REVIEW ARTICLE

Review of Influenza A Virus in Swine Worldwide: A Call for Increased Surveillance and Research

A. Vincent[1], L. Awada[2], I. Brown[3], H. Chen[4], F. Claes[5], G. Dauphin[5], R. Donis[6], M. Culhane[7], K. Hamilton[2], N. Lewis[8], E. Mumford[9], T. Nguyen[10], S. Parchariyanon[11], J. Pasick[12], G. Pavade[2], A. Pereda[13], M. Peiris[14], T. Saito[15], S. Swenson[16], K. Van Reeth[17], R. Webby[18], F. Wong[19] and J. Ciacci-Zanella[20]

[1] Virus and Prion Research Unit,USDA-ARS NADC, Ames, IA, USA
[2] World Organization for Animal Health (OIE), Paris, France
[3] Animal Health and Veterinary Laboratories Agency, Weybridge, UK
[4] Harbin Veterinary Research Institute, Harbin, China
[5] Food and Agriculture Organization of the United Nations (FAO), Rome, Italy
[6] CDC, Atlanta, GA, USA
[7] University of Minnesota Veterinary Diagnostic Lab, St. Paul, MN, USA
[8] Department of Zoology, University of Cambridge, Cambridge, UK
[9] World Health Organization (WHO), Geneva, Switzerland
[10] Department of Animal Health, National Centre for Veterinary Diagnostics, Hanoi, Vietnam
[11] National Institute of Animal Health, Bangkok, Thailand
[12] Canadian Food Inspection Agency, Winnepeg, Canada
[13] Instituto de Virología – INTA, Buenos Aires, Argentina
[14] Hong Kong University, Hong Kong City, Hong Kong
[15] National Institute of Animal Health, Ibaraki, Japan
[16] USDA-APHIS NVSL, Ames, IA, USA
[17] Ghent University, Ghent, Belgium
[18] St. Jude Children's Research Hospital, Memphis, TN, USA
[19] Australian Animal Health Laboratory, CSIRO Livestock Industries, Geelong, Vic., Australia
[20] EMBRAPA Swine and Poultry, Concordia, SC, Brazil

Impacts

- Influenza A viruses (IAV) are shared between humans and animals and many diverse IAV circulate in swine populations around the world. Despite the paucity of data revealed after the emergence of the H1N1 pandemic in 2009, many gaps in swine surveillance remain today.
- Collaboration on influenza between the international organizations the World Organization for Animal Health (OIE), Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO) was established, and networks of laboratories engaged in human and animal IAV surveillance and research were created for sharing information and resources.
- Closing the information gap on IAV in swine requires additional support from governments, practicing veterinarians and the swine industry and, ultimately requires significant increases in funding and infrastructure.

Keywords:
Influenza A virus; swine; one health; surveillance

Correspondence:
A. Vincent. Virus and Prion Research Unit, USDA-ARS NADC, 1920 Dayton Avenue, Ames, IA 50010, USA. Tel.: +1-515-337-7557; Fax: +1-515-337-7428; E-mail: amy.vincent@ars.usda.gov

Summary

Pigs and humans have shared influenza A viruses (IAV) since at least 1918, and many interspecies transmission events have been documented since that time. However, despite this interplay, relatively little is known regarding IAV circulating in swine around the world compared with the avian and human knowledge base. This gap in knowledge impedes our understanding of how viruses adapted to swine or man impacts the ecology and evolution of IAV as a whole and the true impact of swine IAV on human health. The pandemic H1N1 that emerged

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in 2009 underscored the need for greater surveillance and sharing of data on IAV in swine. In this paper, we review the current state of IAV in swine around the world, highlight the collaboration between international organizations and a network of laboratories engaged in human and animal IAV surveillance and research, and emphasize the need to increase information in high-priority regions. The need for global integration and rapid sharing of data and resources to fight IAV in swine and other animal species is apparent, but this effort requires grassroots support from governments, practicing veterinarians and the swine industry and, ultimately, requires significant increases in funding and infrastructure.

Introduction

Surveillance for influenza A viruses (IAV) circulating in pigs and other non-human mammals has been chronically underfunded and virtually non-existent in many areas of the world (Editorial, 2009). This deficit continues in spite of our knowledge that influenza is a disease that has been shared between humans and pigs since at least the 1918 Spanish flu pandemic. In March–April 2009, a novel pandemic H1N1 virus (H1N1pdm09) emerged in the human population in North America (Garten et al., 2009) exposing the paucity of data on IAV in swine. Scrutiny of this surveillance gap continues. The gene constellation of the emerging virus was demonstrated to consist of a combination of genes from IAV of North American and Eurasian swine lineages that had never before been identified in any species. The emergent H1N1pdm09 quickly spread in the human population, and the World Health Organization (WHO) declared on June 11, 2009, that the outbreak had reached pandemic phase 6. Although the sequences of the virus's eight gene segments are similar to those of corresponding genes from swine IAV from North America and Eurasia, no closely related ancestral IAV with this gene combination had been previously identified anywhere in the world (Smith et al., 2009; Trifonov et al., 2009).

Although prior to 2009 there were approximately 100 reported events of sporadic transmission of swine-adapted IAV to humans since the Spanish flu pandemic (Myers et al., 2007; Shinde et al., 2009; Shu et al., 2012), swine IAV of the H1N1 subtype have been historically distinct from avian H1N1 and from other mammalian H1N1 IAV in qualities such as serologic cross-reactivity, nucleotide sequence and host specificity. The exception to this tendency for host specificity of the swine IAV is the susceptibility of domestic turkeys to swine origin viruses (Nfon et al., 2011a,b). The emergence of H1N1pdm09 brought a heightened, global, cross-sector awareness to the evolution and epidemiology of IAV in swine and started a new era of challenges and opportunities in monitoring, understanding and controlling influenza in pigs. Unfortunately, these opportunities have yet to be significantly realized, and a satisfactory understanding of the ecology and evolution of IAV in swine does not yet exist, despite greater interest and resource investments in the past 3 years.

An Overview of Pandemic H1N1 (2009) and Its Worldwide Emergence

H1N1pdm09 has a unique genome, with six gene segments (PB2, PB1, PA, HA, NP and NS) descending from the triple-reassortant IAV of the North American swine lineage and the M and NA genes derived from a Eurasian lineage of swine IAV (Dawood et al., 2009). H1N1pdm09's sequence does not contain many of the known human adaptation or virulence markers, yet it became a highly transmissible virus in the human population. It does, however, contain the human-like α-2,6 sialic acid receptor–binding preference, as do other swine IAV that have been evaluated (Chen et al., 2011), but this similarity alone cannot explain why this particular virus became transmissible in humans and other swine-adapted viruses had not. The pandemic influenza became infamously known as 'swine flu' because of the phylogenetic origin of its gene segments. However, the unique combination of gene segments had never before been recognized in swine, and exposure to swine was not a common feature of human cases of pandemic influenza. It is also clear that the epidemiology of the pandemic in humans has not been affected by the subsequent human-to-pig transmission and outbreaks in pigs (Dawood et al., 2009). The initially documented swine outbreaks due to H1N1pdm09 were preceded by reported human influenza-like illness (Howden et al., 2009; Pereda et al., 2010; Holyoake et al., 2011), and the rapid global appearance of H1N1pdm09 in pigs can be attributed to spread via humans rather than to spread mediated by the international or domestic movement of pigs. H1N1pdm09 replicates efficiently in the lower and upper respiratory tract of experimentally infected pigs, causing a clinical disease similar to that caused by common enzootic IAV (Brookes et al., 2009; Lange et al., 2009; Vincent et al., 2010).

Early reference to the H1N1pdm09 as 'swine flu' contributed to unfounded public alarm over the safety of pork meat products and culminated in the ban on importation of pork products and live pigs from North America by

several countries. The ban, coupled with an initial loss in consumer confidence, resulted in reduced consumption of pork products and billions of dollars in estimated lost revenue for the swine industry. These fears were allayed, however, when virus was not detected in fresh pork meat of swine experimentally infected with H1N1pdm09 virus (Brookes et al., 2009; Lange et al., 2009; Vincent et al., 2009a). The World Organization for Animal Health (OIE), the Food and Agriculture Organization of the United Nations (FAO) and the WHO collaborated on the human–animal interface aspects of H1N1pdm09. The OIE, FAO, WHO and World Trade Organization (WTO) issued a statement supporting the conclusion that pork does not pose a risk of transmission of H1N1pdm09 to humans. The OIE also made statements warning against scientifically unjustified trade restrictions on live pigs or pork products in relation to H1N1pdm09 infection in humans and pigs. Such trade bans breached the OIE standards for trade, which are recognized by the WTO Agreement on the Application of Sanitary and Phytosanitary Measures (SPS Agreement) and oblige WTO Members to base their sanitary measures on scientific risk assessment. The WHO, OIE and FAO also worked together to agree on the name pandemic H1N1 (2009) as an alternative to swine flu. In addition to the consumer and trade impacts, the name swine flu may have contributed towards inappropriate actions with implications for animal welfare, such as the culling of pigs or other ineffective actions targeting swine to limit the spread of H1N1pdm09 in humans. Likewise, these actions fuelled distrust by some in the swine industry and harmed surveillance efforts in the swine population. These issues have led to subsequent discussions between OIE, FAO and WHO to seek a solution to the naming issue so that common names chosen for diseases or pathogens are unlikely to have unnecessary adverse consequences.

Immediately after the sustained spread of H1N1pdm09 in human populations, outbreaks in pigs were reported in many countries around the world. The first case was detected on April 28, 2009, on a farm in Canada in pigs that were not previously vaccinated against swine IAV (Howden et al., 2009; Weingartl et al., 2010). The virus was transmitted from humans to susceptible pigs, with subsequent pig-to-pig transmission. H1N1pdm09 infection was even reported in pigs in Australia, one of the few countries reportedly free of swine IAV prior to 2009 (Deng et al., 2012), and in Norway, where swine IAV status was previously undetermined (Hofshagen et al., 2009). According to the international reporting obligations in the OIE Terrestrial Animal Health Code, H1N1pdm09 infection in pigs was reportable to OIE as a new and emerging disease until September 2010, when requirement was dropped because the disease was generally accepted to be circulating widely in pigs. OIE Member Countries responded to this by showing solidarity and transparency: between April 2009 and September 2010, a total of 25 countries reported occurrences of H1N1pdm09 to OIE.

H1N1pdm09, a virus shared between people and pigs, has the potential to bring additional diversity to the genetic complexity of IAV in human and swine populations. Numerous reports document reassortment between enzootic swine IAV and H1N1pdm09, (Vijaykrishna et al., 2010; Ducatez et al., 2011; Howard et al., 2011; Kitikoon et al., 2011; Lam et al., 2011; Moreno et al., 2011; Pereda et al., 2011; Starick et al., 2011; Tremblay et al., 2011; Zhu et al., 2011b), further escalating the potential for evolution of IAV in swine (Fig. 1). Complicating matters further, swine H3N2 viruses bearing four gene segments from H1N1pdm09 have recently been isolated from a breeder turkey flock in Canada (Berhane et al., 2012). Evidence that genetic and antigenic drift had taken place in the H3 of these isolates, combined with the potential for turkeys to be infected with other avian IAV, suggest that an additional evolutionary pathway exists for the emergence of novel viruses that can infect swine.

None of H1N1pdm09's genes cluster tightly with those of the swine IAV that were characterized prior to the outbreak in humans (Smith et al., 2009). Phylogenetic analyses of each gene segment show that H1N1pdm09 forms an independent branch that is distinct from that of swine viruses collected before 2009. This separation suggests that neither H1N1pdm09 nor closely related progenitor viral genes were present in U.S. swine IAV before 2009. Although a swine virus isolated in China in 2004 had a triple-reassortant gene constellation with a matrix gene segment of Eurasian avian and swine origin (i.e. seven of eight genes of the gene constellation of the H1N1pdm2009 virus), each gene segment had a temporal gap of more than 10 years of evolution from the H1N1pdm09 virus (Smith et al., 2009). The long period of unsampled ancestry before the emergence of the pandemic virus in 2009 highlights the need for improved surveillance in animal hosts worldwide and in human hosts in poorly represented parts of the world.

Influenza A Viruses Identified in North American Swine Populations

Swine influenza was first recognized in pigs in the Midwestern U.S. in 1918 as a respiratory disease that coincided with the human Spanish flu pandemic. Since then, influenza has become an important disease to the swine industry throughout the world. The first IAV was isolated from swine in 1930 by Shope (Shope, 1931) and was shown to cause respiratory disease in swine that was similar to human influenza. This classical swine lineage H1N1 virus derived from the 1918 pandemic virus was relatively stable

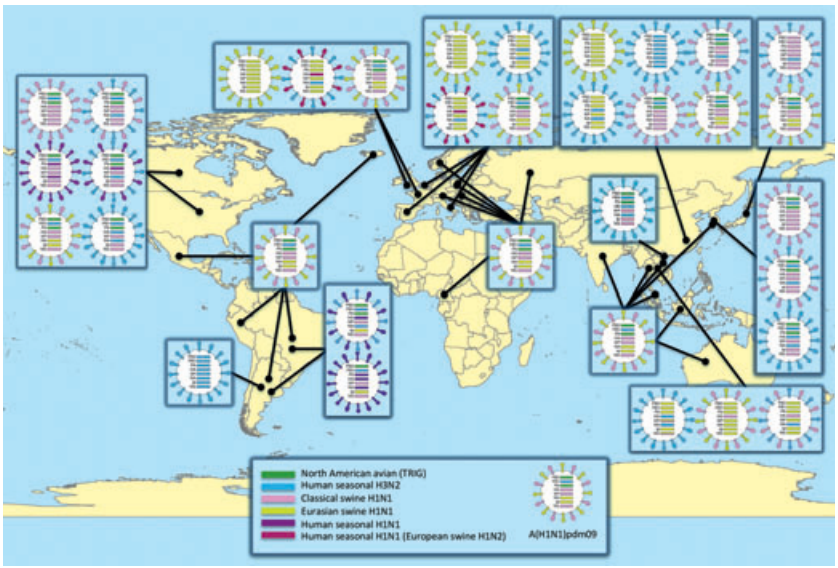

Fig. 1. Predominant influenza A viruses (IAV) endemic in swine populations around the world. Although all IAV established in swine populations are of the H1N1, H1N2 or H3N2 subtypes, multiple lineages and whole genome constellations distinguish viruses from different countries and regions. The major lineages include swine-adapted viruses of North America, Europe, Asia and human seasonal viruses that have spilled over into swine and become established including the H1N1pdm09.

at the genetic and antigenic levels in U.S. swine for nearly 80 years.

The epidemiology of IAV in U.S. pigs dramatically changed after 1998 when triple-reassortant H3N2 viruses containing gene segments from the classical swine virus (NP, M, NS), H3N2 human seasonal IAV (PB1, HA, NA) and avian IAV (PB2, PA) (Zhou et al., 1999) became successfully established in the pig population (Webby et al., 2000). This genome composition of swine IAV is referred to as the triple-reassortant internal gene (TRIG) cassette (Vincent et al., 2008). After their emergence, the H3N2 viruses reassorted with classical H1N1 swine IAV acquiring the H1N1 or H1N2 subtypes (Karasin et al., 2002; Webby et al., 2004). Reassortant H1 TRIG viruses are enzootic along with the H3N2 viruses in most major swine-producing regions of the U.S. and Canada; since early in the new millennium, the vast majority of the fully characterized swine viruses contain the TRIG cassette, regardless of subtype (Evseenko et al., 2011; Lorusso et al., 2011; Nfon et al., 2011a,b). Outside of North America, genetically related swine viruses that contain the TRIG have been identified in Korea, Vietnam and China (Pascua et al., 2008; Zhu et al., 2011a; Ngo et al., 2012). North American TRIG-containing swine viruses can readily infect turkeys, an ability that may play an unidentified role in the epidemiology of IAV in swine and human hosts (Yassine et al., 2007).

Since 2005, H1N1 and H1N2 viruses with either HA, NA or both derived from human seasonal IAV have emerged and spread across the U.S. in swine herds (Vincent et al., 2009b). The HAs from the human-like swine H1 viruses are genetically and antigenically distinct from those of classical swine–lineage H1 viruses. However, their TRIG genes are similar to those found in contemporary swine triple-reassortant viruses. To represent the evolution of the currently circulating North American H1 viruses, a cluster classification was established. Viruses with the HA gene of the classical H1N1 viruses that have circulated in swine since 1918 evolved into the contemporary α-, β- and γ-clusters, whereas H1 subtype isolates with HA genes most similar to those of human seasonal H1 viruses circulating in the early 2000s evolved into the δ-cluster (Vincent et al., 2009b). All four HA gene cluster types (α, β, γ, and δ) can be found with NA genes of either the N1 or N2 subtype. The HA genes from the δ-cluster viruses most likely emerged from at least two separate introductions of human seasonal HAs from H1N2 and H1N1 viruses, differentiated phylogenetically into two distinct sub-clusters, δ1 and δ2, respectively (Lorusso et al., 2011). Both sub-clusters have evolved extensively in swine (Nelson et al., 2011). During investigations of 2008–2010 viruses, the HAs of the δ-cluster were paired with either an N1 or N2 gene of human virus lineage but not with an N1 gene of swine lineage. Prior to 2009, the H1 IAV evolved by drift and reassortment while maintaining the TRIG backbone and giving rise to viruses differing genetically and antigenically, thus having consequences for vaccine and diagnostic test efficacy (Lorusso et al., 2011). Since that time, H1N1pdm09 has become established in the U.S. pig population with subsequent second-generation reassortants emerging (Ducatez et al., 2011; Kitikoon et al., 2012).

Influenza Viruses Identified in European Swine Populations

Multiple swine IAV with genetic lineages that are distinct from the North American TRIG viruses evolved in Europe and Asia (reviewed in Van Reeth et al., 2012). Although classical H1N1 swine viruses previously circulated in Europe, Asia and many other parts of the world for many years, they were eventually replaced by a new lineage in Europe, a wholly avian H1N1 that entered the swine population around 1979. Human lineage H3N2 viruses descending from the pandemic A/Hong Kong/1/68 (H3N2) human virus and, thus, distinct from the H3N2 IAV in North America, emerged in Europe in the 1970s. In Europe, these H3N2 viruses reassorted with the avian-like H1N1 swine IAV, from which they acquired the internal gene cassette in the mid-1980s (Castrucci et al., 1993; de Jong et al., 1999). Additionally, a reassortant H1N2 virus emerged in pigs in Great Britain in 1994 (Brown et al., 1995) and subsequently spread to other countries in Western Europe, retaining most of the genotype of the reassortant H3N2 virus but having acquired the HA gene of a human H1N1 virus found in the 1980s. Thus, the three major virus lineages share common internal protein genes, but they have clearly distinguishable HAs. A recent European surveillance study reported the continued circulation of Eurasian avian-like H1N1, human-like H3N2 and human-like H1N2 viruses in swine. All three subtypes were detected in Belgium, Italy and Spain, but only H1N1 and H1N2 viruses were found in the UK and north-western France (Kyriakis et al., 2011). Since November 2010, structured, coordinated and harmonized passive surveillance within Europe has detected a 30% incidence of IAV, primarily in pigs with acute respiratory disease, from 14 countries after investigating 3500 herds. The results have revealed the continued circulation of previously identified subtypes and the presence of H1N1pdm09 in at least seven of these countries, although continued circulation of H1N1pdm09 in some countries remains uncertain. Additionally, next-generation reassortants arising from H1N1pdm09 with endemic strains in European pigs have been detected. Additional second-generation reassortants – H1N2 viruses with an avian-like H1 and H1N1 viruses with a human-like H1 derived from the prototype H1N2 viruses – have also been detected (Marozin et al., 2002; Balint et al., 2009; Howard et al., 2011; Kyriakis et al.,2011; Moreno et al., 2011), but they remain relatively rare.

Influenza Viruses Identified in Asian Swine Populations

In 2010, China and Vietnam produced 476.2 and 27.3 million pigs, respectively, collectively accounting for 53% of global pig production (FAOSTAT-www.faostat.fao.org). The existence of highly dense populations of swine, poultry and waterfowl in China, Vietnam, Thailand and other Asian countries indicates the need to intensify surveillance for IAV in the region. Classical swine H1N1 viruses (first detected in China in 1974 but probably present for many decades before) are enzootic in swine in China and co-circulated with H1N2 viruses that acquired an N2 of contemporary human origin. Human H3N2 viruses (A/Hong Kong/1/68-like; A/Port Chalmers/1/73-like; A/Sydney/05/97-like) were repeatedly transmitted to pigs and circulated in pigs long after the parent human virus had been replaced in the human population (Shortridge et al., 1977; Peiris et al., 2001). Avian H1N1 viruses were detected in swine in China in 1993. However, these were not descendants of Eurasian avian-like H1N1 viruses and probably represented an independent interspecies transmission from the Asian avian reservoir to swine (Guan et al., 1996). European H3N2 and H1N1 viruses were first detected in China in 1999 and 2001, respectively, and North American triple-reassortant viruses were first found in 2002, indicating intercontinental movement of swine viruses, possibly via importation of swine (Vijaykrishna et al., 2011). Co-circulation of different swine IAV lineages was associated with the appearance of reassortants during the intercessory time period between emergence of each new lineage (Vijaykrishna et al., 2011). More recently, H1N1pdm09 and its reassortants have also been detected in swine (Vijaykrishna et al., 2010). Although much of this surveillance has been carried out in slaughterhouses in Hong Kong, the swine slaughtered in Hong Kong are imported from many provinces in China; therefore, these data probably give an indication of swine influenza ecology in the wider region. In addition, H9N2, H5N1, H4N8 and H6N6 avian IAV have been sporadically detected in pigs in some Asian countries (Xu et al., 2004; Trevennec et al., 2011; Zhang et al., 2011; Su et al., 2012) .

Classical swine H1N1 virus probably appeared in the Japanese swine population around 1977 (Miwa et al., 1986) and then reassorted with a human seasonal H3N2 virus to emerge in 1980 as an H1N2 virus possessing all the segments from classical swine IAV except the NA gene (Sugimura et al., 1980). Swine IAV of this genotype have been the predominant isolates from pigs in Japan (Ito et al., 1998; Saito et al., 2008; Yoneyama et al., 2010). The results of serologic examination (Goto et al., 1992) and of virus isolation from swabs taken at slaughterhouses (T. Saito, S. Kasuo, Y. Yamashita, K. Goto, H. Kubo, unpublished data) confirmed that H3N2 viruses of human lineage have occasionally entered Japanese pig populations. After the emergence of the H1N1pdm09 virus in humans, the virus infected pig populations in Japan and reassorted with H1N2 IAV (Matsuu et al., 2012).

H1N1 and H3N2 viruses have been found in swine in Thailand since the early 1980s (Nerome et al., 1981, 1982). In general, the H3N2 and H1N1 IAV circulating in Thai pigs are related to the lineages found in the Eurasian H3N2 and H1N1 viruses and in the classical H1N1 virus. Swine H3N2 viruses from early 2000 to 2007 (Takemae et al., 2008) contain human lineage HA and NA genes, with internal genes from the Eurasian (PB1, PB2, PA and M genes) and classical H1N1 (NP and NS genes) swine lineages. The Thai swine H1N1 viruses (ThH1N1) circulating from 2000 until the emergence of the H1N1pdm09 virus are unique reassortants of classical and Eurasian swine lineage. These ThH1N1 viruses could be grouped into classical-HA and Eurasian-NA swine lineages, with internal genes being either all Eurasian swine (7 + 1) or Eurasian swine with the classical swine NS gene (6 + 2) (Takemae et al., 2008). The NA genes of ThH1N1 and H1N1pdm09 have <90% nucleotide similarity, indicating that although the NA genes originated from the same Eurasian H1N1 ancestor, they have evolved separately. The third subtype, H1N2, was first isolated from pigs in 2005 (Chutinimitkul et al., 2008) and contained combinations of genes from the endemic human-like H3N2 and ThH1N1 viruses that were circulating in swine herds (Takemae et al., 2008). Reassortant H1N1 containing seven genes of H1N1pdm09 and the NA gene of endemic ThH1N1 virus were detected repeatedly in a commercial swine herd with IAV-associated respiratory disease (Kitikoon et al., 2011), suggesting an increasing genetic diversity among future circulating IAV in Thai pigs.

In Vietnam, reassortant H3N2 viruses with the North American TRIG were detected in swine (Ngo et al., 2012). The H3 and N2 genes were acquired by reassortment with a human seasonal virus circulating in humans around 2004–2006. The H3 and N2 genes were very similar to those of H3N2pdm09 swine reassortant viruses isolated in China (Fan et al., 2012). In South Korea, the classical swine H1N1, H1N2 and H3N2 IAV of the North American triple-reassortant lineage co-circulate (Lee et al., 2008) with an additional human lineage H3N2 virus that is distinct from the lineages in Vietnam and China (Pascua et al., 2008). Serologic screening of commercial pigs in Malaysia has detected H3N2 and H1N1 in 41.4% of the farms surveyed (Suriya et al., 2008), but information about the distribution and gene flow of swine IAV in most other countries in the Asia-Pacific region is unavailable.

Influenza A Viruses Identified in South American Swine Populations

Few published reports about virus isolations or sequences are available to document the presence of IAV in swine in many countries in Central and South America. Argentina has recently reported the presence of distinct human lineage viruses of the H1N1 and H3N2 subtypes (Cappuccio et al., 2011; Pereda et al., 2011). The Argentinean viruses are distinguishable from similar subtypes in North America and represent independent human-to-swine transmission events. In late 2008, a wholly human H3N2 was isolated from pigs with clinical signs of respiratory disease and fever typical of influenza. Experimentally reproduced infections showed that the virus was transmitted efficiently between pigs and that the inoculated pigs had characteristic lesions of influenza, suggesting that this virus was completely adapted to swine and could be maintained in the swine population (Pereda et al., 2011).

In 2009 and 2010, Argentina reported the isolation of reassortant viruses with internal genes from H1N1pdm09 and surface genes (HA and NA) from human-like (North American δ2-like) H1 swine IAV. Therefore, some indirect evidence of circulation of this δ2 H1 cluster in Argentina exists (Pereda et al., 2011). In 2011, another reassortant virus was isolated, with surface genes from the wholly human H3N2 virus first isolated in 2008 and internal genes from the H1N1pdm09 virus. The clinical signs observed in all these cases were typical for influenza (e.g. fever, dyspnoea, coughing and sneezing). It is postulated that absence of vaccines and the characteristics of pig production in Argentina (e.g. presence of all ages of pigs from neonatal to adult at one site) may have contributed to the emergence of these new reassortants.

In Brazil, few reports of IAV infection in pigs existed before 2010. Recently, coinciding with the H1N1pdm09 in humans, numerous outbreaks of acute respiratory infection in pigs of different age groups were reported in Brazil, and the H1N1pdm09 virus was identified as being the cause (Schaefer et al., 2011). In addition, a new H1N2 IAV identified in a recent study contains H1 and N2 genes of human seasonal origin (similar to North American Swine δ-cluster) and internal genes (M, NP, PB1, PB2 and PA) from H1N1pdm09. ELISA testing (IDEXX Influenza MultiS-Screen ELISA) of sera collected from 106 commercial farms from July 2009 to December 2011 had a 60% positivity rate (1889 positive/3150 total sera). Furthermore, analyses of sera collected from pigs in Brazil revealed that HI antibodies against H1N1pdm09 were not detected in pigs in Brazil prior to 2009 (Ciacci-Zanella et al., 2011). A separate study, which involved serologic detection, virus isolation, genomic sequencing and study of the dynamics of infection, detected IAV in pigs in six Brazilian states. It was concluded that the IAV was circulating in at least 64.7% of the farms studied. The HA gene of the isolates was very similar to H1N1pdm09. Samples collected from those farms before 2009 showed IAV infection but not H1N1pdm09 infection (J. R. Ciacci-Zanella, unpublished). The population of wild *Suidae*, including both captive wild boars reared under intensive management and free-range feral pigs of the Pantanal Region in Brazil,

was also evaluated. IAV were detected in 11 of 60 lungs with macroscopic pneumonia lesions using quantitative PCR. The M gene sequence was 98–99% identical to that of H1N1pdm09 (Ciacci-Zanella, unpublished). Of the 141 feral pigs tested, IAV antibodies were found in 105 (74.5%).

Influenza A Viruses Identified in African Swine Populations

One published report of IAV detection in pigs in Africa was found, the isolation of an H1N1pdm09 in Cameroon (Njabo KY, 2011). Sequence for an additional H1N1 pdm09 from Nigeria was also found in GenBank. Although there was a limited serologic survey in pigs from Nigeria suggesting high seroprevalance to human seasonal IAV (Adeola OA, 2010), this is in contrast to the extremely low seroprevalence reported in Cote d'Ivoire, Benin and Togo (Couacy-Hymann E, 2012), and the status of endemic IAV in swine in Africa remains unclear. The population of pigs is Africa is low compared with other continents, and the sparse population density may contribute to the relatively low incidence of IAV reported in swine from this region.

The Relevance of IAV to Swine Health and Implications for Control

Influenza A viruses is a major cause of viral pneumonia in pigs and a significant contributor to the porcine respiratory disease complex. In addition to causing primary disease, this virus is well documented to predispose pigs and many other host species to subsequent secondary bacterial pneumonia, the effects of which may last long after IAV can be detected. Because of the complicated nature of IAV evolution in all major host species, inactivated vaccines have become a suboptimal method to control IAV. No single inactivated swine influenza vaccine fits all situations in a single geographical region, and the issue is more problematic from a global perspective. In an epidemiological investigation of the occurrence of influenza disease in swine farms in the Midwestern U.S., sow vaccination status did not change the number of herds with IAV respiratory disease diagnoses (Beaudoin et al., 2012). Studies have shown not only partial protection from disease but also an enhancement of disease and pathologic changes in the lungs of pigs, most recently in pigs vaccinated with a virus with the H1 HA derived from human seasonal IAV (δ-cluster H1) and challenged with H1N1pdm09 in laboratory settings (Gauger et al., 2011). These experimentally induced events may have implications for commercially raised pigs because of the multiple antigenic variants circulating in pigs worldwide and the use of inactivated vaccines in some regions.

The Relevance of Swine IAV to Human Health

Influenza A viruses from swine can cause sporadic zoonotic infections, representing a potential and continuous threat to human health, especially when the human population immunity acquired from vaccination or natural infection with human seasonal viruses lacks cross-reactivity to the swine viruses. In rare occasions, IAV from pigs can acquire the ability to cause human pandemics. The H1N1pdm09 virus replaced the previously circulating human seasonal H1N1 IAV and is now the only H1N1 virus circulating in the human population. Therefore, it has become the H1N1 component in the trivalent human seasonal influenza vaccine since 2010. Consequently, exposure to the H1N1pdm09 virus in the majority of the human population is expected to have induced significant cross-reactive immunity to alpha-, beta- and gamma-cluster swine H1 viruses that may be circulating in swine in different regions of the world (Lorusso et al., 2011). However, because of the poor antigenic cross-reactivity between the H1N1pdm09 virus and the swine δ-cluster H1N1 and H1N2 viruses, younger people have no specific humoral immunity to these δ-cluster H1 viruses elicited by either vaccination or natural infection. Similarly, the Eurasian avian-like H1N1 IAV that has circulated in Europe and Asia since 1979 has caused zoonotic infections in Spain and Switzerland. The antigenic properties of these viruses indicate poor cross-protection by immunity to the human seasonal IAV or H1N1pdm09. Likewise, the antigenic characteristics of human lineage H3N2 viruses that entered the swine population of North America in the late 1990s have become increasingly divergent from the human seasonal H3N2 and will continue to do so. Indeed, a dozen zoonotic human infections with swine H3N2 viruses (termed H3N2v) were identified in the fall of 2011 (Lindstrom et al., 2012). The genome of these viruses contained seven genes from the North American H3N2 and the M gene from H1N1pdm09 (Lindstrom et al., 2012). The number of cases of H3N2v rose dramatically in the late summer of 2012, with over 300 cases detected to date, most with extensive contact with swine at agricultural fair exhibitions (http://www.cdc.gov/flu/swineflu/variant-cases-us.htm). A limited number of cases reported no swine contact, indicating limited human-to-human transmission may occur. The current European H3N2 swine viruses still have some serologic cross-reactivity with human viruses from the 1970s and 1980s but not with human viruses isolated after 1990 (de Jong et al., 2007; Kyriakis et al., 2011).

The pig population now serves as a reservoir of IAV genes that have circulated in humans: the surface glycoprotein genes HA and NA from the U.S. δ-cluster H1s and other human-like H1 viruses from Europe, Asia and South America; H3N2 surface genes from Europe, Asia and North

Table 1. Influenza A viruses (IAV) in swine by region with genetic origins of hemagglutinin (HA) or neuraminidase (NA) from human seasonal IAV with year of isolation of most closely related ancestor by gene segment[a]

Country or region	Subtype	HA	NA
North America	H3N2	1996	1996
			2003
	H1N2	2003	2003
	H1N1	2003	2003
Argentina	H3N2	2001	2002
	H1N2	2003	2003
	H1N1	2003	2003
Brazil	H1N2	2002	1997
Europe	H3N2	1973	1973
	H1N2	1980	1973
China	H3N2	1968	1968
		1973	1973
		1997	1997
		2004	2004
Thailand	H3N2	1976	1976
		1997	1997
Japan	H3N2	1968	1968
Vietnam	H3N2	2004	2004
South Korea	H3N2	1995	1995

[a]Does not include H1N1pdm09 transmitted from people to pigs during and following the pandemic.

and South American lineages (Table 1); HA and NA from H1N1pdm09; the M gene from H1N1pdm09; the PB1 gene of human origin found in the North American TRIG constellation and H1N1pdm09 viruses; and genes from swine lineage viruses from other global regions (Nelson et al., 2012 and co-authors' data). This virus reservoir status, combined with sporadic interspecies infections in pigs with human-, equine- and avian-lineage viruses, may provide the opportunity for continued IAV reassortment and emergence of new variants in pigs that may potentially infect the human population.

The potential for further zoonotic transmission of existing and emerging novel viruses from pigs to people is difficult to quantify. Influenza A viruses can be shared among species, but the interplay between human and swine viruses seems to be detected with greater frequency. These reports may be expected to continue to increase with improved surveillance, PCR diagnostic assays targeting multiple genes, and readily available, more affordable sequencing technology. The risk of swine origin viruses to public health is not a new concept in veterinary medicine, and there are many examples of animal pathogens and diseases that are controlled because of their effect on human health rather than that on animal health (e.g. rabies, trichinella, brucellosis, tuberculosis, *E. coli* O157H7, bovine spongiform encephalopathy, *Salmonella enterica*) (Pappaioanou et al., 2004). Indeed, swine IAV have been infecting occupationally exposed humans for more than 40 years (Myers et al., 2006). Good animal health management, biosecurity and hygiene practices are essential on farms, but these control measures are much more difficult to extrapolate to points of concentration at the human–animal interface, such as live-animal markets, fairs, exhibits and petting zoos. Furthermore, the phenomena of reverse zoonoses whereby infected humans transmit IAV to pigs should also be considered as a risk to pig health. Research, routine surveillance and careful epidemiological investigations are needed to fully understand the role that these environments play in the overall transmission of IAV between humans and pigs. Improved vaccine strategies, combined with other preventive practices in both species, are essential to the control of IAV. The frequency with which endemic human viruses have become adapted to pigs only underscores the need for improved influenza control programs (including vaccination) to protect both humans and pigs.

Implementing successful control programs for IAV depends on proper research and virological surveillance. To preserve resources, the focus of surveillance and research must be guided by well-structured analyses of risk factors to ensure meaningful results. In an effort to provide such guidance, we conducted a systematic review of published literature and ranked putative risk factors associated with emergence and dissemination of IAV in swine to determine high-priority countries for targeted surveillance. The analysis was targeted to the 27 countries with the highest pig populations using the software ArcGIS and the command 'sort on natural breaks'. Factors associated with pig, poultry and wild waterfowl density; IAV infection in pigs and birds; live-pig exports; husbandry; market practices in the swine and poultry industries; and international trade practices were considered. Details on the methods of this analysis can be found as Appendix S1 and Tables S1–S6 in Supporting information.

Figure 2 summarizes those countries identified in the analysis as being a high-priority for targeted surveillance for IAV in swine. The analysis shows that the highest ranking for spread and emergence of IAV occurs in East and Southeast Asia, followed by Western Europe and North America. This priority ranking does not imply that lower-ranked countries should not conduct IAV surveillance in swine, but merely emphasizes those regions that should have the highest priority and subsequent allocation of resources. Although many of these countries currently survey swine for IAV, albeit on a limited basis, others have relatively little information about the status of IAV in the pig population. Until the status of IAV in the pig populations in these high-priority countries is revealed, we cannot fully understand the complicated nature of IAV evolution.

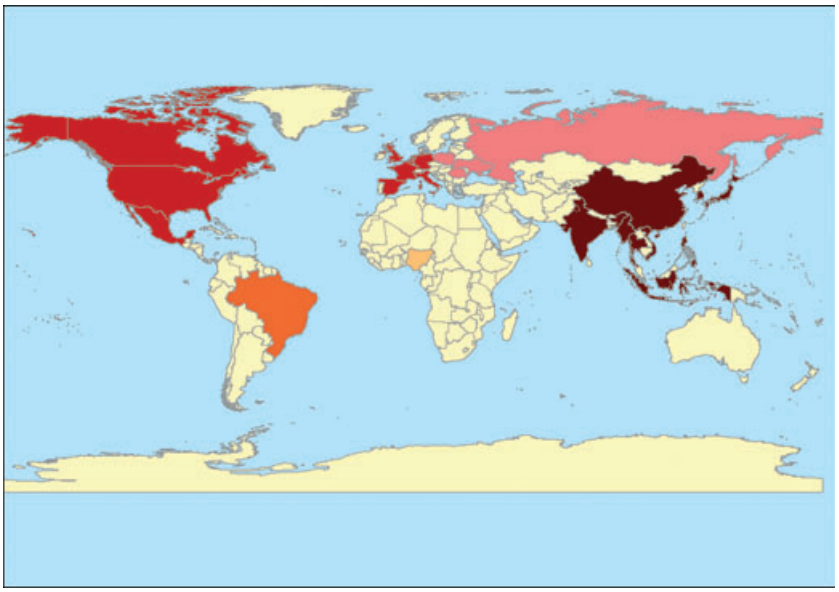

Fig. 2. Countries with priority for targeted surveillance for influenza A virus (IAV) evolution or circulation in swine. Brown, the highest priority for surveillance in swine is in countries of East and Southeast Asia due to higher rankings for spread and emergence of IAV in swine, with high population numbers of pig, poultry, and waterfowl, risk factors for outbreaks, and potential for reassortment between viruses circulating in pigs and birds due to husbandry methods. Red, Western Europe and North America have higher ranking with risk factors for outbreaks in pigs and continental and global spread through movement and export. Pink, countries of Eastern Europe are ranked for potential reassortment between viruses circulating in pigs and birds plus global spread. Light orange, Nigeria ranked for potential reassortment between viruses circulating in pigs and birds. Dark orange, Brazil ranked for outbreaks in pigs, but with lower risk of global spread by export. This priority ranking does not imply that lower-ranked countries should not conduct IAV surveillance in swine, but emphasizes those regions that should receive priority focus.

Conclusions

Surveillance, epidemiological investigations and genetic characterization of IAV associated with respiratory disease outbreaks in pigs are necessary to monitor the evolution of viruses in the pig population and to aid in the development of sensitive and specific diagnostic tests. Additionally, antigenic characterization is needed to fully understand the relevance of genetic changes for vaccine antigen selection, and vaccine efficacy must be evaluated in the context of serologic cross-reactivity when new variants arise. The H1N1pdm09 virus underscores the potential risk to human and animal populations of IAV subtypes and genotypes that may evolve with the swine IAV TRIG backbone and other virus lineages. Increased surveillance for IAV and new variants of H1N1pdm09 and endemic swine IAV in the swine and human populations is essential to understanding the dynamic ecology of IAV in susceptible host populations. A cycle of human-to-swine transmission, followed by evolution in swine, then re-entry into the human population has been established in contemporary human and swine IAV (Nelson et al., 2012). The bidirectional interspecies transmission of IAV and ongoing evolution of these viruses in swine and humans are unprecedented in the history of this virus. In addition to wild waterfowl and other avian species, swine serve as reservoir host for IAV, and other susceptible species, including turkey, quail, mink and small mammals (Gagnon et al., 2009; Nfon et al., 2011a; Tremblay et al., 2011), may play a direct role in the evolution and ecology of IAV in swine and subsequently humans.

To address the many knowledge gaps in our understanding of this virus, the OIE and the FAO formed OFFLU (OIE-FAO network of expertise on animal influenza) in 2005. OFFLU started as a network of laboratories formally organized to provide expertise in the animal health sector for surveillance, diagnostics, research and control of highly pathogenic avian influenza. It was established to coordinate and improve the sharing of biological materials and influenza data within the public health sector, particularly with the WHO. In 2009, expertise on IAV circulating in swine, equine and other animal hosts was added to OFFLU's mission. The mission of OFFLU is accomplished although collaboration by exchange of information, biological materials and resources between all influenza sectors. Although pigs may support the emergence of new viral reassortants, increasing evidence suggests that they are more often the recipients of viruses containing gene segments from human or avian viruses than they are the source of new viruses that infect other hosts.

Cross-species transmission and the true directionality of virus movement cannot be fully understood without surveillance combined with virological and epidemiological investigation. A global influenza surveillance system in pigs has not yet come to fruition despite the existence of several successful local and regional surveillance programs. A global approach enables integration and sharing of data and resources across regions, increasing the effectiveness of surveillance efforts. Furthermore, unless a wide variety of pigs and geographical locations are continuously sampled, the information may be biased and lead to inaccurate interpretation or decisions. Building trust between the swine industry and animal and public health officials to conduct more thorough investigations in swine is critical for accomplishing these surveillance efforts. The OFFLU network and WHO are addressing the need for global integration and rapid sharing of data and resources to fight IAV in swine and other animal species including humans, but this effort also requires grassroots support from government, practicing veterinarians and the swine industry and, ultimately, requires significant increases in funding and infrastructure. Facing the swine and human health issues caused by influenza proactively using science, transparency and cooperation is our challenge and opportunity now and in the coming years.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Appendix S1. Materials and methods.

Table S1. Factors positively associated with the prevalence of antibodies to H1N1 and/or H3N2 SIV.

Table S2. Factors positively associated with clinical outbreaks of highly pathogenic avian influenza in domestic birds.

Table S3. Swine population and density by country (WAHID 2009 and FAO 2007 Data).

Table S4. Countries with higher national risk factors associated with influenza infection in swine.

Table S5. Countries with higher risk factors associated with influenza infection in birds and potential for interspecies transmission and/or reassortment between viruses circulating in pigs and in birds.

Table S6. Density of exported pigs and destination.