However, neither these tests nor real time RT-PCRs based on H3 or N2 genes are able to specifically identify the H3N2pM as being different from European strains. The panel of serological reagents for conventional typing will reliably type all H3N2 strains and the H3N2pM will raise a different reactivity profile in such assays, due to its antigenic differences when compared to European H3N2 SIVs.

Only by combining currently applied diagnostic approaches with gene sequencing will it be possible to identify H3N2pM should it occur in Europe either in pigs or in humans. All of these diagnostic approaches are relevant to the timely identification of variant viruses or new strains that may appear in European pigs. This combination is not done on a routine basis and there is no official surveillance for SIV as this is not a listed disease. It is recommended to reinforce the monitoring of influenza strains circulating in pigs in EU.

With respect to the implications and consequences of the possible evolution of H3N2v virus on pig health such as clinical manifestation and transmission between pigs, it is considered likely that the H3N2pM virus would have the potential to cause disease, to spread and to become endemic. As seen with other SIVs, host selection pressures may drive genetic evolution of the strain, especially in the gene segments encoding the external glycoproteins (HA and NA).

According to the risk assessment developed, given that a first holding has become infected, the likelihood of spread of H3N2pM virus to second holdings was expected to be high assuming a frequent movement of pigs between holdings of the same Member State, and there is a high likelihood for pigs in a second holding to be susceptible.

With respect to the risk that animals from a herd which was infected with influenza A (H3N2v) virus spreads the virus after the last clinical signs of disease have been observed, it is concluded that, independent of whether clinical signs are present or not, the virus excretion in individual pigs may last up to 7 days post infection. Furthermore, clinical signs, when present, do not entirely cover the period of virus excretion. Consequently, an absence of clinical signs cannot be used as evidence of absence of virus excretion. At farm level SIV infections can be maintained with a continuous introduction of susceptible pigs. Therefore, the risk of spread from holdings can remain high for an extended period of time even after cessation of clinical signs. This takes place particularly when susceptible pigs continuously enter the fattening unit.

With respect to the possibility, efficacy and efficiency of vaccination in pigs, using the existing vaccines or newly developed vaccines against influenza A(H3N2v) virus, immunity resulting from vaccination with commercially available European SIV vaccines is expected to provide no or only a low level of cross-protection against infection with the H3N2pM influenza viruses, whereas vaccines based on North American swine H3N2 viruses would offer superior protection. Such vaccines may significantly reduce H3N2pM replication in the lungs of vaccinated animals. However, voluntary vaccination of pigs with existing vaccines has not succeeded in halting the circulation of SIV in the swine population and this limitation is also considered valid for H3N2pM.

According to the available data, H3N2pM/H3N2v is not present in the European swine population and no measures are needed with regard to vaccination. If such a virus should enter Europe and spread in the pig population, then use of US licensed vaccines based on closely related H3N2 strains could be useful.

With respect to the use of vaccines in relation to the possible evolution of variants of influenza viruses posing a risk to public and animal health, vaccination might increase antigenic drift of

circulating influenza strains, and newly appearing variant might not be neutralized by vaccine-induced antibodies. However, based on current knowledge there is no indication that the latter has happened with the use of the available SIV vaccines in the European pig population. Furthermore, based on the likely divergent evolution of H3N2pM in pigs compared to humans, it is unlikely that virus with increased transmissibility to humans would evolve.

With respect to the most important factors to be monitored that would suggest a risk for the emergence of a new pandemic influenza strain from the influenza A(H3N2v) virus, the new influenza strains emerge through natural reassortment and/or mutations and past experience has shown that reassortment events involving inter-species transmission are necessary steps in the evolution of new pandemic strains. However, it is not always clear in which species these events occur. Monitoring for reassortant viruses should therefore include as important target species both pigs and poultry. Several molecular markers in influenza virus genes have been reported to be associated with biological properties related to virulence and transmission. However, these associations have been inconsistent between strains and virulence traits appear to be polygenic.

Currently, the number and type of mutations, as well as the genetic constellation that would be needed for efficient human-to-human transmission of H3N2v is unknown.

It is currently not possible to predict which changes (mutations or reassortments) within the H3N2v could enable it become a new pandemic influenza virus. Hence it is not possible to set up a system to monitor "the most important factors (…) that would suggest a risk for the emergence of a new pandemic influenza strain from the influenza A (H3N2v)".

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BACKGROUND AS PROVIDED BY EUROPEAN COMMISSION

Swine are an important host in influenza virus ecology since they are susceptible to infections with both avian and human influenza A viruses and are often involved in interspecies transmission. The maintenance of these viruses in pigs and the exchange of viruses between pigs and other species are facilitated by certain husbandry practices that have a limited biosecurity level and regular contact with humans. This cross species transfer of virus can lead to co-infections involving viruses of swine, human or avian origin with subsequent opportunities for genetic reassortment of influenza A viruses; as a result a new virus can emerge. Following interspecies transmission to pigs, some influenza viruses may be genetically extremely unstable, giving rise to variants able to again breach the species barrier.

In 2011 the United States of America (US) - International Health Regulation National Focal Point reported a cluster of cases of human infection with an influenza A(H3N2) variant virus (H3N2v) containing the matrix (M) gene from the 2009 H1N1 pandemic virus (A(H1N1)pdm09). This M gene might confer increased transmissibility to and among humans. The same influenza A(H3N2v) virus has been detected in swine in several US states.

In 2012, between August and October, 307[5] influenza A (H3N2v) infections in humans were identified in the US. Ten States reported confirmed human cases. Currently the state of Indiana, with 138 human cases, is the most affected. 16 persons were hospitalised and one person with underlying risk factors died from the infection. It is reported that most of the human infections occurred in persons that had contact with live pigs at county fairs, especially children. Though limited person-to-person spread with this virus has occurred, no sustained community spread of influenza A (H3N2v) virus has been detected at this time.

Influenza A viruses of subtype H1N1 and H3N2 have been reported world-wide in pigs, associated sometimes with mild clinical disease. At present there is no evidence suggesting that in pigs the influenza A (H3N2v) virus behaves in a different way from the other influenza viruses, even though influenza viruses are notorious for their unpredictability. What makes this virus of particular concern is not only its zoonotic nature but also its zoonotic potential due to the fact that its M gene originates from the 2009 H1N1 human pandemic influenza virus strain. In addition, current CDC (US Centers for Disease Control and Prevention) data indicate that seasonal vaccines formulated in accordance with the latest recommendations of the WHO[6] may only provide limited protection against infection with the influenza A (H3N2v) virus among adults and no protection in children.

A quite comprehensive scientific monitoring programme in pigs is on-going in the EU in the framework of research projects such as ESNIP3 (preceded by ESNIP and ESNIP2) and FLUPIG on influenza viruses. At present, the influenza A (H3N2v) virus strain currently causing human infections in some parts of the United States, has not been detected in EU pig herds or reported from other European countries.

In order to be prepared for a possible emergence and to enable limiting of the spread of the influenza A(H3N2v) virus in an effective and proportionate manner, the Commission needs scientific advice and a risk assessment concerning the potential spread and the implications for animal and human health of this zoonotic virus showing increased pandemic potential. It should further be examined which factors may contribute to the emergence of this influenza virus and which of these factors need to be monitored.

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[5] CDC, Centre for Disease Control and Prevention, Atlanta, USA: http://www.cdc.gov/flu/swineflu/h3n2v-case-count.htm (H3N2v) human case count (as of 05/10/2012).
[6] http://www.who.int/influenza/vaccines/virus/recommendations/2012_13_north/en/index.html

TERMS OF REFERENCE AS PROVIDED BY EUROPEAN COMMISSION

In view of the above, and in accordance with Article 29 of Regulation (EC) No 178/2002, the Commission asks EFSA for a scientific opinion and to specifically assess:

1. the significance for the health of pigs of the occurrence of influenza A (H3N2v) virus in a naïve pig population;

2. the current situation in the EU as regards the risk of a possible introduction of influenza A (H3N2v) virus in particular to EU pig herds and the diagnostic capabilities to early detect an incursion;

3. the implications and consequences of the possible evolution of the influenza A (H3N2v) virus on pig health such as clinical manifestation, transmission between pigs and specifically the risk that animals from a herd which was infected with influenza A (H3N2v) virus spreads the virus after the last clinical signs of disease have been observed;

4. the possibility, efficacy and efficiency of vaccination in pigs, using the existing vaccines or newly developed vaccines against influenza A(H3N2v) virus, also in relation with the possible evolution of variants of influenza viruses posing a risk to public and animal health;

5. which are the most important factors to be monitored that would suggest a risk for the emergence of a new pandemic influenza strain from the influenza A(H3N2v) virus.

ASSESSMENT

1. Introduction and assessment approach

Infection of pigs with influenza A virus (swine influenza virus, SIV) is common worldwide, often causing severe respiratory disease in non-immune animals. Infection is maintained in endemic cycles without clear seasonality. Currently, H1N1, H1N2 and H3N2 are the predominant subtypes in pigs worldwide, but other subtypes have also been found in pigs in some parts of the world, e.g. H9N2 and H5N1.

In Europe, swine H3N2 viruses were occasionally detected in pigs in the early 1970s as a result of cross-species transmission from humans to pigs of viruses derived from the 1968 pandemic Hong Kong influenza virus. Around 1984, reassortment between the H3N2 viruses and the avian-like Eurasian H1N1 virus gave rise to H3N2 viruses with much greater potential to spread in the European pig population (de Jong et al., 2007).

Influenza H3N2 viruses were uncommon in US pigs until 1998, when triple-reassortant (TR) viruses containing segments from human seasonal, avian and classical swine H1N1 viruses emerged and became endemic in the pig population (Zhou et al., 1999).

Pigs have long been hypothesised as mixing vessels for influenza A viruses of mammalian and avian origin. This hypothesis was corroborated by the emergence of the swine-origin pandemic A(H1N1)pdm09 virus in 2009. A substantial amount of evidence has now accumulated that clearly demonstrates that pigs do not constitute a closed environment for influenza viruses but rather a platform that supports the persistence of typical swine-adapted viruses while allowing for a dynamic, bi-directional exchange of viruses with both avian and other mammalian species that may eventually lead to the generation of viruses with increased potential for transmission in pigs and/or humans.

The approach followed to reply to the five terms of reference (ToRs) had to consider that:

- The terms of reference cover biologically linked areas (e.g. significance of disease in ToR1 with implications and consequences from ToR3, the risk of spread in ToR2 with the consequences of the possible evolution in ToR3).

- The general information in this document supports the reply to one or more ToRs.

- The risk assessment developed to reply to the question in ToR2, "current situation in the EU as regards the risk of a possible introduction of influenza A (H3N2v) virus in particular to EU pig herds" needs information from several sections of the general information and data.

- The reader needs a clear understanding from where each of the conclusions and recommendations were taken and what is the scientific evidence.

It was therefore decided to write the general information common to all ToRs at the beginning of this document. After the descriptive chapters, the ToRs are addressed in each of their chapters. ToR1, ToR3, ToR4 and ToR5 are answered in a descriptive way based on the general information. For ToR2, regarding risk of introduction, a qualitative risk assessment has been carried out. The assumptions, methodology, risk flow pathways and results are described in a specific chapter (Chapter 10). In order to facilitate the link between this general text and the final conclusions and recommendations, a summary of the main aspects related to the ToR extracted from each section were highlighted inside text boxes included at the end of each descriptive chapter.

2. Origin and characterisation of H3N2v virus

From 1998 until 2009 the majority of H3N2 viruses isolated from swine in the USA contained the triple-reassortant internal genes (TRIGs). Multiple distinct lines of TR H3N2 viruses have been identified in swine (Richt et al., 2003), suggesting that several introductions of different human seasonal H3N2 viruses into swine had taken place. During this period human infections with swine H3N2 viruses were only occasionally reported (Cox, 2011).

Following the pandemic expansion in humans of the A(H1N1)pdm09, this virus has been shown to be frequently transferred back to swine worldwide (Nelson et al., 2012a). This has given rise to new reassortants between A(H1N1)pdm09 and existing H1N1 and H3N2 swine viruses, including the US H3N2v strain that has acquired the M gene from A(H1N1)pdm09. Reassortant A(H1N1)pdm09/H3N2 viruses were first detected in pigs in 2009 (Dukatez, 2011) and in human cases of influenza as of July 2011 (Liu et al., 2012a; Nelson et al., 2012b). Reassortants between A(H1N1)pdm09 and swine H3N2 viruses have also been described from other parts of the world, e.g. Canada (Tremblay et al., 2011), China (Fan et al., 2012) and Europe (Starick et al., 2012).

In the present document, the following nomenclature will be followed to indicate the H3N2 viruses under discussion, either when isolated from pigs or when having been transmitted to and isolated from humans:

a) Triple-reassortant (TR) H3N2. Swine influenza viruses originated around 1998 in the USA as a result of reassortment between avian, human and swine influenza viruses. These strains carry the following gene combination: human HA, NA and PB1; swine NS, NP and M; and avian PB2 and PA (see Glossary).

b) Triple reassortant internal gene (TRIG) cassette. This acronym stands for the internal set of genes (PA, PB1, PB2, NP, NS and M) derived from the original TR H3N2 viruses. This genetic constellation is found combined with various haemagglutinins (HAs) and neuraminidases (NAs), forming TR H1N1, H1N2 and H3N2 lineages currently circulating in the pig population in the USA.

c) rH3N2p. This represents all strains isolated from swine and characterised by a genetic constellation derived from the enzootic US swine TR H3N2 genetic reassortment events with A(H1N1)pdm09 (the number of A(H1N1)pdm09 genes contained in these isolates varies according to the genotype). This group includes H3N2pM isolates.

d) H3N2pM. This represents the US swine TR H3N2 virus which has reassorted with the pandemic A(H1N1)pdm09 virus from which only the M gene has been acquired. It represents one of the rH3N2p genotypes isolated from swine. The H3N2pM isolates carry seven genes from TR H3N2 and only the M gene from A(H1N1)pdm09.

e) H3N2v. This denotes the porcine H3N2pM virus strain after it has been transmitted from pigs to humans and has been isolated from infected humans. The gene segments of A H3N2v are thus considered as being the same as of H3N2pM.

Since 2009, the pandemic M gene (pM) has frequently reassorted with endemic SIVs, both H1N1 and H3N2. Nelson et al. (2012b) examined an extensive set of sequence data from swine influenza viruses isolated during 2009–2011 and found that the frequency of the pM gene increased significantly during this period, being present in approximately half of all H3N2 strains by the end of the period. Over the same period a significant increase in the relative frequency of isolation of H3N2 over H1N1 subtypes was observed.

3. Description of the H3N2v/H3N2pM natural infections in the USA

Laboratory characterisation showed that the matrix gene was derived from the A(H1N1)pdm09 virus. Although this was the first identification of this virus from people in the USA, this virus had previously been isolated from pigs in the USA in November 2010 (CDC, 2012a).

3.1. Influenza virus infection in pigs

3.1.1. Clinical signs of influenza in pigs in general

Swine influenza is a disease of the respiratory tract. The onset of disease is typically sudden, and general signs include anorexia, inactivity, fever, respiratory distress, coughing, conjunctivitis, nasal discharge and weight loss (Olsen et al., 2006). The disease incubation period is between one and three days, with rapid recovery beginning five to seven days after onset. Swine influenza is a herd disease characterised by high morbidity and generally low mortality rates. However, the H3N2 viruses introduced into North American swine herds in the late 1990s initially induced severe disease in the naive population, which resulted in abortion and an unusually high mortality rate in mature sows (Richt et al., 2003).

The severity of disease differs depending on the size and density of the population on the farm and the age of the pigs. Fattening pigs, particularly when experiencing a first influenza virus infection in the second half of the fattening period, may show the most severe signs with fever, inappetence, coughing and severe dyspnoea. Secondary bacterial infections can prolong the disease. Morbidity approaches 100 % and mortality 2–3 %. Recovery follows after four to seven days.

The virus may persist at the farm level. This takes place particularly when susceptible pigs, often pigs with declining maternal immunity, continuously enter the fattening unit. However, there are indications that SIVs frequently disappear from farrow-to-finish herds to be reintroduced at a later time (Kyriakis et al., 2013). Endemic SIVs may also become part of the multi-aetiological so-called porcine respiratory disease complex in feeder pigs (Van Reeth et al., 1996).

3.1.2. Clinical signs of H3N2pM infection in pigs

In field infections with H3N2pM in pigs in the USA (agricultural fairs) a subclinical course was very common, and when clinical signs were observed (coughing, fever), they were generally mild, with low morbidity and no mortality.

Clinical signs of SIV-associated disease in general are variable and may include anorexia, fever and respiratory distress, and the duration of clinical signs, if present, is variable.

Field infections with H3N2pM in pigs in the USA had a subclinical course with low morbidity and no mortality.

3.1.3. Surveillance in pigs in the USA

The United States Department of Agriculture (USDA), in cooperation with US administration and industry, conducts voluntary surveillance for SIV in the USA. This surveillance is not conducted to define prevalence—the goal is to identify viruses that may be circulating in pigs, and gain knowledge to contribute to improved animal health diagnostics and vaccines. The agency first identified H3N2pM virus isolates collected in late 2010 and have continued to find them across the USA since then.

USDA's SIV Surveillance Program has tested 12 662 samples from 3 766 swine diagnostic laboratory submissions collected from October 2010 until July 2012. Over that time period, 1 488 case submissions were identified as positive for influenza A infection. Overall, 73 H3N2-positive submissions were detected between October 2010 to September 2011 and 138 from October 2011 to July 2012. Of the 138 H3N2 cases identified and tested to date, 57 contain the pandemic M gene and

were classified as H3N2pM (USDA, online). The USDA SIV Surveillance Program continues to collect and test samples to monitor for the type and distribution of all influenza viruses in pigs.

There is currently no information on the prevalence of endemic swine influenza and A(H1N1)pdm09 viruses in wild boar. No experimental studies have been performed on the susceptibility of wild boar to A(H1N1)pdm09.

Surveillance in the USA is conducted not to define prevalence but to identify viruses that may be circulating in pigs, and gain knowledge to contribute to improved animal health diagnostics and vaccines.

From all samples collected and analysed in the USA between October 2010 and July 2012, there were 211 H3N2-positive out of 1 488 influenza-positive submissions, and H3N2pM was identified in 57 of 138 H3N2 isolates tested (41 %) indicating that H3N2pM is quite prevalent among the H3N2 strains circulating in the USA.

3.2. Infection with H3N2v in humans

Detection of novel influenza A viruses in humans has been notifiable in the USA since 2007 and suspected cases are reportable to the Centers for Disease Control and Prevention (CDC). Between July and December 2011, the CDC confirmed 12 human cases of a novel swine-origin influenza A H3N2 variant virus (H3N2v) (Nelson, 2012a).

Infection with swine influenza A viruses has occasionally been detected in humans since the 1950s (Myers et al., 2007; Krueger and Gray, 2013). Cases of swine influenza in humans occur after a history of exposure to pigs with direct, close or indirect contact (Van Reeth, 2007). Novel influenza viruses appearing in humans have to be reported by countries under the World Health Organization (WHO) International Health Regulations of 2005.

In 2012, 309 cases of H3N2v virus were identified. Two of the cases were reported between April and July 2012. Both infected individuals were found to have been exposed to swine prior to onset of illness. A total of 305 cases of H3N2v were reported between July and September 2012. Two more cases were reported in November 2012.

Exposure data for the cases identified that 273 of the 305 (90 %) had attended an agricultural fair and that 73 % (205/279) of those for whom information was available had direct contact with swine prior to onset of clinical symptoms (Jhung M, Epperson S, Biggerstaff et al., CDC, personal communication). These reports coincided with information from animal health officials that febrile illness had been identified among some pigs at one of the first implicated fair events. The preliminary results showed that samples collected from some of these ill animals were positive for H3N2pM (Bowman et al., 2012). This SIV was found to be similar to virus later isolated from the human cases.

In the USA, exposure data for the human cases of H3N2v identified that 273 of the 305 (90 %) had attended an agricultural fair and that 73 % (205/279) of those for whom information was available had direct contact with swine prior to the onset of clinical symptoms.

The symptoms and severity of H3N2v infection were similar to those of seasonal influenza, with frequent reports of fever, cough and fatigue, often in combination. The incubation period was approximately two to three days, and in most cases the duration of illness was five days. Sixteen of the 309 cases were hospitalised; one died (CDC, 2012b). People at high risk include children younger than five years, 65 years and older, pregnant women and people with certain long-term health conditions.

In 2013, 17 influenza H3N2v infections in humans were reported until August 30 in USA. All new cases had been in close contact with pigs.

The Epidemiology and Prevention Branch in the Influenza Division at CDC collects, compiles and analyses information on influenza activity year round in the USA and produces FluView[7], a weekly online influenza surveillance report. The US influenza surveillance system is a collaborative effort between CDC and its many partners in state, local and territorial health departments, public health and clinical laboratories, vital statistics offices, health care providers, clinics and emergency departments.

4. Pathogenesis of influenza virus infections in pigs

4.1. Pathogenesis of swine influenza in general

In general, replication of SIV is limited to epithelial cells of the upper and lower respiratory tract of pigs—the nasal mucosa, tonsils, trachea and lungs—and virus excretion and transmission occur exclusively via the respiratory route. Infectious virus can thus be isolated from the tissues mentioned, as well as from bronchoalveolar lavage (BAL) fluid, and nasal, tonsillar or oropharyngeal swabs (Brown et al., 1993; Heinen et al., 2001; Landolt et al., 2003; Richt et al., 2003; De Vleeschauwer et al., 2009; Khatri et al., 2010). In most experimental studies, the virus can be isolated from one day post infection (dpi) onwards and becomes undetectable after day 7. SIV has a preference for the lungs over the upper respiratory tract (De Vleeschauwer et al., 2009; Khatri et al., 2010). The virus is unlikely to spread beyond the respiratory tract and there is generally no detectable viraemia.

In experimental SIV infection studies, the kinetics of virus replication and nasal virus excretion, as well as the viral loads in various parts of the respiratory tract, are dependent upon the inoculation route and dose, and so are the severity of lung inflammation and disease. That is, virus can be recovered from the nasal mucosa and nasal swabs from day 1 after intranasal inoculation, but only at day 2 or 3 and at lower titers (De Vleeschauwer et al., 2009). Lung virus titers, in contrast, peak more rapidly and are generally higher after intratracheal inoculation. This route reproducibly leads to typical swine flu symptoms - tachypnea and dyspnoea with a forced abdominal respiration, fever exceeding 41 °C, dullness and loss of appetite (Haesebrouck et al., 1985; Van Reeth et al., 1998, 2002; De Vleeschauwer et al., 2009).

In experimental SIV infection studies, nasal virus excretion becomes undetectable a few days after the disappearance of clinical signs. SIV shedding is generally associated with the clinical signs. However, clinical signs are often absent or mild and their duration is variable (i.e. until 3 dpi in mild cases and 5–6 dpi in severe cases) (Van Reeth and Ma, 2012). If other viruses or bacteria cause super-/co-infection, the duration of clinical signs may be prolonged, without increasing the period of virus shedding. In pigs with post-infection immunity from previous exposure to different European SIV subtypes (European H3N2 SIV in particular), or in SIV-vaccinated pigs, the duration of virus excretion is likely to be shortened (see Chapter 8).

4.2. Pathogenesis of H3N2v in pigs

The pathogenesis of the infection with H3N2v in pigs is based on one study carried out with this strain (Kitikoon et al., 2012) and is very similar to that upon infection with known SIV strains. Kitikoon et al. (2012) examined, in experimentally infected pigs, the pathogenesis and transmission of the novel H3N2v isolated from humans and compared different aspects of those induced by two H3N2 isolates from pigs collected in 2010–2011. The three viruses were (1) a representative of TR H3N2 SIV, (2) a representative of rH3N2p, containing three gene segments from the TR H3N2 (HA, NA, PB2) and five genes from the 2009 A(H1N1)pdm09 virus (M, NP, NS, PA, PB1) and (3) an H3N2v virus isolated from humans. All isolates induced mild illness with fever, coughing and inappetence, similar to that obtained with other SIVs. Lung lesions were most pronounced for TR H3N2, intermediate for rH3N2p and least severe for H3N2v. Virus titers were, in BAL and nasal swabs, highest with TR H3N2 and lowest with rH3N2p. All three viruses were transmitted in a similar pattern to contact-naive pigs, but TR H3N2 exhibited the most efficient transmission. The novel H3N2v virus isolated from a human appeared to be the least pathogenic.

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[7] http://www.cdc.gov/flu/weekly/

By analogy with other SIVs and based on the single pathogenesis study with H3N2v in pigs, it can be assumed that the virus excretion in individual pigs lasts up to 7 dpi, whether or not they show clinical signs (Van Reeth et al., 2006; De Vleeschauer et al., 2009).

Pathogenicity studies in pigs experimentally inoculated with H3N2v, which has the same gene constellation as H3N2pM, show that the infection is purely of respiratory nature and shows a variable but relatively mild course with fever, coughing and inappetence similar to that of the endemic SIVs currently circulating in the swine populations worldwide.

By analogy with other SIVs and based on the limited pathogenesis studies with H3N2v in pigs, it can be assumed that the virus excretion in individual pigs lasts up to 7 dpi, whether or not they show clinical signs.

Clinical signs are generally associated with viral shedding; however, virus excretion does not entirely coincide with presence of clinical signs (may start earlier or last longer than clinical signs). Consequently, an absence of clinical signs cannot be used as evidence that pigs are not infectious.

4.3. Virus distribution in organs and tissues

Influenza A infection in swine is respiratory with no virus dissemination to muscles or edible organs (Vincent, 2009; EFSA, 2010). It is safe to accept that, with regard to food safety, the information for the A(H1N1)pdm09 virus also applies to other strains of SIV including H3N2pM. However, low-level virus contamination of meat by respiratory secretions from infected pigs may be possible at slaughter or processing. If ingested with food, the virus has to overcome several hurdles such as acidic pH in the stomach and bile salts in the duodenum, which reduce the infectivity. As oropharyngeal tissues are known ports of entry for mammalian influenza viruses, food that passes such tissues, if contaminated with influenza virus, could hypothetically transmit a respiratory infection to humans. So far, there is no epidemiological evidence that this theoretical possibility has contributed to the zoonotic spread of this infection. Normal cooking procedures inactivate the virus in food. Commercially available disinfectants used for cleaning of equipment after contact with meat products rapidly destroy influenza viruses. Since these statements are generally accepted to apply to SIVs, there is no reason to change them for H3N2pM.

5. Transmission of H3N2pM/H3N2v virus

5.1. Transmission between pigs (within herds and between herds)

As described in Chapter 4, transmission of H3N2v from experimentally infected naive pigs to contact naive pigs has been demonstrated. In addition, detection of H3N2pM in several farms in the USA indicates that the virus has spread between pigs and between herds.

Infection with H3N2pM in pigs is essentially not different from infection with other SIV subtypes in the US and EU swine population. This implies that infection with H3N2pM in swine is respiratory in nature and that the route of transmission can be assumed to be mostly, if not exclusively via the respiratory route (contact or aerosol).

5.2. Transmission from pigs to humans

Early reports in July 2012 associated with the 305 human cases of H3N2v indicated that many affected persons had attended agricultural fairs at which pigs were present. These reports coincided with information from animal health officials of febrile illness among some pigs at one of the first implicated fair events. Preliminary results from samples collected from some of these ill animals were positive for H3N2 virus containing the A(H1N1)pdm09 matrix gene (CDC, 2012c). This strain (H3N2pM) was found to be identical to virus later isolated from the human cases (H3N2v). Additional swine and human sampling associated with a fair in Ohio documented similar findings (Bowman et

al., 2012). Of those reporting direct or indirect exposure to pigs (see Section 3.1), 65 % reported exposures on multiple days during the seven days prior to illness onset. Ultimately, the investigation identified 37 fairs in the nine states associated with the cases, although some cases had attended more than one fair. In 2013, 12 influenza H3N2v infections in humans were reported in June in Indiana, USA. All new cases had been in close contact with pigs.

As of 9 October 2013, no influenza H3N2v virus infections have been reported among humans in the EU. Novel influenza viruses appearing in humans have to be reported by countries under the WHO International Health Regulations of 2005 (WHO, 2005). Infection with swine influenza A viruses have occasionally been detected in humans since the 1950s (Myers et al., 2007). There are only five recent reports of human infection with swine-origin influenza A infections in Europe (ECDC, 2012c). Cases of swine influenza in humans occur after a history of exposure to pigs with direct, close or indirect contact (Van Reeth, 2007).

5.3. Transmission from humans to pigs

Transmission of influenza A viruses from humans to pigs has been described both for seasonal human influenza strains and in particular for the pandemic A(H1N1)pdm09 strain (Nelson et al., 2012a). No documented transfers of H3N2v to pigs have been reported, but, given that this strain is a swine-adapted virus that has been transmitted to humans, back-transmission from humans to pigs must be considered possible.

5.4. Human-to-human transmission

There has been some evidence of limited (sporadic) person-to-person transmission of H3N2v but none for sustained human-to-human transmission. The investigations in 2011 identified two clusters of children with probable person-to-person transmission; one cluster of two cases and the second cluster of three cases (CDC, 2011a, b). However, in the former instance the second case experienced onset of symptoms 10 days after the onset of clinical symptoms in the index case. The investigations in 2012 identified 15 cases of possible human-to-human transmission, all in children less than 10 years old (CDC, 2012c). Fourteen of these cases reported one or more contacts, including contact with swine, contact with another ill household member or contact with an extended family member who reported influenza-like illness. One case identified an extended family member who had contact with swine, although the case did not.

5.5. Transmission in experimental models

In ferrets, H3N2v shows the capacity for efficient replication and transmission and these mammals are considered good models for how influenza behaves in humans (Pearce et al., 2012). Pearce et al., (2012) analysed the virulence and transmissibility in ferrets of four swine-origin influenza H3N2 viruses isolated from humans: one from 2009, two from 2010 and on from 2011. The isolates obtained in 2009 and 2010 were TR H3N2 viruses, while the 2011 isolate was an H3N2v. All four isolates replicated to high titres and were transmitted through direct contact. Furthermore, both the 2010 TR H3N2 and 2011 H3N2v isolates showed efficient respiratory droplet transmission, comparable to that observed with seasonal influenza viruses in ferrets, although only the latter contained the pM segment.

Although a few cases of human-to-human transmission have been reported, there are no reports of sustained human-to-human transmission of influenza H3N2v virus in the USA. Most human cases have been associated with direct exposure to pigs.

6. Epidemiology of H3N2 influenza viruses in pigs in the EU

Surveillance data issued by the European Surveillance Network for Influenza in Pigs (ESNIP) since 2000 have shown that avian-like swine H1N1, human-like reassortant swine H1N2 and human-like reassortant swine H3N2 subtypes, as well as A(H1N1)pdm09 since 2009, constitute the dominant lineages in Europe (Van Reeth et al., 2008; Kuntz-Simon and Madec, 2009; Kyriakis et al., 2011; Kyriakis et al., 2013).

The human-like swine H3N2 viruses isolated from European pigs some years after the Hong Kong pandemic reassorted in 1984 with the avian-like swine H1N1 virus, acquiring six internal protein genes from latter strain. This human-like reassortant swine H3N2 has now become the dominant genotype of H3N2 virus in European swine populations (Castrucci et al., 1993; de Jong et al., 2007).

The current virological passive surveillance (targeted to pig farms where acute respiratory symptoms were observed) conducted from November 2010 to October 2012 (see Table 1) under ESNIP 3 (FP7-funded project due to complete 31 October 2013) allowed the detection of 1 533 influenza A virus-positive farms out of 4 413 farms examined in 13 countries (35 % of farms were positive). A total of 1 062 viruses have been subtyped, revealing that the H1N1 and H1N2 subtypes are the most prevalent viruses. Influenza A(H1N1)pdm09 viruses were isolated at an increasing frequency in some countries, probably indicating that this subtype has become established in the European pig population. In contrast, the H3N2 subtype has been isolated less frequently or not at all in some regions, whereas it remains prevalent in other parts of Europe.

Table 1: Overview of swine influenza viruses subtyped in 13 European countries from November 2010 to October 2012—ESNIP 3 consortium

Country	Number of subtyped viruses	Influenza A subtypes and lineages within subtypes						
		H1N1		pdm-like swine H1N1	H3N2	H1N2		Others
		Avian-like swine H1N1	Reassortant swine H1N1 (human-like HA)		Human-like reassorted swine H3N2	Human-like reassortant swine H1N2	Reassortant swine H1N2 (avian-like HA)	Reassortant pdm-like swine HxNx
UK	39	2	?	27	0	10	?	?
Belgium	20	11	?	0	8	1	?	?
Netherlands	30	16	?	0	7	3	?	?
France	185	128	1	5	1	44	6	0
Italy	121	57	1	6	24	29	4	0
Denmark	170	44	0	50	0	0	63	10
Poland	13	11	0	0	1	1	0	0
Slovakia	1	1	0	0	0	0	0	0
Spain	19	6	0	0	9	4	0	0
Germany	443	273	2	15	41	75	7	30
Finland	2	1	0	1	0	0	0	0
Hungary	16	11	0	2	2	0	0	1
Greece	3	0	0	0	3	0	0	0
Total	**1 062**	**561**	**4**	**106**	**96**	**167**	**80**	**41**
%		**52.8**	**0.4**	**10.0**	**9.0**	**15.7**	**7.5**	**3.9**

pdm: A(H1N1)pdm09

Thus, the European pig population has variable immune status to H3N2 viruses. The European swine H3N2 viruses are antigenically (by haemagglutination inhibition test - HI) closely related to H3N2 viruses that circulated in the human population in the early 1970s (see Chapter 8). They have undergone a lower rate of evolution than their counterparts in humans and currently show highly significant antigenic differences from contemporary human H3N2 viruses, and from H3N2v.

Furthermore, levels of specific immunity to the EU H3N2 are likely to be low or even absent in swine populations in the regions of Europe where this virus has been absent for some years (see Table 1).

New reassortant viruses within the three main enzootic SIVs or between SIVs and A(H1N1)pdm09 or seasonal human influenza viruses have recently been detected in several countries, with evidence of further spread through the swine population for some of them. Co-circulation of enzootic SIVs with A(H1N1)pdm09 has resulted in various reassortants that have mainly exchanged HA and/or NA genes (Howard et al., 2011; Moreno et al., 2011; Bányai et al., 2012; Starick et al., 2012, 2011).

Most recently, reassortant viruses very similar to H3N2pM have been isolated from healthy slaughter pigs in Korea (Pascua et al. 2013). Both the North American TR H3N2 and A(H1N1)pdm09 have also been recovered from pigs in Korea and it is therefore not possible to conclude whether the H3N2pM has been generated locally or whether it was brought in by import of live pigs. This study also highlights the importance of including healthy pigs in a systematic monitoring.

 Based on all these experiences with SIVs, it is to be predicted that an H3N2pM virus has a chance to persist in the European swine population after entry. However, it remains to be seen if all these influenza virus types can co-circulate in a pig population. If H3N2pM becomes endemic, it can be expected that, as seen with other SIVs, host selection pressures will drive this strain to evolve whereby changes in the gene segments, especially those encoding the external glycoproteins (HA and NA), will appear.

In some European countries or in some geographical areas of some European countries with intensive pig production the European H3N2 virus has been circulating at low levels or has been absent in recent years. Therefore, the European pig population has variable immune status to European H3N2 viruses. These above-mentioned areas might have herds which are more susceptible to H3N2pM than regions where all three European (H1N1, H3N2, H1N2) SIVs are prevalent.

In predicting the potential evolution of H3N2pM on the health of the European pig populations, it is relevant to make a comparison with similar events that have occurred historically after emergence or transmission of influenza viruses (avian H1N1, A(H1N1)pdm09, H3N2, H1N2) in pig populations. It has been observed that these influenza viruses, upon adaptation to swine, have become endemic and thus persist in the population despite some degree of existing population immunity.

If H3N2pM is introduced into the European swine populations, it is likely that the virus will be maintained in the swine population together with the current endemic European SIVs.

7. Influenza surveillance and diagnostic capabilities in Europe

7.1. Surveillance in pigs

There are no rules for control of influenza in pigs in the EU legislation. However, under the auspices of ESNIP 3, guidelines have been prepared to attempt to harmonise SIV surveillance following the spread of A(H1N1)pdm09 to pigs in most EU/European Economic Area (EEA) countries (www.esnip3.com)

The level of surveillance programmes varies between EU/EEA countries. However, all countries within Europe that test for influenza in pigs use a passive surveillance system based on reporting of acute respiratory disease in pigs. Monitoring as defined by Hoinville et al. (2013) of healthy pigs is extremely limited. The case identification varies between countries. Sampling for virus detection includes numerous specimen types, such as nasal swabs from sick animals or, where morbidity/mortality is recorded, tissue sampled from the lungs and/or the upper respiratory tract. These samples may also be accompanied by acute and/or convalescent sera to support the virological surveillance.

In some countries the passive surveillance is organised at a national level, i.e. the UK, France and Finland, with private practitioners liaising with specified laboratories over the description and identification of cases meeting a set of clinical criteria that will result in the submission of samples. Vaccine manufacturers also contribute to the network by enhancing the flow of material from the field to the laboratories.

It is important to remember that not all strains of influenza A virus will necessarily induce clinical signs in all production systems. Therefore, the currently applied sampling will probably not give a full picture of circulating virus strains. This is relevant when considering the ability to detect early incursion of a virus.

Previous incursions of novel influenza A viruses into pigs probably occurred some years before their detection due to the type of surveillance used and the need for virus adaptation before they are associated with more severe disease/infection kinetics (Brown, 2000). Some steps towards early detection of new strains have been made in Europe by means of the consecutive concerted action projects, ESNIP 1, 2 and 3 (ESNIP, 1999, 2006, 2010). Although this network is predominantly based on voluntary submission of diagnostic samples, the data collected from the surveillance programmes provide a greater understanding of the epidemiology of SIVs at the global level and alert to the emergence of new reassortants. However, the project is based on research funding and therefore represents only a temporary solution to the long-term need to monitor influenza viruses in animals.

> Surveillance for influenza in pigs within Europe uses a passive surveillance system based on reporting of acute respiratory disease in pigs. Monitoring of healthy pigs is extremely limited.

7.2. Diagnostic capabilities for surveillance of SIVs

Standardised diagnostic tools have been established for the detection and diagnosis of influenza A viruses in pigs through ESNIP 1 and ESNIP 2. Further harmonisation and proof of laboratory test standardisation has been conducted in ESNIP 3. Essentially, clinical material submitted from virological passive surveillance is screened using PCR technology. Most partners are using standard real time RT-PCRs that are capable of detecting all of the influenza A viruses known to be endemic in European pigs plus emergent strains such as rH3N2p from North America. These assays are largely based on the M or the NP gene, which are highly conserved across these viruses and known to be fit for the purpose of detection of endemic SIVs, including A(H1N1)pdm09-like strains, and therefore have direct relevance to the detection of H3N2pM/H3N2v (Munch et al., 2001; Slomka et al., 2010; Pol et al., 2011).

Testing algorithms have been developed following initial screening by M or NP gene RT-PCR. Several laboratories run RT-PCRs, specific for HA and NA genes, for a rapid molecular subtyping. Thus, real-time RT-PCRs have been developed to specifically detect H1 and N1 genes of A(H1N1)pdm09 (Hoffman et al., 2010; Slomka et al., 2010; Pol et al., 2011) and conventional multiplex RT-PCR assays allow the identification of HA and NA genes of European enzootic strains, i.e. avian-like swine H1N1, human-like reassortant swine H1N2 and human-like reassortant swine H3N2 (Chiapponi et al., 2012). When combined, these specific RT-PCR molecular tools also allow users to rapidly detect reassortant viruses that would have exchanged their HA or NA genes (reassortant between endemic strains or between endemic strains and A(H1N1)pdm09).

Clinical materials are subject to more detailed analyses, primarily through the culture of virus in either cells or embryonated fowl's eggs. Amplified viruses from these *in vitro* systems are then characterised using a range of tools, including standard typing through haemagglutination inhibition test (HI) using panels of sera developed through the various ESNIP programmes over the last 14 years and recently reviewed to ensure fitness for purpose and relevance to the accurate identification of virus subtypes circulating in European pigs (H1N1, H3N2, H1N2 and A(H1N1)pdm09). Thus, reference panels of sera and antigens have been made available to ESNIP 3 partners. Finally, preliminary subtyping using

these molecular and serological tools is supported through targeted gene sequencing, primarily of the HA and NA genes, but also internal gene sequencing, to better understand the potential emergence of novel genotypic variance that may be indicative of reassortment between contemporary and/or new/novel viruses.

Within ESNIP 3 a ring trial for M (or NP) gene RT-PCR was organised and has demonstrated that most partners participating in surveillance programmes deployed tests that were capable of detecting all of the relevant circulating SIV subtypes. Subsequent work done within the network has demonstrated that H3N2pM viruses obtained from colleagues in North America are also reliably detected with these assays. This is perhaps not surprising given that the H3N2pM virus from North America possesses the M gene from the pandemic strain, which was itself acquired from an ancestral virus believed to have circulated in Europe. Therefore, the utility of the assays being deployed within the ESNIP 3 consortium are demonstrated as being fit for the purpose of detecting H3N2pM virus should it occur in European pig herds.

From *in silico* analyses, it appears that primers designed to detect H3 and N2 genes from European SIVs in multiplex RT-PCR assays should also match with H3 and N2 genes from H3N2pM (and rH3N2p), as they were designed in conserved regions from these HA and NA subtypes. Experimental demonstration remains to be done, but it can be hypothesised that H3N2pM would be detected as a European H3N2 SIV. As genetic lineages within the H3N2 subtype could not be differentiated at this analysis step, further H3 and N2 gene sequencing would be necessary to identify H3N2pM amongst endemic H3N2 SIVs. Development of molecular tools specific to H3N2pM, especially targeting H3, N2 and/or M genes from this strain, would be necessary for rapid discrimination.

The panel of serological reagents for conventional typing will reliably type H3N2. However, the H3N2pM will raise different reactivity profiles in such assays, owing to its antigenic differences when compared with European H3N2 SIVs. Currently, the ESNIP 3 network is preparing appropriate reagents specific for H3N2pM in order to enhance speed and accuracy of detection.

Thus, it should be stressed that all these approaches, together with gene sequencing, would identify the emergence of H3N2pM should it occur in Europe. Furthermore, all of these diagnostic approaches are relevant to the timely and appropriate identification of variant viruses or new strains that may appear in European pigs. This potentially includes second-generation reassortants from the endemically co-circulating strains that also include H3N2, H1N1 and H1N2.

Currently applied real time RT-PCRs based on the matrix (M) or the nucleoprotein (NP) gene are capable of detecting all of the influenza A viruses known to be endemic in European pigs plus emergent strains such as rH3N2p from North America. However, neither these tests nor real time RT-PCR based on H3 or N2 are able to specifically identify the H3N2pM as being different from European H3N2 strains.

The panel of serological reagents for conventional typing will reliably type all H3N2 strains. The H3N2pM will exhibit a different reactivity profile in such assays, owing to its antigenic differences when compared with European H3N2 SIVs. Specific reagents for detecting H3N2pM are being raised.

Currently applied diagnostic approaches, together with gene sequencing, will identify the emergence of H3N2pM should it occur in Europe either in pigs or in humans. Furthermore, all of these diagnostic approaches are relevant to the timely identification of variant viruses or new strains that may appear in European pigs.

A series of EU-funded networks through the ESNIP programme has greatly strengthened and enabled harmonisation of approaches for diagnosis of and surveillance for swine influenza.

7.3.   Influenza surveillance in humans in the EU/EEA countries

The surveillance of influenza activity in Europe relies on virological and syndromic surveillance (influenza-like illness (ILI) and acute respiratory infections (ARIs). The population under surveillance consist of patients seeking care in sentinel networks of primary care. The surveillance is not targeted to any specific group, such as people in contact with pigs. The human influenza surveillance results are reported weekly to The European Surveillance System (TESSy) hosted at the European Centre for Disease Prevention and Control (ECDC). ECDC publishes weekly influenza surveillance overviews during the influenza season and fortnightly overviews during the inter-seasons.

Following the detection of H3N2v in humans infected from pigs in the USA in 2012, ECDC and European reference laboratories (the Community Network of Reference Laboratories for human influenza in Europe, CNRL) ensured that there was at least the capacity in national reference laboratories for human influenza (National Influenza Centres) to readily detect H3N2v should it appear in humans in Europe. Although surveillance is in place, it is unlikely to detect sporadic cases infected with H3N2v if not presenting with influenza-like illness and notified through a sentinel surveillance general practitioner (GP). The surveillance systems in Europe will certainly be able to detect outbreaks like those observed in the USA. Enhanced surveillance of humans in close and frequent contact with animals would be needed for a more risk-based surveillance. However, this might not be feasible at the country level and would need to be reviewed strategically.

To ensure that novel influenza A infections can be detected in European primary diagnostic and influenza reference laboratories, further capacity building will be needed. Based on a survey done in 2012, the detection capacity of the H3N2v virus as influenza A viruses in the EU/EEA countries is good, but the subtyping capability is significantly reduced compared to type A-specific detection (ECDC, 2012a).

The survey indicates that with current capabilities, the variant viruses would be detected as influenza A viruses; however some of them would not be subtyped and identified as H3N2v viruses other than by sequencing (ECDC, 2012b).

If a human infection with H3N2v were to be detected in the EU, a vigorous response would be triggered, including activation of the laboratory network to increase the national capability for detection, distribution of standard and control material, shipment of the variant viruses to the WHO Collaborating Centres, etc. For the serological detection of past or present cases, probably the appropriate Consortium for the Standardization of Influenza Seroepidemiology (CONSISE; http://consise.tghn.org/about/) protocols would be deployed. However, it should be noted that it is likely that such infections would be detected with some delay. The epidemiological response would include rapid studies to assess the severity, risk factors in humans and transmissibility of the virus at an early stage.

The human population under surveillance consist of patients seeking care in sentinel networks of primary care. The surveillance is not targeted to any specific group, such as people in contact with pigs.

ECDC and European reference laboratories (CNRL) ensured that there was at least the capacity in National Influenza Centres to readily detect H3N2v should it appear in humans in Europe. Although surveillance is in place, it is unlikely to detect sporadic cases infected with H3N2v if not presenting with influenza-like illness and notified through a sentinel surveillance general practitioner.

The survey indicates that, with current capabilities, the variant viruses would be detected as influenza A viruses but H3N2v, if present, would not be identified as such by routine diagnostic methods.

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8.   Cross-immunity to North American H3N2 swine influenza viruses in European pigs

There are no experimental cross-protection studies in which pigs are first inoculated with a European H3N2 SIV and then challenged with the H3N2pM virus. Therefore, we can only make assumptions about cross-protection based on (1) data about the antigenic and genetic relatedness of these viruses; (2) data about serologic cross-reaction between these viruses; and (3) extrapolations from cross-protection studies with other antigenically distinct H3N2 influenza viruses from pigs. The available data regarding these three points are summarised below.

1) Data about the genetic relationship in the HA and NA of relevant H3N2 viruses are shown in Tables 2 and 3 respectively (Kristien Van Reeth, unpublished data). The selected virus strains are representative of an endemic European H3N2 SIV, a North American TR H3N2 SIV (cluster IV) and an H3N2v virus (similar gene constellation as TR H3N2 SIV, but with pandemic M gene). Both the HA and NA of the H3N2v virus are closely related to those of the cluster IV TR H3N2 SIV, whereas there is relatively low homology between the first two viruses and the European H3N2 SIV.

Table 2:   Comparison of the HA genes of a European H3N2 SIV (sw/Gent/172/08), a North American cluster IV TR H3N2 SIV (sw/Ontario/33853/05) and H3N2v (A/Indiana/08/11)

	sw/Gent/172/08		sw/Ontario/33853/05		A/Indiana/08/11	
	nt	aa	nt	aa	nt	Aa
sw/Gent/172/08	100*	100				
sw/Ontario/33853/05	85	85	100	100		
A/Indiana/08/11	83	83	97	97	100	100

nt, nucleotide; aa, amino acid. * % homology.

Table 3:   Comparison of the NA genes of a European H3N2 SIV (sw/Gent/172/08), a North American cluster IV triple reassortant H3N2 SIV (sw/Ontario/33853/05) and H3N2v (A/Indiana/08/11)

	sw/Gent/172/08		sw/Ontario/33853/05		A/Indiana/08/11	
	nt	aa	nt	aa	nt	Aa
sw/Gent/172/08	100*	100				
sw/Ontario/33853/05	85	86	100	100		
A/Indiana/08/11	84	83	96	96	100	100

nt, nucleotide; aa, amino acid. * % homology.

2) Table 4 shows the antigenic relationship in cross-HI tests between the three viruses that were compared at the genetic level (unpublished data, Kristien Van Reeth). There was minimal cross-reactivity between the European H3N2 SIV and each of the other two H3N2 viruses. The low antigenic cross-reactivity between endemic European H3N2 SIVs on the one hand and H3N2v on the other hand has also been confirmed in HI tests with hyperimmune swine sera against the European H3N2 SIVs (Table 5). The sera had antibody titres of < 10, 10, 20, 40 or 80 against H3N2v, compared with titres of 320–5 120 against the homologous virus.

Table 4: Serological cross-reactivity between H3N2 virus of three different lineages

	HI titres with hyperimmune/post-infection serum to			
	sw/Gent/172/08 (Sᵃ)	sw/Gent/172/08 (Sᵇ)	sw/Ontario/33853/05 (Sᵇ)	A/Indiana/08/11 (F)
sw/Gent/172/08	1 280	320	< 10	10
sw/Ontario/33853/05	20	10	320	40
A/Indiana/08/11	20	10	40	640

Sᵃ, swine hyperimmune serum; Sᵇ, swine post-infection serum; F, ferret post-infection sera.
nd, not determined; sw/Gent, European SIV; sw/Ontario, TR H3N2; A/Indiana, H3N2v

Table 5: HI antibody titers to European H3N2 swine influenza viruses (SIVs) with swine hyperimmune or post-vaccination sera

Virus	HI antibody titer										
	Hyperimmune serum							Post-vaccination serum			
	sw/Gent/1/84 V-02-519 10/09/02	sw/Flanders/1/98 V-01-363 17/12/01	sw/Gent/83/00 V-01-355 26/11/01	sw/Gent/80/01 V-01-351 10/12/01	sw/Gent/131/05 V-68-06 02/06/06	sw/Gent/172/08 V-09-1042 28/01/10	A/Indiana/08/11 Ferret 38/11	Port Chalmers/73 Gripovac Pig 506 (07/01/08)	Port Chalmers/73 Suvaxyn Flu Pig 514 (07/01/08)	Sw/Belgium/220/92 Respipork Flu Pig 516 (08/01/08)	Sw/Bakum/1769/03 Respipork Flu3 Pig 522 (08/01/08)
H3N2 SIV field isolates											
sw/Gent/1/84	<u>5120</u>	5 120	640	160	5 120	2 560		640	1 280	160	40
sw/Flanders/1/98	640	<u>2 560</u>	320	160	1 280	1 280		80	320	160	40
sw/Gent/83/00	640	2 560	<u>320</u>	160	1 280	1 280		160	320	160	40
sw/Gent/80/01	1 280	2 560	640	<u>320</u>	2 560	2 560		160	320	320	80
sw/Gent/131/05	1 280	2 560	640	160	<u>2 560</u>	2 560		160	640	320	80
sw/Gent/172/08	1 280	5 120	640	160	2 560	<u>2 560</u>		160	640	320	80
sw/Gent/538/10	1 280	5 120	640	320	1 280	1 280		160	320	160	40
sw/Gent/167/12	640	2 560	320	160	1 280	1 280		80	320	160	40
A/Indiana/08/11*	10	80	10	<10	40	20	<u>1 280</u>	< 10	160	< 10	< 10
H3N2 vaccine strains											
A/Port Chalmers/73	320	640	80	20	320	160		<u>640</u>	<u>640</u>	20	10
sw/Belgium/220/92	640	2560	320	160	1 280	1 280		80	320	<u>160</u>	40
sw/Bakum/1769/03	1 280	5 120	640	160	2 560	2 560		160	320	160	<u>80</u>

*HI tests against A/Indiana/08/11 were performed with turkey red blood cells instead of chicken red blood cells.

3) The Laboratory of Virology at Ghent University has performed a preliminary experiment to examine the extent of cross-protection between H3N2 SIVs from Europe and the North American TR H3N2 (cluster IV). A group of four conventional, influenza-negative pigs was inoculated intranasally with sw/Gent/172/08 at the age of six weeks. Eleven weeks later, the pigs were challenged with sw/Ontario/33853/05 by the intranasal route. Table 6 shows the serological results at the time of challenge with the North American H3N2 SIV.

Table 6: Serological profile 11 weeks after inoculation with sw/Gent/172/08

| Pig no | Antibody titers to | | | | | |
| | sw/Gent/172/08 | | | sw/Ontario/33853/05 | | |
	HI	VN	NI	HI	VN	NI
1	80	128	320	10	2	< 10
2	160	384	640	10	3	< 10
3	80	128	80	<10	< 2	< 10
4	80	128	80	20	< 2	< 10
Geomean	95	168	190	10	2	< 10

HI: haemagglutination inhibition test; VN: virus neutralisation test; NI: neuraminidase inhibition test

Table 7 shows virus titers in nasal swabs of the individual pigs during the first week post challenge. There was no challenge control group in this preliminary study, but TR H3N2 SIV and other SIVs are usually detected in nasal secretions from day 1 through to day 5 or 6 post challenge. Although the pigs had minimal cross-reactive antibody titers against the challenge virus before challenge, excretion of the challenge virus was undetectable in three of the four pigs.

Table 7: Nasal virus excretion after challenge with sw/Ontario/33853/05 in pigs with infection immunity to sw/Gent/172/08

| | Day post challenge | | | | | | | |
Pig no.	0	1	2	3	4	5	6	7
1	< 1.7*	< 1.7	1.7	3.5	1.8	< 1.7	< 1.7	< 1.7
2	< 1.7	< 1.7	< 1.7	< 1.7	< 1.7	< 1.7	< 1.7	< 1.7
3	< 1.7	< 1.7	< 1.7	< 1.7	< 1.7	< 1.7	< 1.7	< 1.7
4	< 1.7	< 1.7	< 1.7	< 1.7	< 1.7	< 1.7	< 1.7	< 1.7
Mean	< 1.7	< 1.7	< 1.7	2.1	< 1.7	< 1.7	< 1.7	< 1.7

* Virus titer (log10 TCID50/100 mg)

These data (Kristien Van Reeth, unpublished data) indicate that cross-protection can occur between two given influenza viruses with important genetic and antigenic differences in the HA and NA and other viral genes, and in the absence of cross-reactive HI antibodies in serum. The immune mechanisms mediating this cross-protection remain to be determined, but they will probably include mucosal antibodies and cell-mediated immune mechanisms.

Based on these results, it appears that a single infection with a European H3N2 SIV will not induce cross-reactive serum antibodies against H3N2v that can be detected in HI, VN or NI tests. However, the data shown in Tables 6 and 7 further support the notion that infection with a European H3N2 SIV can, in the absence of detectable cross-reactive antibodies in serum, induce partial cross-protection against the North American TR H3N2 SIV and H3N2v.

It is unlikely that a single infection with a European H3N2 SIV would induce cross-reactive antibodies (detectable by HI, VN or NI tests) against TR H3N2 or the closely related H3N2pM.

Preliminary data, however, support the notion that infection with a European H3N2 SIV may induce partial cross-protection against the North American TR H3N2 SIV and H3N2pM, even in the absence of detectable cross-reactive antibodies in serum, indicating that cell-mediated immunity or mucosal

immunity can also contribute to protection.

It is expected that a history of previous SIV infection in a herd would reduce the disease impact of an infection with H3N2v/H3N2pM. However, because of the antigenic differences between H3N2pM and the H3N2 viruses that have been circulating in European pigs since the mid-1980s it is considered likely that H3N2pM would have the potential to cause disease and to become endemic in the European pig population,

8.1. Cross-protection against H3N2pM using current European swine influenza vaccines in EU pigs

8.1.1. General information on SIV vaccines

Commercially available SIV vaccines are inactivated vaccines with an adjuvant. Most vaccines are whole virus preparations with an oil-based adjuvant. However, unlike human influenza vaccines, SIV vaccines are not standardised for antigenic dose and vaccine strains. In keeping with the antigenic and genetic differences between SIVs in Europe and North America, the vaccines for each geographic region are produced locally and they contain entirely different strains. Within each continent, the vaccine strains may differ among different products; the exact adjuvant formulation and antigen dose may also vary. Autogenous vaccines are used in the USA but not in Europe.

The immune response induced by these killed influenza vaccines is fundamentally different from that induced by infection with live influenza virus and it consists mainly of serum antibody to the viral HA. In theory, the vaccines should also induce antibodies to the NA, but the NA antibody response to human influenza vaccines appears to be inconsistent (Dormitzer et al., 2011) and there are no data for SIV vaccines. Unlike an infection with live influenza virus, the vaccines fail to induce mucosal antibodies or virus-specific CD8+ T cells and a cytotoxic T lymphocyte response. On the other hand, serum HI and VN antibody titers are generally higher after a double vaccination of SIV-naive pigs than after infection (reviewed in Van Reeth and Ma, 2012). The antibodies are passively transferred to the mucosae of the respiratory tract by transudation, where they can contact and neutralise influenza virus. The process of transudation of serum IgG is supposed to be more efficient in the lung than in the nasal mucosa (reviewed in Graham and Crowe, 2007). It is therefore believed that killed SIV vaccines mainly reduce pulmonary virus replication and the associated disease, whereas reduction of virus replication in the upper respiratory tract and prevention of virus transmission are more difficult to achieve. In general, post-vaccination HI antibody titers in the serum of individual pigs correlate with the reduction in lung virus titers upon intratracheal challenge, provided that HI tests are performed against the challenge strain (reviewed in Van Reeth and Ma, 2012). High HI titers may completely block virus replication in the lungs, while lower titers reduce lung virus replication sufficiently to prevent the typical symptoms, which are highly dependent on the viral load in the lungs. In some SIV vaccination-challenge studies (using various challenge methods), nasal virus excretion was also reduced (Macklin et al., 1998; Kitikoon et al., 2006; Lee et al., 2007) or blocked (Larsen et al. 2001; Kitikoon et al., 2009). Virus transmission to or from vaccinated pigs is not traditionally assessed, but one recent study showed a significant reduction of transmission from unvaccinated, challenged pigs to pigs vaccinated with a commercial SIV vaccine (Romagosa et al., 2011).

Table 8 presents an overview of the major commercial SIV vaccines in Europe in 2011. The genetic relationship between the HA of the H3N2 strains used in European SIV vaccines and H3N2v is shown in Table 9.

Table 8: Commercially available swine influenza virus vaccines in Europe

Manufacturer	Product name	Influenza virus strains	Adjuvant	Antigenic content[d] per vaccine dose
Merial	Gripovac[a]	A/New Jersey/8/76 (H1N1) A/Port Chalmers/1/73 (H3N2)	Oil	H1N1: $\geq$ 1.7 HIU H3N2: $\geq$ 2.2 HIU
Pfizer Olot	Suvaxyn Flu	sw/Netherlands/25/80 (H1N1) A/Port Chalmers/1/73 (H3N2)	Oil	H1N1: 4 µg HA H3N2: 4 µg HA
Hipra	Gripork	sw/Olost/84 (H1N1) A/Port Chalmers/1/73 (H3N2)	Oil	H1N1: 3×10^7 EID$_{50}$ H3N2: 2.5×10^7 EID$_{50}$
Impfstoffwerk Dessau-Tornau	Respiporc Flu[b]	sw/Belgium/230/92 (H1N1) sw/Belgium/220/92 (H3N2)	Aluminium hydroxide - oil	H1N1: $\geq$ 256 HAU H3N2: $\geq$ 256 HAU
Impfstoffwerk Dessau-Tornau	Respiporc Flu3[b],[c]	sw/Haselunne/2617/03 (H1N1) sw/Bakum/1769/03 (H3N2) sw/Bakum/1832/00 (H1N2)	Carbomer	H1N1: $\geq 10^7$ TCID$_{50}$ H3N2: $\geq 10^7$ TCID$_{50}$ H1N2: $\geq 10^7$ TCID$_{50}$

[a] Production stopped in 2010.
[b] Produced in cell culture; other vaccines are produced in eggs.
[c] The vaccine is marketed by Merial under the trademark Gripovac 3.
[d] HIU, haemagglutination inhibiting units as determined by measuring the HI antibody response after administration of the vaccine to pigs.
HAU, haemagglutinating units before inactivation as determined in a haemagglutination assay with chicken red blood cells; TCID$_{50}$, tissue culture infectious dose 50 % before inactivation; EID$_{50}$, egg infectious dose 50 % before inactivation.

8.1.2. Protection against H3N2pM viruses using commercially available North American and European SIV vaccines

A study was performed to evaluate the efficacy of the US commercial inactivated SIV vaccines against challenge with a swine reassortant virus rH3N2p that contains the A(H1N1)pdm09 matrix gene (Loving et al., 2013). The effectiveness was evaluated by the capacity by which vaccinated-challenged pigs prevented aerosol transmission to naive pigs in an indirect contact model. One vaccine provided significant partial protection, as measured by reduction of nasal virus titers in vaccinated as compared with non-vaccinated pigs. Two other vaccines provided limited efficacy. None of the vaccines was able to prevent transmission of virus to naive pigs in an indirect contact model. Clinical signs were not observed either in the vaccinated-challenged pigs or in the unvaccinated controls. It was concluded that the vaccines may reduce viral shedding, but none was able to prevent indirect transmission of rH3N2p challenge virus to naive pigs.

Table 16 in Appendix A gives a detailed overview of the major licensed SIV vaccines in the USA in 2011. The vaccines contain at least one H3N2 strain and one vaccine contains two different H3N2 strains. The H3N2 vaccine strains belong to the so-called cluster I or cluster IV of the North American swine H3N2 lineage. Compared with the European vaccines, the H3 of these vaccine strains and of cluster IV strains in particular is much more related to H3N2pM. The H3 of the cluster IV strains shows about 97 % amino acid identity with that of the H3N2pM virus.

Experimental vaccination-challenge studies with European SIV vaccines and H3N2pM virus have so far not been performed. Therefore one can only try to estimate the extent of cross-protection based on 1) serological examinations of pigs vaccinated with European vaccines and 2) extrapolation from vaccination-challenge studies with European vaccines and heterologous H3N2 SIVs.

1) Table 5 shows antibody titers against different H3N2 viruses, including H3N2pM, in serum from pigs vaccinated with different European SIV vaccines. Sera were collected two weeks after a double vaccination (three- to four-week interval) of conventional influenza negative pigs. Only one vaccine, based on Port Chalmers/73 (Suvaxyn Flu), induced cross-reactive HI antibodies against H3N2pM. Cross-reactive antibodies were undetectable with the other Port Chalmers/73-based commercial vaccine. This demonstrates that the potency of commercial

SIV vaccines is determined not only by the vaccine strain, but also by other factors such as the adjuvant and antigen amount, which differ between different commercial products.

2) In challenge studies with European SIVs, several of these commercial vaccines have shown the ability to provide protection against H1N1 and H3N2 SIVs isolated over many years and with considerable antigenic and genetic drift compared to the vaccine strains. Independent studies with Port Chalmers/73-based vaccines showed significant virological protection against challenge with H3N2 SIVs isolated in 1984, 1996, 1998, and 2008 (reviewed in Van Reeth and Ma, 2012). The challenge viruses were 84–92 % similar to the Port Chalmers strain in amino acid sequence of their HA1. As shown in Table 9, the H3N2v virus shows only 79–83 % identity with the European H3N2 vaccine strains.

Table 9: Genetic relationship between the H3N2 strains in European SIV vaccines and H3N2v

| | % identity with H3 of A/Indiana/08/11 | |
Virus strain	Nt	aa
A/Port Chalmers/1/73	86	83
sw/Belgium/220/92	82	81
sw/Bakum/IDT1769/2003	82	79

nt, nucleotide; aa, amino acid.

In summary, the HA of the H3N2pM is genetically and antigenically very distinct from that of H3N2 viruses strains present in the European SIV vaccines. It is therefore to be expected that the latter vaccines will provide suboptimal, if any, protection against H3N2pM. Still, some protection cannot be excluded, as demonstrated by the finding that one of the European vaccines based on the Port Chalmers strain/73 was able to induce a cross-reactive HI antibody titer of 160 (see Table 5). Difference in efficacy between vaccines based on the same strain may be due to aspects at preparation such as antigen quantity and type of adjuvant.

Cross-infection studies with the well-known endemic European H3N2 SIVs indicate that prior infection with these viruses may confer some cross-protection against infection with the H3N2pM/H3N2v viruses.

Immunity resulting from vaccination with commercially available European SIV vaccines is expected to provide no or only a low level of cross-protection against infection with the H3N2pM influenza viruses whereas vaccines based on the North American swine H3N2 viruses would offer superior protection. Such vaccines may significantly reduce H3N2 replication in the lungs and disease in the individual animal. However, voluntary vaccination of pigs with existing vaccines has not succeeded in halting the circulation of SIV in the swine population and this limitation is also considered valid for H3N2pM.

According to the available data, H3N2pM and H3N2v are not present in the European pig population and no measures are needed with regard to vaccination.

8.2. Cross-immunity in humans

The H3N2v virus differs antigenically from currently circulating H3N2 human viruses, thus seasonal influenza vaccines are not expected to provide significant protection. Only modest increases in cross-reactive antibodies to H3N2v viruses were detected in individuals of any age who received seasonal trivalent inactivated vaccine (CDC, 2012; Skowroski et al., 2012). The HA of H3N2v has an amino acid homology of 89 % compared with current human seasonal influenza H3N2 vaccine virus (A/Perth/16/2009) (Lindstrom et al., 2012). Lina et al. (2011) compared the HA1, which contains all HA antigenic sites, of various 2011 human isolates of H3N2v to all human H3N2 vaccine reference strains since 1972 to 2011 and a representative swine TR H3N2 isolate from 2010. The group of human strains isolated between 1986 and 1998 presented the highest homology to H3N2v, with

A/Wuhan/359/95 being the most closely related strain (5.5 % nucleotide divergence, consistent with the time of emergence of TR H3N2 in the USA). Human strains isolated before 1983 and after 1999 had nucleotide divergences ranging from 8 to 11 %, with current human H3N2 vaccine strain A/Perth/16/2009 having a 9.3 % nucleotide divergence with H3N2v. Lindstrom et al. (2012) evidenced no measurable inhibition to H3N2v by ferret antiserum primed against the current human seasonal influenza H3N2 vaccine virus (A/Perth/16/2009). Houser et al. (2013) challenged ferrets, previously vaccinated with the 2011–2012 trivalent inactivated influenza vaccine (TIV), with a 2011 H3N2v human isolate and suggested that this vaccine may provide minimal to no cross-protection against H3N2v virus.

Evidence from serological studies indicates that young children can be expected to have little immunity to these H3N2v viruses (CDC, 2012d; Skowronski et al., 2012; Waalen et al., 2012). However there is evidence to suggest that young adults, aged 20 to 40 years, may have cross-reactive antibodies to H3N2v, most likely due to exposure to human H3N2 viruses that circulated in the early to mid-1990s. Middle-aged (> 50 years old) and older adults have lower levels of cross-protective antibodies and may therefore be more susceptible.

8.3. Influence of H3N2 naivety or immune status in the European swine population related to possible H3N2pM entry

The level of immunity to H3N2 virus in European pigs varies across regions and the following three scenarios are the most likely, based on the current geographical prevalence of SIVs:

1) A totally SIV-naive pig population that has not been infected with any of the European SIVs circulating in pigs in Europe.

It is expected that the introduction of H3N2pM would cause a typical clinical picture as has been observed when the European pig population was for the first time confronted in 1979 with the H1N1 avian-like influenza virus or in the mid-1980s with the human Port Chalmers/73-like H3N2 reassortant. In this case, influenza symptoms are observed, characterised by an acute and a rapidly spreading respiratory disease, high fever, anorexia, inactivity, tachypnea, dyspnea and coughing.

2) An H3N2-naive pig population which has not been infected with the European H3N2 subtype but which has been infected with the other endemic SIV subtypes (H1N1 and/or A(H1N1)pdm09 and/or H1N2). The European H3N2 subtype is presently not detected in some Member States or in regions within some Member States (see Table 1 from ESNIP 3).

It is to be expected, in such cases, that a limited cross-protection might be observed despite the absence of HI or VN antibodies to H3N2. Limited cross-protection has previously been documented between European H1N1, H1N2 and H3N2 viruses (Heinen et al., 2001; Van Reeth et al., 2003, 2006; reviewed in Van Reeth and Ma, 2012)

If the H3N2pM were to enter such pig populations, the clinical disease would be expected to be milder than that described above for naive pigs, with less virus being produced in the respiratory tract, and for a shorter time.

3) A pig population which is immune after infection with all SIVs (H3N2 virus included) endemically present in Europe and/or a population which has been vaccinated with European vaccines.

If H3N2pM were to enter such a population, infection immunity would be expected to provide a certain but variable and unreliable degree of cross-protection. Earlier studies (reviewed in Van Reeth and Ma, 2012) and data presented in Table 7 confirm that, in the absence of cross-reactive HI antibodies in serum, cross-protection can occur between the US TR H3N2 and the EU H3N2 influenza viruses, despite substantial genetic and antigenic differences in the HA and NA and in other viral

genes. However, no challenge experiments have been performed to evaluate the degree of cross-protection against H3N2pM. On the other hand, when only vaccination immunity induced by European SIV vaccines is present, and thus in the absence of infection immunity, no or only a very low level of cross-protection against H3N2pM is to be expected.

In this scenario 3, with European H3N2 infection immunity, it is to be anticipated that the H3N2pM in the European swine population will cause infections with possible reduced virus replication in the respiratory tract and mild to subclinical respiratory disease with some fever, variable morbidity and no mortality.

It should also be stressed that the level and effectiveness of cross-protection is expected to be very variable from one country to the other and from one farm to the other depending on the prevalence of SIV infections, the subtypes/lineages involved and the frequency of reinfections.

Upon entry of H3N2pM into Europe, scenario 1 (no immunity to any of the SIVs) would lead to a typical influenza outbreak; scenario 2 (immunity only to SIV subtypes other than H3N2) would be anticipated to afford very limited cross-protection; and scenario 3 (immunity to one or more SIVs including H3N2 and/or immunity after vaccination) would be expected to provide a significant but varying degree of cross-protection.

Because of the antigenic differences between H3N2pM and the H3N2 viruses that have been circulating in European pigs since the mid-1980s, it is considered likely that, should H3N2pM enter the European pig population, it would have the potential to cause disease, to spread and to become endemic.

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9. Emergence of a new pandemic strain from H3N2v

A large array of viral, host and environmental factors influence intra- and inter-species transmission of influenza viruses. HA receptor-binding specificity, in conjunction with cellular receptor expression, influences host and tissue tropism (upper respiratory versus intestinal tract). However, other structural and non-structural proteins involved in replication and transcription of the viral genome also affect the host range, level of viral replication, temperature permissiveness and excreted titre (Neumann and Kawaoka, 2006; Yassine et al., 2010). Transmission and establishment of an infection in a population can also be influenced by the prevailing specific immunity of the population.

Following the establishment of A(H1N1)pdm09 virus in domestic pigs worldwide (originating from an as yet unidentified source), a significant number of A(H1N1)pdm09 and enzootic swine influenza reassortants (involving both H1 and H3 subtypes) has been described over the last three years in the USA (Ducatez et al., 2011; Kitikoon et al., 2012; Nelson et al., 2012b), Canada (Tremblay et al., 2011), Europe (Starick et al., 2012) and Asia (Fan et al., 2012; Liu et al., 2012a). H3N2pM is one example of a reassortant that has become established in US swine herds. This strain carries seven genes originating from TR H3N2 SIVs and the M gene from A(H1N1)pdm09, which originally derives from the Eurasian swine lineage (Garten et al., 2009). Thus, the presence of A(H1N1)pdm09 in pigs constitutes a public health risk for the emergence of new influenza strains with pandemic potential (Chou et al., 2011). This strain is able to provide one or more genes to new reassortants that might increase transmissibility to humans of any subtype occurring in pigs, including H1, H3, H5 or H9 (Vijaykrishna et al., 2010; Liu et al., 2012).

At present, two simultaneous and collaborating projects are developing risk assessment framework/tools for ranking influenza A viruses circulating in animals; namely the influenza risk assessment framework (IRAF) within the EFSA-funded project FLURISK and the CDC influenza risk assessment tool (IRAT). Data on epidemiological and virological risk factors are being gathered (through literature reviews and expert knowledge) to contribute to developing these evidence-based

tools. Components of these under-development assessments are presented in this document to contribute to the response to ToR5.

9.1. Inter-species transmission

Influenza A viruses infect a large variety of species, including humans, wild and domestic birds, pigs, horses, seals, whales, cats, ferrets, dogs and mink. Generally, each strain shows a certain level of restriction to the host species in which it is circulating (Neumann and Kawaoka, 2006). Inter-species transmission occurs readily among avian species and, more seldom, from the avian to the mammalian host.

In order for a virus to transmit from an animal to humans, it has to be able to attach, replicate, counteract the host immune response and efficiently exit this new host. And in order to spread from human to human it has to be airborne. Each of these steps depends on polygenic interactions. Therefore, adaptation to a new host species usually requires a series of genetic adaptations to become successful, and these adaptations are the result of accumulating genetic changes (mutations and/or reassortment) that represent a complex polygenic viral trait (Forrest and Webster, 2010; Yassine et al., 2010), which may take several years to occur (Smith et al., 2009).

Most of the studies on influenza A adaptation to other species than their natural reservoir have been focused on the avian-to-human species jump and the mutations and reassortment deemed necessary for this event. Notwithstanding the probable emergence of A(H1N1)pdm09 from pigs, very few published studies are found regarding adaptation of swine viruses to humans, and data in this field of study are scarce. It is important to highlight, nonetheless, that human and swine influenzas share some common molecular markers (i.e. similar mutations arise in avian strains when replicating in swine and humans, e.g. HA mutations in the receptor-binding site (RBS), mutations in PB2).

It is important to highlight that SIVs of different subtypes have been isolated not only from humans, but also from mink, waterfowl and turkeys. Proximity between farms or presence of other animals than swine (e.g. turkeys) in the farm have been theorised on several occasions to be the reason for the inter-species transmission, but no risk factors have been quantified (e.g. Olsen et al., 2003; Yassine et al., 2007; Gagnon et al., 2009; Tremblay et al., 2011). Few case–control studies, conducted either in Europe or in the USA, have been carried out to elucidate risk factors associated with SIV transmission to humans and occupational exposure has repeatedly been identified as a risk factor (Olsen et al., 2002; Myers et al., 2006; Gerloff et al., 2009). So far, H3N2pM viruses have been isolated only from humans and the virological mechanisms behind this event or potential human-to-human transmission in the future are currently unknown.

The CDC influenza risk assessment tool (IRAT) and the influenza risk assessment framework (IRAF) within the EFSA-funded project FLURISK take into account a range of virological characteristics as risk elements which are described below.

9.2. Cellular receptors

Influenza A viruses bind through the viral HA to *N*-acetylneuraminic acid, a type of sialic acid (SA) found on the glycan chains of host cellular receptors. Differential binding is observed depending on whether the SA is linked through the hydroxyl group of carbon-3 ($\alpha2–3$) or carbon-6 ($\alpha2–6$) of the last galactose on the glycan chain. Avian and equine viruses preferentially bind to receptors having the $\alpha2–3$ glycosidic linkage, whereas swine and human viruses preferentially bind to receptors with $\alpha2–6$ linkage (Connor et al., 1994; Matrosovich et al., 1997). Mutations in or near the RBS of the virus may change their binding preference. Similar RBS mutations that shift receptor preference from $\alpha2–3$ SAs towards $\alpha2–6$ SAs are seen in human and swine strains, as both species present similar receptor distribution, $\alpha2–6$ SAs being the predominant type in their upper respiratory tract. It has been suggested that an increase and/or shift in receptor preference towards $\alpha2–6$ SAs is a prerequisite for human-to-human transmission (Matrosovich et al., 2000). Other receptor characteristics can influence binding affinity, such as the glycan species linked to the SA and glycan topology. The HA segment in

TR SIVs and thus in H3N2pM/v circulating in the USA derives from a human seasonal H3N2 virus and has retained the amino acid motif which confers α2–6 SA receptor preference, typically found in human seasonal strains (Pearce et al., 2012). Currently it is not known if the HA of SIV lineages needs further modifications to attach efficiently to human receptors. Nevertheless, it is important to highlight that several *in vitro* studies have evidenced similar attachment patterns to synthetic receptors between SIVs and seasonal human influenza viruses (both H1 and H3 subtype) (Bateman et al., 2010; Chen et al., 2011; Pearce et al., 2012).

9.3. Replication and release

Following binding of influenza virus to the host cell SA receptor, the virion is internalised in an endocytic vesicle and, as a result of several conformational modifications the ribonucleoprotein complex (RNP, i.e. viral RNA segments coated with the nucleoprotein (NP) and the RNA-dependent RNA polymerase: PB1, PB2 and PA; Bouvier and Palese, 2008), is released into the cytoplasm. Viruses rely on the host's intracellular machinery for replication, and the viral RNP requires adaption to numerous intracellular factors (Moncorgé et al., 2010).

Like most swine and human strains, TR H3N2, including H3N2pM, carries mutations in the PB2 gene that are important for mammalian replication (Liu et al., 2012b). It is currently not known how, and if, swine strains need further mutations to gain overall efficient replication in humans.

Progeny virions assemble and bud at the plasma membrane (Neumann et al., 2009). The NA contributes to the release of progeny virus from the host cell and prevents viral particle aggregation (Bouvier and Palese, 2008). The released viral progeny can then infect neighbouring cells or exit the host to infect a new organism. NA's functions are correlated with HA, and its substrate preference is in accordance with HA binding preference. Kobasa et al. (1999) identified a residue in the NA of N2 human and European swine subtypes that increases preference towards α2–6 SA without affecting α2–3 activity. As the NA in TR SIVs originates from a seasonal human influenza virus, and this residue is conserved in circulating H3N2 SIVs, including H3N2pM, it is reasonable to suggest that it serves the same function in H3N2v/H3N2pM. Nonetheless, the relative importance of this mutation in species jump is still to be ascertained.

Virological characteristics governing airborne transmissibility are so far largely unknown. It has recently been proposed that the presence of the M gene from the Eurasian swine H1N1 lineage is possibly associated with the airborne transmission of A(H1N1)pdm09 virus (Chou et al., 2011). Additionally, both NA and M Eurasian genes may contribute to transmissibility and release of these viruses (Lakdawala et al., 2012). Point mutating the A(H1N1)pdm09 M gene to residues found in Eurasian and/or American TR swine lineages reduced plaque size, growth rate and overall virus replication (Bialas et al., 2012). On the other hand, an *in vivo* transmission study in ferrets infected with H3N2 strains isolated from human cases of swine influenza did not evidence a transmission ability difference between a human H3N2 isolate carrying the M gene of A(H1N1)pdm09 and two other H3N2 human isolates bearing a TR swine lineage M gene (see study by Pearce et al. (2012) in Section 5.4). Therefore, it is difficult to ascertain the role of the A(H1N1)pdm09-M gene within the H3N2v genetic background.

HA, PB2 and NA and M mutations, mentioned throughout these sections, have not been studied in an H3N2v/pM genetic background; thus, their possible effect on the phenotype of these strains, as well as their possible contribution to potential human-to-human transmission, is currently unknown. Nevertheless, an H3N2v isolate has been shown to be able to replicate efficiently in human cells *in vitro* and in ferrets *in vivo*, and to be transmitted through respiratory droplets to naive ferrets (Pearce et al., 2012). Further work, using a broader spectrum of H3N2v, H3N2pM and TR H3N2 isolates, is needed to clarify the transmission phenotype of this strain (both using naive ferrets and ferrets primed against human H3N2 seasonal strains).

In addition, all of these molecular markers (or similar) are found in all swine strains, except for the A(H1N1)pdm09-M gene, which is found only in rH3N2p viruses circulating in swine in the USA (and in a subset of isolates in Europe and Asia). Therefore, it is currently unknown what makes H3N2pM more prone to jump to humans than other rH3N2p or TR H3N2 strains. It is not possible to state which modifications (in terms of further reassortment and/or mutations) this strain needs for acquiring human-to-human transmission (owing to the intricacy of the issue and scientific data paucity) and whether its evolution will ever lead to such an event.

It is currently unknown if H3N2v (and SIVs in general) is able to modulate the human innate immune response as efficiently as a human seasonal strain. Regarding adaptive immune response, as already described in Chapter 8 (Section 8.4), there is an age-related presence of cross-protective antibodies against H3N2v in the human population, with children < 10 years old being most susceptible and, to a lesser extent, adults > 50 years old. Less vulnerable are people aged 20–40 years (CDC, 2012d; Skowronski et al., 2012; Waalen et al., 2012). Therefore, this may represent a barrier to the human-to-human transmission of this virus.

9.4. Animal models for human disease

At present, detailed studies of the relevance of the various molecular markers to pathogenesis and transmission in influenza virus can be undertaken only in animal models. As a model for influenza in man, the ferret is regarded as the most appropriate because (1) it is naturally susceptible to all strains of influenza virus without the need for prior adaptation, (2) clinical signs and illness parallel those which are produced in humans infected with the same strains and (3) the distribution of receptor types in the respiratory tract is similar to that in humans (i.e. predominance of α2–6 receptors in the upper tract and both α2–3 and α2–6 receptors in the lower tract). As already described in Section 5.4 and referred to in Section 9.3, an *in vivo* study in ferrets evidenced both TR H3N2 and H3N2v human isolates as capable of respiratory transmission between ferrets. This does not reflect exactly what has been seen in the field at the animal–human interface and highlights that aerosol transmission is a polygenic trait and the complexity of studying influenza transmission in the laboratory.

9.5. Significance of molecular markers

A large number of mutations and molecular markers of the influenza virus genes have been linked to various properties of the virus, such as receptor binding, host and tissue tropism, virulence, modulation of the host immune response, and efficiency of replication and transmission (Reperant et al., 2012). In most cases, however, the phenotypic manifestation of particular characteristics is polygenic and does not rely on single mutations. Therefore, it cannot be concluded that a specific mutation will have exactly the same phenotypic consequence regardless of the genetic make-up of the strain within which it is found or that the same mutation will be essential in each and every transmission event (e.g. it was commonly believed that the PB2-627K mutation was a fundamental marker of mammalian adaptation and the emergence of A(H1N1)pdm09, carrying other mutations in the PB2 than 627, demonstrated the unpredictability of these viruses and the difficulty of applying molecular markers as paradigms to a broad influenza spectrum). Although some human-adaptive signature amino acids can be found in H3N2v (see previous paragraphs for reference), as has been formerly highlighted, the phenotypic consequences of these genetic markers have been studied in genetic backgrounds other than H3N2v and we cannot be certain that these mutations will have the same effect on this strain. Neither can it be assumed that the emergence of mutations not yet seen in H3N2v and which have been recognised as important for human adaptation in other zoonotic strains (e.g. PB2-627K) will be of any significance for the emergence of a pandemic virus from H3N2v.

9.6. Reassortment

Since the genome of influenza A consists of eight separate RNA molecules (the genome segments), mixed infection by two viral strains within the same cell can lead to reassortment. The rules that govern reassortment dynamics are largely unknown. The emergence and characteristics of progeny viruses are unpredictable and may therefore contain previously unknown genetic profiles.

Viruses possessing the triple reassortant internal gene (TRIG) cassette with several combinations of surface proteins (H1, H3, N1, N2) have been circulating in swine in the USA since 1998, and their emergence led to an end to the 80 years of predominance of classical swine H1N1 virus circulation. This has led Ma et al. (2009) and Kimble et al. (2011) to suggest that this genomic combination allows for easy surface protein reassortment and may endow an infection/transmission advantage on swine viruses possessing the TRIG cassette. H3N2v possess all TRIG except for the M gene, which derives from A(H1N1)pdm09. The role of the A(H1N1)pdm09-M gene within the H3N2v/pM genetic background and of potential further reassortment is currently not known.

New influenza virus strains emerge through natural genetic reassortment and/or mutations.

Past experience has shown that reassortment events involving inter-species transmission are necessary steps in the evolution of new pandemic strains. Studies in animal models have shown that the correlation between the molecular markers and biological properties is not absolute. Virulence and pathogenesis are not the properties of a single gene or protein. They are polygenic and the specific gene constellation is also important.

Currently, the number and type of mutations, as well as the genetic constellation needed for efficient human-to-human transmission, are unknown. Consequently, no single genetic marker or genetic constellation can be reliably associated with increased pathogenicity or transmissibility of influenza virus strains in humans.

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10. Risk of introduction of H3N2v/H3N3pM in EU

EU legislation on imports from third countries of ungulates including breeding pigs is laid down in Regulation (EU) No 206/2010.[8] The USA was not included in the list of third countries from where imports of breeding pigs into the EU may be authorised until a recent amendment of the import rules by Regulation 102/2013.[9] This amendment introduced specific provisions in relation to vesicular stomatitis, which include a pre-export quarantine of pigs intended for export of at least 30 days in a quarantine station, during which time the pigs must be tested for that disease. The USA was thus included in 2013 in the list of third countries from where imports can take place provided the supplementary guarantees for vesicular stomatitis are fulfilled.

In addition, after importation the animals must be conveyed without delay to the holding of destination, where they shall remain for a minimum period of 30 days before further movement outside the holding, except in the case of animal dispatched directly to the slaughterhouse or of animals transiting the EU en route from one third country to another third country. However, in case pigs are moved to another Member State official animal health examination and certification before dispatch is required.

There are no legal requirements determining if, during this 30-day period or part thereof, imported pigs must be kept separate from pigs already present on the holding of destination.

ToR2 concerns the following questions: What is "the current situation in the EU as regards the risk of a possible introduction of influenza A (H3N2v) virus in particular to EU pig herds and the diagnostic capabilities to early detect an incursion?". The question on risk of introduction was split into two parts and the risk is understood as likelihood and does not include consequences:

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[8] Commission Regulation (EU) No 206/2010 of 12 March 2010 laying down lists of third countries, territories or parts thereof authorised for the introduction into the European Union of certain animals and fresh meat and the veterinary certification requirements. OJ L 73, 20.3.2010, p. 1-121.

[9] Commission Implementing Regulation (EU) No 102/2013 of 4 February 2013 amending Regulation (EU) No 206/2010 as regards the entry for the United States in the list of third countries, territories or parts thereof authorised for the introduction of live ungulates into the Union, the model veterinary certificate 'POR-X' and the protocols for testing for vesicular stomatitis. OJ L 34, 5.2.2013, p. 4-11.

Part 1: What is the likelihood of viable H3N2v (humans) or H3N2pM (pigs) virus entering the EU, followed by exposure and infection of the first pig holding?

Part 2: What is the likelihood of H3N2pM (pigs) virus spreading through the movement of infected pigs from the first infected holding followed by exposure and infection of the second holding?

The consecutive events that may lead to the infection of a first holding (Part 1) and spread to a second holding (Part 2) in the EU are combined into two risk pathways (Section 10.2), the first for two scenarios, after taking into account several assumptions (Section 10.1.1).

10.1. Methodology

Owing to data gaps and the fact that some events in the risk pathway are hypothetical, since the import of breeding pigs is only newly implemented, it was decided to use a qualitative risk assessment approach. Qualitative approaches for risk assessment have proved useful for many examples of animal health-related questions (EFSA, 2011, 2013) and provide a useful tool for risk managers to identify ways to mitigate the risk and to communicate their decisions.

10.1.1. Assumptions

The following assumptions were made while developing this risk assessment:

- It is expected that only a limited number of breeding pigs will be imported, as they are primarily imported for genetic improvement. These breeding pigs are moving from holdings in the USA that are usually of a high biosecurity standard to pig breeding holdings in the EU of comparable biosecurity standard. From these holdings, after having remained at the holding of destination for a minimum period of 30 days, imported pigs can be moved to pig holdings of any biosecurity level.

- It is assumed that both infectious humans and infectious pigs would be moved from the USA by aeroplane, and the entire duration of the journey was expected to take only one or two days. Infectious humans can possibly infect pigs in holdings with a high biosecurity level, or pigs in holdings with a lower level of biosecurity, including free-range holdings.

- There is no exposure posed by pig products (no viraemia), semen, wild boar or migratory birds.

- The likelihood of introduction of the virus into the EU through illegal import of live breeding pigs cannot be excluded a priori (even though we can reasonably assume it to be highly unlikely), but there is no information available and therefore this path is not included in the risk assessment pathway.

- Surveillance for detection of infectious humans at the airport checks is not feasible (according to ECDC) and therefore this event was not included in the risk assessment pathway.

- Although a few cases of human-to-human transmission have been reported, no sustained human to human transmission has been observed (see Section 5.4). Therefore, amplification of the infection among humans was not considered in the model.

- Humans travel directly from the USA to the EU without intermediate stops.

10.1.2. Model input parameters

The *likelihood* that the several events in the risk pathway will occur is known to depend on multiple risk factors. The estimates of these likelihoods were initially assessed by considering available relevant published data or information. Whenever there were no published data or information, those

likelihoods were estimated based on experts' opinion. Experts were asked to give likelihood estimates for the events in the risk pathway, in response to specific questions, as presented in Table 17 in Appendix B, according to the categories presented in Table 10. Then, the final estimate of the likelihood of each event was based on the consensus value agreed between the Working Group members. These likelihood estimates are indicated in Table 17 in Appendix B and also in the risk pathway presented in Figures 1, 2 and 3, inside the pink boxes (L).

Table 10: Definition of likelihood categories

Likelihood category	Interpretation
Negligible (N)	Probability of event sufficiently low to be ignored or event only possible in exceptional circumstances
Low (L)	Occurrence of event is a possibility in rare cases
Moderate (M)	Occurrence of event is a possibility
High (H)	Occurrence of event is clearly a possibility

Besides estimating the likelihoods for each event to happen, the *uncertainty* associated with the estimate considered was given to prevent misinterpretation and over-confidence in the outcomes of the risk assessment and to highlight areas with poor data quality or disagreement between experts. Definitions of these uncertainty categories are presented in Table 11. These uncertainty values are indicated in Table 17 in Appendix B and also in the risk pathway presented in Figures 1, 2 and 3, inside the blue boxes in the risk pathway presented in Figures 1 and 2 (H).

Table 11: Definition of uncertainty categories

Uncertainty category	Interpretation
Low (L)	Solid and complete data available; strong evidence provided in multiple references; authors report similar conclusions
Medium (M)	Some but no complete data available; evidence provided in small number of references; authors report conclusions that vary from one another
High (H)	Scarce or no data available; evidence is provided not in references but rather in unpublished reports, based on observations, or personal communication; authors report conclusions that vary considerably between them

10.1.3. Combination of likelihood estimates

The risk model formalises the combination of likelihood estimates downstream through the events of the risk pathway (hierarchical model). To construct the general model, three components were defined:

- deriving total likelihood estimates of events influenced by multiple independent horizontally related events in the pathway (horizontally related events indicate that these events may happen at the same time);

- combining likelihood estimates of dependent vertically related events in the pathway (vertically related events indicate that the events happen chronologically subsequent of each other);

- combining likelihood estimates of non-dependent vertically related events in the pathway.

A combination matrix is used to combine vertically related events in the pathway. It uniquely defines the resulting likelihood estimate for any binary combination of input likelihoods. Combination matrices standardise the evaluation of resulting risk along events constituting a specific pathway. Hence, they may contribute to the reliability/repeatability of the overall risk estimation. Once the rules in the matrix are established, application of this approach to sequentially combine the likelihoods may limit the need to interpret the outcomes. Therefore, the specific combination rules implemented in the model by combination matrices (Tables 13 and 15) was discussed and agreed by the Working Group.

10.1.3.1. Deriving total likelihood estimates of events influenced by multiple independent, horizontally related events in the pathway

If several independent events which could happen at the same time contribute to the likelihood estimate of a given event in the risk assessment, the greatest likelihood estimate of these independent events was used in the downstream calculations and determined the likelihood estimate of the given event.

Table 12: Example 1: Exposure of the first pig holding in the EU to H3N2pM/H3N2v

Event in risk pathway	Event	Probability that event will occur
Exposure of the first pig holding in the EU to H3N2pM/H3N2v	Non-professional in contact with pigs of first pig holding	N
	Professional in contact with pigs of first pig holding	L
	Breeding pig of US in contact with pigs of first pig holding	N

Three independent horizontally related events in the pathway may contribute to the event in the risk pathway "*the exposure of pigs at the first pig holding in the EU to H3N2pM/H3N2v*", namely contact between infected humans (non-professionals and/or professionals) and pigs and/or contact between an infected imported pig and a susceptible pig (in a EU pig holding). These events could happen at the same time. To determine the risk estimate of the event 'exposure of the first pig holding', the greatest likelihood is used to define the overall risk. In the example below, the likelihood for exposure of susceptible pigs is low (N + L + N = L).

10.1.3.2. Combination of likelihood estimates of dependent vertically related events in the pathway

If a risk pathway consists of events that are completely dependent on the outcome of the previous event, then the principles of conditional probabilities can be applied. There are animal health-related examples where such combination matrices were applied (EFSA, 2011, 2013) and the risk matrices described by Wieland et al. (2011) were used to combine pairs of events in the risk pathway that described an exclusive cascade of events (e.g. "exposure of the first holding" followed by "susceptibility of pigs from first holding" leads into "first infected holding"). Table 13 provides the matrix applied to combine likelihood estimates of such cascading, or dependent, events.

With this matrix, increase of likelihood along a pathway is not possible. The matrix principle transfers the multiplication of conditional probabilities to combinations of qualitative likelihood levels.

Table 13: Combination matrix 1, used to evaluate two likelihood estimates based on the assumption that the second event is conditional on the first event

Event 1	Event 2			
	Negligible	**Low**	**Moderate**	**High**
Negligible	Negligible	Negligible	Negligible	Negligible
Low	Negligible	Low	Low	Low
Moderate	Low	Low	Moderate	Moderate
High	Low	Moderate	Moderate	High

Matrix 1, based on Wieland et al. (2011), was adapted to four likelihood categories, and agreed by the Working Group. Application: if event 1 has an estimate "low" and event 2 an estimate "moderate", the combined estimate of the sequence event1 and event 2 will be "low".

The matrix was also applied to combine events assessing the efficacy of risk mitigation measures. Usually these measures have to be assessed in a logical order (clinical signs, identification animal by vet at border inspection post and effective measures to mitigate the further progression along the pathway) leading to an effective case detection and response. The corresponding likelihood estimates of those consecutive events are combined with matrix 1 (Table 13).

Table 14: Example 2: Detection and response at BIP exporting country

Event in risk pathway	Event	Probability that event will occur
Detection and response at BIP exporting country	Infectious pig to be exported shows clinical signs	L
	Animal with clinical signs is/gets identified	H
	Effective measures in place to prevent infected pig to be exported	H

It is assumed that there are three events representing the effectiveness of confirming a case and every event depends on a previous one (identifying a case, reporting a case and confirming a case). According to the model, combination matrix 1 is applied stepwise to combine the estimates. In the example shown above, the probability of being effective in confirming a case was "low". In this case the risk estimate for detection and effective response at the border inspection post (BIP) of the exporting country will be L x H x H = L.

10.1.3.3. Combination of likelihood estimates of non-dependent events that are vertically related in the risk pathway

This approach considers pairs of events in the risk pathway that describe independent events.

Where an increase in the overall risk is possible between events, for example because of an increased number of infected animals (spread of disease), there is a need to reflect such a scenario in the combination matrix used to combine the likelihood estimates of non-dependent events. An example of such a matrix was presented by Zepeda-Sein (1998) (Table 15).

Table 15 provides the matrix applied to combine likelihood estimates of such non-dependent events. If the risk estimate of one event is "low" but that of the second event is "high", the combined likelihood will be "moderate". Hence, the overall risk is assumed to be between "low" and "high". The matrix principle transfers the average of independent probabilities to combinations of qualitative risk levels.

Table 15: Combination matrix 2 is used to evaluate two likelihood estimates that were independent of each other.

Event 1	Event 2			
	Negligible	Low	Moderate	High
Negligible	Negligible	Low	Low	Moderate
Low	Low	Low	Moderate	Moderate
Moderate	Low	Moderate	Moderate	High
High	Moderate	Moderate	High	High

Matrix 2 was based on Zepeda *et al*. (1998) and agreed by the working group. Application: if event 1 has estimate "low" and event 2 has estimate "moderate", the combined estimate of event 1 or event 2 worsening the situation will be "moderate".

Event 1: How likely is it that the first infected holding is not detected and therefore without an effective response?	H

Event 2: How likely is it that pigs from first infected holding move to other holdings in the same Member State?	H

$$H \times H = H$$
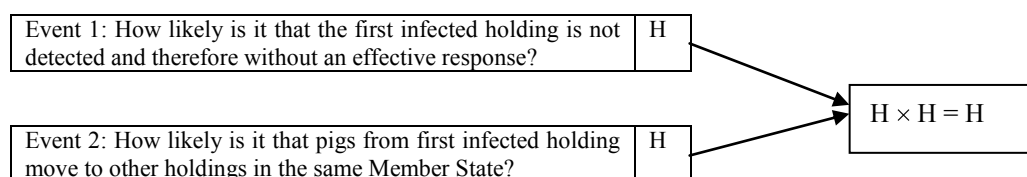
Figure 1: Example 3: Spread of H3N2pM/H3N2v into unaffected areas

It is assumed that two events influence the likelihood of further spread of the disease into unaffected areas: 'no detection of first infected holding and no response'; and 'infected pig(s) moves to second holding'. The events independently influence the risk for unaffected areas. Therefore, combination matrix 2 has to be applied to combine the estimates. In this example, the probability of a pig from a second holding being exposed to pigs infected would be "H".

In the risk flow pathway developed for the current risk assessment (Figures 2, 3 and 4), if the event in the risk pathway relates to a human involvement, the boxes are coloured yellow and if they involve pigs are coloured purple. The estimates of the basic events are combined using either matrix 1 (Table 13), indicated by green, dashed arrows chaining consecutive events, or matrix 2 (Table 15), indicated by orange dotted arrows coupling events that represent independent events.

The estimate for the effectiveness of a mitigation measure related to an event is first converted into the opposite outcome (e.g. "effective response" → "non-effective response", shown by two adjacent boxes linked with a black arrow). The corresponding effectiveness estimate is converted into a risk estimate by taking the reminder of 1 (e.g. "low" → "1 – low = high") (see Table 4 of Wieland et al., 2011).

10.2. Results

Part 1: *What is the likelihood of viable H3N2v (humans) or H3N2pM (pigs) virus entering the EU, followed by exposure and infection of the first pig holding?*

Two risk pathways were developed, considering two possible scenarios for **Part 1** in terms of biosecurity and/or management measures implemented by the farmer during the initial 30 days that pigs imported into the EU must remain at the holding of destination. Details of the likelihood estimates given by the experts are provided in Table 17 in Appendix B.

The **first scenario** (Figure 2) assumed application of adequate biosecurity and an efficient separation of the imported pigs from those already present in the holding during the whole 30-day period in which these pigs are required to remain at the holding before being allowed to move from the holding. During the separation the imported pigs would always be kept in isolation without direct or indirect contact with other pigs to prevent the transmission of pathogens while the animals were undergoing observation and, if the farmers decided that it would be needed, testing and treatment. It must be

noted, as mentioned above, that legislation requires only that imported pigs remain for 30 days on the holding, but no requirements are laid down for what measures are to be applied during this period.
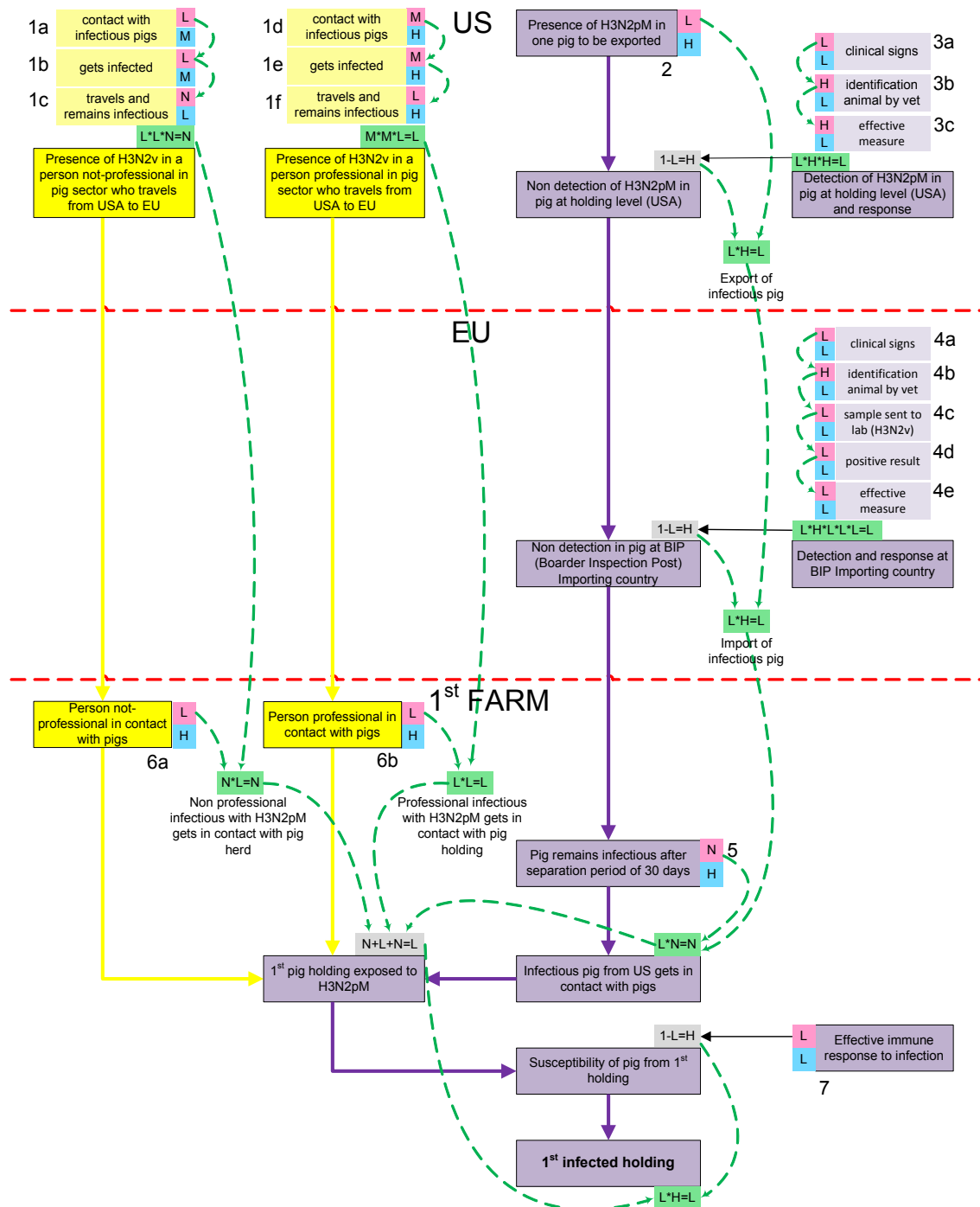
Figure 2: Part 1, scenario 1. Risk pathway for entry of influenza A (H3N2pM/H3N2v) virus in the EU and exposure and infection of the first pig herd in the EU, when the imported pigs have to remain in the holdings in effective separation for 30 days after import.

The risk assessment differentiated the likelihood of *entry of H3N2v through travelling of two categories of people* to the EU from the USA. The first category comprised people who are not working in the pig sector and without a specific interest in pig fairs. The second category comprised people professionally involved in the pig sector or people regularly visiting pig fairs. The likelihood that H3N2v would be present within the first category of people travelling to the EU was considered

negligible compared with a low likelihood of H3N2v presence within the second category of people. This difference would be mainly due to the more frequent and intense contact with pigs expected from the second category of people.

Besides the possible entry of H3N2v through infectious people travelling from USA to the EU, import of *infectious breeding pigs* was also considered as a potential pathway for *entry of H3N2pM*. Considering the low likelihood of H3N2pM being present in pigs to be exported from the USA, the fact that H3N2pM infection does not necessarily induce clinical signs in pigs, the fact that the duration of the journey assumed will allow some pigs to get clear from the infection, and the high likelihood that infectious breeding pigs pass through the visual inspections both at the exporting holding and at the importing BIP, the overall risk of importing infectious pigs was considered to be low.

Given that infectious breeding pigs would pass undetected with a low likelihood of being detected through the visual inspections both at the exporting holding and at the importing BIP, and assuming that breeding pigs imported from any third country, including the USA, are kept in effective separation for 30 days upon decision of the farmer at the first pig holding in the EU, the likelihood that the breeding pigs in separation will remain infectious at the end of that time was considered to be negligible. Following this, the likelihood of consequent exposure of the pigs in the first EU holding to H3N2pM would be negligible.

The overall likelihood of a *first holding* in the EU being infected after exposure to H3N2pM/H3N2v through either *imported infectious pigs* or *humans* coming from the USA was expected to be low.

The experts judged that the pigs in the EU would be highly susceptible to H3N2pM as only limited cross-immunity would be present.

The **second scenario** (Figure 3) included only one difference, namely there was no initial separation of the imported pigs in the holding and so the imported pigs would be immediately housed together with the other pigs in the holding. This resulted in a low likelihood that pigs in EU would be exposed to H3N2pM, compared with the negligible likelihood of exposure of pigs in the EU to H3N2pM through contact with infectious breeding pigs from US pigs in scenario 1 (Figure 2), where a separation of the imported pigs was considered.
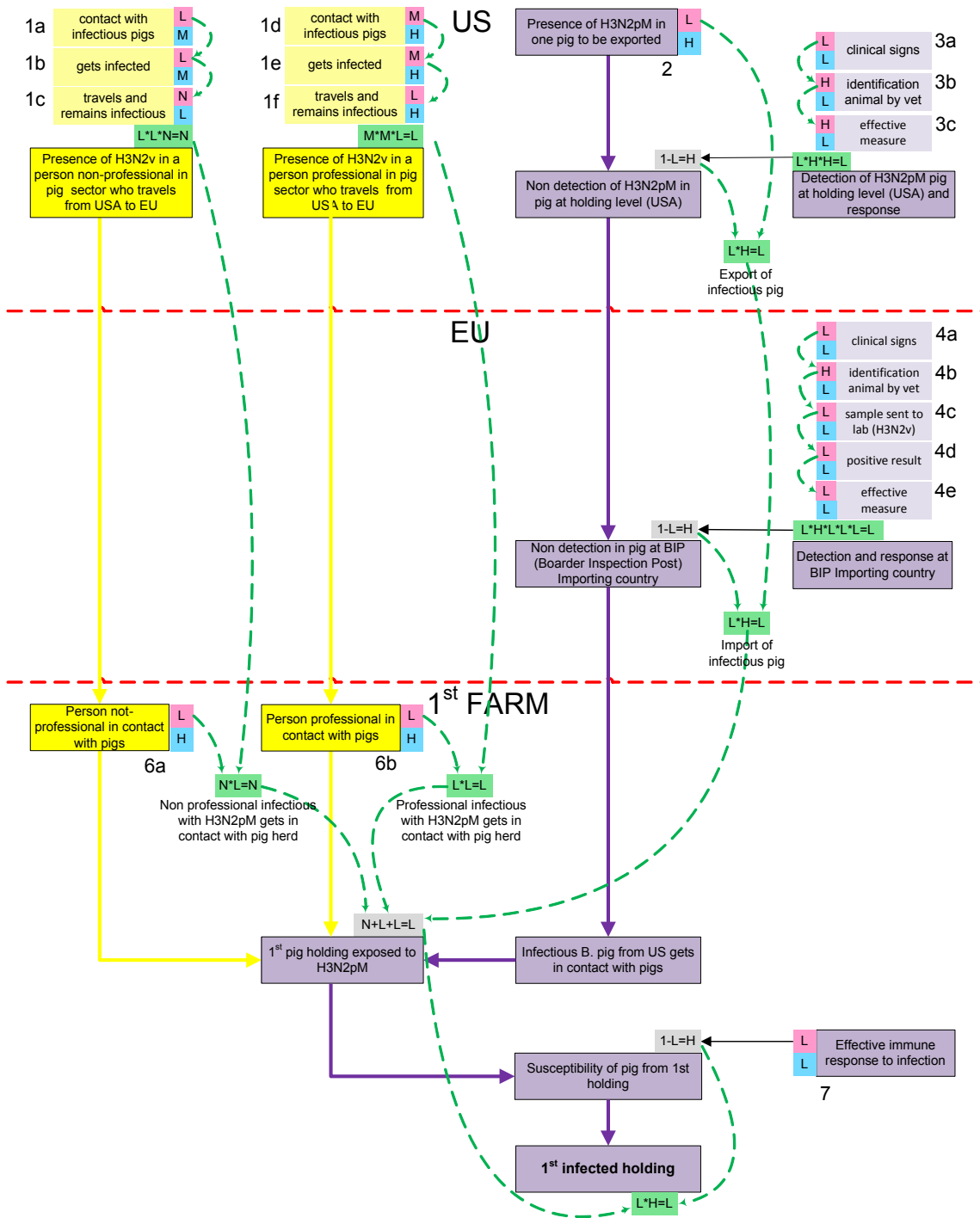
Figure 3: Part 1, scenario 2. Risk pathway for entry of influenza A (H3N2pM/H3N2v) virus in the EU and exposure and infection of the first pig holding in the EU, without prior separation of the imported pigs

It should be noted that the model is highly sensitive to the estimated prevalence of H3N2pM/H3N2v in imported pigs and travelling humans; however, this estimated prevalence is associated with a high uncertainty. Increasing this estimate would increase the likelihood of exposure of the pigs in the first holding, especially when there is no initial separation of the imported pigs at the farm level.

The use of matrix 1 for combining the likelihoods of dependent events that are vertically related may have led to an underestimation of the risk of a first EU holding to become infected, as this matrix was designed to evaluate the effectiveness of actions taken to mitigate risk of introduction only.

Part 2: *What is the likelihood of H3N2pM (pigs) virus spreading through the movement of infected pigs from the first infected holding followed by exposure and infection of a second holding?*

The likelihood of spread of H3N2pM by pigs to *second holdings* (in the same Member State) from the first holding was expected to be high since an intense movement of pigs from the first holding is expected, and there is a high likelihood that pigs in a second holding will be susceptible (Figure 4).
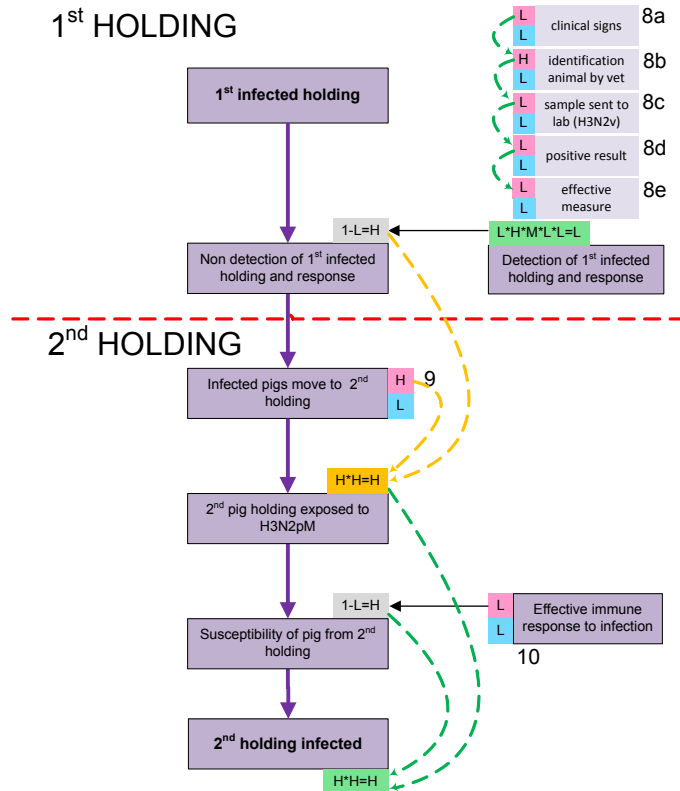
Figure 4: Part 2. Risk pathway for the spread of influenza A (H3N2pM/H3N2v) virus, from an infected holding to second pig holdings in the same Member State (applicable for both scenarios of Part 1)

Legislation for movements of pigs to another Member State requires official animal health examination and certification prior to movement which will increase the likelihood of detection of infection in the first holding and lead to a response (prohibit dispatch) in case clinical signs are observed. The overall likelihood of a first holding in the EU being infected after exposure to H3N2pM/H3N2v through either imported infectious pigs or infectious humans was considered to be low. Effective separation of imported pigs from other pigs at farm level for a period of 30 days reduces the likelihood of exposure to newly entered influenza strains.

When no initial separation of the imported pigs is considered (scenario 2), the likelihood of pigs from the first holding being exposed to H3N2pM through imported infectious pigs would be low (compared with negligible in scenario 1, where a separation of the imported pigs is considered).

Should the virus be introduced in a first pig holding, the likelihood of spread of H3N2pM by pigs to second holdings from the first holding is expected to be high. The model is highly sensitive to the estimated prevalence of H3N2pM/H3N2v in imported pigs and travelling humans; however, this

estimated prevalence is associated with a high uncertainty. Increasing this estimate would increase the likelihood of exposure of the pigs in the first holding, especially when there is no initial separation of the imported pigs at the farm level.

11. ToR1 - the significance for the health of pigs of the occurrence of influenza A (H3N2v) virus in a naive population

Three different scenarios of H3N2 naivety and/or immune status that represent most current situations in the European pig population and the significance for the health of pigs upon entry of A(H3N2)/H3N3pM virus are considered in Section 8.4.

The scientific background to evaluate the significance of an infection with this strain for the health of naïve pigs can be found in Section 3.1 (infection with H3N2pM in pigs), Chapter 4 (pathogenesis of influenza H3N2v in pigs), Chapter 6 (epidemiology of H3N2 influenza viruses in pigs in EU including detection of reassortants) and Chapter 8 (cross-immunity to North American H3N2 swine influenza viruses in Europe).

11.1. Conclusions

- The clinical significance of infection with H3N2pM virus is comparable to that of infection with other SIVs, as seen in the past in Europe in a naive pig population.

- Pathogenicity studies in pigs experimentally inoculated with H3N2v show that the infection is of purely respiratory nature and shows a variable but relatively mild course with fever, coughing and inappetence similar to that of the endemic SIVs currently circulating in swine populations worldwide.

- In field infections with H3N2pM in pigs in the USA (agricultural fairs), a subclinical course was very common, and when clinical signs were observed (coughing, fever), they were generally mild, with low morbidity and no mortality. These signs can be exacerbated by other factors such as secondary bacterial infections so the clinical spectrum may be variable.

- Infection with a European H3N2 SIV will not induce cross-reactive serum antibodies against H3N2v; however, a certain level of cross protection may be achieved via cell mediated immunity and mucosal immunity

- Due to low-level circulation or absence of H3N2 in some areas in recent years, the European pig population has variable immune status to European H3N2 viruses. Such areas might have holdings that are more susceptible to H3N2pM than holdings in regions where H3N2 is prevalent.

- It is expected that previous infection with either of the European SIV virus subtypes (H1N1, H3N2, H1N2) would reduce the negative impact on the health of pigs following an infection with H3N2pM.

12. ToR2 - the current situation in the EU as regards the risk of a possible introduction of influenza A (H3N2v/H3n2pM) virus in particular to EU pig herds and the diagnostic capabilities to early detect an incursion

The risk question included in the first part of the ToR, "*the current situation in the EU as regards the risk of a possible introduction of influenza A (H3N2v/H3N2pM) virus in particular to EU pig holdings*", was split into two parts:

- *Part 1:* What is the likelihood of viable H3N2v (humans) or H3N2pM (pigs) virus entering the EU, followed by exposure and infection of the first pig holding?

- *Part 2:* What is the likelihood of H3N2pM (pigs) virus spreading through the movement of infected pigs from the first infected holding followed by exposure and infection of the second holding?

Two risk pathways were developed, one for each of these two parts. Two possible scenarios were developed for Part 1, considering different biosecurity and/or management measures that the farmer takes during the 30 days that pigs imported into the EU must remain on the holding of destination (Section 10.2). Assumptions made are described in Section 10.1.1, the model parameters in Section 10.1.2 and the combination of risk estimates in Section 10.1.3.

The second part of the ToR, asks for a "*current situation in the EU as regards…the diagnostic capabilities to early detect an incursion*". The reply to the second part of this ToR is supported by the scientific background presented in Section 7.1.1 (diagnostic capabilities).

12.1. Conclusions on the risk of introduction of H3N2v/H3N2pM into EU pig holdings

- Taking into account the possible contact with infectious pigs in the USA, the duration of the journey to travel to the EU and the length of the infectious period, the likelihood of pigs in the EU being exposed to H3N2v by persons working in the pig sector or visiting pig fairs regularly was considered to be low.

- Taking into account the possible contact with infectious pigs in the USA, the assumed duration of the journey to travel to the EU and the length of the infectious period, the likelihood of pigs in the EU being exposed to H3N2v by persons not working in the pig sector or visiting pig fairs regularly was considered to be negligible.

- Considering the low likelihood of H3N2pM being present in pigs to be exported from the USA, the fact that H3N2pM infection does not necessarily induce clinical signs in pigs and the high likelihood that infectious breeding pigs pass through the visual inspections both at the exporting holding and at the importing border inspection post, the overall risk of importing infectious pigs in the EU was considered to be low.

- When breeding pigs imported from any third country, including the USA, are kept in effective separation for 30 days, upon decision of the farmer of the first pig holding in the EU, the likelihood that the breeding pigs in separation would remain infectious at the end of that time would be negligible and the likelihood that the pigs in the importing holding would be exposed to H3N2pM would be negligible.

- When the imported pigs are not kept separate for 30 days, the likelihood that the pigs in the importing holding in the EU would be exposed to H3N2pM would be low.

- Effective separation at farm level of pigs imported from affected countries for a period of 30 days reduces the probability of EU pigs being exposed to this new influenza strain through imported infected pigs.

- Given that a first holding is infected, the likelihood of spread of H3N2pM by pigs to second holdings in the same Member State was expected to be high, assuming frequent movement of pigs between holdings and a high likelihood for pigs in a second holding to be susceptible.

12.2. Conclusions on diagnostic capabilities to detect at an early stage an incursion of H3N2v in the EU

- Early detection of H3N2pM/H3N2v in the EU is not likely owing to the currently limited surveillance efforts in combination with routine diagnostic methods which are not able to specifically identify this new strain.

- Currently applied real time RT-PCRs based on the matrix (M) or the nucleoprotein (NP) gene are capable of detecting all of the influenza A viruses known to be endemic in European pigs plus emergent strains such as rH3N2p from North America. However, neither these tests nor real time RT-PCRs based on H3 or N2 genes are able to specifically identify the H3N2pM as being different from European strains.

- The panel of serological reagents for conventional typing will reliably type all H3N2 strains. The H3N2pM will raise a different reactivity profile in such assays, owing to its antigenic differences when compared with European H3N2 SIVs.

- Only by combining currently applied diagnostic approaches with gene sequencing will it be possible to identify H3N2pM should it occur in Europe either in pigs or in humans. All of these diagnostic approaches are relevant to the timely identification of variant viruses or new strains that may appear in European pigs. This combination is not done on a routine basis and there is no official surveillance for SIV as this is not a listed disease.

- A series of EU-funded networks through the ESNIP programme has greatly strengthened and enabled harmonisation of approaches for diagnosis of and surveillance for swine influenza within many EU laboratories.

12.3. Recommendations on diagnostic capabilities to early detect an incursion of H3N2v in the EU

- Further increase the capability of the national reference laboratories to detect variant viruses.

- Expand the use of standardized methods and reagents including controls for the diagnosis of influenza to all EU/EEA Member States.

- Establish proficiency trials for swine influenza testing in all EU/EEA Member States' reference laboratories.

- Considering the role that pigs play as mixing vessels for influenza viruses, and hence their importance in terms of public health, reinforce monitoring of SIVs on pig farms and link this to surveillance of influenza viruses in humans.

- Consider both passive surveillance and monitoring. Passive surveillance is important for early detection of new emerging viruses, focusing on holdings with clinical signs of respiratory diseases and conducting full characterisation of isolated SIVs. In addition, a broader picture of the circulating influenza virus strains in pig holdings in EU should be obtained from well-designed monitoring, including healthy pigs.

13. ToR3 - the implications and consequences of the possible evolution of the influenza A (H3N2v) virus on pig health such as clinical manifestation, transmission between pigs and specially the risk that animals from a herd which was infected with influenza A (H3N2v) virus spreads the virus after the last clinical signs of disease have been observed

This ToR is considered in two parts (A and B):

A) *Implications and consequences of the possible evolution of the influenza A (H3N2v) virus on pig health such as clinical manifestation and transmission between pigs*

Different scenarios can be foreseen in terms of immune status after previous infection with the contemporary European SIVs H1N1, H1N2 H3N2, as well as the A(H1N1)pdm09 virus, and/or possible vaccination with European swine influenza vaccines described in ToR1, with different clinical implications in case H3N2pM/H3N2v would enter the European swine population.

The possible evolution of the infection in the pig population (transmission, pig health) is described and the possible impact on the pig population considering the current population immunity of the EU is assessed (see Chapter 8). Some information can also be found in Chapters 4 (pathogenesis of influenza H3N2v in pigs) and 6 (epidemiology of H3N2 influenza viruses in pigs in EU including detection of reassortants).

The assessment of possible spread of H3N2pM is described in Chapter 10.

> B) *Risk that animals from a holding which was infected with influenza A (H3N2v) virus spread the virus after the last clinical signs of disease have been observed*

The scientific background to evaluate this risk can be found in Chapter 4 (pathogenesis of influenza H3N2v infection in pigs), and in the Chapter 8 (cross-immunity to H3N2v in Europe).

13.1. Conclusions on the implications and consequences of the possible evolution of the H3N2v virus on pig

- Because of the antigenic differences between H3N2pM and the H3N2 viruses that have been circulating in European pigs since the mid-1980s it is considered likely that, should H3N2pM enter the European pig population, it would have the potential to cause disease, to spread and to become endemic. It can then be expected that, as seen with other SIVs, host selection pressures will drive this strain to evolve whereby changes in the gene segments, especially those encoding the external glycoproteins (HA and NA), will appear.

- Based on previous experiences with influenza viruses that emerged in the past and adapted to swine, the risk that a H3N2v/H3N2pM virus becomes endemic in some areas after entering the European swine population is high (see ToR2).

- Cross-infection studies with the well-known endemic European H3N2 SIVs indicate that prior infection with these viruses may confer some degree of cross-protection against infection with the H3N2pM/H3N2v viruses.

- According to the risk assessment developed in Chapter 10, given that a first holding is infected, the likelihood of spread of H3N2pM by pigs to second holdings is expected to be high in the context of unrestricted movement of pigs from the first holding and a high likelihood that pigs in a second holding are susceptible.

13.2. Conclusions on the virus spread after the last clinical signs have disappeared

- Clinical signs after SIV infection, H3N2V/H3N2pM infection included, are often absent or mild and their duration is variable (i.e. until 3 dpi in mild cases, until 5–6 dpi in severe cases). By analogy with other SIVs and based on the limited pathogenesis studies with H3N2v in pigs, it can be inferred that the virus excretion in individual pigs can last up to 7 dpi. Clinical signs are generally associated with viral shedding; however, virus excretion does not entirely coincide with presence of clinical signs (may start earlier or last longer than clinical signs). Consequently, an absence of clinical signs cannot be used as evidence that pigs are not infectious.

- At farm level SIV infections can be maintained with a continuous introduction of susceptible pigs. Therefore, the risk of spread from holdings can remain high for an extended period of time even after cessation of clinical signs. This takes place particularly when susceptible pigs continuously enter the fattening unit.

13.3.   Recommendations on the virus spread after last clinical signs have disappeared

- Since the absence of clinical signs is not a reliable basis on which to decide that H3N2pM virus is no longer circulating in an infected holding where no new pigs have entered, it is recommended that, when the health status with regard to excretion of such viruses from a farm needs to be known, nasal/oropharyngeal swabs focussing on pigs of 8–12 weeks of age should be tested by RT-PCR to confirm the absence of H3N2pM virus.

14.   ToR4 - the possibility, efficacy and efficiency of vaccination in pigs, using the existing vaccines or newly developed vaccines against influenza A (H3N2v) virus, also in relation with the possible evolution of variants of influenza viruses posing a risk to public and animal health

In this ToR, the term "efficiency" is interpreted as effectiveness. Below is a brief assessment of each of the specific questions mentioned in ToR4. The background information used to answer these questions can be found under Chapters 4 and 8 of this document. For clarity, vaccination in pigs using existing vaccines or newly developed vaccines against influenza A (H3N2v) is treated separately. The terms "possibility", "efficacy" and "effectiveness" are defined as follows:

- The "possibility" of vaccination means availability of the vaccine.

- The "efficacy" of vaccination means, according to the European Pharmacopoeia, the ability of the vaccine to offer significant virological protection in an experimental setting, i.e. after a double vaccination of influenza-naive pigs and challenge with H3N2v virus three weeks after the second vaccination. Virological protection is defined as a significant reduction in virus titres in the lungs of vaccinated pigs compared with unvaccinated challenge control pigs.

- The "effectiveness" of vaccination means the ability of the vaccine to reduce virus circulation under field conditions.

14.1.   Existing vaccines

- *Possibility of vaccination*: Inactivated vaccines based on the endemic SIVs are commercially available in many, but not all, European countries. Most vaccines are bivalent and contain H1N1 and H3N2 virus strains; one vaccine is trivalent and also includes the recently emerged H1N2 strain. However, the H3N2 virus strains present in European vaccines show substantial genetic and antigenic differences compared with the H3N2 virus that forms part of the SIV vaccines in the USA. There has been no need to update H3N2 vaccine strains in European SIV vaccines since strain evolution and variation has been minor and the original vaccine strains have, until now, induced sufficient efficacy.

- *Efficacy of vaccination*: No challenge studies have been done to examine to what extent SIV vaccines in Europe protect against H3N2pM. Serological investigations of pigs vaccinated with various existing European SIV vaccines allow the expectation that these vaccines will induce no or few cross-reactive HI antibodies against H3N2pM and thus may not reduce H3N2v virus replication in the lungs to a significant degree. This assumption is based on the fact that influenza virus replication in the lungs of vaccinated pigs correlates negatively with post-vaccination HI antibody titres against the challenge virus.

  The European situation is not at all comparable with that in the USA, where H3N2 strains included in the licensed US vaccines show a much closer antigenic match with the H3N2pM virus and these vaccines have been demonstrated to offer significant cross-protection against rH3N2p viruses.

- *Effectiveness of vaccination*: Data about the effectiveness of vaccination against the H3N2pM virus are lacking. As European vaccines, in general, are not expected to be efficacious against

H3N2pM, effectiveness of vaccination will likely be very low. Furthermore, it must be remembered that vaccination is voluntary, that vaccination rates vary in different countries and that H3N2 virus seroprevalence in swine populations in different European countries, which may influence effectiveness , is highly variable (see Table 1, Chapter 6, ESNIP 3). Voluntary vaccination of swine with the existing SIV vaccines has not succeeded in halting the circulation of SIV in the swine population, neither in Europe nor in the USA. It is unknown if, and to what degree, obligatory SIV vaccination could reduce the virus circulation.

14.2. New vaccines

- *Possibility of vaccination*: A monovalent inactivated vaccine specifically based on H3N2pM virus for use in pigs is not licensed neither in the USA nor in Europe. It would, however, be possible to develop such a vaccine in a rather short time interval based on the experience of development and production of other SIV vaccines.

- *Efficacy of vaccination*: If such vaccine were to be available, it would be expected to induce immunity and cross-protection similar to that induced by existing European SIV vaccines against the endemic European SIVs.

- *Effectiveness of vaccination*: There are no data about the effectiveness of widespread vaccination with H3N2v vaccines in pigs in the field. Based on the experience with existing SIV vaccines and endemic SIVs, voluntary vaccination is unlikely to halt the circulation of H3N2v virus in the swine population. It is unknown if, and to what degree, obligatory vaccination could reduce virus circulation.

14.3. Potential evolution of variants of influenza viruses posing a serious risk to public and animal health

- *Evolution of variants of H3N2pM virus posing a serious risk to animal health*: As mentioned above, vaccination is unlikely to prevent the spread of H3N2pM virus in European pig populations in the event that this virus enters and become endemic in Europe. The emergence of novel reassortants of influenza viruses is unpredictable, but it will probably increase if H3N2pM enters the European pig population, and such events are unlikely to be prevented by vaccination. In addition, it cannot be excluded that vaccination increases the risk of antigenic drift by selection of neutralisation-resistant escape mutants by vaccine-induced antibodies. The latter phenomenon, however, has not yet been observed with the existing vaccines and endemic SIVs.

- *Evolution of variants of H3N2pM virus posing a serious risk to human health*: For the same reasons mentioned above, vaccination of swine, even if able to reduce virus replication in the respiratory tract, is unlikely to prevent the emergence of variants of H3N2pM virus with increased transmissibility to humans. However, based on previous experience, a divergent evolution of H3N2pM in pigs compared with that in humans is likely, thus making it unlikely that virus with increased transmissibility to humans would evolve.

14.4. Conclusions

- Immunity resulting from vaccination with commercially available European SIV vaccines is expected to provide no or only a low level of cross-protection against infection with the H3N2pM influenza viruses, whereas vaccines based on North American swine H3N2 viruses would offer superior protection. Such vaccines may significantly reduce H3N2pM replication in the lungs in vaccinated animals. Voluntary vaccination of pigs with existing vaccines has not succeeded in halting the circulation of SIV in the swine population and this limitation is also considered valid for H3N2pM.

- According to the available data, H3N2pM is not present in the European swine population and no measures are needed with regard to vaccination. If such a virus were to enter into Europe and spread in the swine population, then the use of vaccines based on the closely related TR H3N2 strains could be useful.

- Vaccination might increase antigenic drift of circulating influenza strains, and newly appearing variants might not be neutralised by vaccine-induced antibodies. However, based on current knowledge there is no indication that this has happened with the use of the available inactivated SIV vaccines in the European pig population.

14.5. Recommendations

- The possibility of using more specific vaccines, based on the closely related US swine TR H3N2 virus strains, should be assessed, as a possible emergency procedure, in the event of a change in the epidemiological situation, i.e. if the H3N2pM/H3N2v virus were to enter the European pig population.

14.6. Recommendations for future research

- Experimental cross-protection studies with H3N2pM challenge in pigs previously infected with the major European SIV subtypes should be carried out.

- Experimental vaccination-challenge studies with the existing European and American SIV vaccines and H3N2pM challenge should be carried out. The extent of protection conferred by different vaccines should be compared.

15. ToR5 - the most important factors to be monitored that would suggest a risk for the emergence of a new pandemic influenza strain from the influenza A (H3N2v) virus

The background information used to answer this question can be found under Chapters 7 (influenza surveillance in Europe including diagnostic capabilities), 8 (Section 8.4, cross-immunity in humans) and 9 (emergence of a new pandemic strain from H3N2v).

15.1. Conclusions

- New influenza strains emerge through natural reassortment and/or mutations.

- Past experience has shown that reassortment events involving inter-species transmission are necessary steps in the evolution of new pandemic strains. However, it is not always clear in which species these events occur. Monitoring for reassortant viruses should therefore include as important target species both pigs and poultry.

- Several molecular markers in influenza virus genes have been reported as being associated with certain biological properties, such as receptor binding, host and tissue tropism, virulence, and modulation of host immune response, as well as efficiency of replication and transmission. These associations have been inconsistent between strains.

- Studies in animal models have reaffirmed and highlighted the importance of individual virus proteins, such as the HA, NA, polymerase and non-structural proteins, and even point mutations within these molecules, for cross-species transmission. They have also emphasised that the correlation between the molecular markers and biological properties is not absolute. Virulence and pathogenesis are not the properties of a single gene or protein. They are polygenic and the specific gene constellation is also important.

- Currently, the number and type of mutations, as well as the genetic constellation needed for efficient human-to-human transmission of H3N2v, is unknown.

- Based on current knowledge, it is not possible to predict which changes (mutations or reassortments) within H3N2v could enable it become a new pandemic influenza virus. Hence, it does not seem possible, at present, to set up a system to monitor "the most important factors that would suggest a risk of emergence of a new pandemic strain from the influenza A (H3N2v) virus.

15.2. Recommendations

- Ensure appropriate monitoring in pigs to permit early identification of newly appearing strains (e.g. novel reassortants) which might cross the animal–human interface.

- Develop a European strategy for appropriate surveillance of humans in close contact with swine.

REFERENCES

Bányai K, Kovács E, Tóth ÁG, Biksi I, Szentpáli-Gavallér K, Bálint Á, Dencső L and Dán Á, 2012. Genome sequence of a monoreassortant H1N1 swine influenza virus isolated from a pig in Hungary. Journal of Virology, 86, 13133–13133.

Bateman AC, Karamanska R, Busch MG, Dell A, Olsen CW and Haslam SM, 2010. Glycan Analysis and Influenza A Virus Infection of Primary Swine Respiratory Epithelial Cells. Journal of Biological Chemistry, 285, 34016–34026.

Bialas KM, Desmet EA and Takimoto T, 2012. Specific residues in the 2009 H1N1 swine-origin influenza matrix protein influence virion morphology and efficiency of viral spread in vitro. PloS One 7, e50595.

Bouvier NM and Palese P, 2008. The biology of influenza viruses. Vaccine, 26, D49–D53.

Bowman AS, Sreevatsan S, Killian ML, Page SL, Nelson SW, Nolting JM, Cardona C and Slemons RD, 2012. Molecular evidence for interspecies transmission of H3N2pM/H3N2v influenza A viruses at an Ohio agricultural fair, July 2012. Emerging Microbes Infection, 1, e33.

Brown IH. 2000. The epidemiology and evolution of influenza viruses in pigs. Veterinary Microbiology, 74(1–2), 29–46.

Brown IH, Done SH, Spencer YI, Cooley WA, Harris PA and Alexander DJ, 1993. Pathogenicity of a swine influenza H1N1 virus antigenically distinguishable from classical and European strains. Veterinary Record, 132, 598–602.

Castrucci MR, Donatelli I, Sidoli L, Barigazzi G, Kawaoka Y and Webster RG.1993. Genetic reassortment between avian and human influenza A viruses in Italian pigs. Virology, 193,503–506.

CDC (Centers for Disease Control and Prevention), online. Overview of influenza surveillance in the United States. Available online: http://www.cdc.gov/flu/weekly/overview.htm

CDC (Centers for Disease Control and Prevention), 2011a. Limited human-to-human transmission of novel influenza A (H3N2) virus—Iowa, November 2011. MMWR, 60(47), 1615–1617.

CDC (Centers for Disease Control and Prevention), 2011b. Swine-origin influenza A (H3N2) virus infections in two children—Indiana and Pennsylvania, July–August, 2011. MMWR, 60(35), 1213–1215.

CDC (Centers for Disease Control and Prevention), 2012a. Update: influenza A (H3N2)v transmission and guidelines—five states, 2011. MMWR, 60(51), 1741–1744.

CDC (Centers for Disease Control and Prevention), 2012b. Influenza A (H3N2) variant virus-related hospitalizations—Ohio, 2012. MMWR, 61(38), 764–767.

CDC (Centers for Disease Control and Prevention), 2012c. Notes from the field: outbreak of influenza A (H3N2) virus among persons and swine at a county fair—Indiana, July 2012. MMWR, 61(29), 561.

CDC (Centers for Disease Control and Prevention), 2012d. Antibodies cross-reactive to influenza A (H3N2) variant virus and impact of 2010–2011 seasonal influenza vaccine on cross-reactive antibodies—United States. MMWR, 61(14), 237–241.

Chen L, Rivailler P, Hossain J, Carney P, Balish A, Perry I, Davis CT, Garten R, Shu B and Xu X, 2011. Receptor specificity of subtype H1 influenza A viruses isolated from swine and humans in the United States. Virology, 412, 401–410.

Chiapponi C, Moreno A, Barbieri I, Merenda M and Foni E, 2012. Multiplex RT-PCR assay for differentiating European swine influenza virus subtypes H1N1, H1N2 and H3N2. Journal of Virological Methods,, 184, 117–120.

Chou Y, Albrecht RA, Pica N, Lowen AC, Richt JA, García-Sastre A, Palese P and Hai R, 2011. The M segment of the 2009 new pandemic H1N1 influenza virus is critical for its high transmission efficiency in the guinea pig model. Journal of Virology, 85, 11235–11241.

Connor RJ, Kawaoka Y, Webster RG and Paulson JC, 1994. Receptor specificity in human, avian, and equine H2 and H3 influenza virus isolates. Virology, 205, 17–23.

Cox CM, Neises D, Garten RJ, Bryant B, Hesse RA, Anderson GA, Trevino-Garrison I, Shu B, Lindstrom S, Klimov AI and Finelli L, 2011. Swine influenza virus A (H3N2) infection in human, Kansas, USA, 2009. Emerging Infectious Diseases, 17, 1143–1144. doi: 10.3201/eid/1706.101488.

Cox LA Jr., 2008. What's wrong with risk matrices? Risk Analysis, 28, 497–512. doi: 10.1111/j.1539-6924.2008.01030.x.

de Jong JC, Smith DJ, Lapedes AS, Donatelli I, Campitelli L, Barigazzi G, Van Reeth K, Jones TC, Rimmelzwaan GF, Osterhaus AD and Fouchier RA, 2007. Antigenic and genetic evolution of swine influenza A (H3N2) viruses in Europe. Journal of Virology, 81, 4315–4322.

De Vleeschauwer A, Atanasova K, Van Borm S, van den Berg T, Rasmussen TB, Uttenthal A and Van Reeth K, 2009. Comparative pathogenesis of an avian H5N2 and a swine H1N1 influenza virus in pigs. PloS One, 4(8), e6662. doi: 10.1371/journal.pone.0006662.

De Vleeschauwer AR, Van Poucke SG, Karasin AL, Olsen CW and Van Reeth K, 2011. Cross-protection between antigenically distinct H1N1 swine influenza viruses from Europe and North America. Influenza and Other Respiratory Viruses, 5, 115–122.

Dormitzer PR, Galli G, Castellino F, Golding H, Khurana S, Del Giudice G and Rappuoli R, 2011. Influenza vaccine immunology. Immunological Reviews, 239, 167–177.

Ducatez MF, Hause B, Stigger-Rosser E, Darnell D, Corzo C, Juleen K, Simonson R, Brockwell-Staats C, Rubrum A, Wang D, Webb A, Crumpton JC, Lowe J, Gramer M and Webby RJ, 2011. Multiple reassortment between pandemic (H1N1) 2009 and endemic influenza viruses in pigs, United States. Emerging Infectious Diseases, 17, 1624–1629. doi: 10.3201/eid1709.110338.

ECDC (European Centre for Disease Prevention and Control), 2012a. ECDC Technical Report - CNRL *in silico* exercise to determine the capabilities of network laboratories to detect triple reassortant swine origin influenza A(H3N2) viruses. Available online: http://ecdc.europa.eu/en/publications/Publications/1204-TER-CNRL-Capability-Exercise.pdf

ECDC (European Centre for Disease Prevention and Control), 2012b. ECDC Technical Report - Influenza A(H3N2)v laboratory detection questionnaire results. Available online: http://ecdc.europa.eu/en/publications/Publications/1204-TER-CNRL-Capability-Exercise.pdf

ECDC (European Centre for Disease Prevention and Control), 2012c. Risk Assessment Update – Swine-origin triple reassortant influenza A(H3N2) variant viruses in North America 17 August 2012.Available online:http://www.ecdc.europa.eu/en/publications/publications/1208-ter-rapid-risk-assessment-influenza-ah3n2-us.pdf

EFSA AHAW Panel (EFSA Panel on Animal Health and Welfare), 2010. Scientific opinion on African swine fever. EFSA Journal 2010;8(3):1556, 149 pp. doi:10.2903/j.efsa.2010.1556

EFSA AHAW Panel (EFSA Panel on Animal Health and Welfare), 2013. Scientific Opinion on the risk of entry of *Aethina tumida* and *Tropilaelaps* spp. in the EU. EFSA Journal 2013;11(3):3128, 127 pp. doi:10.2903/j.efsa.2013.3128

EFSA BIOHAZ Panel (EFSA Panel on Biological Hazards), 2010. EFSA statement on food safety considerations of novel H1N1 influenza virus infections in humans. EFSA Journal 2010;8(6):1629, 43 pp. doi:10.2903/j.efsa.2010.1629

Fan X, Zhu H, Zhou B, Smith DK, Chen X, Lam TT-Y, Poon LLM, Peiris M and Guan Y, 2012. Emergence and dissemination of a swine H3N2 reassortant influenza virus with 2009 pandemic H1N1 genes in pigs in China. Journal of Virology, 86, 2375–2378.

Forrest HL and Webster RG, 2010. Perspectives on influenza evolution and the role of research. Animal Health Research Reviews, 11(1), 3–18. doi: 10.1017/S1466252310000071

Garten RJ, Davis CT, Russell CA, Shu B, Lindstrom S, Balish A, Sessions WM, Xu X, Skepner E, Deyde V, Okomo-Adhiambo M, Gubareva L, Barnes J, Smith CB, Emery SL, Hillman MJ, Rivailler P, Smagala J, de Graaf M, Burke DF, Fouchier RA, Pappas C, Alpuche-Aranda CM, López-Gatell H, Olivera H, López I, Myers CA, Faix D, Blair PJ, Yu C, Keene KM, Dotson PD Jr., Boxrud D, Sambol AR, Abid SH, St George K, Bannerman T, Moore AL, Stringer DJ, Blevins P, Demmler-Harrison GJ, Ginsberg M, Kriner P, Waterman S, Smole S, Guevara HF, Belongia EA, Clark PA, Beatrice ST, Donis R, Katz J, Finelli L, Bridges CB, Shaw M, Jernigan DB, Uyeki TM, Smith DJ, Klimov AI and Cox NJ, 2009. Antigenic and genetic characteristics of swine-origin 2009 A (H1N1) influenza viruses circulating in humans. Science, 325, 197–201.

Gerloff NA, Kremer JR, Charpentier E, Sausy A, Olinger CM, Weicherding P, Schuh J, Van Reeth K and Muller CP, 2011. Swine influenza virus antibodies in humans, Western Europe, 2009. Emerging Infectious Diseases, 17, 403.

Graham BS and Crowe JE Jr., 2007. Immunization against viral diseases. In: Field's virology, vol. 1, 5th edn. Eds Knipe DM and Howley PM. Lippincott Williams & Wilkins, Philadelphia, PA, USA, 487–538.

Gravenor MB and Kao RR, 2003. Risk assessments on BSE. Veterinary Record, 152, 816.

Haesebrouck F, Biront P, Pensaert MR and Leunen J, 1985. Epizootics of respiratory tract disease in swine in Belgium due to H3N2 influenza virus and experimental reproduction of disease. American Journal of Veterinary Research, 46, 1926–1928.

Heinen PP, van Nieuwstadt AP, de Boer-Luijtze EA and Bianchi AT, 2001. Analysis of the quality of protection induced by a porcine influenza A vaccine to challenge with an H3N2 virus. Veterinary Immunology and Immunopathology, 82, 39–56.

Heim D, Gardner I, Mumford E and Kihm U. 2006. Risk assessment and surveillance for bovine spongiform encephalopathy. Revue scientifique et technique, 25, 937–950.

Hoffmann B, Harder T, Lange E, Kalthoff D, Reimann I, Grund C, Oehme R, Vahlenkamp TW and Beer M, 2010. New real-time reverse transcriptase polymerase chain reactions facilitate detection and differentiation of novel A/H1N1 influenza virus in porcine and human samples. Berliner und Münchener tierärztliche Wochenschrift, 123, 286–292.

Hoinville, L.J., Alban, L., Drewe, J.A., Gibbens, J.C., Gustafson, L., Häsler, B., Saegerman, C., Salman, M., Stärk, K.D.C., 2013. Proposed terms and concepts for describing and evaluating animal-health surveillance systems. Preventive Veterinary Medicine 112, 1–12.

Houser KV, Katz JM and Tumpey TM, 2013. Seasonal trivalent inactivated influenza vaccine does not protect against newly emerging variants of influenza A (H3N2v) virus in ferrets. Journal of Virology, 87, 1261–1263.

Howard WA, Essen SC, Strugnell BW, Russell C, Barrass L, Reid SM and Brown IH, 2011. Reassortant pandemic (H1N1) 2009 virus in pigs, United Kingdom. Emerging Infectious Diseases, 17, 1049–1052.

Khatri M, Dwivedi V, Krakowka S, Manickam C, Ali A, Wang L, Qin Z, Renukaradhya GJ and Lee CW, 2010. Swine influenza H1N1 virus induces acute inflammatory immune responses in pig lungs: a potential animal model for human H1N1 influenza virus. Journal of Virology, 84, 11210–11218.

Kimble JB, Sorrell E, Shao H, Martin PL and Perez DR, 2011. Compatibility of H9N2 avian influenza surface genes and 2009 pandemic H1N1 internal genes for transmission in the ferret model. Proceedings of the National Academy of Sciences of the USA, 108, 12084.

Kitikoon P, Nilubol D, Erickson BJ, Janke BH, Hoover TC, Sornsen SA and Thacker EL, 2006. The immune response and maternal antibody interference to a heterologous H1N1 swine influenza virus infection following vaccination. Veterinary Immunology and Immunopathology, 112, 117–128.

Kitikoon P, Vincent AL, Jones KR, Nilubol D, Yu S, Janke BH, Thacker BJ and Thacker EL, 2009. Vaccine efficacy and immune response to swine influenza virus challenge in pigs infected with porcine reproductive and respiratory syndrome virus at the time of SIV vaccination. Veterinary Microbiology, 139, 235–244.

Kitikoon P, Vincent AL, Gauger PC, Schlink SN, Bayles DO, Gramer MR, Darnell D, Webby RJ, Lager KM and Swenson SL, 2012. Pathogenicity and transmission in pigs of the novel A (H3N2) v influenza virus isolated from humans and characterization of swine H3N2 viruses isolated in 2010–2011. Journal of Virology, 86, 6804–6814.

Kobasa D, Kodihalli S, Luo M, Castrucci MR, Donatelli I, Suzuki Y, Suzuki T and Kawaoka Y, 1999. Amino acid residues contributing to the substrate specificity of the influenza A virus neuraminidase. Journal of Virology, 73, 6743–6751.

Krueger WS and Gray GC, 2013. Swine influenza virus infections in man. In: Swine influenza, current topics in microbiology and immunology. Eds Richt JA and Webby RJ. Springer, Berlin Germany, 201–225.

Kuntz-Simon G and Madec F, 2009. Genetic and antigenic evolution of swine influenza viruses in Europe and evaluation of their zoonotic potential. Zoonoses Public Health, 56, 310–325. doi: 10.1111/j.1863-2378.2009.01236.x.

Kyriakis CS, Brown IH, Foni E, Kuntz-Simon G, Maldonado J, Madec F, Essen SC, Chiapponi C and Van Reeth K, 2011. Virological surveillance and preliminary antigenic characterization of influenza viruses in pigs in five European countries from 2006 to 2008. Zoonoses Public Health, 58, 93–101. doi: 10.1111/j.1863-2378.2009.01301.x.

Kyriakis CS, Rose N, Foni E, Maldonado J, Loeffen WL, Madec F, Simon G and Van Reeth K, 2013 Influenza A virus infection dynamics in swine farms in Belgium, France, Italy and Spain, 2006–2008. Veterinary Microbiology, 162, 543–550.

Lakdawala SS, Lamirande EW, Suguitan AL, Wang W, Santos CP, Vogel L, Matsuoka Y, Lindsley WG, Jin H and Subbarao K, 2011. Eurasian-origin gene segments contribute to the transmissibility, aerosol release, and morphology of the 2009 pandemic H1N1 influenza virus. PLoS Pathogens, 7, e1002443.

Larsen DL, Karasin A and Olsen CW, 2001. Immunization of pigs against influenza virus infection by DNA vaccine priming followed by killed-virus vaccine boosting. Vaccine, 19, 2842–2853.

Lee JH, Gramer MR and Joo HS, 2007. Efficacy of swine influenza A virus vaccines against an H3N2 virus variant. Canadian Journal of Veterinary Research, 71, 207–212.

Lina B, Bouscambert M, Enouf V, Rousset D, Valette M and van der Werf S, 2011. S-OtrH3N2 viruses: Use of sequence data for description of the molecular characteristics of the viruses and their relatedness to previously circulating H3N2 human viruses. Euro Surveillance, 16, 50.

Lindstrom S, Garten R, Balish A, Shu B, Emery S, Berman L, Barnes N, Sleeman K, Gubareva L, Villanueva J and Klimov A, 2011. Human infections with novel reassortant influenza A(H3N2)v viruses, United States, 2011. Emerging Infectious Diseases, 18, 834–837. doi: 10.3201/eid1805.111922.

Liu Q, Ma J, Liu H, Qi W, Anderson J, Henry S, Hesse R, Richt J and Ma W, 2012a. Emergence of novel reassortant H3N2 swine influenza viruses with the 2009 pandemic H1N1 genes in the United States. Archives of Virology, 157, 555–562.

Liu Q, Qiao C, Marjuki H, Bawa B, Ma J, Guillossou S, Webby RJ, Richt JA and Ma W, 2012b. Combination of PB2 271A and SR polymorphism at positions 590/591 is critical for viral

replication and virulence of swine influenza virus in cultured cells and in vivo. Journal of Virology, 86, 1233–1237.

Loving CL, Lager KM, Vincent AL, Brockmeier SL, Gauger PC, Anderson TK, Kitikoon P, Perez DR and Kehrli ME Jr., 2013. Efficacy of inactivated and live-attenuated influenza virus vaccines in pigs against infection and transmission of emerging H3N2 similar to the 2011–2012 H3N2v. Journal of Virology, 2013 Jul 3 [Epub ahead of print].

Ma W, Lager KM, Vincent AL, Janke BH, Gramer MR and Richt, JA, 2009. The role of swine in the generation of novel influenza viruses. Zoonoses Public Health, 56, 326–337.

Macklin MD, McCabe D, McGregor MW, Neumann V, Meyer T, Callan R, Hinshaw VS and Swain WF, 1998. Immunization of pigs with a particle-mediated DNA vaccine to influenza A virus protects against challenge with homologous virus. Journal of Virology, 72, 1491–1496.

Matrosovich M, Gambaryan A, Teneberg S, Piskarev V, Yamnikova S, Lvov D, Robertson J nd Karlsson KA, 1997. Avian influenza A viruses differ from human viruses by recognition of sialyloligosaccharides and gangliosides and by a higher conservation of the HA receptor-binding Site. Virology, 233, 224–234.

Matrosovich M, Tuzikov A, Bovin N, Gambaryan A, Klimov A, Castrucci MR, Donatelli I and Kawaoka, Y, 2000. Early alterations of the receptor-binding properties of H1, H2, and H3 avian influenza virus hemagglutinins after their introduction into mammals. Journal of Virology, 74, 8502–8512.

Moncorgé O, Mura M and Barclay WS. 2010. Evidence for avian and human host cell factors that affect the activity of influenza virus polymerase. Journal of Virology, 84, 9978–9986.

Moreno A, Di Trani L, Faccini S, Vaccari G, Nigrelli D, Boniotti MB, Falcone E, Boni A, Chiapponi C, Sozzi E and Cordioli P, 2011. Novel H1N2 swine influenza reassortant strain in pigs derived from the pandemic H1N1/2009 virus. Veterinary Microbiology, 149, 472–477.

Munch M, Nielsen LP, Handberg KJ and Jørgensen PH, 2001. Detection and subtyping (H5 and H7) of avian type A influenza virus by reverse transcription-PCR and PCR-ELISA. Archives of Virology, 146, 87–97.

Myers KP, Olsen CW, Setterquist SF, Capuano AW, Donham KJ, Thacker EL, Merchant JA and Gray, GC. 2006. Are swine workers in the United States at increased risk of infection with zoonotic influenza virus? Clinical Infectious Diseases, 42, 14–20.

Myers KP, Olsen CW and Gray GC. 2007. Cases of swine influenza in humans: a review of the literature. Clinical Infectious Diseases, 44, 1084–1088.

Nelson MI, Gramer MR, Vincent AL and Holmes EC, 2012a. Global transmission of influenza viruses from humans to swine. Journal of General Virology. 93, 2195–2203.

Nelson M, Detmer SE, Wentworth DE, Tan Y, Schwartzbard A, Halpin RA, Stockwell T, Lin X, Vincent A and Gramer MR, 2012b. Genomic reassortment of influenza A virus in North American swine, 1998–2011 Journal of General Virology, 93, 2584–2589.

Neumann G and Kawaoka Y, 2006. Host range restriction and pathogenicity in the context of influenza pandemic. Emerging Infectious Diseases, 12, 881–886.

Neumann G, Noda T and Kawaoka Y. 2009. Emergence and pandemic potential of swine-origin H1N1 influenza virus. Nature, 459, 931–939. doi: 10.1038/nature08157.

Olsen CW, Brammer L, Easterday BC, Arden N, Belay E, Baker I and Cox NJ. 2002. Serologic evidence of H1 swine influenza virus infection in swine farm residents and employees. Emerging Infectious Diseases, 8, 814.

Olsen CW, Karasin A and Erickson G, 2003. Characterization of a swine-like reassortant H1N2 Influenza virus isolated from a wild duck in the United States. Virus Research, 93, 115–121.

Olsen C, Brown I, Easterday B and Van Reeth K, 2006. Swine influenza. In: Diseases of swine. Eds Zimmerman J, Karriker L, Ramirez A, Kent Schwartz and Stevenson G. John Wiley & Sons, AASV,USA 469–482.

Pascua, P.N.Q., Lim, G.-J., Kwon, H., Park, S.-J., Kim, E.-H., Song, M.-S., Kim, C.J., Choi, Y.-K., 2013. Emergence of H3N2pM-like and novel reassortant H3N1 swine viruses possessing segments derived from the A (H1N1)pdm09 influenza virus, Korea. Influenza and Other Respiratory Viruses n/a–n/a. Published online August 30.

Pearce MB, Jayaraman A, Pappas C, Belser JA, Zeng H, Gustin KM, Maines TR, Sun X, Raman R, Cox NJ, Sasisekharan R, Katz JM and Tumpey TM, 2012. Pathogenesis and transmission of swine origin A(H3N2)v influenza viruses in ferrets. Proceedings of the National Academy of Sciences of the USA,109, 3944–3949. doi: 10.1073/pnas.1119945109.

Pol F, Quéguiner S, Gorin S, Deblanc C and Simon G, 2011. Validation of commercial real-time RT-PCR kits for detection of influenza A viruses in porcine samples and differentiation of pandemic (H1N1) 2009 virus in pigs. Journal of Virological Methods, 171, 241–247. doi: 10.1016/j.jviromet.2010.11.007.

Reperant LA, Kuiken T and Osterhaus AD, 2012. Adaptive pathways of zoonotic influenza viruses: from exposure to establishment in humans. Vaccine, 30, 4419–4434.

Richt JA, Lager KM, Janke BH, Woods RD, Webster RG and Webby RJ, 2003. Pathogenic and antigenic properties of phylogenetically distinct reassortant H3N2 swine influenza viruses cocirculating in the United States. Journal of Clinical Microbiology, 41, 3198–3205.

Romagosa A, Allerson M, Gramer M, Joo HS, Deen J, Detmer S and Torremorell M, 2011. Vaccination of influenza a virus decreases transmission rates in pigs. Veterinary Research, 42, 120.

Skowronski DM, Janjua NZ, De Serres G, Purych D, Gilca V, Scheifele DW, Dionne M, Sabaiduc S, Gardy JL, Li G, Bastien N, Petric M, Boivin G and Li Y, 2012. Cross-reactive and vaccine-induced antibody to an emerging swine-origin variant of influenza A virus subtype H3N2 (H3N2v). Journal of Infectious Diseases, 206, 1852–1861. doi: 10.1093/infdis/jis500.

Slomka MJ, Densham AL, Coward VJ, Essen S, Brookes SM, Irvine RM, Spackman E, Ridgeon J, Gardner R, Hanna A, Suarez DL and Brown IH. 2010. Real time reverse transcription (RRT)-polymerase chain reaction (PCR) methods for detection of pandemic (H1N1) 2009 influenza virus and European swine influenza A virus infections in pigs. Influenza and Other Respiratory Viruses, 4, 277–293. doi: 10.1111/j.1750-2659.2010.00149.x.

Smith G, Bahl J, Vijaykrishna D, Zhang J, Poon LLM, Chen H, Webster RG, Peiris JSM and Guan, Y, 2009. Dating the emergence of pandemic influenza viruses. Proceedings of the National Academy of Sciences of the USA 106, 11709–11712.

Starick E, Lange E, Fereidouni S, Bunzenthal C, Höveler R, Kuczka A, Grosse Beilage E, Hamann, H-P, Klingelhöfer I, Steinhauer D, Vahlenkamp T, Beer M and Harder T, 2011. Reassorted pandemic (H1N1) 2009 influenza A virus discovered from pigs in Germany. Journal of General Virology, 92, 1184–1188.

Starick E, Lange E, Grund C, Grosse Beilage E, Döhring S, Maas A, Noé T, Beer M and Harder TC. 2012. Reassortants of pandemic influenza A virus H1N1/2009 and endemic porcine HxN2 viruses emerge in swine populations in Germany. Journal of General Virology, 93, 1658–1663.

Tremblay D, Allard V, Doyon J, Bellehumeur C, Spearman JG, Harel J and Gagnon CA, 2011. Emergence of a New Swine H3N2 and Pandemic (H1N1) 2009 Influenza A virus reassortant in two Canadian animal populations, mink and swine. Journal of Clinical Microbiology, 49, 4386–4390.

USDA (United States Department of Agriculture). Online. Swine Influenza Surveillance Update. Available online: http://www.aphis.usda.gov/animal_health/animal_dis_spec/swine/siv_surveillance.shtml

Van Reeth K and Ma W, 2012. Swine influenza virus vaccines: to change or not to change—that's the question. Current Topics in Microbiology And immunology, 370, 173–200.

Van Reeth K, Brown IH, Dürrwald R, Foni E, Labarque G, Lenihan P, Maldonado J, Markowska-Daniel I, Pensaert M, Pospisil Z and Koch G, 2008. Seroprevalence of H1N1, H3N2 and H1N2 influenza viruses in pigs in seven European countries in 2002–2003. Influenza and Other Respiratory Viruses, 2, 99≠105.

Van Reeth K, Gregory V, Hay A and Pensaert M, 2003. Protection against a European H1N2 swine influenza virus in pigs previously infected with H1N1 and/or H3N2 subtypes. Vaccine, 21, 1375–1381.

Van Reeth K, Labarque G and Pensaert M, 2006. Serological profiles after consecutive experimental infections of pigs with European H1N1, H3N2, and H1N2 swine influenza viruses. Viral Immunology, 19, 373–82. Erratum in Viral Immunology, 19, 775.

Van Reeth K, Nauwynck H and Pensaert M. 1996. Dual infections of feeder pigs with porcine reproductive and respiratory syndrome virus followed by porcine respiratory coronavirus or swine influenza virus: a clinical and virological study. Veterinary Microbiology, 48, 325–335.

Van Reeth K, 2007. Avian and swine influenza viruses: our current understanding of the zoonotic risk. Veterinary Research, 38, 243–260.

Vijaykrishna D, Poon LLM, Zhu HC, Ma SK, Li OTW, Cheung CL, Smith GJD, Peiris JSM and Guan Y, 2010. Reassortment of pandemic H1N1/2009 influenza a virus in swine. Science. 328, 1529.

Vincent AL, Lager KM, Harland M, Loursso A, Zanella E, Zanella-Ciacci JR, Kehrli Jr. ME and Klimov A, 2009. Absence of 2009 pandemic H1N1 influenza A virus in fresh pork. PloS One 4(12), e8367. doi: 10.1371/journal.pone.0008367.

Waalen K, Kilander A, Dudman SG, Ramos-Ocao R and Hungnes O, 2012. Age-dependant prevalence of antibodies cross-reactive to the influenza A (H3N2) variant virus in sera collected in Norway in 2011. Euro Surveillance, 17 (19), 10 May 2012.

WHO (World Health Organisation), 2005. International Health Regulations of 2005. Available online. http://www.who.int/ihr/9789241596664/en/

Wieland B, Dhollander S, Salman M and Koenen F, 2011. Qualitative risk assessment in a data-scarce environment: a model to assess the impact of control measures on spread of African swine fever. Preventive Veterinary Medicine, 99, 4–14. doi: 10.1016/j.prevetmed.2011.01.001

Yassine HM, Al-Natour MQ, Lee C and Saif YM, 2007. Interspecies and intraspecies transmission of triple reassortant H3N2 influenza A viruses. Virology Journal, 4, 129.

Yassine HM, Lee CW, Gourapura R and Saif YM, 2010. Interspecies and intraspecies transmission of influenza A viruses: viral, host and environmental factors. Animal Health Research Reviews, 11, 53–72. doi: 10.1017/S1466252310000137.

Zamarin D, Ortigoza MB and Palese P, 2006. Influenza A virus PB1-F2 protein contributes to viral pathogenesis in mice. Journal of Virology, 80, 7976–7983.

Zepeda-Sein C, 1998. Methodes d'evaluation des risques zoosanitaires lors des echanges internationaux. OIE seminaire sur la securite zoosanitaires des echanges dans les Caraibes, 2–17.

Zhou NN, Senne DA, Landgraf JS, Swenson SL, Erickson G, Rossow K, Liu L, Yoon K, Krauss S and Webster RG, 1999. Genetic reassortment of avian, swine, and human influenza A viruses in American pigs. Journal of Virology, 73, 8851–8856.

APPENDIX A: COMMERCIALLY AVAILABLE VACCINES IN THE USA

Table 16: Overview of the major licensed SIV vaccines in the USA in 2011

Manufacturer	Product name	Influenza virus strains[c]	Adjuvant[d]
Novartis	PneumoStar[a] SIV	α-cluster H1N1 cluster I H3N2	Immunstar®
Intervet/Schering-Plough Animal Health	MaxiVac Excell[b] 3.0	α-cluster H1N1 β-cluster rH1N1 cluster I H3N2	Emunade®
Intervet/Schering-Plough Animal Health	MaxiVac Excell 5.0	β-cluster H1N1 γ-cluster H1N1 δ-cluster H1N1 cluster I H3N2 cluster IV H3N2	Emunade®
Zoetis	FluSure Legacy	α-cluster H1N1 cluster I H3N2	Amphigen®
Zoetis	FluSure XP	γ-cluster H1N1 δ-cluster H1N1 cluster IV H3N2	Amphigen®
Zoetis	FluSure Pandemic	A(H1N1)pdm09	Amphigen®
Zoetis	FluSure XP	γ-cluster H1N1 δ2-cluster H1N1 cluster IV H3N2 δ1-cluster H1N2	Amphigen®

(a): Pneumostar is the only single-dose SIV vaccine.
(b): MaxiVac Excell is a registered trademark of Intervet/Schering-Plough Animal Health.
(c): Exact strain names and antigen dose are proprietary for most vaccines.
(d): All adjuvants are oil-in-water emulsions, except for Immunstar®, which is water-in-oil-in-water.

APPENDIX B: ESTIMATES OF LIKELIHOODS OF EVENTS

Table 17: Estimates of likelihoods of events based on expert opinion, factors to be considered, and their rationale

Question no	Risk/events	*What is the likelihood that…?*	Facts to be considered	Assessment	Reason	Uncertainty	Reference in opinion
Presence of H3N2v in a human travelling from the USA to the EU							
Presence of H3N2v in a "non-professional" in the pig sector who travels from the USA to the EU							
1a	Contact with infectious pigs	… out of all "non-professional" persons, one will come in contact with an infectious pig in US?	Prevalence in pigs, contact frequency	L	Contact is expected to be occasional, for instance visiting a fair once and by chance	M	Section 5.2. Transmission from pigs to humans
1b	Gets infected	... this person will get infected?	Immunity of person, contact type and intensity, infectious doses	L	Immunity is considered the same for human populations. Low intensity of contact	M	Section 5.2. Transmission from pigs to humans
1c	Travels and remains infectious	… this person will travel from the USA to the EU and remains infectious until a possible visit to a farm?	Infectious period, duration of journey	N	There are many people travelling but there is a short infectious period and it is very unlikely that this person will travel tot he EU within a few days	L	Section 3.2. Clinical signs in humans
Presence of H3N2v in a "professional" in the pig production sector who travels from the USA to the EU (*including people usually attending pig fairs and family members of pig producers, if they come in contact with pigs*)							
1d	Contact with infectious pigs	.. a "professional" will come in contact with an infectious pig in the USA?	Prevalence in pigs, contact frequency	M	It is very likely that there is a direct intense contact; however, the prevalence is unknown and may change	H	Section 5.2. Transmission from pigs to humans
1e	Gets infectious	...this person will get infected?	Immunity of person, contact type and intensity, infectious doses	M	Immunity is considered the same for human populations. Medium intensity of contact	M	Section 5.2. Transmission from pigs to humans
1f	Travels while infectious	... this person will travel from the USA to the EU and remains infectious until a possible visit to a farm?	Infectious period, duration of journey	L	May be more likely than 1c as EU "professionals" who visit the USA may return to the EU a short time after being in contact with the infectious pigs	M	Section 3.2. Clinical signs in humans
Presence of H3N2pM in 1 breeding pig to be exported							
2		… a breeding pig infected with H3N2pM is exported from the USA to the EU?	Prevalence in breeding pigs to be exported, type of surveillance (active/passive), prevention and control (vaccination)	L	According to the data provided from US surveillance there is a low percentage of positive H3N2pM samples	H	Section 3.1.3. Surveillance in swine in the USA
Detection of H3N2pM pig at farm level (US) and response							
3a	Clinical signs	… the breeding pig for export, infected with H3N2pM, has clinical signs?	Age, infectious dose, viral load, immune status, infection pressure, etc.	L	It is known that the majority of the cases are asymptomatic	L	Section 4.1. Pathogenesis of swine influenza in general

Question no	Risk/events	*What is the likelihood that…?*	Facts to be considered	Assessment	Reason	Uncertainty	Reference in opinion
3b	Identification of animal by vet	… the infected breeding pig with clinical signs is identified at the farm level of the exporting country?	Awareness of farmer and veterinarian at the farm level	H		L	No references
3c	Effective measures	… the identified infected breeding pig will not be allowed to be exported?	Biosecurity measures in place, contingency plan	H		L	No references
Detection and response at BIP (Boarder Inspection Post) at the Importing country							
4a	Clinical signs	… an imported breeding pig, infected with H3N2pM, has clinical signs?	Age, infectious doses, viral load, immune status, infection pressure, etc.	L	The majority of the cases are asymptomatic	L	4.1. Pathogenesis of swine influenza in general
4b	Identification of animal by vet	… the infected breeding pig with clinical signs, it is identified at the BIP of the Importing country?	Awareness of Inspector at the BIP	H		L	No references
4c	Sample sent to lab (H3N2pM)	… from that pig with clinical signs, a sample is properly taken and sent to a lab?	Sampling protocols, laboratory capacity	L	BIPs that are approved for ungulates should have facilities to house a consignment in case it needs to be detained and have possibilities for further examination and testing. However, currently, no sampling is done for H3N2pM	L	No references
4d	Positive result	… a positive result is obtained from a sample taken from that identified infected breeding pig?	Test characteristics	L	BIPs that are approved for ungulates should have facilities to house a consignment in case it needs to be detained and have possibilities for further examination and testing. However, currently, no sampling is done for H3N2pM	L	No references
4e	Effective measures	… the identified infected breeding pig will not be allowed to move to farm?	Legislation on imports	L	BIPs that are approved for ungulates should have facilities to house a consignment in case it needs to be detained and have possibilities for further examination and testing. However, currently, no sampling is done for H3N2pM	L	No references
Imported pigs with a separation period at the first holding of 30 days before joining the other animals of the first holding—scenario 1							
5	Pig remains infectious after separation period	… the influenza strain from the infected imported pigs is not cleared when the imported animals are initially separated before joining the holding at the first holding (duration of separation is 30 days)?	Biosecurity measures in place for pig diseases	N	Farmer decides to separate imported pigs by applying high biosecurity measures, ensuring that the imported pigs are always maintained in separation without direct or indirect contact with other animals, preventing the transmission of diseases. The infection will be cleared from the	H	No references

Question no	Risk/events	*What is the likelihood that…?*	Facts to be considered	Assessment	Reason	Uncertainty	Reference in opinion
					imported breeding pigs hat were set apart		
First pig holding exposed to H3N2v by humans							
6a	Non-professionals in contact with pigs	… a non-professional travelling from the USA immediately comes in contact with the pigs in the first farm?	Biosecurity measures in place, contact pattern	L	As per 1c	H	No references
6b	Professionals in contact with pigs	… a human travelling from US gets immediately in contact with the pigs in the 1st farm?	Biosecurity measures in place, contact pattern	L	Awareness in biosecurity and good farm practice	H	No references
Effective immune response to infection							
7	Effective immune response to infection	… that non-infected pigs that are exposed to H3N2pM have enough immunity to avoid the infection?	Known preimmune status of the EU pig population, herd immunity, cross-immunity with other SIV virus including H3N2 or vaccine virus,	L	There is a big variation in the prevalence of EU H3N2 SIV (can vary from none to low). In some MS it is not circulating. Vaccination status is also very variable. Previous contact with H3N2 combined with vaccination gives the best possible protection. The naivety and immunity situation may vary among European countries	M	Chapter 6, Table 1: Overview of swine influenza viruses subtyped in 13 European countries from November 2010 to October 2012
Detection of first infected holding and response during (*residency period at the holding of destination—pigs must be conveyed without delay to the holding of destination where they shall remain for a minimum period of 30 days before further movement outside the holding*)							
8a	Clinical signs	… that one breeding pig infected with H3N2pM has clinical signs in the 1st farm?	Virulence of H3N2pM	L	The majority of the cases are asymptomatic	L	4.1. Pathogenesis of swine influenza in general
8b	Identification of animal by vet	… that the infected breeding pig with clinical signs, it is identified in the 1st farm?	Awareness of vet at the farm and farmer ,	H	Farmer is aware of clinical signs and may call the vet	M	No references
8c	Sample sent to lab (H3N2pM)	… that from that pig with clinical signs, a sample is properly taken and sent to a lab?	Sampling protocols, laboratory capacity,	M	Vet takes samples	M	No references
8d	Positive result	…that a H3N2pM-positive result is obtained from a sample taken from that identified infected breeding pig?	Test characteristics	L	Influenza A is screened initially, no further typification	M	No references
8e	Effective measures	…that the identified infected breeding pig will not be allowed to further spread the strain?	Biosecurity measures in place, contingency plan	L	Animals may spread the virus to other farm	M	No references
Infected pigs move to 2nd holding							

Question no	Risk/events	*What is the likelihood that…?*	Facts to be considered	Assessment	Reason	Uncertainty	Reference in opinion
9	Infected pigs move to second holding	... that a second farm is exposed to a pig that has been infected in the first farm?	Biosecurity measures in place, movement pattern, residency period in place in the first farm	H	The second farm may have any level of biosecurity implemented A lot of animal movement is assumed	L	No references
Effective immune response to infection							
10	Effective immune response to infection	… that non-infected pigs of the second farm, that are exposed to H3N2pM, have enough immunity to avoid the infection?	Known pre-immune status of the EU pig population, herd immunity, cross-immunity with other SIVs including H3N2 or vaccine virus	L	There is a big variation in the prevalence of EU H3N2 SIV (can vary from none to low). In some MS it is not circulating. Vaccination status is also very variable. Previous contact with H3N2 combined with vaccination gives the best possible protection.	L	Chapter 6, Table 1: Overview of swine influenza viruses subtyped in 13 European countries from November 2010 to October 2012

GLOSSARY AND ABBREVIATIONS

BAL	Bronchoalveolar lavage fluid
Genetic constellation	Set/combination/constitution of genes and/or mutations and the interaction between these components
H3N2pM	Represents the US swine TR H3N2 virus which has reassorted with the pandemic A(H1N1)pdm09 virus from which only the M gene has been acquired. It represents one of the rH3N2p genotypes isolated from swine. The H3N2pM isolates carry seven genes from TR H3N2 and only the M gene from A(H1N1)pdm09
H3N2v	Represents this porcine H3N2pM virus strain once it has been transmitted from pigs to humans and has been isolated from infected humans. The gene constellation of A H3N2v is thus considered as being the same as H3N2pM
HA	Influenza A RNA segment that encodes haemagglutinin
M	Influenza A RNA segment that encodes two matrix proteins (M1 and M2)
NA	Influenza A RNA segment that encodes neuraminidase
NP	Influenza A RNA segment that encodes nucleoprotein
NS	Influenza A RNA segment that encodes two distinct structural proteins (NS1 and NEP)
PA	Influenza A RNA segment that encodes an RNA polymerase acidic
PB1	Influenza A RNA segment that encodes an RNA polymerase basic 1 and PB1-F2 protein
PB2	Influenza A RNA segment that encodes an RNA polymerase basic 2
rH3N2p	Represents all strains isolated from swine and characterized by a genetic constellation derived from the enzootic US swine TR H3N2 genetic reassortment events with A (H1N1)pdm09 (the number of A(H1N1)pdm09 genes contained in these isolates varies according to the genotype). This group includes H3N2pM isolates
SIV	Swine influenza virus
TRIG	Triple reassortant internal gene (cassette). This acronym stands for the internal set of genes (PA, PB1, PB2, NP, NS and M) derived from the original TR H3N2 viruses. This genetic constellation is found combined with various HAs and NAs, forming TR H1N1, H1N2 and H3N2 lineages currently circulating in the pig population in the USA
Triple reassortant (TR) H3N2	Swine influenza viruses originated around 1998 in the USA as a result of reassortment between avian, human and swine influenza viruses. These strains carry the following gene combination: human HA, NA and PB1; swine NS, NP, and M; and avian PB2 and PA