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SCIENTIFIC OPINION

Scientific Opinion on the pandemic (H1N1) 2009 influenza and its potential implications for animal health[1]

EFSA Panel Animal Health and Welfare (AHAW)[2, 3]

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This opinion, published on 7 October 2010, replaces the earlier version published on 4 October 2010[4].

ABSTRACT

Analysis of the recent pandemic (H1N1) 2009 (pH1N1) virus indicates a probable origin in pigs. However, it was not reported in pigs prior to its detection in humans. Several cases of pH1N1 virus infections in animals have been reported, mainly in pigs but also in other animals including turkeys. Occasionally, pigs have been infected following exposure to pH1N1 infected humans. In pigs, a subclinical course was common and when clinical signs were seen (coughing, fever) they were generally mild. Presently, the clinical impact of pH1N1virus on the EU pig population is considered minimal. In poultry, outbreaks of pH1N1 have been reported only in turkey breeder flocks. So far, there is no evidence that pH1N1 virus is able to spread horizontally among turkeys. Awareness should be raised about the risk of infecting breeder turkeys with pH1N1 virus during artificial insemination. To date, no infection of wild birds with pH1N1 virus has been reported. From an animal health perspective, no specific disease control measures are considered necessary. Vaccines based on the pH1N1 virus appear to induce protection in swine similar to that induced by the existing swine influenza virus (SIV) vaccines. Such vaccines efficiently prevent disease by reducing virus replication in the lungs. However, voluntary vaccination of swine with these vaccines has not halted the circulation of SIV in swine. There is no urgency for vaccination of pigs against pH1N1 virus. Currently, no vaccines against H1 viruses for poultry are available but at present, there is no need to vaccinate poultry against pH1N1 virus. Monitoring of circulating influenza viruses in swine and poultry populations should be instigated to monitor the evolution of the pH1N1 virus including changes in virulence.

KEY WORDS

Pandemic H1N1, pigs, poultry, turkey, vaccines, control measures, virus evolution.

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1 On request from European Commission, Question No EFSA-Q-2009-00935, adopted on 9 September 2010.

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4 In 10 August 2010, WHO announced that the H1N1 influenza virus has moved into the post-pandemic period.

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SUMMARY

Following a request from The European Commission, the Panel on Animal Health and Welfare was asked to deliver a scientific opinion on the pandemic (H1N1) 2009 influenza and its potential implications for animal health and thus:

1. To assess the significance for the health of animals of different species (specially pigs and different poultry species) of the occurrence of pandemic (H1N1) 2009 influenza virus in the EU and elsewhere;

2. To assess the implications and consequences of the possible evolution of the pandemic (H1N1) 2009 influenza virus on animal health;

3. To assess the effectiveness and efficiency of disease control options such as establishing animal movement restrictions in protection and surveillance zones, culling of infected pig herds and contact herds for pandemic (H1N1) 2009 influenza virus, as it is common practice for notifiable diseases, e.g. CSF, AI, FMD;

4. To assess the risk that animals from a herd/ flock which was infected with pandemic (H1N1) 2009 influenza virus spread the virus after the last clinical signs of disease have been observed;

5. To assess the possibility, efficacy and efficiency of vaccination, using existing vaccines or newly developed vaccines against pandemic (H1N1) 2009 influenza virus, in pig and poultry populations also in relation with possible evolution of variants of influenza viruses posing a serious risk to public and animal health;

6. To assess the role of wildlife, in particular wild boar and wild birds in the epidemiology of pandemic (H1N1) 2009 influenza virus, if any.

Analysis of the recent pandemic (H1N1) 2009 (pH1N1) virus shows that it contains gene segments from pig, bird and human influenza viruses in a combination that has never been observed before. It appears probable that the pH1N1 virus originates from pigs, however, the pH1N1 virus has not been detected in pigs prior to its emergence in humans.

In addition to human infections, several cases of pH1N1 virus infections in animals have been reported worldwide, predominantly in pigs but also in other animals including turkeys and cats.

Occasionally pigs in the field have been infected following exposure to pH1N1 virus infected humans. Virus spread between and within herds has been observed, but the prevalence of pH1N1 in the swine populations worldwide is not known as no comprehensive epidemiological surveillance has been performed except in Norway.

In field infections a subclinical course was very common, and when clinical signs were seen (coughing, fever), they were generally mild, the morbidity was low, and there was no mortality

Pig to pig transmission passages with the pH1N1 virus have occurred but no increase in virulence of the virus has been observed, even in herds with naïve pigs.

At present, the overall impact of pH1N1virus for the health of the EU pig population is considered minimal, and there is no indication that the situation is different elsewhere.

Pathogenic features of pH1N1 virus in experimentally inoculated pigs indicate that the infection is purely of a respiratory nature and shows a course similar to that of the endemic swine influenza viruses (SIVs) currently circulating in the swine populations worldwide. Thus, clinical signs, in experimentally infected pigs, are variable but relatively mild with fever, coughing and inappetence.

In poultry, outbreaks of pH1N1 have been reported only in turkeys specifically in breeder flocks. The most likely cause of outbreaks in turkey breeder flocks is transmission of pH1N1 virus from infected poultry workers carrying out artificial insemination. Currently, there is no evidence that pH1N1 virus is able to spread horizontally among turkeys within a flock. Drop in egg production and decreased shell quality are the main clinical signs of pH1N1 virus infection of turkeys.

Turkeys, chickens and ducks are refractory to experimental infections with pH1N1 virus via the respiratory tract. However, turkeys can be infected experimentally with pH1N1 virus by the intrauterine and intracloacal route.

From the animal health point of view, no specific control measures against pH1N1 are considered necessary.

The use of clinical signs as temporal proxy for termination of virus excretion within an infected epidemiological unit is of little value in either pigs or poultry. In these species the duration of virus excretion is not sufficiently associated with the appearance of clinical signs to allow epidemiological decision making based on their temporal order of occurrence. In consequence, using the time of cessation of clinical signs and a preset time interval at the level of epidemiological units to establish clearance from infectiousness (virus shedding) is lacking any scientific basis.

Immunity resulting from vaccination of pigs with SIV vaccines existing on the European market will provide some extent of cross-protection against infection with the pH1N1 influenza virus but specific pH1N1 vaccines will offer superior protection. Such vaccines will significantly reduce or even completely prevent pH1N1 replication and disease in the individual animal.

At present, from the available data, the epidemiological situation of pH1N1 in pigs does not justify their vaccination with pH1N1 vaccine. Vaccination on a voluntary basis will likely protect the vaccinated animals but it will not prevent the spread of the pandemic H1N1 virus in swine populations, unless sufficient proportion of farms coverage is reached.

Currently, no vaccines against H1 viruses for poultry are available.

Wild boar may be susceptible to pH1N1 but, if so, they are not expected to play any significant epidemiological role. No pH1N1 virus infections have been reported in wild boar or in wild birds despite the extensive surveillance programmes for influenza viruses conducted since the start of the H5N1 epidemic in poultry in 2004.

RECOMMENDATIONS:

- Place strong emphasis on information to (a) increase disease awareness and (b) ensure that biosecurity is implemented to contribute to the reduction of potential spread of pH1N1 within and between animal units and also from humans to animals and back.

- Awareness should be raised about the risk of infecting breeder turkeys with pH1N1 virus during artificial insemination. Specific guidelines should be developed to lower the risk of transmission of pH1N1 during AI.

- Clinical signs are not reliable as a basis to decide on the end of an infection with pH1N1 virus in an infected herd/flock because pH1N1 induced signs are variable, non-specific or absent. Therefore, when the health status in regard to excretion of pH1N1 virus from a farm/flock needs to be known, it is recommended to test a number of nasal/oro-pharyngeal swabs (swine) or oropharyngeal and cloacal swabs (poultry) according to the expected within herd/flock prevalence for pH1N1 virus by a specific pH1N1 PCR. Testing should start 14 days after the diagnosis is established and continue at 2 week intervals until no excretion of virus can be demonstrated. In pigs, the focus should be on animals of 8-12 weeks of age.

- Inclusion of diagnostic procedures for the detection of pH1N1 might be considered in the event of the detection of non-notifiable influenza A viruses in existing syndromic surveillance programmes for H5/H7 in poultry to provide some baseline data should the virus change its tropism and pathogenicity for poultry.

- There is no urgency for vaccination of pigs against pH1N1 virus. It could be useful, however, to have a specific vaccine, based on the pH1N1 virus, in case of change of the epidemiological situation of the virus in the pig population.

- At present, there is no need to vaccinate poultry against pH1N1 virus.

- Monitoring of circulating influenza viruses in swine and poultry populations should be instigated to obtain data to characterize the circulating influenza viruses for further evolution of the pH1N1 virus including changes in virulence etc can be assessed. This information should be shared and analyzed together with similar information from the human health area.

RECOMMENDATIONS FOR FUTURE RESEARCH:

- Available/stored swine influenza viruses detected in surveillance programs in a variety of countries in the ten years prior to the pandemic should be sequenced as far as possible to provide valuable scientific data that may improve understanding of the factors involved and led to the emergence of pH1N1.

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BACKGROUND AS PROVIDED BY COMMISSION

The present influenza pandemic (H1N1) 2009 influenza virus is a new virus subtype of influenza A (H1N1) viruses that spreads from human to human and is causing a human influenza pandemic in accordance with the declaration made by the WHO on June 11 2009.

The pandemic virus contains gene segments from pig, bird and human influenza viruses in a combination that has never been observed before. Apart from humans, the virus has also infected pigs in Canada, Argentina, Australia, Singapore (pigs from Indonesia), Norway, UK (Northern Ireland), the Republic of Ireland and Iceland and turkeys in Chile. The epidemiological situation appears to be in evolution.

In contrast, the classical swine influenza viruses circulate widely in many pig populations around the world, including the EU. In relation to these viruses, a quite comprehensive monitoring programme has been ongoing under EU research programmes in the context of research on influenza viruses. Valuable experience in this regard has been made available through networks of expertise such as OFFLU and research initiatives under a specific call for avian and human influenza: Framework 6 (FP6) Projects, FP7 Projects, and Preparedness and capacity building for emerging epidemics, swine focused projects like ESNIP2 and influenza network enhancing projects such as Flulabnet. The EU has been particularly active on research on human influenza. Some examples are FLUPAN which developed the first candidate H7N1 vaccine and NOVAFLU which developed the computer algorithm now incorporated by WHO in the vaccine strain selection process.

There is no evidence suggesting that the novel virus behaves in pigs in a different way from the other classical influenza viruses of pigs that only cause a mild respiratory disease.

As regards poultry, the pandemic influenza virus was identified in August 2009 in two turkey breeder holdings in Chile. The clinical symptoms had started in mid July with a sudden drop in egg laying and altered egg shells. No increased mortality was observed. Normal egg production was again reached after 20 days of the infection. The symptoms were very much alike an infection with an LPAI virus. By the time of the virus detection in turkeys there had been extensive human to human transmission of the pandemic influenza virus in Chile which makes occasional transmission from man to bird the most likely scenario. Some birds had been in contact with persons with respiratory disease.

Genetic sequencing of the HA gene from the pandemic influenza virus isolated from the turkeys showed 99.5% similarity to the Californian human strain and a 100% match to the human strain currently circulating in Chile. Mutations that might explain an increased capability of the virus to infect turkeys have not been detected, but work to further characterise the virus is needed. No turkey-to-human transmission has been reported so far.

However, the finding of the pandemic influenza virus in turkey holdings in Chile is unexpected as attempts to date in the USA and Europe to infect turkeys experimentally with the pandemic influenza virus have been unsuccessful. The significance of the pandemic (HN) 2009 influenza virus for different animal species remains unclear.

Surveillance for avian influenza is currently carried out in member States (MS) in poultry and wild birds. The objectives for AI surveillance are currently laid down in the official guidelines adopted in 2007 by Commission Decision 2007/268/EC Surveillance in poultry aims in particular at detecting sub-clinical infections with LPAI of these subtypes thereby complementing other early detection systems, in order to determine genetic characteristics of influenza viruses and subsequently preventing possible mutation of these viruses to HPAI. It should be noted that surveillance for HPAI

H5N1 subtype virus in wild bird populations by testing live birds and those found dead has become more important to protect domestic poultry from becoming infected.

In a longer term, there is a need for comprehensive monitoring of influenza virus genotypes to follow the state of play and the emergence and evolution of possible virus reassortants (virus monitoring) in pigs and other animal species, with the final aim to protect public and animal health.

In order to limit the emergence and spread of influenza viruses with pandemic potential in an effective and proportionate way, the risk manager will require a better scientific understanding of influenza viruses and in particular of the underlying factors that most strongly contribute to the emergence on influenza viruses. It is also necessary to develop better methods and criteria to asses the risk such viruses may pose to people and animals.

During recent years it has emerged that cooperation between public health and animal health experts from different fields such as virology and epidemiology is necessary to address such a complex issue. Furthermore, full and immediate sharing of research results and data between the scientific community and health authorities are essential to reap the full public health benefits of these research efforts. Scientific advice and risk assessment provides also for gathering and exchange of relevant information.

In general the potential control measures to be taken in case of pandemic (H1N1) 2009 influenza outbreaks or infection(s) in farms should be proportionate to i) the risk posed by animals, in particular pigs and different poultry species, in the transmission of the pandemic virus to humans, if any, compared to the role played by human-to-human transmission, and ii) the severity of the disease in animals and humans.

From an animal disease control point of view it is considered that certain movement restrictions should be implemented for animal showing signs of influenza such as clinical respiratory illness. The main measure should be the movement controls of live animals to the other farms. The farm movement controls (quarantine) should be in place until a certain number of days (i.e. seven) after the last clinical signs of disease have been observed in the epidemiological unit and influenza is no longer considered a veterinary risk.

Pre-existing immunity induced due to a previous influenza infection of following conventional influenza vaccination may not protect animals and specially pigs against the infection with pandemic influenza virus, but it is not excluded that it may provide partial protection. Partial protection has been observed in some experimental studies with piglets having maternal antibodies but not with sufficient challenge studies to provide confidence in these findings. Vaccines currently used in the EU or elsewhere to protect pigs against influenza may not be effective against the pandemic influenza virus. Therefore it is unclear weather vaccination is an appropriate tool to control pandemic (H1N1) 2009 influenza virus in different animal species.

As regards food safety, the statements made by the OIE/WHO/FAO/WTO/ ECDC/EFSA adequately address the issue of safety of meat such as pork and pork products for human consumption in relation to influenza.

However, the Commission is in need of further scientific advice and risk assessment, as regards of the pandemic (H1N1) 2009 influenza virus in animals.

EFSA Reply letter

Given that the original nine terms of reference (ToR) of the mandate were very comprehensive and also required different kinds of expertise, it was considered to be more efficient to split the mandate in two and to allocate the tasks to two working groups according to expertise and urgency. The first mandate, considered more urgent, dealt with this opinion replied to the six ToR presented below. The second mandate should cover those proposed ToR having a longer perspective [5]will be dealt with separately. EMEA was invited to collaborate when responding to ToR 5.

TERMS OF REFERENCE AS PROVIDED BY COMMISSION

1. To assess the significance for the health of animals of different species (specially pigs and different poultry species) of the occurrence of pandemic (H1N1) 2009 influenza virus in the EU and elsewhere;

2. To assess the implications and consequences of the possible evolution of the pandemic (H1N1) 2009 influenza virus on animal health;

3. To assess the effectiveness and efficiency of disease control options such as establishing animal movement restrictions in protection and surveillance zones, culling of infected pig herds and contact herds for pandemic (H1N1) 2009 influenza virus, as it is common practice for notifiable diseases, e.g. CSF, AI, FMD;

4. To assess the risk that animals from a herd/ flock which was infected with pandemic (H1N1) 2009 influenza virus spread the virus after the last clinical signs of disease have been observed;

5. To assess the possibility, efficacy and efficiency of vaccination, using existing vaccines or newly developed vaccines against pandemic (H1N1) 2009 influenza virus, in pig and poultry populations also in relation with possible evolution of variants of influenza viruses posing a serious risk to public and animal health;

6. To assess the role of wildlife, in particular wild boar and wild birds in the epidemiology of pandemic (H1N1) 2009 influenza virus, if any.

[5] 1.To indicate the most important factors to be monitored in animals that would suggest a risk of emergence of a new pandemic influenza strain;

2.To assess possible options of monitoring for the presence of the most important factors that would suggest a risk of emergence of influenza viruses (potentially leading to a pandemic) in different animal populations, that could act as reservoir, mixing vessels or otherwise contribute to the risk posed to humans and animals by influenza viruses;

3.To assess the possible predictability of the emergence of a new pandemic influenza strain by monitoring the molecular evolution and development of influenza viruses in different animal populations.

ASSESSMENT

1. Introduction

Pigs are an important host in influenza virus ecology since they are susceptible to infections with both avian and human influenza A viruses, often being involved in interspecies transmission, facilitated by regular close contact with humans or birds. This cross species transfer of virus to pigs can lead to co-infections involving swine, human or avian influenza viruses with subsequent opportunities for genetic reassortment (Kuiken et al., 2006; Olsen et al., 2006; De Vleeschauwer et al., 2009) of influenza A viruses and as a result new viruses can emerge.

2. Origin and characterization of virus causing the pandemic (H1N1) 2009

Phylogenetic studies of influenza A viruses have revealed species specific lineages of viral genes and have demonstrated that the prevalence of interspecies transmission depends on the animal species, the virus and its genetic characteristics. The molecular epidemiology of influenza viruses in pigs is complex with differences both at continental level (especially between Europe and North America) but also at regional level within continents. Genotypic characterisation of viruses is therefore essential to understand ecology and evolution of influenza viruses in different animal species and is central to determining the origin of new and emerging strains, such as pandemic (H1N1) 2009 (pH1N1) virus (pH1N1).

Detailed analysis of early isolates of pH1N1 virus revealed that it closely resembled several viruses known to be circulating in pigs and that the initial transmission to humans probably occurred several months before recognition of the outbreak. Genetic analysis of each gene segment revealed that they derived from both North American and Eurasian swine lineages (Garten et al. 2009). The pH1N1 contained a unique combination of gene segments that had not been previously reported. The neuraminidase and M gene segments derived from Eurasian swine viruses, that themselves evolved in pigs following the transmission of the 'whole' avian influenza virus to European pigs in 1979 (Pensaert et al. 1981). The haemagglutinin, nucleoprotein and non-structural gene segments derived from a classical lineage known to be contemporaneously circulating in pigs in North America. These classical swine viruses themselves were descendants of the 1918 pandemic H1N1 virus. These classical swine viruses underwent a series of reassortant events during the late 1990s and early 2000s in North America leading to a wide genetic diversity resulting in the so called swine 'triple reassortant' lineage becoming established that comprised genes from both classical swine, avian and human viruses. These triple reassortant viruses derived PB2 and PA gene segments from avian viruses and PB1 from a human virus. The so called 'TRIG' cassette of genes (PB1, PB2, PA, NP, M & NS) commonly found in these contemporary North American viruses (Zhou et al.1999) minus the M gene forms the backbone of the pandemic virus.

In addition, pH1N1 shows genetic and antigenic distance in all gene segments of the virus, compared to contemporaneous strains (Smith et al. 2009). It has been postulated that the uncertainty in the phylogenetic estimate for the precise time of origin reveals a long period of un-sampled ancestry of swine viruses before the pandemic in April 2009 and could indicate reassortment of swine lineages may have occurred some years before emergence of the virus in humans (Smith et al., 2009). To date a single influenza virus from pigs has not possessed this unique genotype and so definitive evidence of the occurrence of this strain in pigs prior to the emergence in humans is still lacking. However, a virus containing seven out of eight genomic segments from the pH1N1 virus has been detected in a single 2004 isolate of H1N2 swine influenza from Hong Kong (Smith, et al. 2009).

In conclusion, therefore, on the balance of evidence an origin in pigs appears probable given the unique genotypic characteristics of pH1N1. Furthermore, there are pig populations in the world where contemporaneous circulation of American and Eurasian viruses does occur providing an opportunity for genetic reassortment in pigs. However, it should be noted that definitive evidence for the origin of pH1N1 in pigs may only be gained through analysis of viruses isolated from pigs and archived in the ten years prior to emergence of the pandemic.

The aspects related to the surveillance of possible emerging influenza strains will be taken into consideration by the report replying to the second mandate.

3. Description of the Pandemic H1N1

3.1. Epidemiological overview of the pandemic in humans and likely scenarios

After early outbreaks in North America in April 2009 the new influenza virus spread rapidly around the world. By the time WHO declared a pandemic in June 2009, a total of 74 countries and territories had reported laboratory confirmed infections. To date, most countries in the world have confirmed infections from the new virus.

ECDC has been developing work in order to inform EU stakeholders of the likely scenarios for influenza transmission (pandemic and inter-pandemic) in Europe in the immediate future (2010/2011), with substantial input from its Advisory Forum, other European experts and WHO ("ECDC Forward look risk assessment for the 2009 pandemic influenza A(H1N1) and future influenza season", ECDC, March 2010). It has also identified the further information that needs to be gathered through surveillance and research in order to determine vaccine strategies. Observations, data, and other information were considered from a number of sources including prior pandemics, the European experience during this pandemic, sero-epidemiology, modelling and especially what happened in the Southern Hemisphere in 2009/10 following their initial autumn/winter wave.

On the basis of the above, it seems unlikely that there will be another spring/summer pandemic wave in Europe unless there are significant unrecognised uninfected populations or the virus changes and becomes more transmissible. According to ECDC, serological surveys (measuring the levels of immunity in the human population) could help reduce this uncertainty. It seems highly likely that even when WHO judged the post-peak and post-pandemic phases to have been reached, Europe will continue to experience low-level transmission and small outbreaks of the pandemic pH1N1 influenza. This is the most likely scenario throughout the whole of 2010. On 10 August 2010 WHO announced that the H1N1 influenza virus has moved into the post-pandemic period. However, localized outbreaks of various magnitudes are likely to continue. However, larger outbreaks cannot be excluded given the lack of information from seroepidemiology.
ECDC anticipates that epidemic transmission of the pandemic virus is highly likely in the next (2010/2011) winter season, at least in very young children and other susceptible individuals. It is also most likely that pH1N1will become the dominant virus in humans in the coming winter season along with influenza B viruses, though the presence of influenza A(H3N2) viruses as well cannot presently be excluded. By then Europe will probably be referring to this combination as the 'new seasonal influenza'.

There is currently no evidence of increased virulence of the circulating pandemic influenza virus, however influenza viruses are notorious for their unpredictability and so this forward look risk assessment must not be seen as representing anything more than the most likely scenario. It will also be updated as relevant and significant data become available.

3.2. Cases of transmission reported between human and animals

Several cases of pH1N1 virus infections in animals have been reported worldwide. These were predominantly documented in pigs but also incidentally reported from turkeys and cats (Hofshagen et al., 2009; Howden et al., 2009; Mathieu et al., 2010; Löhr et al., 2010; Pasma et al., 2010; Pereda et al., 2010; Sponseller et al., 2010; Song et al., 2010). Infections in other species (cheetah, ferrets and dogs) have so far been mentioned only in other media (meetings, internet). Table 1 summarizes the pH1N1 infections in different species that were officially recorded to the OIE and/or EU.

Occasionally, pigs in the field have been infected following contact with humans showing influenza-like symptoms. Influenza infections in these contact persons, however, which probably transmitted the infection to pigs, have only been laboratory confirmed in relative few individual cases, e.g. in Norway, Australia, United Kingdom, (Eurosurveillance, 2009, online). The zoonotic transmission of a virus from animals to humans is in this case reversed, which led to the term 'reverse zoonosis' as a result of virus transmission events from humans to animals. It can not be ruled out that in addition to humans displaying clinical symptoms also subclinical infections in humans resulted in incidental infections in pigs. Furthermore, there is a report from Ireland about 2 veterinarians showing influenza-like illness two days after sampling in a pH1N1 infected pig herd. Both have since been confirmed positive for pH1N1 virus (OIE, 2009, online). Currently comparative sequence analyses of pH1N1 viruses detected in humans and pigs show very high homologies. Sequence analysis can at the moment give no additional information on the source of cross-species transmission of pH1N1 between humans and animals in either direction. After introduction of pH1N1 into pig populations, pig to pig transmission seems to be the main route of virus spread (Reports on further transmissions of pH1N1 virus from humans to pigs are given in 4.1.2.1.). In addition, epidemiological investigations suggest humans (farm workers) as the likely source of pH1N1 infection in turkey breeder flocks (see section 4.2.2.).

Table 1: First Occurrence of Pandemic (H1N1) 2009 Influenza infections in Different Animal Species Reported to the OIE and/or EU (until July 2010)

Country	*Date of first notification*	*Reported to*	*Animal species (number of herds/flocks; number of cases for dogs and cats)*
Argentina	25/06/09	OIE	Pig herd (2)
Australia	31/07/09	OIE	Pig herd (1)
Canada	02/05/09	OIE	Pig herd (several)
	23/10/09	OIE	Turkey breeding flock (1)
Chile	21/08/09	OIE	Turkey breeding flock (2)
China (People's Rep. of)	17/12/09	OIE	Pig herd (1)
	17/12/09	OIE	Dog (2)
Chinese Taipei	05/11/09	OIE	Pig herd (1)
Denmark	11/01/10	OIE	Pig herd (4)
Finland	30/11/09	EU	Pig herd (1)
France	19/01/10	OIE and EU	Turkey breeding flock (1)
Germany	10/12/09	OIE and EU	Pig herd (1)
Iceland	27/10/09	OIE and EU	Pig herd (2)
Indonesia	26/11/09	OIE	Pig herd (1)
Ireland	29/09/09	EU	Pig herd (2)
Italy	04/12/09	OIE and EU	Pig herd (4)
	22/12/09	OIE and EU	Cat (1)
Japan	21/10/09	OIE	Pig herd (2)
Mexico	10/12/09	OIE	Pig herd (1)
Norway	12/10/09	OIE and EU	Pig herd (around 80)
Russia	24/12/09	OIE	Pig herd (1)
Serbia	27/01/10	OIE	Pig herd (1)
Thailand	17/12/09	OIE	Pig herd (1)
United Kingdom	18/09/09	OIE and EU	Pig herd (19)
USA	03/11/09	OIE	Pig herd (1)
	30/11/09	OIE	Turkey breeding flock (1)
Korea	23/12/09	OIE	Pig herd (20)

4. Influenza in animals

4.1. Influenza in Pigs

4.1.1. Epidemiology of endemic SIV in pigs in EU

Influenza viruses of H1N1, H3N2 and H1N2 subtypes are endemic in swine populations worldwide, but there has been a clear genetic distinction between North American and Eurasian lineages of swine influenza viruses (SIVs) (Olsen et al., 2006). The predominant H1N1 viruses in Europe have an entirely avian genome and were introduced from wild ducks to pigs in 1979 (Pensaert et al., 1981). These "avian-like" H1N1 viruses have established a stable lineage and are currently co-circulating with H3N2 and H1N2 SIVs. The European swine H3N2 viruses contain haemagglutinin (HA) and neuraminidase (NA) genes similar to those of A/Hong Kong/68-like human influenza viruses, while the genes encoding internal proteins are of avian-like swine H1N1 origin. The dominant H1N2 viruses are considered triple reassortant viruses between a human H1N1 virus from the 1980s from which they obtained the HA protein, the swine H3N2 virus from which they obtained the NA and the avian-like swine H1N1 virus, from which they inherited their internal genes (Brown et al., 1998). A serological survey study in unvaccinated sows in 2002-03 demonstrated high (≥30%) to very high (≥50%) seroprevalence to each of the 3 SIV subtypes in swine-dense regions of Belgium, Germany, Italy and Spain, except for a lower H1N2 seroprevalence in Italy (Van Reeth et al., 2008). In addition, most sows in these countries with high pig populations had antibodies to two or three subtypes. In Ireland, the Czech Republic and Poland, in contrast, H1N1 seroprevalence were lower (8-11.7% seropositives) and H1N2 and H3N2 antibodies were rare (0-4.2% seropositives). In North America, viruses of the classical swine H1N1 lineage were the dominant cause of influenza among pigs until the late 1990s. Beginning in 1998, H3N2 viruses with genes of classical swine, avian and/or human origin became established in the swine population. These viruses further reassorted with classical swine H1N1 viruses, leading to H1N2 and reassortant H1N1 viruses and resulting in a very complex picture (Vincent et al., 2008). Thus, the SIVs in Europe differ significantly in their antigenic and genetic make-up from those circulating in North America, while still other variants are circulating in various Asian countries (Brockwell-Staats et al., 2009).

It should be mentioned, however, that surveillance for influenza in pigs is voluntary and inconsistent.

4.1.2. Infections with pH1N1 virus in pigs

Though the pH1N1 influenza virus is accepted to have emerged from pigs, it was not reported in pigs anywhere prior to its detection in humans. On the basis of phylogenetic analyses, it has been suggested that the virus must have circulated in pigs and its transmission to humans is the result of genetic events (Smith et al., 2009), the basis of which is not yet understood. This presumably one time transfer and adaptation to humans has resulted in a sustained chain of transmissions in humans leading to the 2009 human pandemic. Transmission to humans has been observed with several of the typical type A endemic swine influenza viruses (SIVs) such as H1N1 and H3N2, which have been endemic in swine populations for many years all over the world. However, this zoonotic feature has been expressed only very occasionally and has been observed in people in direct contact with pigs, most often through professional activities. Also, these swine viruses have failed to spread further between humans (Van Reeth, 2007; Van Reeth and Nicholl, 2009; Myers et al., 2007). So, the pH1N1 virus has been exceptional in that it has shown sustained human to human transmission in contrast to the endemic SIVs. In addition the infection has also been transmitted to other mammalian species and poultry which support a rather unique biological feature of the pH1N1 virus.

The assumed pig origin of the pH1N1 virus has quickly stimulated researchers to perform virus inoculation experiments in swine using isolates obtained from infected humans and special attention has also been given to possible field infections on swine farms. Humans, especially those over 60, regularly have cross-reactive antibodies against pH1N1 while such cross-reactive titres were lower or undetectable in younger age groups before the start of the pandemic (Miller et al., 2010; Labrosse et al., 2010). The emergence of the pH1N1 virus in humans was thus rather visible especially in children and young adults. This epidemiological situation is different in swine where partial cross protection against the pH1N1 virus is demonstrated after infection or vaccination with SIVs such as H1N1 and H1N2 (see.5.1.1. and 5.1.2.). Therefore, contrary to the epidemiological situation in humans, disease outbreaks in swine will be less manifest and circulation of the pH1N1 virus in the swine population, if existing, may only be recognized by coincidence or after systematic and structured surveillance.

The relatively few cases of pH1N1 virus infection in pigs in the field which have been reported worldwide, have offered the possibility to observe the impact of the virus on a limited basis and has raised questions on the possible role of pH1N1 virus infections for animal health and the possible role of the pig as source of zoonotic infections.

In this section of the present report, attention will be given to the effect of pH1N1 in pigs in the field after reverse zoonosis and to pathogenetic aspects after experimental inoculation.

4.1.2.1. Field infections in pigs including clinical signs and epidemiology

Reverse zoonosis i.e. spread of H1N1 virus from infected humans to pigs has been reported.

In the first stages of the pandemic in humans and when the first pig herds were infected, epidemiological data and field observations had shown that humans were the most likely source of the infection of pigs herds with no other sources of infection identified. However, from the phylogenetic point of view, the direction of transmission of the viruses isolated from pigs and humans was not definitely established.

The first field infection in swine was diagnosed in Canada but such infections were later also reported in many different countries including Argentina, USA, Japan, Ireland, Iceland, Australia, Singapore, UK, Taiwan, Indonesia, Finland, Mexico, Germany, Denmark , Norway and others (see Table 1). In many of these countries, the infection in swine has been detected either by coincidence or via an epidemiological link when pig caretakers were ill with pandemic flu. Some cases were detected by surveillance or by suspicion and no link with ill persons was established such as in farms where pigs showed acute influenza like clinical signs despite previous vaccination against the endemic SIVs. Detailed reports of the clinical disease are often not given. An outbreak which occurred in Manitoba Canada in February 2010 and in which no epidemiological association with infected humans was described, can be given as example (Pig Progress, 2009, online). The pH1N1 virus infection was first suspected in a sow barn when sows that had been vaccinated against common strains of influenza, exhibited influenza-like symptoms and pH1N1 virus were identified as the cause. The herd where the virus has been diagnosed experienced a very mild disease with pigs showing only slight signs of respiratory illness, mild cough and nasal discharge, decreased feed intake and rectal temperatures up to 40.5°C. No deaths were reported and animals recovered without further problems within 4 to 7 days after the onset of the illness. The infection subsequently moved to production units with nursery, feeder and finishing pigs. Biosecurity protocols were installed to reduce the possible spread into the barn and between barns and measures were taken to safeguard the health of pig producers and animal caretakers in the herd. It is interesting that the Canadian Food Inspection Agency agreed that farms where pigs have been diagnosed with pH1N1 virus infection do not require quarantine or culling of pigs. The reason was that the pandemic virus was not considered to behave differently in pigs than

other influenza viruses commonly detected in swine herds and that there was no evidence that pigs play a significant role in the spread of the virus in the human population.

The introduction of pH1N1 virus in Norwegian pig herds is rather interesting since the Norwegian pig population has been, prior to this outbreak, free from H1N1 swine influenza viruses (Lium et al., 2010).The first Norwegian report dates from November 12 (Hofshagen et al., 2009). In October 2009, coughing was observed in a sow in a pig herd of 85 sows and 850 growers and fatteners. No other signs were observed on the farm. A farm staff member had been ill with influenza-like symptoms diagnosed as pH1N1 before the pigs showed clinical signs. The pH1N1 virus was suspected and virus isolated from 12 of 20 pigs tested. Another herd in the proximity with only fattening pigs was infected soon thereafter presumably by an animal handler working on the first infected farm and this second infected herd was depopulated. Four more herds tested positive in the next few days. Surveillance programs were set up and, by the end of October, a total of 23 herds were found to be positive for pH1N1, 20 of which had confirmed or suspected contacts with humans with influenza-like disease while in 3 others the origin of infection was unclear. Clinical signs were described as moderate in some herds (fever, coughing) and mild to non-existing in others. According to the report (Hofshagen et al., 2009), all the initially infected herds had been in contact with humans with influenza like symptoms prior to the onset of disease in pigs. There was, in the early stage, no evidence of infection through pig-to pig contacts or fomites and the possibility of airborne infection was ruled out due to long distances between the first and the second round of infected farms. These observations therefore suggested that humans infected by pH1N1 virus were the likely source of infection for the pigs.

In a recently published annual report by the National Veterinary Institute in Norway, in which the results of the 2009 national surveillance and control programme for specific viral infections in swine were presented, it was reported that a total of 20 swine herds were positive for antibodies against pH1N1 virus. Also, other 71 swine herds were positive for such antibodies in a targeted surveillance programme carried out between October 10 and December 31 2009 (Lium et al., 2010). These data show that pH1N1 virus, by the end of 2009, had become established in swine herds in Norway. The report stated that it remains to be seen whether the virus will become endemic in the Norwegian swine population.

Recently, the clinical, epidemiological, and virological findings from the first three pig farms found to be infected with pH1N1 virus in England were reported in detail (Williamson et al., 2010). The truck driver was the suspected source of virus in a breeding farm which was detected first. Infected pigs from this farm appear to have introduced the virus into 2 nursing-finishing farms. The infection spread readily from one pig source to another and, in one of the 2 nursing-finishing farms, infection was detected for an 8 week period. Active infection in the first breeding farm was present over at least a seven-week period. These cases showed that pH1N1 virus, after its introduction, can indeed become established on pig farms and spread through movement of pigs. It remains to be determined by structured epidemiological surveys if the virus will become endemic in swine populations as a whole, and in different countries or continents.

In conclusion, the impact of the infections with pH1N1 in pigs is difficult to assess in herds or regions where the endemic SIVs circulate and where vaccination against endemic SIVs is performed since cross-immunity and partial cross-protection are likely to occur. However, the outbreak in Norway in herds with naïve pigs has shown that pH1N1 infection in several herds was subclinical or showed low morbidity (e.g. only one sow with cough in the first detected herd). If clinical signs were seen, they were mild to moderate and characterized by fever and coughing. No mortality has been described. Similar observations were made in other countries (Williamson et al., 2010).

4.1.2.2. Experimental infection in pigs including clinical signs and virus excretion

Six studies have been published in which pH1N1 virus was inoculated in pigs experimentally (Lange et al., 2009; Brookes et al., 2010; Weingartl et al., 2010; Itoh et al., 2009; Vincent et al., 2009, 2010a, b). Only three of the above studies refer to virus excretion (see Table 2). Four of these have studied different pathogenetic aspects of the infection and will be discussed in more detail.

In the study by Brookes et al. (2010) (Table 2) 4 to 5 week old pigs were inoculated by intranasal aerosol with the human California/0709 isolate and followed for clinical signs, virus dissemination and excretion. "In-contact" pigs were followed through 4 successive transmission cycles. Clinical signs were observed both in all inoculated and in all the contact pigs. They were generally mild (range mild to severe at the level of individual pigs), characterized by fever, nasal and ocular discharge, coughing, lethargy and inappetence. Clinical signs in individual animals lasted up to about 6-8 days post inoculation (dpi). Virus was detected by isolation in turbinates, nasopharynx, lung lobes and occasionally in respiratory associated lymph nodes but not in plasma, spleen, liver, kidney, ileum and muscle. Viral RNA was found consistently in nasal, ocular and oral swabs and rarely in faecal swabs. Titers and duration of viral excretion are given in Table 2. Nasal swabs in some animals were positive until 15 dpi. Contact animals maintained the virus during the successive cycles and their clinical signs, virus excretion pattern and profile of infection dynamics were similar to those of the directly inoculated pigs. These results indicate that pH1N1 virus would have the ability to become established in global pig populations particularly in immunologically naïve circumstances.

The study performed by Lange et al. (2009) (Table 2) yielded results that were highly comparable to those of Brookes et al. (2010). Here, the human Regensburg/D6/09 pH1N1 isolate was inoculated intranasally in 10 week old pigs and naïve contact pigs were housed in direct contact. Clinical signs were mild, consisting largely of nasal discharge, sneezing and fever and lasted 4 days with a peak at 4 to 5 dpi. Contact pigs became readily infected and showed similar signs of disease. Diarrhoea was observed in some inoculated and in some contact pigs but was not considered as being directly virus induced since pH1N1 virus was not shown to have intestinal tropism. Virus excretion was followed by RT-PCR and virus isolation. Oropharyngeal swabs were consistently positive and excretion lasted until 11 dpi in infected and contact animals as presented in Table 2. No viral RNA was detected in plasma samples. Lesions of bronchopneumonia were observed.

In the Canadian study (Weingartl et al., 2010), (Table 2) 2 different pH1N1 isolates were used the human- derived Mexican isolate and the swine/Alberta OTH-33_2009 isolate which was obtained from pigs that had been infected in the field after contact with infected humans. Pigs were inoculated at 3 weeks of age either intranasally or intratracheally. With both isolates, inoculated animals developed mild clinical signs consisting of sneezing and transient increase in body temperature for 2 to 3 days with much individual variation between animals. Virus excretion was followed by virus isolation and RT-PCR in nasal and pharyngeal swabs. Most pigs started to shed virus at 1 dpi and shedding lasted until 8 dpi by virus isolation and until 9 dpi by RT-PCR and no differences were observed between the 2 isolates (see Table 2).Virus was also demonstrated in lungs. The swine-derived isolate was less frequently isolated from the lungs and at lower titres (peak titre 1.9 log 10 TCID50 per 0.1 g) than the human-derived isolate (peak titre 5.7), whereas RNA copy numbers and numbers of viral antigen positive cells were similar for both isolates. It should also be noted, however, that conclusions about the differences in lung virus titres were based on 2-3 pigs only and that statistical evaluation was not possible. Blood, rectal swabs, muscle and submandibular lymph nodes were negative for viral RNA with both isolates.

Other studies were carried out in the USA (Vincent et al., 2009, 2010a) with pigs inoculated intratracheally (age not mentioned) with either the human pH1N1 California or the human Mexican isolate at a dose of 2 X 5 log 10 TCID50. In a first study, pigs were euthanized at different intervals

until 7 dpi and examined for virus in tissues by virus isolation and RT-PCR (Vincent et al., 2009). Clinical disease was induced in all the pigs but no details were given. Virus was shown in lungs in all the pigs with both methods and in tonsils of only 2 out of 30 pigs by virus isolation only. The single extra-respiratory sample that tested positive by RT-PCR was the inguinal lymph node in only one pig. Serum, spleen, liver, kidney and muscle were negative in all animals. Viral shedding was not studied. In a second study (Vincent et al., 2010a), 4 pigs inoculated with A/California/04/2009 were euthanized 5 dpi for virological examinations of the same tissues as in the first study. All pigs developed clinical signs and elevated rectal temperatures beginning as early as 24 dpi and extending to 4-5 dpi. Clinical signs included lethargy, inappetence, and increased respiration rate and respiratory effort. Virus was first detected day 1, 2 or 3 pi, when both RT-PCR and virus isolation yielded positive results. Only samples from the respiratory tract (lung, tonsil, broncho-alveolar lavage fluid) tested positive by both RT-PCR and virus isolation. The inguinal lymph node from one pig and serum from two pigs were positive for viral RNA only. Macroscopic and microscopic lesions typical of influenza infection were seen in all pigs.

In a very limited study (Itoh et al., 2009), miniature pigs were inoculated with the pH1N1 California isolate and it was only reported that efficient replication was observed in the respiratory tract and that no clinical signs were produced.

The experimental inoculation experiments enable the conclusion that pH1N1 virus readily induces an infection of the respiratory tract in pigs inducing mild clinical signs which last, on the average, not longer than 3 to 6 days. Marked variation in clinical signs was observed between individual animals. Based on the group observations, clinical signs did not appear to differ after nasal-aerosol, intranasal or intratracheal inoculations but groups were rather small to make a solid evaluation and the results of individual pigs were not given. Clinical signs and infection profiles were similar in "in-contact" animals compared to in directly inoculated ones. No mortality was observed and pigs recovered uneventfully. Pathogenesis studies showed that pH1N1 virus typically causes infection of respiratory tract tissues. The virus infects the entire respiratory tract including the lungs and induces broncho-pneumonia. No infectious virus was detected outside the respiratory tract. Virus excretion was consistently detected in nasal, pharyngeal, oral and ocular excretions with highest values obtained for nasal swabs. Nasal excretion was most consistent and sustained and infectious virus was detected until 8 dpi in one study, and even until 11 dpi in another (see Table 2). PCR was positive until 15 dpi.

Table 2: Duration (in days post inoculation) of clinical symptoms and Virus excretion as detected in various types of swabs after experimental infection of pigs with pH1N1 virus

Study	Age of pigs, inoculation route, inoculation dose*	Clinical signs (dpi)	Virus excretion (methods and dpi)			
			type of swabs	method	duration in days post inoculation	peak titre**
Brookes et al. 2009,2010	4-5 wks IN 5.8	7-8	nasal	PCR	1-10 (consistent) 11-15 (intermittent)	3-4 REU 1-2 REU
			oral	PCR	1-7	1.5-2,0 REU
			ocular	PCR	1-13	0.5-1.0 REU
			rectal	PCR	2-5 (rare)	2.3 REU
Lange et al. 2009	10 wks IN 6.0	7	oropharyngeal	VI PCR	3-11 1-11	ND ND
Weingartl et al. 2010	3 wks	3	nasal (s) ***	VI	1-7	5 $TCID_{50}$
	IN or IT		nasal (h) ***	VI	2-8	5 $TCID_{50}$
	5.6		pharyngeal (s)	VI	1-6	3.7 $TCID_{50}$
			pharyngeal (h)	VI	1-6	4.5 $TCID_{50}$
			nasal (s)	PCR	2-9	6-.5 RNA copies
			nasal (h)	PCR	1-9	6.2
			pharyngeal (s)	PCR	1-6	4.5
			pharyngeal (h)	PCR	1-9	5-5.2

IN: intranasal; IT: intratracheal; VI: virus isolation; PCR: real-time polymerase chain reaction; ND: not determined
*inoculation dose in log_{10} TCID50
**virus titres are expressed as log_{10} tissue culture infectious dose 50%(TCID50), amounts of viral RNA are expressed as log_{10} RNA copies or relative equivalent units (REU)
***pigs were inoculated with pH1N1 isolates of swine (s) or human (h) origin

4.1.3. Comparative aspects of pH1N1 virus infection and endemic SIV infections in pigs (particularly swine H1N1)

A brief comparison is made between infections in pigs with the endemic SIVs and pH1N1 virus, based on the information presently available. It can be stated that the infection dynamics upon experimental inoculation are similar for all these viruses. All established subtypes (H1N1, H1N2, H3N2) and genotypes examined cause a typical respiratory infection with no tropism shown for extra-pulmonary organs. Disease signs are mild and transitory. Several factors, even upon experimental inoculation, are known to influence the severity of disease such as route and dose of inoculation, age and physiological status of the animals. Intratracheal inoculation with a high virus dose (7 log_{10} EID_{50}) for example, results in more severe disease characterized by high fever for several days and difficult breathing in all the animals inoculated, which was not always the case in animals inoculated intranasally with a similar dose (De Vleeschauwer et al., 2009). Still, experimental inoculation experiments with pH1N1 virus (Weingartl et al., 2009) in young pigs showed that the infection course and disease were not different with two virus isolates administered either intranasally or intratracheally at a dose of 5.6 log_{10} TCID50. However, in this study, too few pigs were used to allow solid conclusions on disease severity.

Pigs at the end of the fattening period have shown to be more severely sick than young pigs and weight loss can be considerable (reviewed by Olsen et al., 2006).

Also, from a pathogenetic point of view, both the endemic SIVs and pH1N1 virus have a similar strong tropism for all respiratory tissues issues including the lungs with virus production to similarly high titers. Excretion of both viruses mainly occurs via nasal excretions. However, in 2 studies with pH1N1, duration of such excretion lasted until 11 and 15 dpi, but was intermittent during the last days. In the study performed by Lange et al. (2009) virus was detected by RT-PCR and virus isolation in individual infected and in contact animals until 11 dpi whereas virus detection in the second study mentioned above was positive by RT-PCR until 15 dpi. Such a long period of virus excretion has not been described for endemic swine H1N1 virus where maximal duration of excretion has been reported up to 7 dpi (Van Reeth et al., 2006; De Vleeschauwer et al., 2009). More experimental data seem to be necessary to verify whether this reflects a real biological difference between the pandemic and endemic influenza viruses in pigs. Some of the investigations were performed in different laboratories. At least from the study descriptions no obvious methodological differences between the investigations performed with the pandemic and endemic influenza viruses do exist.

Overall, infections with both types of H1N1 virus have a highly similar course in controlled experimental circumstances.

The occasional pH1N1 virus infections described on pig farms in the field, presumably after reverse zoonosis, have been very mild even though different age groups were involved. Interestingly the Norwegian outbreaks occurred in a naïve pig population and remained moderate to mild to subclinical.

Infections with endemic SIVs may sometimes be more severe under field situations, particularly in older fattening pigs and pregnant sows (reviewed by Olsen et al., 2006). Fattening pigs, when experiencing a first influenza virus infection in the second half of the fattening period may be severely sick with fever, inappetence, coughing and severe dyspnoea. Secondary bacterial infections can then play a role in prolonging the disease and mortality of 2 to 3 percent can be encountered. Weight losses may amount to 5 to 6 kilograms. Also, endemic SIVs may be part of the multi-etiological so called porcine respiratory disease complex in feeder pigs (Van Reeth et al., 1996). Sometimes, sows may abort (reviewed by Olsen et al., 2006), not because of possible fetotropic characteristics of SIVs, but very likely as a result of high fever. These severe influenza clinical signs or complications were not reported with pH1N1 in Norway.

4.1.4. Infections with Pandemic Influenza in wild boar

There is currently no knowledge on the prevalence of endemic swine influenza and pH1N1 viruses in wild boar. No experimental studies have been performed on the susceptibility of wild boar to pH1N1.

4.2. Influenza in Poultry

4.2.1. Epidemiology of Avian Influenza virus

Avian influenza viruses can be divided in low pathogenicity or mildly pathogenic (LPAI) viruses that cause mucosal infection of respiratory and or enteric tract and highly pathogenic (HPAI) viruses that cause systemic infections.

In poultry, LPAI viruses cause sub-clinical infections or mild respiratory disease. Decrease in water and feed consumption is another sign of infection but is only apparent when keeping consumption records. In egg laying chicken and turkey flocks, production losses can be severe or flocks never come to maximal production as is common in turkey breeders infected with swine-like influenza viruses

(Hinshaw et al., 1983; Mohan et al., 1981). LPAI infection may contribute to increase in daily mortality.

All HPAI viruses described up to now all are of H5 and H7 subtype although not all H5 and H7 viruses are of high pathogenicity. Sometimes LPAI H5 and H7 evolve into highly HPAI viruses. The mechanism which drives this evolution is unknown but probably is driven by stochastic events with increasing risk when these LPAI viruses are allowed to circulate in poultry. For this reason both LPAI and HPAI H5 and H7 viruses are notifiable. Control measures have been laid down in the Manual of Diagnostic Tests and Vaccines (OIE, 2009, online, Chapter 2.3.4.), the Terrestrial code of the OIE (OIE, 2009, online, Chapter 10.4.) and Council Directive 2005/94/EC (EU, 2005)[6]. Infections of influenza subtypes other than H5 and H7 are not notifiable.

Most of outbreaks of LPAI viruses other than H5 and H7 have a limited spread, remained unnoticed or are not recorded (Alexander, 2006). Moreover, serological surveys that are obligatory in EU member states are directed at detecting H5 and H7 subtype viruses and generic tests that detect antibodies against all influenza A viruses can only be used when properly validated.

Up to now humans have been infected with LPAI and HPAI H5 and H7 viruses and LPAI H9N2 virus, (EFSA, 2008). H9N2 is now endemic in many countries in the Middle East and Asia and has been detected in pigs in China (Cong et al., 2004; Yu et al., 2008).

4.2.2. Field observations including clinical signs and epidemiology

Infection of poultry with human influenza viruses have not been reported before the emergence of pH1N1. Cases of infection of turkeys with H1N1 swine viruses have been reported in the USA (Mohan et al. 1981) and Europe (Andral et al., 1985; Ludwig et al., 1994). Infection of turkeys with swine, H1N2 (Suarez et al., 2002) and H3N2 (Tang, 2005; Choi et al., 2004; Pillai et al., 2009; Kapczynski et al., 2009) viruses were also reported. The clinical signs reported were drop in egg production and or respiratory symptoms and are similar to those found after infection of turkeys with LPAI viruses (Homme, 1970). Where infection of turkeys with swine influenza occurred, no major spread to other flocks was observed. Based on this knowledge it is not very surprising that infection with pH1N1 of poultry species in particular turkeys could occur after exposure to infected workers or pigs. However, up to now only cases of pH1N1 infections of poultry (all in turkey breeding flocks) have been reported in Chile, in Canada, in United States of America and in France. Infection of poultry with pH1N1 is not an OIE listed disease because it is not, at this time, a significant threat to poultry and therefore reporting of pH1N1 virus infections to the OIE is not obligatory although it should be reported as a new and emerging disease. It is unclear whether all countries do report pH1N1 and therefore the actual number of outbreaks may be higher.

On August 21, 2009 Chile reported two outbreaks of pH1N1 in turkeys. The outbreaks occurred on two farms housing turkey breeding flocks. The farms belonged to the same company, and were vertically integrated applying appropriate biosecurity measures. The first outbreak involved a farm with 5 breeding flocks comprising 29782 birds of which 24337 were reported affected. The outbreak started in flock no. 1 and reportedly through horizontal transmission, it reached 3 other flocks. The second outbreak involved a farm of the same company with 5 flocks. Egg production dropped by more than 50%. Egg shell quality was reduced also. The average morbidity was reported as 61.4% (81.7% for outbreak 1 and 41.3% for outbreak 2). The morbidity is based on the egg production and similar

[6] Council Directive 2005/94/EC of 20 December 2005 on Community measures for the control of avian influenza and repealing Directive 92/40/EEC, *Journal of the European Union* L 10, 14.1.2006, p. 16–65.

prevalence is found when serum samples were tested in the laboratory (Mathieu et al. 2010). In the first outbreak neither respiratory signs nor increased mortality were observed. Necroscopy of the affected birds showed salpingitis, peritonitis and an interruption of the follicular development. No other lesions were observed. Samples of embryonated eggs collected at the hatchery scored negative in the real-time PCR. Twenty days after the beginning of the event, a recovery in the laying rate was observed. (http://www.oie.int/wahis/public.php?page=event_summary&reportid=8389).

In Ontario, a grandparent turkey flock was suspected to be infected on October 9, 2009. The farm comprised two barns with in total 7,300 hens. In barn 1 the birds of 52 weeks of age showed a drop in egg production from about 1,800 eggs/ day, to about 300 eggs/day, a drop of about 80%. No spread to other farms was detected. A slight increase in mortality was also reported (about 10 birds over 3 day period) but it is stated that this increase may not be associated with the pH1N1 infections (OIE, 2009, online).

In Virginia pH1N1 virus was detected in a turkey farm. Again the flock was investigated because of a drop in egg production on November 16, 2009. The infection did not spread to flocks in the other barns of the farm (OIE, 2009, online).

Also in France, a turkey flock showing a significant drop in egg production has been observed to be infected with pH1N1. The drop was observed starting on January 11, 2010. The morbidity was reported as 35% which probably is based on the number of animals that stopped laying (OIE, 2009, online).

In summary, all outbreaks occurred in breeder flocks and a drop in egg production and reduced quality of egg shells seems to be the predominant clinical sign that was observed in the field. Where reported, the morbidity, estimated as the percentage of birds that stopped laying, varied between 35 and 82% at flock level (OIE WAHID database). Other signs were not reported or could not be attributed to the infection with certainty.

Epidemiological data of the outbreaks in poultry are scarce. Strikingly, in all cases breeding turkeys were involved suggesting that these type of birds are more susceptible or that handling practices unique to these type of flocks caused the outbreaks (i.e. artificial insemination). In most outbreaks it was assumed that turkeys were exposed to pH1N1 virus-infected workers. Investigation into the possible source of pH1N1 virus infection of the turkey flocks in Chile pointed to workers showing respiratory symptoms although none of the staff working on the farm reportedly showed clinical signs consistent with influenza like illness (Mathieu et al., 2010). Coincidently, in the week before the drop in egg production occurred, the peak of human cases of pH1N1 was recorded in the area where the infected poultry farms are located (in the epidemiological week 28). The secondary spread to other flocks did not occur in Chile. However, both flocks were of the same owner. Viral dissemination might have occurred either from the same infectious source, i.e., a farm worker or through fomites transported between premises (Mathieu et al., 2010). This view is compatible with high degree of homology of all genes of the Chilean turkey and human isolates. Complete genome sequences revealed similarity percentages ranging from 99.7% (PA, NP and M) to 100% (HA).

4.2.3. Experimental infection in poultry

Several groups addressed the question of the susceptibility of major poultry species to the pH1N1 virus and performed challenge experiments with high doses (up to 10^6 EID_{50}) using turkeys (Swayne et al., 2009; Terregino et al., 2009; Russell et al., 2009); chickens, ducks and quails (Swayne et al., 2009). The overall conclusions of all these experiments are that chickens and turkeys are not susceptible to infection via the nasal or oro-nasal route. Swayne et al. 2009 used turkey poults 3

weeks of age and reproductively active turkeys of 73 weeks of age. No virus could be isolated from swabs and none of the turkeys developed antibodies detectable in the HI test. Terregino et al. (2009) used turkeys at the age of 21 and 70 days of age. Birds did not shed virus but some of the younger birds did develop antibodies that were detectable in competitive antibody ELISA and the HI tests.

To determine the virus pathotype, 4-week-old chickens were inoculated intravenously. The intravenous route of infection is routinely used to assess the pathogenicity of avian influenza viruses (OIE, 2009, online). None of the 4 week-old chickens that were inoculated intravenously died or developed disease indicating that the pH1N1 virus is not pathogenic for chickens. This is not a surprising outcome as none of chickens infected by the intravenous or intranasal route developed measurable antibodies suggesting that chickens are resistant to infection. In contrast one of the intranasally infected ducks developed an antibody titre of 1:16 in the HI test. Swabs collected from 2 of 5 quails infected intranasally were positive in virus isolation at day 2 and all 5 at day 4 after infection and these quail seroconverted (Swayne et al., 2009).

The field observation encouraged Pantin-Jackwood et al. (2010) to study whether turkeys could be infected via the intracloacal or intrauterine route. In these experiments the authors confirmed that turkeys were resistant to infection via the intranasal route but could be successfully infected via cloacal and intrauterine route. Birds did seroconvert and developed high antibody titers in the HI test.

4.2.3.1. Clinical signs

Infected quails showed heterophilic to lymphocytic rhinitis. Influenza virus was visualized by immunohistochemical analysis of epithelium and macrophages within the mucosa of the nasal cavity; but neither lesions nor antigen were detected in other respiratory and non- respiratory tissues. Infected ducks and chickens did not develop disease. The clinical signs reported by Terregino et al. (2009) in the 21 day-old infected turkeys could not be attributed with certainty to viral activity of pH1N1 virus.

Turkeys infected via this intrauterine route stopped egg production at day 5 after infection and intracloacally from day 9 after infection. In contrast intranasal infection of turkeys had no effect on egg production or on the clinical condition of the infected birds. Virus was isolated from the oviduct and viral antigen was detected in ovary and luminal epithelium lining the oviduct (Pantin-Jackwood et al., 2010).

4.2.3.2. Excretion of virus

Virus excretion was not demonstrated in turkeys (Swayne et al., 2009; Russell et al., 2009; Terregino et al., 2009), or in chickens, or ducks infected intranasally (Swayne et al., 2009). All infected quails did shed virus via the respiratory tract at day 2-4 after infection at a mean titre of 0.9 log10 and 2.8 log10 EID50/ml. However, the infected quails did not infect in contact naive quails through direct contact. No virus was isolated from and antibodies were not detected in the contact turkeys exposed to infected birds (Swayne et al., 2009, Russell et al., 2009) and quails (Swayne et al., 2009).

When turkeys are infected via the uterus virus can be detected in oro-pharyngeal and cloacal swabs between day 2 to 14 after infection. In contrast only 1 bird that was infected intracloacally shed virus at day 4 only. Unfortunately, contact turkeys were not used and therefore we do not know whether virus shedding is contributing to the bird to bird transmission when considering the resistance of birds to infection via the respiratory tract (Pantin-Jackwood et al. 2010). In the field, virus was detected in swabs from turkeys for 2-4 weeks after egg production began progressively recovering in flocks (Mathieu et al., 2010).

The uterine and cloaca route of exposure is realistic in modern turkey production systems. Current breeds of male turkeys are unable to fertilise females by natural mating because of their large breast muscles. Therefore, female turkeys are inseminated once a week. For artificial insemination, workers pick up hens individually and to locate the vagina they expose the cloaca manually. The straw with semen is than introduced into the uterus. The whole process provides ample opportunities for initiating infection by either large droplet exposure during human sneezing activities or direct inoculation from infectious fomites on contaminated hands. Bird-to-bird transmission could occur through inserting contaminated instruments to the cloaca or reproductive tract by the inseminators. The observations are in agreement with observation in the field that outbreaks are only reported for turkey breeders and the limited secondary spread to other flocks or farms (Mathieu et al., 2010).

4.2.4. Infections with Pandemic Influenza virus in wild birds

Wild birds are the reservoirs of low pathogenicity avian influenza (LPAI) viruses. These LPAI viruses can be transmitted to poultry, and from poultry to other domesticated animals and humans. To assess the risk posed by this wild bird reservoir for human and animal health, it is important to understand the ecology of avian influenza viruses. Therefore surveillance of wild birds is conducted in many regions (Munster, 2009). To date no infection with pH1N1 virus of wild birds has been reported despite several surveillance programmes that are conducted in wild birds in particular since the start of H5N1 epidemic in poultry in 2004. In any case, mammalian viruses (e.g. from swine, human and equine) have never been detected in wild birds, and therefore such a finding would seem exceptional.

4.3. Infections with Pandemic Influenza virus in other species

4.3.1. Infection with pH1N1

Transmissions of pH1N1 virus presumably from infected owners to their influenza naïve indoor cats living in the same households have been reported (Sponseller et al., 2010; Loehr et al., 2010). Family members showed signs of influenza-like illness 4-6 days before the cats developed clinical signs. Infected cats developed disease signs of depression, inappetence and respiratory signs of 4 days duration. From the reports two animals developed fatal pneumonia (Löhr et al., 2010), the other animals recovered from the infection (Sponseller et al., 2010). Cats were of different age and showed a healthy status before the infection. No immunosuppressive virus infections (e.g. FeLV, FIV) have been documented in the affected cats as underlying infection which could have facilitated an increased likelihood for becoming infected. A rather limited study to estimate the prevalence of pH1N1 in cats was performed in France (Pingret et al., 2010). A total of 99 oropharyngeal swab, conjunctival swab, bronchioalveolar fluid and organ samples from cats displaying signs of upper respiratory tract disease and/or acute ocular disease were collected during the peak season of pH1N1 in France and subjected to RT-PCR analysis. None of the samples were found positive.

Similarly to the situation in cats, pH1N1 infections have also been detected in pet ferrets (Slavec et al., 2010). In nine animals clinical signs of dyspnea, naso-ocular discharge, sneezing, coughing or fever were observed for about 3 days. The pet owners showed clinical signs of influenza-like illness seven days before the animals became ill. Pharyngeal swabs, conjunctival swabs and nasal swabs from the pet ferrets were analysed by RT-PCR and found to be positive for pH1N1. All animals survived the infection.

In a serological investigation for pH1N1 specific antibodies which included more than 964 sera from dogs collected just prior to or coinciding with the peak of the pH1N1 epidemic in humans in Italy in 2009, 7 samples (= 0.7%) showed evidence of exposure to pH1N1 (Dundon et al., 2010).

As a first documented infection in wildlife animals the virus has been detected in two striped skunks in Canada (Britton et al., 2010). The animals developed severe fatal interstitial pneumonia. The source of the pH1N1 infection in these animals is unclear. Because the animals had frequent visits to a mink farm on which animals showed nasal discharge, transmission of pH1N1 from farm workers to minks to skunks is a possibility.

Although not officially reported to public authorities and not yet published several additional Pro-med reports of pH1N1 cases in species like ferret, dog or cheetah and cats have been made public on the internet. These are summarized in Table in Appendix 1.

So far no reports on experimental or natural pH1N1 infections in horses have been published.

4.4. Evolution of Influenza in pigs and poultry

Successful cross species transmission of influenza viruses is dependent on both host and virus genetic factors and subsequent spread within the new host population requires a period of adaptation of the virus to the new host (Webster et al., 1992; Kida et al., 1994). The frequent transmission of human influenza viruses to pigs is well documented although the establishment of stable lineages is not always a consequence of such transmission (Brown, 2008; Klenk et al., 2008). However, the close contact between humans or birds and pigs facilitates interspecies transmission. The factors which fully govern whether these viruses can establish stable infection in pigs are unknown but it is likely to be multifactoral, dependent on both host and virus strain. In order to establish stable lineages, newly transmitted viruses need to be able to compete with viruses already endemic in the swine population and therefore by definition highly adapted to produce efficient infection and transmission. Host range is a polygenic trait with compatibility between gene segments in a given host cell. Successful transmission between species can also follow genetic reassortment with the progeny virus containing a gene constellation having the ability to replicate in a new host. Once the virus has successfully transmitted and been maintained within the global swine population, the long term endemnicity of the virus may be facilitated through the continual availability of susceptible pigs. The precise mechanisms whereby an avian or human virus is able to establish a new lineage in pigs remains unknown.

The spread of human pandemic strains to pigs has occurred in three out of the last four pandemics including the present pH1N1. In 1918, the virus associated with the human pandemic H1N1 transmitted to pigs and has been maintained to the present day (classical swine influenza). In 1968 shortly after the appearance of H3N2 in the human population there was spread to pigs at a global level, showing many similarities to events that have occurred since the emergence of pH1N1 in the human population. Very rapidly, the H3N2 virus became well established in pig populations and continues to circulate widely at a global level, including contributing genes to frequent reassortment events leading to the emergence of new virus genotypes which have high efficiency for maintenance in pig populations.

Presently, avian populations including poultry appear largely to provide a host barrier that restricts transmission of pH1N1 to such populations with relatively few occurrences of infection reported in contrast to other hosts such as pigs. In contrast to pigs, historically pandemic viruses have not been described in poultry populations and so adaptation and endemnicity with these viruses has not occurred. However, it should be noted that classical swine H1N1 viruses that have established stable lineages in North American pigs for nearly 100 years have been associated with frequent transfer to poultry populations especially including turkeys (Hinshaw et al., 1983). In addition, periodically spread of other subtypes from pigs such as H1N2 and H3N2 have been reported especially in North America (Suarez et al., 2002; Choi et al., 2004; Tang et al., 2005).

4.5. Evolution of Influenza viruses in some other species

Although infections in species like cats and skunks have been published and additional infections in species like dogs, ferrets, and cheetahs have been reported in other media, the limited epidemiological data on cats (Pingret et al., 2010) and dogs (Dundon et al., 2010) currently suggest that pH1N1 transmissions from humans to these animals and the further spread of the virus in these species does not often occur. Due to the limited efficiency of pH1N1 replication in these animals it seems unlikely that these animal species play a role in pH1N1 evolution.

5. Immunological response and Vaccines

5.1. Pigs

5.1.1. Immunological response against pH1N1 virus after infection with endemic SIVs.

Experimental infection studies have shown that influenza naïve pigs are susceptible to the pH1N1 virus and that the virus readily transmits between such pigs (Brookes et al. 2010, Lange et al. 2009). One crucial question, however, is whether prior infection with endemic European SIVs may offer some cross-protection against the pandemic virus. At the time of writing this report, there were no published data on the extent of cross-protection between enzootic European SIVs and the pandemic virus. But we can extrapolate from a recent experimental study in which pigs infected with a European avian-like H1N1 SIV showed a solid protection against intranasal challenge with a North American triple reassortant H1N1 SIV 4 weeks later (De Vleeschauwer et al., 2010). Most pigs tested negative for the challenge virus in nasal swabs and respiratory tissues, and none had lung lesions. All previously uninfected challenge control pigs, in contrast, showed nasal virus excretion during 6 consecutive days, high virus titres in the entire respiratory tract, and macroscopic lung lesions. The pigs previously infected with the European H1N1 SIV lacked cross-reactive HI antibodies against the North American H1N1 SIV at challenge, but they had low levels of cross-reactive VN and NI antibodies. The pH1N1 virus closely resembles the triple reassortant virus used for challenge in this study, but it is more closely related to the avian-like H1N1 virus in its NA and M genes. It is therefore rational to expect an even more solid cross-protection against the pandemic virus than to North American H1N1 SIVs in response to prior infection with European H1N1 SIV. Very recently, Busquets et al. have examined the extent of protection against challenge with the human pH1N1 isolate A/Catalonia/63/2009 in pigs experimentally infected with an avian-like H1N1 SIV 3 weeks earlier (Busquets et al., 2010). The challenge virus was undetectable in nasal swabs and lungs of these pigs by RT-PCR, whereas all challenge control pigs tested positive. This study shows that prior infection with an avian-like H1N1 SIV provided cross-protection against an infection with the pH1N1 virus.

Cross-protection has also been documented between European H1N1, H1N2 and H3N2 viruses (Heinen et al., 2001; Van Reeth et al., 2003, 2006). These 3 virus subtypes show greater genetic heterogeneity in their HA than the European and North American H1N1 SIVs mentioned above. Consequently, infection with one subtype completely fails to induce HI and VN antibodies against another subtype. Still, experimental studies have shown a partial, though relatively weak, cross-protection against any subtype in pigs previously infected with a serologically distinct subtype. Nasal excretion of the challenge virus was on the average 2 days shorter in such pigs than in influenza naïve challenge control pigs. Furthermore, the extent of cross-protection increased dramatically if pigs were sequentially infected with two European SIV subtypes and challenge with the third, remaining subtype one month later (Van Reeth et al. 2006). These pigs were usually completely protected against nasal virus excretion. All these studies convincingly demonstrate that cross-protection

between SIVs with very distinct HAs a) can occur in the absence of cross-reactive serum HI antibody and b) strongly increases in pigs that have been exposed to multiple SIV subtypes.

The occurrence of serologic cross-reactivity with the pH1N1 influenza virus after infection of pigs with European SIVs has been examined in more detail by Kyriakis et al. (2010a). They used sera from pigs that had been experimentally infected with a single SIV subtype, or with a combination of two SIV subtypes at a 4-week interval. The sera had been collected 3-4 weeks after the last virus inoculation and were examined in HI tests against a pH1N1 virus (A/California/07/09), and related North American H1 SIVs. Antibodies to the pH1N1 virus were undetectable after a single infection with European SIVs, but they were found in all dually infected pigs. Cross-reactive HI antibody titres were highest in the pigs that had been sequentially infected with H1N2 followed by H1N1 (20-160). These data suggest that pigs with infection-induced immunity to two European SIV subtypes may be at least partially immune against the pandemic virus. Conversely, the absence of cross-reactive HI antibodies in pigs that have been previously infected with only one European SIV does not mean that such pigs are fully susceptible to the pandemic virus, because an infection with influenza virus stimulates a complex and broad immune response, including serum antibodies as well as mucosal and cell-mediated immunity. The studies mentioned above further support the notion that a prior infection with SIV may partially protect pigs against an antigenically unrelated SIV in the absence of cross-reactive serum HI antibody

Additional evidence for broad serologic cross-reactivity with the pH1N1 virus in European pigs in the field comes from HI tests on 1,559 pig serum samples from 195 German pig herds collected from mid-June through mid- September 2009 (Dürrwald et al., 2010). Seroprevalence estimates for individual pigs were as high against the pH1N1 virus (52%) as against the avian-like H1N1 (53%) or H3N2 SIV (52%), compared to a 28% seroprovalence against H1N2.

5.1.2. Immunological response and cross-protection against pH1N1 virus by inactivated vaccines based on endemic SIVs

5.1.2.1. Characteristics of existing SIV vaccines and their efficacy against endemic SIVs

SIV vaccines in Europe have been licensed since the mid 1980's. All vaccines contain inactivated whole-virus or split antigens in combination with an adjuvant. Most vaccines are bivalent and contain older or more recent H1N1 and H3N2 influenza virus isolates. Since 2010 a trivalent vaccine including an H1N2 SIV has become commercially available. The protection offered by such inactivated influenza vaccines is almost entirely dependent on serum HI antibody titres, which are a recognized correlate of protection against challenge. Primary vaccination consists of two intramuscular injections 3 to 4 weeks apart and bi-annual booster vaccinations are recommended for sows. An essential difference between influenza vaccines for swine and those for humans is that most SIV vaccines contain potent oil adjuvants. Also, SIV vaccines are not standardized with respect to the substrates used for production (eggs versus cell culture), inactivation methods, antigenic content, and type of adjuvant. Furthermore, SIV vaccine strain composition differs in Europe and the US, because of antigenic and genetic differences in the circulating SIVs. Table 3 presents an overview of the composition of the commercial SIV vaccines in Europe. Unlike for the human vaccines, the strains included in the SIV vaccines have not been regularly updated. Antigenic drift is much slower with swine than with human influenza viruses. Experimental vaccination-challenge studies have shown that vaccines based on H1N1 and H3N2 antigens from the 1970's can still protect against SIVs from the late 1990's, if antibody titres against the challenge virus are sufficiently high (Van Reeth et al. 2001a,b; Heinen et al., 2001). While there has to be antigenic overlap between vaccine strains of SIV and those circulating in the field, experimental data indicate that factors such as the antigenic content

and nature of the adjuvant may be more important for the potency of SIV vaccines than the choice of the vaccine strain (Kyriakis et al., 2010b).

Most SIV vaccine efficacy data are from experimental vaccination-challenge studies in which SIV seronegative pigs are vaccinated twice with commercial vaccine and challenged with a virulent, usually heterologous SIV 2-6 weeks after the second vaccination. The pigs are challenged via the intranasal, aerosol or intratracheal inoculation routes. The typical clinical picture of SI, however, only results when pigs are inoculated with high virus doses ($\geq$ 7.5 $\log_{10}$ EID50) directly into the trachea. Studies with European SIV vaccines mainly used intratracheal challenge and lung virus titres as the major parameter to evaluate protection. Several studies have been performed with the first SIV vaccine on the European market, based on A/New Jersey/8/76 (H1N1) and A/Port Chalmers/1/73 (H3N2). In studies with challenge viruses from the 1980s and 90s this vaccine could either completely prevent virus replication in the lungs and disease, or significantly reduce lung virus replication and thereby prevent disease (Vandeputte et al., 1986; Haesebrouck and Pensaert, 1986; Van Reeth et al., 2001a,b). In a challenge study with a Belgian H1N1 SIV from 2007, the same vaccine conferred a suboptimal protection, whereas two out of 4 commercial vaccines examined significantly reduced virus replication in the lungs (Kyriakis et al., 2010b).

Only one study has evaluated the effect of vaccination on virus excretion (Heinen et al., 2001). Pigs were vaccinated with the New Jersey- and Port Chalmers-based vaccine, and challenged by aerosol with an H3N2 SIV isolated in the Netherlands in 1996. Vaccination strongly reduced virus titres in oropharyngeal swabs: the challenge virus was isolated from only 2 out of 5 vaccinated pigs, at barely detectable levels, for 3 and 2 days respectively. In contrast, virus was isolated from all 5 unvaccinated control pigs during 4 to 6 consecutive days. The vaccinated pigs also failed to transmit the virus to a group of vaccinated in-contact pigs, but transmission from vaccinated to influenza naive pigs was not examined.

In studies with US SIV vaccines, challenge was performed by the intranasal or intratracheal route. In contrast with the European studies, nasal virus shedding is one of the main parameters used to evaluate protection, next to clinical signs and lung pathology, whereas lung virus titres are only rarely examined. Still, independent studies with the same commercial monovalent H1N1 SIV vaccine and intranasal challenge with a classical H1N1 SIV from 1988 yielded conflicting results regarding nasal virus excretion. Excretion was undetectable in the study by Larsen et al. (2001), while there was only a 1-2 $\log_{10}$ reduction in the level of virus shedding in the study by Macklin et al. (1998). The latter findings were in agreement with the initial efficacy trials conducted by the vaccine manufacturer (Brown and McMillen, 1994), in which nasal virus shedding was prevented in only 50% of vaccinated pigs. Virus infection of the lungs, on the other hand, was undetectable in 95% of the vaccinated pigs, and clinical signs and macroscopic lung lesions were also reduced. Kitikoon et al. (2006, 2009) have performed experiments with 2 different bivalent US vaccines, in which the pigs were challenged intratracheally with the same classical H1N1 SIV from 1992. Virus titres in nasal swabs of vaccinated pigs were reduced with one vaccine (Kitikoon et al., 2009) and undetectable with the other (Kitikoon et al., 2006). Both vaccines reduced clinical signs and macroscopic and microscopic lung lesions. Lee et al. (2007) have compared the efficacy of 3 commercial, bivalent SIV vaccines and an experimental homologous vaccine against challenge with an H3N2 SIV from 2004. Only the experimental homologous vaccine completely prevented nasal virus excretion. The suboptimal results with the commercial vaccines were ascribed to the antigenic heterogeneity of the challenge virus.

In summary, most experimental challenge studies with commercial SIV vaccines show a reduction or prevention of clinical signs, with reduced virus replication in the lungs and/or lung lesions. The effect on nasal virus shedding appears to be more variable, ranging from prevention of shedding to a minimal effect or no effect at all. Some studies suggest that inactivated SIV vaccines reduce virus

titres in the lungs to a greater extent than virus titres in the upper airways or nasal shedding (Brown and McMillen, 1994; Lee et al., 2007), whereas the reverse was found in other studies (Kitikoon et al., 2006). In several studies, nasal excretion of SIV was completely blocked in pigs that had been previously infected with live influenza virus, but not in vaccinated pigs (Heinen et al., 2001). This is in line with the fact that inactivated vaccines, in contrast to infection with field virus, fail to induce mucosal IgA antibody and induce serum antibodies only. Unfortunately, true comparative studies of virus replication in the lungs versus the upper airways of vaccinated pigs have not been performed.

In the field the efficacy of SIV vaccination may be hampered by several factors: maternal antibody interference with vaccination, a short duration of vaccine-induced immunity, and insufficient antigenic match between vaccine and field strains.

5.1.2.2. Cross-protection of existing SIV vaccines against pandemic H1N1

The results of vaccination-challenge studies with the pH1N1 virus have been published for SIV vaccines available in North America. The H1N1 components in these vaccines differ from those in most European vaccines and they are more closely related to the pandemic virus. Vincent et al. (2009) have performed efficacy studies with the pH1N1 virus and 3 commercial SIV vaccines available in North America. The vaccines did not induce a complete protection, but 2 out of 3 reduced one or more of the parameters assessed, including clinical signs, lung lesions and challenge virus titres in nasal swabs or bronchoalveolar lavage fluids. The authors conclude that "Based on cross-protection demonstrated with the vaccines evaluated in this study, the US swine herd likely has significant immunity to the 2009 pandemic virus from prior vaccination or natural exposure."

As for the European SIV vaccines, challenge experiments with the pH1N1 virus in pigs vaccinated with European SIV vaccines have not yet been published. As mentioned, however, serum HI antibody titres induced by the vaccines correlate with protection against challenge, and cross-reactivity with the pandemic virus in sera from pigs vaccinated with European vaccines has been examined (Kyriakis et al., 2010a). The pigs had received a double intramuscular vaccination, with a 4-week interval, with 1 of the first 4 vaccines listed in Table 3. HI antibody titres against the pH1N1 virus and related North American SIVs (not further discussed) were determined in sera collected 4 weeks after the second vaccination. Two vaccines induced antibody titres ≥20 to pH1N1 virus in most pigs, which may protect against virus replication following challenge with SIV in pigs (Kyriakis et al., 2010a). Similar results were obtained in another experimental study in which sera collected 7 days after a double vaccination of pigs with commercial European SIV vaccines were examined in VN tests against the pH1N1 virus (Dürrwald et al., 2010). Most but not all vaccines induced antibodies against pH1N1, but not all pigs responded and antibody titres were lower than after vaccination with an experimental pH1N1 vaccine. Several studies point towards a major role of the adjuvant for SIV vaccine potency and serologic cross-reactivity with the pH1N1 virus (Kyriakis et al., 2010b; Dürrwald et al., 2010). As an example, a trivalent SIV vaccine with a carbomer adjuvant did not induce cross-reactive antibodies against the pH1N1 virus, whereas the same vaccine in combination with an oil adjuvant did. Both serologic studies indicate that some commercial SIV vaccines may offer some protection against the pandemic virus.

5.1.3. Newly developed vaccines based on pH1N1 virus

So far, a conditionally-licensed vaccine for pH1N1 influenza virus for use in pigs is available in the US but not in Europe (Rapp-Gabrielson et al., 2010). In Europe, two vaccine manufacturers have expressed the intention to develop a monovalent vaccine based on pandemic H1N1 SIV There are thus limited data about the efficacy of vaccines based on the pH1N1 influenza virus in pigs.

Vincent et al. (2010) have performed vaccine efficacy experiments with an experimental pH1N1 vaccine in combination with an oil adjuvant. The pigs were challenged intratracheally (5.0 $\log_{10}$ TCID50) with the prototype pandemic strain A/California/04/2009 3 weeks after the second vaccination. The vaccine provided complete protection in all parameters examined, including clinical signs, macroscopic and microscopic pneumonia, and virus isolation from nasal swabs and bronchoalveolar lavage fluid. The commercial vaccine available in the US has been shown to induce serum HI antibody titres ≥40, which are considered as protective, after a double vaccination of pigs (Rapp-Gabrielson et al., 2010).

Table 3: Commercially available SIV vaccines in Europe

Manufacturer	Product name	Influenza virus strains	Substrate for production	Type of vaccine	Adjuvant	Antigenic content per vaccine dose
Merial	Gripovac	A/New Jersey/8/76 (H1N1) A/Port Chalmers/1/73 (H3N2)	eggs	inactivated split vaccine	Oil adjuvant	H1N1: ≥ 1.7 HIU H3N2: ≥ 2.2 HIU
Fort Dodge	Suvaxyn Flu	Sw/Netherlands/25/80 (H1N1) A/Port Chalmers/1/73 (H3N2)	eggs	inactivated whole virus vaccine	Oil adjuvant	H1N1: 4 µg HA H3N2: 4 µg HA
Impfstoffwerk Dessau-Tornau	Respiporc Flu	Sw/Belgium/230/92 (H1N1) Sw/Belgium/220/92 (H3N2)	MDBK* cells	inactivated	Aluminium hydroxide - mineral oil	H1N1: ≥256 HAU H3N2: ≥256 HAU
Impfstoffwerk Dessau-Tornau	Respiporc Flu3	Sw/Haselunne/2617/03 (H1N1) Sw/Bakum/1769/03 (H3N2) Sw/Bakum/1832/00 (H1N2)	MDBK* cells	inactivated	Carbomer	H1N1: ≥ $10^{7.0}$TCID$_{50}$ H3N2: ≥ $10^{7.0}$TCID$_{50}$ H1N2: ≥ $10^{7.0}$TCID$_{50}$
Hipra	Gripork	Sw/Olost/84 (H1N1) A/Port Chalmers/1/73 (H3N2)	eggs	inactivated whole virus vaccine	Oil adjuvant	H1N1: 3 x 10^7 EID$_{50}$ H3N2: 2.5 x 10^7 EID$_{50}$

* MDBK: Madin-Darby bovine kidney; HIU: haemagglutination inhibiting units as determined by measuring the HI antibody response after the 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 infectious dose 50% before inactivation; EID$_{50}$: egg infectious dose 50% before inactivation

5.2. Poultry

5.2.1. Use of vaccination and current availability of pH1N1 vaccines

Vaccination of poultry against strains of influenza that do not fall under the definition of Notifiable Avian Influenza (OIE, 2009) or Avian influenza (EU, 2005)[7] is not restricted and therefore technically and formally unregulated. Historically autogenous vaccines including viruses of the H6 and H9 subtype have been used in poultry in Europe and in the US, particularly in turkeys and in laying hens. The use of these vaccines was generally limited to a time span in which viruses of the same subtypes were circulating in the field.

Despite the occurrence of cases of H1N1 in turkeys between the mid '80s and '90's, vaccine to control this infection was never applied. The infection was self limiting and no additional intervention was foreseen. At the time of writing there appears to be no commercially available product against avian H1 viruses for use in poultry. In any case it would be surprising if avian-origin H1 viruses would afford protection against a swine-origin-human adapted virus such as pH1N1. In this regard, data generated prior to the emergence of the pH1N1 virus, had shown no cross reactivity between avian H1 viruses and antibodies generated against human seasonal H1N1 virus in vaccinated individuals (Capua et al., 2009) using conventional tests.

5.2.2. Vaccines against pH1N1

Currently there is no vaccine based on pH1N1 virus for use in poultry in Europe.

6. ToR 1 - To assess the significance for the health of animals of different species (specially pigs and different poultry species) of the occurrence of pandemic (H1N1) 2009 influenza virus in the EU and elsewhere;

6.1. Pigs

Considering the data presented on pH1N1 influenza virus infections in pigs in the previous sections of the report, both in experimentally inoculated pigs and in cases or outbreaks diagnosed the field, it can be concluded that disease signs with this pandemic virus are certainly not more severe than those observed with endemic swine influenza viruses (H1N1, H1N2, H3N2) presently circulating in most dense swine populations worldwide. Most reports of infections with pH1N1 virus in swine populations describe sporadic outbreaks.

In Norway, however, where the swine population was naïve for SIV's, introduction of pH1N1 in this population has led to widespread dissemination. So far, no structured epidemiological studies have been performed in other countries or continents to determine its current prevalence.

Experimental inoculation of pH1N1 in naïve pigs at the age between 4 and 10 weeks via different routes resulted in pathogenesis, infection dynamics and clinical signs (fever, inappetence, coughing) which are highly similar to those obtained with the endemic SIVs. The virus shows a tropism only for respiratory tissues and the infection is of pure respiratory nature with no direct involvement of other organs.

[7] Council Directive 2005/94/EC of 20 December 2005 on Community measures for the control of avian influenza and repealing Directive 92/40/EEC.
http://eur-lex.europa.eu/LexUriServ/site/en/oj/2006/l_010/l_01020060114en00160065.pdf

In the field, pigs have occasionally been infected apparently through contact with pH1N1 virus infected humans, and disease signs were either absent or morbidity was low. When signs were present, they were characterized by fever and cough and showed a mild to moderate course, even in herds with naïve pigs.

No evidence of increase in virulence of the virus has so far been observed on a clinical basis in infected pig herds where the virus had spread throughout the herds despite the occurrence of numerous pig to pig transmissions.

It remains to be seen if the pH1N1 virus will spread further in the swine population. The epidemiological behaviour of pH1N1 in the swine population is unpredictable as it may disappear as well as become endemic in the future. These observations are not only applicable for the EU but also for other parts of the world. If a previous infection or vaccination with endemic SIVs has a beneficial effect on the course of infection with pH1N1 virus in the field, such impact might not be the same in Europe as that in other parts of the world where other SIV subtypes circulate or are used as active components in vaccines, as will be explained further in ToR 5.

6.1.1. Conclusions

- At present, the impact of pH1N1virus for the overall health of the EU pig population is considered minimal. There is no indication that the situation is different elsewhere.
- Influenza naïve pigs are fully susceptible to infection with human- or swine- derived strains of pH1N1 virus upon experimental inoculation and contact transmission among pigs readily occurs.
- Pathogenetic features in pH1N1 virus experimentally inoculated pigs show that the infection is purely of respiratory nature and shows a course similar to that of the endemic SIVs currently circulating in the swine populations worldwide;
- Clinical signs, in experimentally infected pigs, are variable but relatively mild with fever, coughing and inappetence.
- Occasionally pigs in the field have been infected subsequent to exposure to pH1N1 virus infected humans. Virus spread between and within herds has been observed.
- The prevalence of pH1N1 in the swine populations worldwide is not known as no comprehensive epidemiological surveillance has been performed except in Norway.
- In field infections a subclinical course was very common, and when clinical signs were seen (coughing, fever), they were generally mild, the morbidity was low, and there was no mortality
- Pig passages with the pH1N1 virus have occurred but no increase in virulence of the virus has been observed, not even in herds with naïve pigs.
- Currently and since the occurrence in the field of pH1N1 virus infections in swine, there is no evidence of increased severity of influenza-like disease in European swine populations.
- pH1N1 infections have, at present, not been reported in wild boar; wild boar are expected to be susceptible for pH1N1 infection as they are for the endemic SIVs but, similar to the situation for the endemic SIVs, they are not expected to play an epidemiological role in the infection in the domestic swine population

6.2. Poultry

Considering the data presented on pH1N1 influenza virus infections of poultry in the previous chapters, both from experimental infections and field observation it can be concluded that except for turkeys and quails most poultry species currently are not susceptible to infection. Infection of turkeys and quails do not cause more severe disease than is observed with non-notifiable avian influenza viruses (OIE, 2009) of influenza. In turkeys loss in egg production and decrease in egg shell quality are the only signs which have only economical consequences for the producer. Successful experimental infection was only possible via artificial intra-uterine infection which however might explain that outbreaks only occurred in turkey breeder flocks. The uterus is exposed during artificial insemination which is routinely practiced in breeder flocks. Up to now there is no evidence that major between flock transmission of pH1N1 did occur and there is even doubt whether major within flock transmission does occur considering the outcome of infection experiments that show that turkeys are resistant to infection via natural routes.

Although no extensive assessment has been performed, the current available data both from outbreaks and experimental infections do not show any mutations of pH1N1 leading to viruses that are more fit or virulent for poultry.

6.2.1. Conclusions

- In poultry, outbreaks of pH1N1 have been reported only in turkeys specifically in breeder flocks.
- Currently, there is no evidence that pH1N1 virus is able to spread horizontally among turkeys within a flock.
- Turkeys, chickens and ducks are refractory to experimental infections with pH1N1 virus via the respiratory tract which is considered to be a natural infection route for influenza A viruses.
- Turkeys can be infected experimentally with pH1N1 virus by the intrauterine and intracloacal route.
- The most likely cause of outbreaks in turkey breeder flocks is transmission of pH1N1 virus from infected poultry workers carrying out artificial insemination.
- Drop in egg production and decreased shell quality is the main clinical sign of pH1N1 virus infection of turkeys. However, egg production and shell quality are not pathognomonic signs for pH1N1 infections and thus pH1N1 infections should be included in differential diagnosis of drop in egg production and decreased shell quality.
- In oro-pharyngeal and cloacal swabs collected from turkeys experimentally infected via the uterus virus was detected for periods up to 14 days. In the field virus was detected in swabs from turkeys for 2-4 weeks after egg production began progressively recovering in flocks.

6.2.2. Recommendations

- Awareness should be raised about the risk of infecting breeder turkeys with pH1N1 virus during artificial insemination. Specific guidelines should be developed to lower the risk of transmission of pH1N1 during artificial insemination.

6.2.3. Recommendations for future research

- Studies to determine population dynamics of infection (among others transmission rate, reproduction ratio) in turkeys after infection with pH1N1 virus via the uterus.

7. ToR 2 - To assess the implications and consequences of the possible evolution of the pandemic (H1N1) 2009 influenza virus on animal health;

In predicting the potential for long term evolution of the pH1N1 virus in pig populations it is relevant to parallel with similar events that have occurred historically after transmission of human pandemic strains to pigs. Some viruses have become established in pigs and undergone independent and parallel evolution to their counterparts in the human population and were maintained many years after the viruses were considered to have become a human seasonal strains. It can be expected that different host selection pressures will result in a different trajectory of evolution whereby changes in the gene segments, especially those encoding the external glycoproteins (HA and NA) will mutate at variable rates under different host selection pressures. Over time, this independent evolution, whilst enabling the establishment of host specific lineages of influenza viruses in pigs also results in considerable antigenic diversity whereby such viruses no longer resemble contemporary strains in the human population. This is well illustrated through the analysis of current H3N2 viruses whereby the strains that are maintained in the human population are antigenically distinguishable, being largely non cross-reactive with the strains that are maintained in global swine populations. Strains of H3N2 subtype in global swine populations retain antigenic characteristics of viruses circulating in the human population in the early 1970s. Since antigenic drift has occurred in human strains at a faster rate compared to swine strains, these two host populations present separate reservoirs of virus that although of the same subtype, are very distinct. In reviewing potential outcomes and courses for pH1N1 in swine populations it would appear likely that the virus, if sustained in swine population, will be maintained independently of the human population and divergent evolution both genetically and antigenically would appear probable as long as the virus is maintained independently in both host populations. Evidence to date is one of frequent detection in pigs and already further reassortment of pH1N1 with endemic swine strains has been reported (Vijaykrishna et al., 2010). This pattern of evolution of virus in pigs through genetic drift and reassortment has occurred following previous pandemics in humans so might be expected to occur if pH1N1 continues to infect pigs. Through selection, such strains appear to have increased fitness in pig populations whilst counterpart strains evolving in the human population are able to occasionally spill over to pigs but appear to be at a disadvantage compared to strains that may have established stable lineages within pig populations.

7.1. Conclusions

– At the time of this report, the pH1N1 has not changed its behaviour. Based on previous spread of pandemic virus to pigs, if pH1N1 continues to circulate in pigs, divergent evolution in pigs compared to those viruses in humans appears likely and there is no evidence that this will lead to the emergence of more virulent or zoonotic viruses.

– It appears probable that the pH1N1 virus originates from pigs. However, the pH1N1 virus has not been detected in pigs prior to its emergence in humans and phylogenetic studies indicate that a reassortment of multiple lineages known to circulate in swine may have occurred within the last 10 years.

7.2. Recommendations

– Inclusion of diagnostic procedures for the detection of pH1N1 might be considered in the event of the detection of non-notifiable influenza A viruses in existing syndromic surveillance programmes for H5/H7 in poultry to provide some baseline data should the virus change its tropism and pathogenicity for poultry.

– Monitoring of circulating influenza viruses in swine and poultry populations should be instigated to obtain data to characterize the circulating influenza viruses from which evolution of the pH1N1

virus including changes in virulence etc can be assessed. This information should be shared and analyzed together with similar information from the human health area.

– Monitoring of circulating influenza viruses in swine and poultry populations should be instigated to obtain data to characterize the circulating influenza viruses for further evolution of the pH1N1 virus including changes in virulence etc can be assessed. This information should be shared and analyzed together with similar information from the human health area.

7.3. Recommendations for future research

– Available/stored swine influenza viruses detected in surveillance programs in a variety of countries in the ten years prior to the pandemic should be sequenced as far as possible to provide valuable scientific data that may improve understanding of the factors involved and led to the emergence of pH1N1.

– Any incidental detections of pH1N1 in poultry should be subjected to genetic analysis and a good data flow ensured with ESNIP3 (a new FP7 project entitled 'European swine network for influenza in pigs, 3') to monitor any change in virus characteristics and tropism.

– The virus and host factors that contribute to the successful transmission and establishment of influenza A viruses in animal populations should be studied.

8. ToR 3 - To assess the effectiveness and efficiency of disease control options such as establishing animal movement restrictions in protection and surveillance zones, culling of infected pig herds and contact herds for pandemic (H1N1) 2009 influenza virus, as it is common practice for notifiable diseases, e.g. CSF, AI, FMD;

The terms "Effectiveness" and "Efficiency" of certain control options, as used in the ToR, were interpreted as follows:

- An effective disease control option is a measure that contributes to the reduction of disease spread within and especially between units, i.e. results in reducing transmission and spread.

- An efficient disease control option is a measure that results in reducing disease spread and in addition its use is feasible and proportionate to achieve the reduction in spread within and between units.

8.1. Regulatory status for influenza in pigs including pH1N1

At time of this report no harmonised control rules were laid down in the EU for control of influenza in pigs, and under current EU legislation for the reporting of infectious diseases in terrestrial animals (Council Directive 82/894/EEC)[8] influenza in pigs (any strain) was not included. However, should the pH1N1 virus change its virulence resulting in (a) increased transmissibility and pathogenicity in pigs (with associated economic losses) or (b) significant new public health threat, then surveillance, protection and control measures may have to be taken.

[8] Council Directive 82/894/EEC of 21 December 1982 on the notification of animal diseases within the Community OJ L 378, 31.12.1982, p. 58–62

8.2. Regulatory status for pH1N1 in poultry

At time of this report pH1N1 outbreaks in poultry do not fall under Directive 82/894/EEC and are not subject to notification of animal diseases. Directive 2005/94/EC, however, will apply to surveillance of pH1N1 since a differential diagnosis for AI must be considered to determine whether H5 or H7 influenza virus is involved. Official investigation and appropriate measures have to be applied on the holding until the presence of an influenza virus falling under the AI definition is ruled out through laboratory analysis (EU, 2005) and as recommended in the Commission guidance document (SANCO/6133/2009-Rev.6).

8.3. Assessment

In the light of the limited field data available, a cross-tabulation approach (Table 4) was used to link stages of diagnosis of pH1N1 in pig herds and poultry flocks (e.g. no evidence of disease, clinical suspicion, confirmation) to the set of control measures that are typically applied in the context of other infectious diseases such as CSF, FMD and AI (e.g. non-specific hygiene measures, diagnostic follow up, movement restrictions, culling and vaccination activities (cf. guidance documents SANCO/6133/2009-Rev 6 in poultry and SANCO/6211/2009 Rev.7 in pigs). For the control applications, these measures are combined to achieve control success, since single measures usually have only the capacity to reduce transmissions (of pH1N1) within or between herds by limited extent.

The assessment, evaluations and resulting conclusions are based on

- the epidemiology of pH1N1 virus infection as known at the time of this report;
- the assumption that the epidemiology of pH1N1 virus infection in pigs and poultry will not undergo substantial changes in the near future;
- the animal health perspective, i.e. excluding the zoonotic component of pH1N1; and
- the main principles of efficacy, proportionality and flexibility of measures.

8.3.1. Method of evaluation of control measures

The preventive and control measures, as applied with notifiable diseases, were identified (columns of Table 4).

Each control measure was evaluated for the particular diagnostic stages (rows of Table 4)

- firstly, whether it would contribute to the reduction in spread of pH1N1 virus (Part A); and
- secondly, whether, in addition to the former, its application would be feasible and proportionate in the context of pH1N1 (Part B).

Results of both evaluation procedures were tabulated separately (Table 4 Parts A and B).

The evaluation was discussed and discrepancies clarified. The discussion refers to Sections 4 and 5 of this report as scientific background as well as the aforementioned working documents of the Commission.

8.3.2. Results of evaluation of control measures

Table 4: Cross tabulation of the evaluation of the outcome of measures applied in control schemes for notifiable diseases (columns) at different diagnostic stages for pH1N1 infection (rows).

The table comprises two parts. In Part A ("Effective"), it was considered whether the individual measure could contribute to the reduction in spread of pH1N1 virus within and between poultry and pigs units at the particular diagnostic stage. In Part B ("Efficient"), it was considered whether the individual measure works well in reducing disease spread and, in addition, whether it is feasible and proportionate to apply it in the context of a pH1N1 infection.

Part A: Effective	**General activities**		**Detection, first Intervention**			**Control without vaccination**		**Control with vaccination**		
Measure / **Epidemic situation**	General (unspecific) hygiene measures to prevent disease spread	Prophylatic / preventive vaccination (sufficient population coverage)	Further diagostic procedures to confirm suspicion, local monitoring	Movement restrictions applied to affected flocks / farms	Additional movement restrictions (other flocks, control zones, surveillance zone)	Stamping out (reactive culling)	Preventive culling (neighboring flocks, control zones etc.)	Targeted vaccination (relevant contacts)	Short term preventive vaccination (around outbreak)	Long term preventive vaccination (supression, freedom)
No suspect cases of pH1N1										
General surveillance (disease awareness) situation, no flock/herd-based supicion										
Suspicion of pH1N1										
Presence of suspicious clinical symptoms indicative of pH1N1 (such as egg drop or coughing)										
Confirmation of infection										
No clinical symptoms, but pH1N1 antibody detection										
Laboratory confirmation of pH1N1-related „outbreak" (virus detection)										

Although in Part A all measures can contribute to some extent to the reduction of spread, even of unconfirmed pH1N1 infections, it was decided to grade the shading of cells. Darker colour was applied to indicate more likely impact (e.g. after virus confirmation), while a lighter colour indicates less importance (e.g. with a suspicion only). Blank cells indicate no practical relevance of the measure when there is no evidence of pH1N1 outbreak.

Part B: Efficient	**General activities**		**Detection, first Intervention**			**Control without vaccination**		**Control with vaccination**		
Measure / **Epidemic situation**	General (unspecific) hygiene measures to prevent disease spread	Prophylatic / preventive vaccination (sufficient population coverage)	Further diagostic procedures to confirm suspicion, local monitoring	Movement restrictions applied to affected flocks / farms	Additional movement restrictions (other flocks, control zones, surveillance zone)	Stamping out (reactive culling)	Preventive culling (neighboring flocks, control zones etc.)	Targeted vaccination (relevant contacts)	Short term preventive vaccination (around outbreak)	Long term preventive vaccination (supression, freedom)
No suspect cases of pH1N1										
General surveillance (disease awareness) situation, no flock/herd-based supicion										
Suspicion of pH1N1										
Presence of suspicious clinical symptoms indicative of pH1N1 (such as egg drop or coughing)			POULTRY							
Confirmation of infection										
No clinical symptoms, but pH1N1 antibody detection										
Laboratory confirmation of pH1N1-related „outbreak" (virus detection)										

Coloured cells indicate measures that contribute to reduction of spread, and are also feasible and proportionate. Blank cells represent measures perceived as not feasible or proportionate. Horizontally hatched cells represent control actions enacted due to legislation in cases of unspecific influenza suspicion in poultry (2005/94/EC) which were not considered proportionate in response to only a pH1N1 suspicion. The diagonally hatched cells in the bottom rows indicate a measure considered feasible to protect the production system after pH1N1 confirmation but not as sufficiently proportionate.

Table 4 Part A indicates that most of the measures applied in the context of notifiable infectious animal diseases were considered able to contribute to a reduction in transmission and thus to reduce spread of pH1N1 within and between pig or poultry units.

General hygiene measures such as voluntary isolation (separation, quarantine) of newly introduced or sick animals or "all-in all-out" procedures would certainly reduce the risk of introducing / spreading pH1N1 within and between units. Limiting access of humans that might be infected with pH1N1 should also minimize reverse zoonotic transmission and thus (human-based) introduction of the virus into susceptible flocks.

According to Table 4 Part B, only the general hygiene measures were considered both useful for reducing spread of pH1N1 infections and also as feasible and proportionate in their use.

Culling of infected herds/flocks will reduce spread of the infection from the identified outbreak farm and indeed, if applied at the diagnostic stages prior to pH1N1 confirmation the measure will perform equally well in reducing spread from actually infected units. However, at the time of writing this report, such measures were not considered proportionate for use in controlling pH1N1 (hence marked as not efficient in Table 4 Part B).The reasoning was based on scientific evidence that pH1N1 infection does not induce substantial clinical signs or economic losses in pigs (Chapter 4.1), and the lack of scientific evidence that pH1N1 infection can lead to direct transmission between poultry (Chapter 4.2),

Furthermore, Table 4 Part B indicates certain exceptions which are marked by horizontally hatched cells although the indicated measures are not directly related to preventing spread of pH1N1. Since a differential diagnosis to AI must be considered in the case of an influenza virus suspicion in poultry (Directive 2005/94/EC), a diagnostic follow up of suspect cases in poultry including movement restrictions of the flock under investigation (mandated by AI regulation for differential diagnosis/ exclusion) has to be enacted.

Moreover, after the presence of pH1N1 is confirmed within a flock / herd, voluntary (industry agreed) movement restrictions could be considered feasible to protect the production system (diagonally hatched cells) but not as sufficiently proportionate to be highlighted for application against pH1N1.

During the discussion it became obvious that evaluating feasibility and proportionality of most measures was strongly related to management aspects and that evaluation was influenced by the minor impact of pH1N1 infections in pig and poultry populations, as detailed in Chapter 4-5 of the report and specifically in response to ToR1.

8.4. Conclusion

- From the animal health point of view no specific control measures against pH1N1 are considered necessary at all.

- Measures similar to those jointly used to control notifiable animal diseases (such as CSF, FMD and AI) were all expected to contribute to some extent to a reduction in spread of pH1N1 infections. However, at the time of writing this report, only general hygiene measures were considered proportionate and feasible to be used to reduce virus spread during pH1N1 outbreaks.

- Diagnostic follow up of suspect influenza cases in poultry including movement restrictions of the flock under investigation is mandatory but only due to required differential diagnosis to H5 / H7.

8.5. Recommendations

– Place strong emphasis on information to (a) increase disease awareness and (b) to ensure that biosecurity is implemented to contribute to the reduction of potential spread of pH1N1 within and between animal units and also from humans to animals and back.

9. ToR 4 - To assess the risk that animals from a herd/ flock which was infected with pandemic (H1N1) 2009 influenza virus spread the virus after the last clinical signs of disease have been observed;

In existing control schemes, e.g. for diseases with decisive and standard clinical signs, a specific time interval following the last detection of clinical signs in an infected epidemiological unit is proposed as the (best available) predictor for the end of virus shedding at the level of the epidemiological unit. The ToR refers to the question whether such a "waiting" period after disappearance of clinical symptoms could be defined for pH1N1 infections after which it can be safely assumed that virus can no longer spread from the epidemiological unit. It specifically addresses the recommendations made in two working documents from 2009 in which for poultry [SANCO/6133/2009-Rev.6] and for pigs [SANCO/6211/2009 Rev.7] farm-level quarantine/movement controls should be maintained until at least seven days after the last clinical signs of disease have been observed in the epidemiological unit and influenza is no longer considered a veterinary risk.

It is known that influenza infected individuals are infectious for a certain time after the end of clinical signs. However, there is an absence of data from well documented outbreaks of pH1N1 in pig herds and poultry flocks in which the infection status, clinical symptoms and virus shedding were closely monitored in a natural setting with a larger number of animals. Therefore, only limited information from a few experimental (infection) studies was available and thus used to address the question in the context of this mandate.

9.1. Summary concerning clinical signs and virus shedding

9.1.1. Pigs

Limited temporal association between clinical signs and virus shedding of pH1N1 was reported in pigs. While a virus shedding period is rather general, associated clinical signs occur highly variably (i.e. until 3 dpi in mild courses, generally until 6-7 dpi but until 8 dpi as the maximum reported; Chapter 4.1.2.2). More importantly, however, field data report a substantial number of subclinical infections in an infected epidemiological unit (Chapter 4.1.2.1). On the other hand, if other viruses or bacteria cause super-/co-infection, the duration of clinical signs may be even further prolonged although without causal link to pH1N1 infection or hence to pH1N1 virus shedding.

9.1.2. Poultry

Clinical signs on the individual level are not available. Observable syndromes of pH1N1 infection in poultry, which so far have been reported only for breeder turkeys, are a drop in egg production (Chapter 4.2.2.1). However, it was reported that when this syndrome started to diminish within infected flocks, virus shedding still could be demonstrated (Chapter 4.2.).

9.2. Evaluation of clinical signs as a predictor for pH1N1 virus shedding

Since there is a large variability in the duration of clinical signs in pigs and that there is a risk that the majority of infected (and potentially infectious) animals will not develop detectable clinical symptoms, the following conclusions were reached by expert judgment:

- The risk that an epidemiological pig unit infected with pH1N1 can spread the virus after a certain period of time without clinical signs of disease is high.

- The risk that an epidemiological breeder turkey unit infected with pH1N1 will spread the virus is low and requires human mediated transmission (handling of the animals during artificial insemination). Hence, the risk that the virus is spread from such units after a certain period of time without clinical syndrome of disease (egg drop) is low. The evaluation is based on the information at the time of writing this report according to which the ability of the virus to infect other turkeys via the respiratory route is negligible.

As a consequence, the recommended waiting period of 7 days between the last observed clinical signs/syndromes in an infected epidemiological unit (SANCO/6133/2009-Rev.6) and SANCO/6211/2009-Rev.7) is likely too short. However, with the data available so far it is not possible to justify any scientifically based alternative waiting period.

9.3. Conclusions

- The exploitation of clinical signs as temporal proxy for termination of virus excretion within an infected epidemiological unit is not valuable in either pigs or poultry. In these species the duration of virus excretion is not consistently associated with the appearance of clinical signs to allow epidemiological decision making based on their temporal order of occurrence. In consequence, using the time of cessation of clinical signs and a preset time interval at the level of epidemiological units to establish clearance from infectiousness (virus shedding) is lacking any scientific basis.

9.4. Recommendations

- It is not recommended, at present, to restrict animal movement during infectious periods as pH1N1 has no more significance for pig health than the presently circulating SIVs. Swine influenza is endemic in the majority if not all global pig populations.

- Clinical signs are not reliable as a basis to decide on the end of an infection with pH1N1 virus in an infected herd/flock because pH1N1 induced signs are variable, non-specific or absent. Therefore, when the health status in regard to excretion of pH1N1 virus from a farm/flock needs to be known, it is recommended to test a number of nasal/oro-pharyngeal swabs (swine) or oropharyngeal and cloacal swabs (poultry) according to the expected within herd/flock prevalence for pH1N1 virus by a specific pH1N1 PCR. Testing should start 14 days after the diagnosis is established and continue at 2 week intervals until no excretion of virus can be demonstrated. In pigs, the focus should be on animals of 8-12 weeks of age.

- Currently it cannot be recommended to use serology to differentiate pH1N1 infection from other endemic SIV infections due to cross-reactions when using the available tests.

- Depending on the evolution of the pH1N1 it could be justifiable to develop serological tests that allow differentiation between the circulating SIV subtypes and the pH1N1 virus.

10. ToR 5 - To assess the possibility, efficacy and efficiency of vaccination, using existing vaccines or newly developed vaccines against pandemic (H1N1) 2009 influenza virus, in pig and poultry populations also in relation with possible evolution of variants of influenza viruses posing a serious risk to public and animal health;

10.1. Pigs

Below is a brief assessment of each of the specific questions mentioned in the ToR. The background information used to answer these questions can be found under chapter 5 of this document. For clarity, vaccination with existing vaccines (10.1.1.1) and vaccination with vaccines based on pH1N1 virus (10.1.1.2) are treated separately. The terms "possibility", "efficacy" and "efficiency" are defined as follows:

- "possibility" of vaccination means availability of vaccine
- "efficacy" of vaccination means according to European Pharmacopoeia the ability of the vaccine to offer significant virological protection in an experimental setting, i.e. after a double vaccination of influenza naïve pigs and challenge with pH1N1 virus 3 weeks after the second vaccination. Virological protection is defined as a significant reduction in virus titres in the lungs of vaccinated pigs compared to unvaccinated challenge control pigs.
- "efficiency" of vaccination means the ability of the vaccine to reduce virus circulation under field conditions.

By "in relation with possible evolution of variants of influenza viruses posing a serious risk to public and animal health", we understand "can vaccination prevent or stimulate the emergence of pH1N1 variants that are more virulent for pigs or that show increased transmissibility from pigs to humans?" This question is also considered separately (10.1.1.3).

10.1.1. 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 an H1N2 strain.
- *Efficacy of vaccination*: Serological investigations of pigs vaccinated with various existing vaccines allow the expectation that these vaccines will reduce pH1N1 virus replication in the lungs. This assumption is based on the fact that influenza virus replication in the lungs of vaccinated pigs correlates with post-vaccination HI antibody titres against the challenge virus.
- *Efficiency of vaccination*: Data about the efficiency of vaccination against the pH1N1 virus are lacking. Vaccination with the existing SIV vaccines is voluntary and vaccination rates vary in different countries. Voluntary vaccination of swine with these existing vaccines has not succeeded in halting the circulation of SIV in the swine population. It is unknown if, and to what degree, obligatory SIV vaccination could reduce pH1N1 virus circulation.

10.1.2. Vaccines based on pH1N1 virus:

- Possibility of vaccination: A monovalent inactivated vaccine based on pH1N1 for use in pigs is available in the US, but not in Europe. Such vaccines can be readily developed based on the experience with other SIV vaccines.

- Efficacy of vaccination: There are no published efficacy data for the commercial vaccine available in the US. An experimental vaccine based on pH1N1 was shown to completely prevent virus isolation from nasal swabs and lung lavage fluids upon challenge with pH1N1. Other pH1N1 vaccines are therefore expected to confer significant or even complete virological and clinical protection, superior to the protection with SIV vaccines already authorized in Europe.

- Efficiency of vaccination: There are no data about the efficiency of widespread vaccination with pH1N1 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 pH1N1 virus in the swine population. It is unknown if, and to what degree, obligatory vaccination could reduce virus circulation.

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

- Evolution of variants of pH1N1 virus posing a serious risk to animal health: As mentioned above, vaccination is unlikely to prevent the spread of pH1N1 in swine populations, supposing that pH1N1 virus is or becomes endemic. The emergence of drift variants of influenza viruses is unpredictable, but it is unlikely to be prevented by vaccination. In addition, it cannot be excluded that vaccination increases the risk for antigenic drift by stimulating the circulating virus to escape from neutralization by vaccine-induced antibodies. The latter phenomenon, however, has never been observed with the existing vaccines and endemic SIVs.

- Evolution of variants of pH1N1 virus posing a serious risk to human health: For the same reasons mentioned above, vaccination is unlikely to prevent the emergence of drift variants of pH1N1 virus with increased transmissibility to humans. However, the likely divergent evolution of pH1N1 in pigs compared to that in humans makes it unlikely that virus with increased transmissibility to humans would evolve.

10.1.4. Conclusions

– Immunity resulting from vaccination with existing SIV vaccines on the European market will provide some extent of cross-protection against infection with the pH1N1 influenza virus but specific pH1N1 vaccines will offer superior protection. Such vaccines will significantly reduce or even completely prevent pH1N1 replication and disease in the individual animal. Vaccination will not, however, prevent pH1N1 circulation in the population if it is applied on a voluntary basis.

– Cross-infection studies with the well-known endemic SIVs indicate that prior infection with these viruses will confer some cross-protection against infection with the pandemic virus.

– Vaccines based on the pH1N1 virus appear to induce a protection similar to that induced by the existing SIV vaccines against the respective endemic SIVs. Such vaccines are generally highly efficient in the prevention of disease caused by influenza viruses because they reduce the extent of virus replication in the lungs. Voluntary vaccination of swine with these existing vaccines has not succeeded in halting the circulation of SIV in the swine population.

– At present and according to the available data, the epidemiological situation of pH1N1 in pigs does not justify their vaccination with pH1N1 vaccine. Vaccination on a voluntary basis will likely protect the vaccinated animals but it will not prevent the spread of the pandemic H1N1 virus in swine populations.

10.1.5. Recommendations

– Compulsory vaccination cannot be justified, because of the mild course of the infection and disease. Similarly, emergency vaccination cannot be justified.

– There is no urgency for vaccination of pigs against pH1N1 virus. It could be useful, however, to have a specific vaccine, based on the pH1N1 virus, in case of change of the epidemiological situation of the virus in the pig population.

10.1.6. Recommendations for future research

– Experimental cross-protection studies with pH1N1 challenge in pigs infected with the major European SIV subtypes, or their combination. Experimental vaccination-challenge studies with the existing European SIV vaccines and pH1N1 challenge; the extent of protection should be compared with that of a pandemic H1N1 vaccine.

– Studies of the immune mechanisms mediating heterovariant and heterosubtypic protection between influenza viruses in pigs. Detailed comparative studies of the immune response after infection with live virus and after vaccination with inactivated vaccines.

– Studies into the population dynamics of the virus in presence and absence of vaccination in order to obtain knowledge on the effect of vaccines on the transmission and spread of the virus.

10.2. Poultry

10.2.1. Conclusions

– Currently, no vaccines against H1 viruses for poultry are available.

10.2.2. Recommendations

– At present, there is no need to vaccinate poultry against pH1N1 virus.

11. ToR 6 - To assess the role of wildlife, in particular wild boar and wild birds in the epidemiology of pandemic (H1N1) 2009 influenza virus, if any.

Data available on the presence of pH1N1 in wildlife is scarce. Only a single case of pH1N1 virus infection in two striped skunks in Canada has been documented so far, as described above. No detailed collection and analysis of samples from wild boar or other mammalian species have been conducted.

11.1. Conclusions

– No pH1N1 virus infections have been reported in wild boar. Although expected to be susceptible for pH1N1, they are not expected to play any epidemiological role

– To date no infection with pH1N1 virus of wild birds have been reported despite the many surveillance programmes on Influenza viruses that are conducted in wild birds in particular since the start of H5N1 epidemic in poultry in 2004.

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APPENDICES

APPENDIX 1- INFLUENZA PH1N1/09 INFECTIONS IN COMPANION REPORTED IN PRO-MED

Country	Date	Animal species	Locations (number of animals)
USA	04/11/09 [Pro-med 20091105.3816]	Cat	Iowa (1)
	01/11/09 [Pro-med 20091101.3777]	Ferret	Nebraska (1)
	10/11/09 [Pro-med 20091114.3936]	Ferret	Oregon (3)
	13/11/09 [promed 20091121.4008]	Cat	Utah (1)
	09/12/09 [Pro-med 20091209.4192]	Cat	Oregon (1)
	09/12/09 [Pro-med 20091209.4192]	Cat	Iowa (1)
	09/12/09 [Pro-med 20091209.4192]	Cat	Pennsylvania (1)
	09/12/09 [Pro-med 20091209.4192]	Cat	Colorado (2)
	09/12/09 [Pro-med 20091211.4213]	Cheetah	California (1)
China	28/11/09 [Pro-med 20091128.4079]	Dog	(2)
USA	22/12/09 [Pro-med 20091222.4305]	Dog	New York (1)

APPENDIX 2- SWINE INFLUENZA VACCINES IN MS (SOURCE – DISCONTOOLS)

Species	Pigs
Aethiologic agent	orthomyxovirus

Country	**Comment**
EMA	Information requested from NCAs
Austria	
Belgium	Several vaccines are authorized in BE: in addition to the centrally authorized RESPIPORC FLU 3 and GRIPOVAC 3, the following vaccines are authorized in BE: GRIPOVAC en SUVAXYN FLU
Bulgaria	No
Cyprus	-
Czech Republic	Central MA at EMA: Gripovac 3, Respiporc Flu3. National MA Gripork (Laboratorios Hipra, S.A. Spain, 97/1274/93-C, immunisation of pigs against swine flu, inactivated vaccine for intramuscular injection, Virus influensis typus A(Hsw1N1) OLL inactivatum, Virus influensis typus A(Hsw3N2) G inactivatum, Virus influensis typus A (Hsw3N2) SH inactivatum, pigs.)
Denmark	Respiporc Flu3 and Gripovac authorised centrally.
Estonia	
Finland	special license: Gripovac (Merial), centralized procedure: Gripovac and Respiporc Flu 3
France	MA for:MERIAL - GRIPOVAC, AKIPOR FLU, GESKYGRIP FORT DODGE - SUVAXYN FLU 3
Germany	DE-PEI: e.g. Respiporc Flu, Respiporc Flu3, Suvaxyn Flu
Greece	yes, Akipor Flu, Griporiffa, Griporc, Gripovac and Geskygrip (+Auj) (all Merial)
Hungary	Only the centrally authorized product(s) is(are) available in Hungary.
Ireland	No national licenses for vaccines against Swine Influenza in IE. Under national legislation, the use of vaccines against Swine Influenza are restricted in Ireland. [Restricted: A person cannot import, sell, supply or administer to an animal a vaccine that may be used to produce active or passive immunity to the disease specified except, under and in accordance with a special licence granted by the Department of Agriculture, Fisheries & Food].
Italy	IZOVAC SUI-FLU – Inactivated swine influenza virus H1N1 A/SW/OMS 2899 and H3N2 A/SW/OMS 3633; IZO s.p.a.; AN: 100025; Pig; s.c.; Reduction of mortality, clinical signs and lesions caused by swine influenza AKIPOR FLU – (Inactivated) swine influenza antigen H1N1 and H3N3 + Live Aujeszky desease virus; Merial Italia s.p.a; AN :102407; Fattening pigs; i.m.; Active immunization against Aujesky Desease and swine influenza AUJINFLU-SUIVAX – Inactivated swine influenza virus H1N1 (H/SW/H1N1- A/New Jersey/8/76 related) and H3N2 (A/Port Chalmers/1/73 related) + inactivated Aujeszky desease virus; FATRO s.p.a.; AN : 100036; pig; s.c.; Active immunization against Aujesky Desease and

	swine influenza viruses FLUEN-SUIVAX – Inactivated swine influenza virus H1N1 (A/SW/H1N1/OMS 2614/84) and H3N2 (A/Sw/H3N2-Sw/OMS 3633/84 related); Fatro s.p.a.; AN: 101818; pig; s.c. o i.m.; Protection against clinical signs cauded by H1N1 and H3N2 viruses GESKYGRIP – (Inactivated) Swine influenza antigen H1N1(A/NEW JERSEY/76) and H3N1(A/port Chalmers/73) + Aujeszky virus sub viral units; Merial Italia s.p.a; AN: 100014; pig; i.m.; Active immunization against Aujeszky desease and Swine influenza IZOVAC AUJESZKY-FLU – Inactivated swine influenza H1N1 A/SW/OMS 2899 and H3N2 A/SW/OMS 3633 + inactivated Aujeszky Desease virus; IZO s.p.a.;AN: 100293; pig; i.m.; Prophylaxis of swine influenza GRIPOVAC – (Inactivated) Swine influenza antigen H1N1(A/NEW JERSEY/76) and H3N1(A/port Chalmers/73) ; Merial Italia s.p.a; AN: 101330; pig; i.m.; Active immunization against Swine influenza
Latvia	Not authorized
Lithuania	No vaccine authorised
Luxembourg	
Malta	
Netherlands	Swine Influenza A Virus (subtypes H1N1 and H3N2) vaccines: SUVAXYN FLU 3 (REG NL 1919; inactivated vaccine), GRIPOVAC (REG NL 9216; subunit vaccine), AKIPOR FLU (REG NL 9244; subunit vaccine, combination with live Aujeszky), SUVAXYN FLU (REG NL 9463; inactivated vaccine) Suvaxyn Flu 3 - Fort Dodge (REG NL 1919), Gripovac – Merial (REG NL 9216), REG Akipor – Merial (NL 9244), Suvaxyn Flu - Fort Dodge (REG NL 9463)
Poland	None authorised
Portugal	-
Romania	-
Slovakia	No vaccine available
Slovenia	no national authorisations
Spain	GRIPORIVEN (IVEN); SUVAXYN FLU (FORT DODGE); GRIPORK (HIPRA); SUIPRAVAC-AD/COLI/FLU (HIPRA)
Sweden	Respiporf Flu3, Gripovac 3
United Kingdom	Respiporc Flu 3 and Gripovac 3 recently authorised centrally
Iceland	
Norway	No information on any vaccines.

DOCUMENTATION PROVIDED TO EFSA

Working document on Surveillance, monitoring and control measures for the pandemic (H1N1) 2009 influenza virus in poultry

http://ec.europa.eu/food/animal/diseases/influenzaAH1N1/docs/pandemic_h1n1_2009_influenza_virus_in_poultry.pdf

Working document on Surveillance/monitoring and control measures for the pandemic (H1N1) 2009 influenza virus in pigs

http://ec.europa.eu/food/animal/diseases/influenzaAH1N1/docs/surveillance_control_H1N1_in_pigs_03112009_en.pdf

GLOSSARY

- Zoonosis : any disease or infection which is naturally transmissible from animals to humans (From OIE - Terrestrial Animal Health Code – glossary)
- Reverse zoonosis: means any infectious disease that can be transmitted from humans to animals

- Monitoring: intermittent performance and analysis of routine measurements and observations, aimed at detecting changes in the environment or health status of a population (From OIE - Terrestrial Animal Health Code – glossary)

- Surveillance: systematic ongoing collection, collation, and analysis of information related to animal health and the timely dissemination of information to those who need to know so that action can be taken (From OIE - Terrestrial Animal Health Code – glossary)

- Horizontal [direct/indirect] transmission: transmission of the infectious agent by excretion and either direct (close) contact to susceptible individuals, or by means of a mechanical of live "vector" that acts as a transport "vehicle"