Epidemiological studies of influenza A(H1N1)pdm09 virus infections in the Norwegian pig population

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Figure 1 Electron micrographs of influenza viruses
(http://www.nationalgeographic.com/125/the-smallest-world/#/5)

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Preface

Abundant research, past and ongoing, has elucidated much on the influenza virus. It is an age-old yet ever evolving pathogen for many species where pigs, poultry and man as hosts have elicited the most attention. New subtypes emerging from time to time, such as that of the recent pandemic 2009 strain, have ushered in new research and surveillance activities in pigs and humans worldwide, including Norway. Subtypes that affect poultry and human health have grabbed headline news because they have been the most devastating in terms of morbidity and mortality. Influenza infections in pigs without coinfections of other respiratory pathogens are usually much less severe clinically. Nevertheless, pigs are regarded widely and uniquely as nature's 'mixing vessel' because RNAs from different strains of influenza virus of avian, human and pigs can be mixed and matched within the pig host to produce new strains including fearsome pandemic strains. Even though the world has well-established influenza surveillance and network alert system for human influenza, sustained and systematic surveillance of influenza virus in pig populations are scant or absent worldwide because animal health sectors do not usually get funding to carry out such work. In some countries, the US for example, there is a disincentive to detect influenza viruses in domestic livestock if clinical disease is absent because it can cause needless concern among consumers. Norway is an exception because influenza A virus infection in pigs is a notifiable disease. Annual influenza A virus serosurveillance for its pig population has been in place since 1997 as part of a greater surveillance for other important viruses that are exotic to pigs in Norway. Before 2009, the Norwegian pig population was naïve to all strains of influenza A

virus. It was the emergence of influenza A(H1N1)pdm09 virus as human cases in April 2009 and the subsequent incursion in pigs in Norway that presented the golden opportunity for a series of exciting observational studies under Norway's unique conditions. The goals have been to uncover aspects in epidemiology related to this new influenza A virus and investigate its impact on the Norwegian pig population. Findings on negative impact on growth performance of pigs in this thesis may have been the first time the adverse effects of influenza A virus infection on growth performance of pigs was quantified in such a large scale and shared with the scientific community. I therefore hope that the endeavours in this thesis have contributed somewhat to the scientific community, a greater understanding of the epidemiology, production impact and ecology of influenza A(H1N1)pdm09 virus infection in pigs. As the investigations had focused on the Norwegian pig population, research findings may be especially valuable for Norwegian pig farmers and the Norwegian Food Safety Authority. Despite all, there remain many exciting aspects of influenza virus infection in pigs waiting for discovery, whereby I hope my work can provide a springboard for further research.

Chiek Er

Oslo, September 2016

"And you will know the truth, and the truth will set you free"

The Gospel of John chapter 8 verse 32.

Acknowledgement

Ultimately and of paramount importance, my thanks go to God. He controls the universe actively or passively allows events to happen for the ultimate good. These include the timely clinical outbreak of influenza A(H1N1)pdm09 at the boar testing station in 2011 and also my unforeseen meeting 15 years ago in Perigueux France, with a certain reader of Stephen Hawking's "A Brief History of Time." Although professor Hawking would surely disagree with me, I believe that without destiny's benign intervention, my path in life would have led me elsewhere, no doubt to a less rosy place on earth. Then I would not be working at the Norwegian Veterinary Institute with its amazingly modern facilities, brilliant fellow colleagues, and kind human beings. I would also be missing out on proudly singing "Ja, vi elsker dette landet" on every 17th of May, which would be indeed the greatest shame.

Chiek Er

Oslo, 19 September 2016

Abbreviations

AB	Antibodies
ELISA	Enzyme-Linked Immunosorbent Assay
EP	Enzootic Pneumonia
FCR	Feed Conversion Ratio
H1N1pdm09	Influenza A(H1N1)pdm09 virus
HA	Haemagglutinin
HI	Haemaglutination Inhibition Test
IAV	Influenza A Virus
ILI	Influenza Like Illness
NA	Neuraminidase
NFSA	Norwegian Food Safety Authority
NVI	Norwegian Veterinary Institute
PRDC	Porcine Respiratory Disease Complex
PRRS	Porcine Reproductive and Respiratory Syndrome
PRCV	Porcine Respiratory Corona Virus
PCV2	Porcine Circovirus type 2 Virus
RCT	Randomised control trial
RNA	Ribonucleic Acid
RT-PCR	Reverse transcription-Polymerase Chain Reaction
SI	Swine Influenza
swIAV	Swine Influenza A Virus
SPF	Specific Pathogen Free

List of papers

Paper 1

Clinical impact of infection with pandemic Influenza (H1N1) 2009 virus in naive nucleus and multiplier pig herds in Norway.

Grontvedt, C. A., C. Er, B. Gjerset, A. Germundsson, T. Framstad, E. Brun, A. Jorgensen and B. Lium (2011).

Influenza Res Treat. 2011: 163745.

Paper 2

Influenza A(H1N1)pdm09 virus infection in Norwegian swine herds 2009/10: the risk of human to swine transmission.

Grontvedt, C. A., C. Er, B. Gjerset, A. G. Hauge, E. Brun, A. Jorgensen, B. Lium and T. Framstad (2013).

Prev Vet Med. 110 (3-4): 429-434.

Paper 3

Adverse effects of Influenza A(H1N1)pdm09 virus infection on growth performance of Norwegian pigs - a longitudinal study at a boar testing station.

Er, C., B. Lium, S. Tavornpanich, P. O. Hofmo, H. Forberg, A. G. Hauge, C. A. Grontvedt, T. Framstad and E. Brun (2014).

BMC Vet Res 2014 Dec 4; 10: 284. doi: 10.1186/s12917-014-0284-6.

Paper 4

Production impact of influenza A(H1N1)pdm09 virus infection on fattening pigs in Norway.

Er, C., E. Skjerve, E. Brun, P. O. Hofmo, T. Framstad and B. Lium (2015).

Journal of Animal Science. 2016 Feb; 94(2):751-9. doi: 10.2527/jas.2015-9251.

Paper 5

Occurrence and spread of influenza A(H1N1)pdm09 virus infection in Norwegian pig herds based on active serosurveillance from 2010 to 2014.

C. Er, E. Skjerve, E. Brun, T. Framstad, and B. Lium. (2016).

Epidemiology and Infection. 2016 July 14; 1-18. doi: 10.1017/S0950268816001424

Summary

On October 10 2009, influenza A(H1N1)pdm09 virus (H1N1pdm09) was confirmed as the first influenza A virus (IAV) to infect the Norwegian pig population since 1998 when H3N2 antibodies were detected in a dead end infection on a pig farm.

One year after the outbreak, a case control study involving 115 breeding herds (47 nucleus herds, 68 multiplier herds) reinforced the belief that infected people had transmitted the virus to the pigs during the initial phase of the outbreak. The ineffective conventional biosecurity measures in preventing pig herds, including closed nucleus herds, from being infected were further corroborating evidence.

Pigs in serologically positive herds did not always have detectable clinical signs. Of the 48 positive herds, only 19 or 40% reported clinical signs of swine influenza.

Although the clinical effects appeared mild or absent in infected herds, a longitudinal study (n=1955 pigs) at a boar testing station demonstrated that the infection had adverse effects on the growth rate of growing pigs. An infected pig consumed between 6 – 8 kg more feed and required additional 1.6 – 2.4 days to grow from 30 kg to 100 kg body weight as compared with uninfected pigs.

In an attempt to extrapolate these results to a commercial farming situation, stochastic models predicted the summed extra feed and protracted production time in an infected batch of 150 fattening pigs. Apart from the variability between individual pigs, time point of batch infection (first pig infected), age of pig at infection, and the final prevalence of infected pigs in the batch were variability and uncertainty factors in the model. Simulations found that an infected batch of 150 pigs could require additional feed of between 0.8 tons (fifth percentile) to 1.4 tons (95th percentile) and

protraction of production time by 194 days (fifth percentile) to 334 days (95th percentile) to grow from 30 kg to 100 kg body weight.

The final part of this thesis was a study on the spread and survival of H1N1pdm09 in the Norwegian pig population by using the data from the active national serosurveillance program for virus infections in pigs from 2010 to 2014. Since its incursion in 2009, temporal, spatial and farming demographics analysis found that the virus has established itself in the Norwegian pig population as evidenced by ongoing new and recurrent herd infections. The overall running mean of national herd seroprevalence, and annual herd incidence risks fluctuated narrowly around the means of 45% and 32%, respectively. In 50 % of positive herd tests, > 60 % of the sampled pigs in each herd had antibodies against H1N1pdm09. Spatially, the levels of herd seroprevalence and incidence risks were highest in the heaviest pig farming counties of Rogaland, Nord-Trøndelag and Hedmark, in descending order. Herd prevalence and incidence risks also varied amongst the five production classes. At the end of 2014, sow pool herds registered the highest herd seroprevalence of >90%, followed by multiplier herds ~ 55%, conventional sow herds ~40%, nucleus ~40% and fattening herds ~9%. This investigation indicated strongly that H1N1pdm09 has established itself as a widespread endemic infection in the Norwegian pig population at the end of 2014.

Sammendrag (summary in Norwegian)

Den 10. oktober 2009 ble (H1N1)pdm09 påvist for første gang i en norsk svinebesetning. Med unntak av ett tilfelle i 1998 da antistoffer mot H3N2 ble påvist hos griser i en enkelt besetning, hadde ikke infeksjon med influensa A virus vært diagnostisert hos norske griser tidligere.

Ett år etter introduksjonen av H1N1pdm09 ble det gjennomført en kasus-kontrollstudie som omfattet 115 avlsbesetninger (47 foredlings- og 68 formeringsbesetninger). Studien viste at infiserte personer var en viktig årsak til introduksjon av H1N1pdm09 til nye besetninger. Tradisjonelle smitteforebyggende tiltak hadde ikke hindret at virus ble spredt til nye besetninger, inkludert til foredlingsbesetninger som ikke hadde hatt kontakt med griser i andre besetninger.

Griser i serologisk positive besetninger viste i liten grad kliniske tegn på sykdom. Fra 48 positive besetninger ble det rapportert kliniske tegn på svineinfluensa fra bare 40 % (n = 19) av besetningene.

Selv om kliniske tegn på influensa var milde eller fraværende i infiserte besetninger, viste en stor longitudinell (n = 1955 griser) på Norsvins testingsstasjon for råner at infeksjon med H1N1pdm09 resulterte i redusert fôrutnyttelse og tilvekst. Infiserte griser spiste 6-8 kg mer fôr og brukte 1,6 til 2,4 dager lengre tid fra 30 kg til 100 kg kroppsvekt sammenlignet med ikke infiserte griser.

I et forsøk på å ekstrapolere disse resultatene til kommersiell svineproduksjon, ble det laget en stokastisk modell som estimerte forventet økning i fôrforbruk og lengre produksjonstid i en gruppe med infiserte slaktegriser. I modellen ble de tatt hensyn til variasjoner mellom individer og usikkerhet og variasjon i tidspunkt for infeksjon, alder på grisene når de ble infiserte og prevalensen av infiserte griser i gruppen. Modellen

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viste at en infisert gruppe på 150 slaktegriser brukte mellom 0,8 tonn (5% percentilen) og 1,4tonn (95% percentilen) mer fôr enn en ikke-infisert gruppe, og trengte mellom 194 (5% percentilen) og 334 (95% percentilen) flere dager for å vokse fra 30 til 100 kg kroppsvekt.

En studie basert på det nasjonale overvåkingsprogrammet for virusinfeksjoner hos gris for årene 2010 til 2014, viste at H1N1pdm09 var vidt utbredt i den norske svinepopulasjonen fem år etter at viruset ble introdusert. Det skyldtes både vedvarende infeksjon i allerede smitta besetninger, og stadig spredning av smitte til nye besetninger. På landsbasis varierte forekomsten av positive besetninger lite fra år til år, med et middel på 45 %. I 50 % av de positive besetningstestene hadde over 60 % av undersøkte griser antistoffer mot H1N1pdm09. Forekomsten av seropositive besetninger var høyest i fylker med størst tetthet av svinebesetninger det vil si Rogaland, Nord-Trøndelag og Hedmark i avtagende rekkefølge. Forekomsten av positive besetninger varierte mellom fem ulike produksjonskategorier. Ved utgangen av 2014 var prevalensen av positive besetninger høyest (>90 %) blant purkeringer, fulgt av formeringsbesetninger (55 %), bruksbesetninger med purker (40 %), foredlingsbesetninger (40 %) og lavest hos slaktegrisbesetninger (9 %). Undersøkelsen viser at H1N1pdm09 var etablert som en utbredt, endemisk infeksjon i den norske svinepopulasjonen ved utgangen av 2014.

Introduction

The Norwegian pig production and the pig health status

In 2014, there were ~ 2000 pig herds registered in Norway. With minimal live imports across its borders, the Norwegian pig production is a pyramid self-sufficient system of unidirectional flow of semen and gilts from breeding herds to conventional sow herds before ending with fattening herds at the base level (Figure 2).
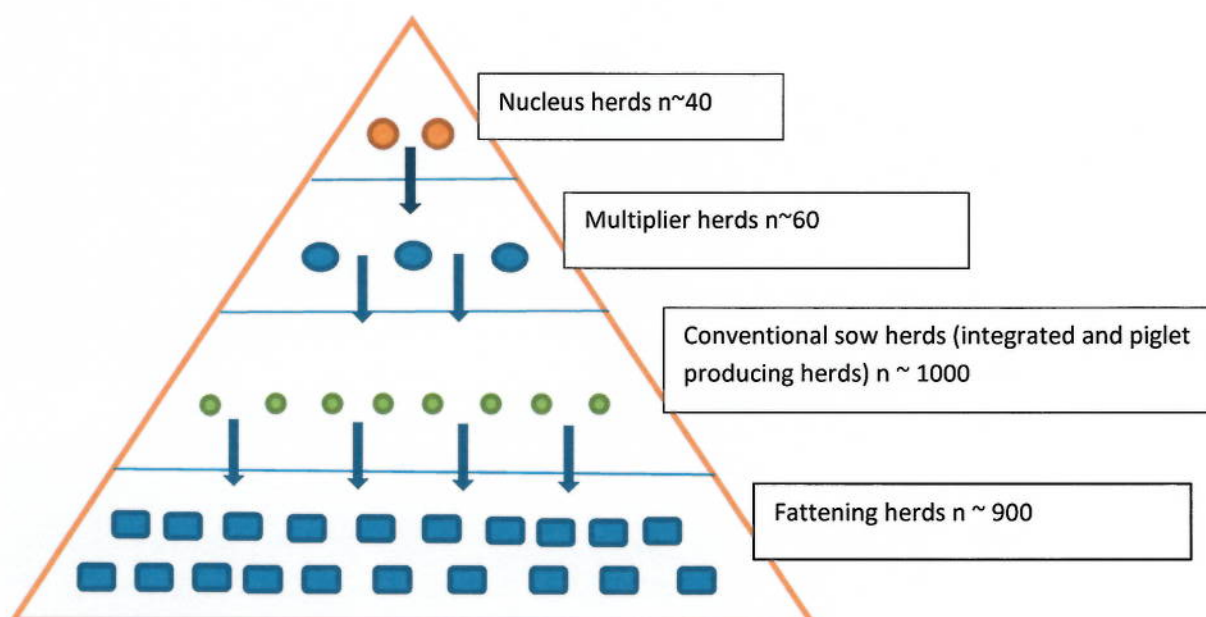
Figure 2. The Norwegian pig production pyramid (n~annual figures in 2014) (original drawing).

Closed nucleus herds are at the top (n ~ 40, of which 4/5 are pure Landrace and 1/5 pure Duroc) with the highest biosecurity amongst production types and where pure genetic lines are constantly improved. Expanding in the next level are multiplier herds (n ~ 60). Some multiplier herds are 'closed' but the majority are associated with one nucleus herd. They produce maternal lines of Landrace-Yorkshire (LY) cross and supply gilts to conventional sow herds (n ~ 1000) which includes both integrated and piglet producing herds.

Unique to Scandinavian countries with their small sow herds, the sow pool system in Norway involves a cooperation of between 10 to 20 pig producers where one central gestation herd supplies other producers (satellite units) with pregnant sows in a leasing system [1].

A few of these breeding herds (including some closed multiplier herds) are specific pathogen free (SPF) herds. SPF pigs are free from important animal diseases such as sarcoptic mange, swine dysentery, pleuropneumonia and enzootic pneumonia (EP). Commercial pig production includes piglet producing herds, farrow-to-finish sow herds (for both types n ~1000), and fattening herds (n~ 900). With ~ 1.5 million fattening pigs produced annually for slaughter, Norway is self-sufficient for pork. On average, each herd has ~ 60 breeding sows, small-scaled in comparison with standards of industrialized pig production in other countries. Piglets weaned at ~ 33 days of age (1 week longer than EU standards) or at body weight of ~ 10kg. Sows have on average three parities before replacement with gilts. Concession limits restrict each piglet-producing herd to 105 breeding sows per year, and the number of fattening pigs to about 2100 per year in a fattening herd.

The Norwegian pig population has a very good health status. Since 1990s, the annual surveillance of all pig herds in Norway has shown them free from Aujeszky's disease, transmissible gastroenteritis, porcine respiratory coronavirus, porcine respiratory and reproductive syndrome (PRRS) and swine influenza A virus (swIAV) [2]. Enzootic pneumonia (EP) caused by *Mycoplasma hyopneumoniae*, was eradicated by 2009. Swine dysentery (*Brachyspira hyodysenteriae*) and scabies (*Sarcoptes scabiei*) are close to eradication. Atrophic rhinitis, rarely reported in pig herds, are absent in nucleus herds and SPF herds. Pleuropneumonia caused by *Actinobacillus pleuroneumoniae*, Glässers disease caused by *Haemophilus parasuis*, proliferative enteropathy (*Lawsonia intracellularis*) and diseases caused by PCV2-infection are sporadically reported at the herd level. The pig population also has a very low prevalence (<0.1%) of Salmonella [3]. The above-mentioned pig diseases are, however, common in the rest of the world. Nevertheless, in spite of having a

closed pyramidal pig farming system and no import of live animals, introduction of new infections may still occur at all levels in the pyramid as shown by the outbreak of H1N1pdm09 during the fall of 2009.

Background on influenza A virus in pigs

H1N1pdm09 belongs to the highly contagious IVAs genus of the Orthomyxoviridae family of viruses that can cause respiratory disease in birds and some mammals including man [4]. Records show that influenza epidemics stretching several centuries back to present times would occur periodically when new strains of influenza evolve molecularly by either antigenic drift or shift [5]. Antigenic drift in the influenza virus, because of a lack of viral RNA polymerase proofreading activity, involves a high rate of mutations within the genes that code for antibody binding sites that are immunologically significant [6]. Antigenic shift occurs when different strains of the virus exchange genetic materials, usually during co-infection in the same host, to produce a new subtype that has a mixture of surface antigens from the progenitor viruses [7]. Polymorphic and enveloped, IAV is a negative-sense, single stranded, segmented RNA virus. It is a highly efficient pathogen because its core eight genetic segment forming the ribonucleoprotein complex can encode all the major proteins needed for productive infection [8]. The eight separate segments in the influenza genome also allow re-assortment between two viruses by exchanging RNA segments during viral replication. The envelope, a lipid membrane derived from the host cell, carries two important glycoproteins that give the virus a distinctive spikey appearance (Figure 3). The hemagglutinin (HA) is the attachment protein that mediates the entry of virus into the host cell by binding to sialic acids receptors on the host cell surface and initiating fusion of the cell and viral membranes. The neuraminidase (NA) is an enzyme that facilitates cell-to-cell spread of the virus by cleaving sialic acids from the

glycan elements on the host cell surface, thereby facilitating the release of budding progeny viruses [9, 10]. The variations in these two nucleoprotein antigens (HA and NA) classify influenza viruses into different subtypes.
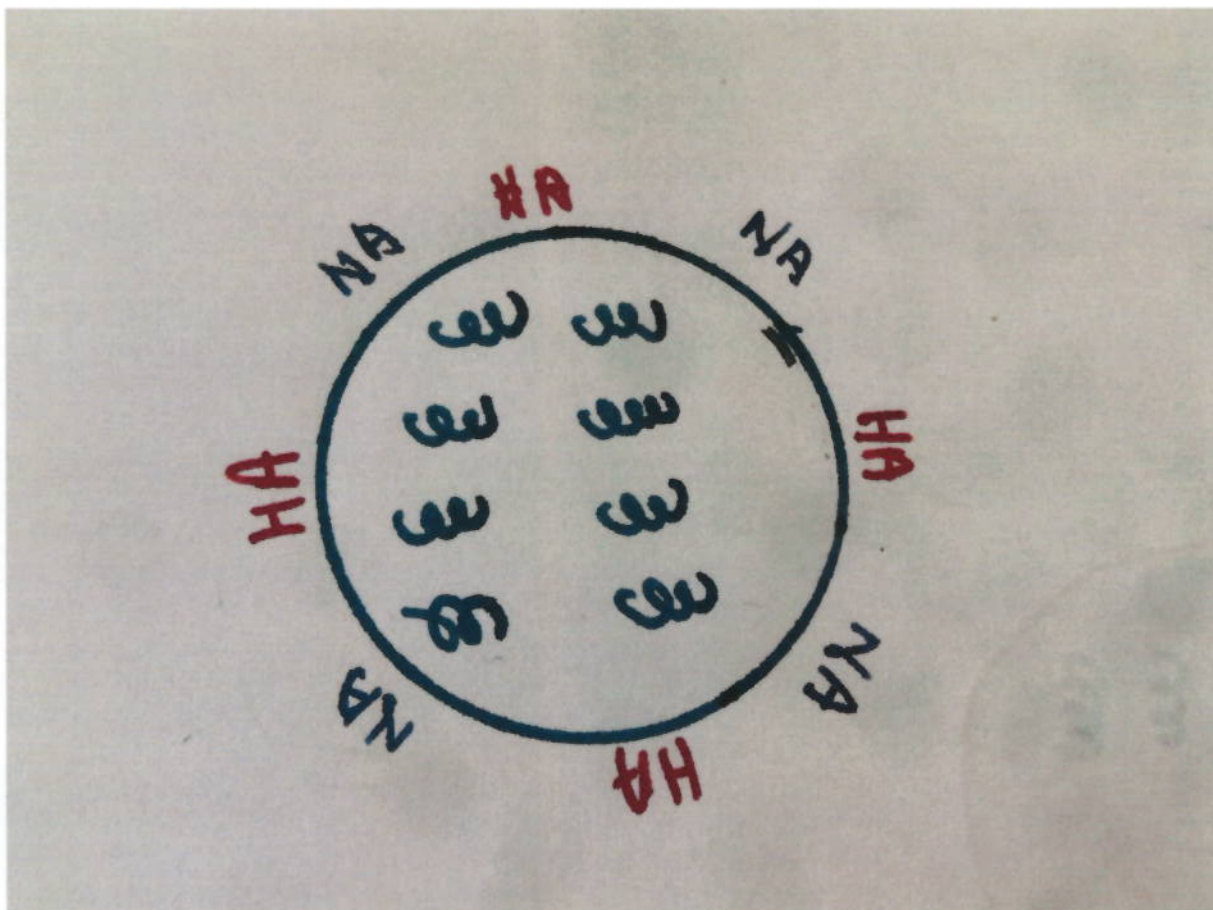
Figure 3 Schematic diagram of an influenza virus (original drawing).

It has a nucleocapsid of two distinctive proteins, Hemagglutinin (HA) and Neuraminidase (NA), which are keys to the nomenclature of subtyping influenza viruses. Encased are eight loose strands of RNA that code information for all the processes for productive infection.

With humans, pigs, birds and horses as the main hosts, Type A influenza viruses (IAVs) have been the most important influenza subtypes. The last four pandemics were all caused by IAVs. Wild aquatic birds (Anseriformes and, Charadriiformes) are nature's reservoir. They carry a diversity of influenza A subtypes designated H1-16 and N1-9 according to their HA and NA proteins. Only IAVs, specifically H1 and H3, can successfully infect the pig hosts and propagate themselves in the pig population

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for long periods of time [11]. Preceding the emergence of H1N1pdm09, there were already three established swIAVs in Europe. The first report of a major swIAV infection in pigs in Europe was in Belgium in late 1970s after transmission from wild ducks to pigs [12, 13]. This 'avian like' H1N1 virus has since become established as the dominant H1NI swIAV strain in the European pig population [14, 15]. Since the early 1970s, the second established swIAV is A/Hong Kong /68-like H3N2 virus of human origin. Reassortant strains between these two subtypes appeared in mid-80s [16]. The third established swIAV in the European pig population is a 'triple re-assortant' H1N2 virus that has been isolated frequently from pigs throughout Europe since the mid-1990s [17]. All three subtypes are endemic in regions of Europe with high pig density, and unvaccinated sows frequently have antibodies (AB) to two or all of the three subtypes [18]. Amongst the three strains, H3N2 has been the more virulent subtype in causing respiratory disease in pigs leading to greater economic impact [19, 20].

Norway's nearest neighbour, Sweden, has also recorded swIAVs infections of the European subtypes in its pigs since 1983 [21]. Even though Norway shares a long border (>1000 km) with Sweden, Norwegian pigs have managed to remain free from all European swIAVs [2]. For the rest of the world, swIAVs infections in pigs are considered to be one of the most important primary pathogens of pig respiratory diseases [17] and are key pathogens in causing porcine respiratory disease complex [22, 23].

Emergence of H1N1pdm09 and introduction into the Norwegian pig population

In April 2009, H1N1pdm09 emerged first in humans in Mexico and North America before its detection in the pig population several weeks later [24]. It was first named 'swine-origin" influenza A (H1N1) virus, because the virus theoretically had evolved

and circulated in pigs before crossing species barriers to infect humans [25]. This was never proven and the name 'swine-origin' was dropped eventually.

Genetic analyses demonstrated that H1N1pdm09 originated evolutionarily from a unique "intercontinental" re-assortment between swine-like influenza viruses of North American and Eurasian lineages [26]. Hence, H1N1pdm09 is a "quadruple-reassortant" virus because it contains gene segments from Eurasian swine influenza viruses, North American swine influenza viruses, human-origin, and avian-origin viruses. The emergence of influenza H1N1pdm09 in 2009 reminded the world that pigs play a pivotal role in influenza virus evolution and generation of potential pandemic strains. However, this remains a theory because of the lack of systematic surveillance of influenza in pigs in most countries to confirm time precedence that the virus had evolved in pigs first before crossing species to infect humans. The initial human cases also had no history of contact with pigs [27]. Systematic surveillance of influenza virus in pigs could have provided evidence that natural mixing of genetic elements in swine had either taken place or not causing the emergence of the virus [28]. It could also rule out notions that human manipulation created the virus under laboratory conditions, an ability that alarmingly has already been proven possible [29].

The first human cases of pandemic influenza in Norway began to surface very quickly in May 2009. It became increasingly clear that the prevailing exposure factor amongst these human cases were recent trips abroad. The number of human cases in Norway increased gradually through the summer and peaked in July 2009. By 26 October 2009, the number of verified human cases in Norway exceeded 3,300 [30].

The first report of H1N1pdm09 in Norwegian pigs was on 10 October 2009 when a farrow-to-finish pig herd with 85 sows and 850 fattening pigs in Nord-Trøndelag County tested positive for the virus [31]. Nord-Trøndelag County is one of the most

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intensive pig producing regions in Norway. On 4 October, the attending local veterinarian at Farm Zero noticed some pigs were coughing in the farrowing unit, but not other sections in the herd. He suspected an influenza infection (list B disease) outbreak and reported to the Norwegian Food Safety Authority (NFSA). NFSA confirmed the suspicion by taking nasal swabs from 20 pigs from the index herd whereby 12 pigs were tested positive for H1N1pdm09 using RT-PCR [32]. Further investigation on six more pig herds in close proximity or with contact history with the index herd revealed a second positive pig herd, a fattening herd with 500 finisher pigs. A critical finding on elucidating the possible route of infection was that a farm worker on the index farm had been ill with influenza-like symptoms (ILI) since 1st October 2009. The second positive herd also had the same owner as the index herd. Suspecting a risk of potential airborne spread of the virus to the neighbouring farms in this heavy pig farming zone [33], NFSA ordered depopulation of this second positive herd by sending the pigs to the slaughterhouse. Depopulation of the index farm was also follow next because the policy of the NFSA was to eradicate the infection and regain Norway's freedom status of all influenza viruses in its pig population. However, NFSA abandoned the eradication strategy when more pig herds in the area tested positive within the next few days. In addition, accumulating data suggested that humans carrying the virus had infected their pig herds rather than by animal contacts or by airborne spread from infected herds, indicating that depopulation of infected herds would have little effect in preventing this initial spread of the infection to pig herds.

Within a few months after 10 October, H1N1pdm09 was detected in more than one third of the swine herds in the country, including closed nucleus herds with the highest levels of biosecurity [34]. This influenza outbreak was the first IAV infection

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detected in Norwegian pigs since 1998 when antibodies for influenza H3N2 virus was detected in a dead-end infection in one pig herd [2]. H1N1pdm09 has so far been the only IAV detected in Norwegian pigs that was able to propagate itself beyond one farm successfully. Six years after the incursion in 2009, the herd prevalence of H1N1pdm09 in Norwegian pigs remained above 40%.
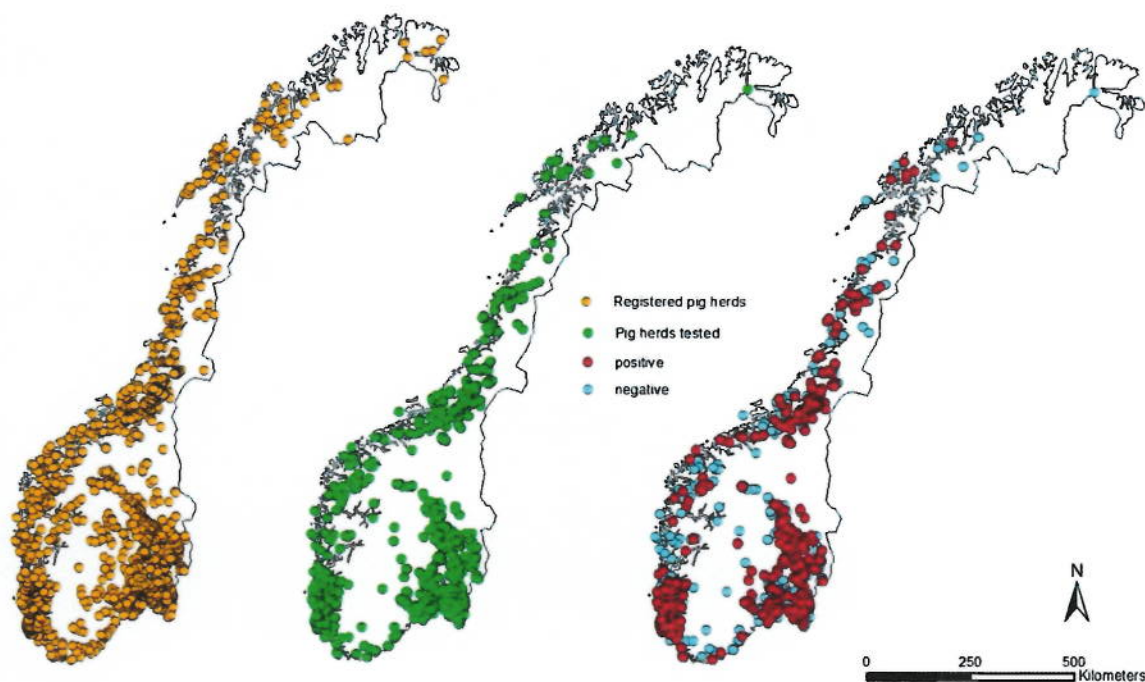
Figure 4 Spatial distribution of pig herds, tested herds and pig herds positive at least once during surveillance from 2010-2014 (original drawing).

Pathogenesis and clinical signs

Generally, the pathogenesis of influenza virus in pigs is similar to that in human infections [35, 36] . Experiments have shown that infecting pigs take place easily via intranasal, aerosol or intratracheal inoculation and the virus can be isolated within 24 hours post inoculation and for up to 7 days. Virus replication takes place chiefly in the

epithelial cells of the respiratory tract of pigs (nasal mucosa, ethmoid, trachea, and lungs) with a predilection for the lower instead of the upper respiratory tract. The inoculating viral dose and the extent of replication in the host thereafter determine the severity of lung inflammation and disease. Lower viral doses during inoculation produce a slower escalation of viral burden on the lungs resulting in milder lung inflammation and clinical signs such as nasal discharge, sneezing, low-grade fever or subclinical infection [37-39]. Conversely, a high enough viral dose can trigger enough release of cytokines during the acute stage of infection to elicit more severe clinical signs. The array of more severe clinical signs an infected pig may display includes high fever (40.5-41.5°C), anorexia, inactivity, huddling, tachypnea, and coughing. Labored abdominal breathing and dyspnea are most typical [19]. The onset of the disease is sudden, after an incubation period of 1-3 days. The infected pig also begins to shed virus in nasal secretions for 4-7 days, during which it is highly contagious. Morbidity in the herd can reach 100%, but mortality is low (<1%) in uncomplicated infections. Recovery is rapid (5-7 days). Acute outbreaks of clinical SI are limited to fully susceptible seronegative pigs of all ages [20]. Infections with H1N1, H1N2, and H3N2 subtype viruses could clinically be similar and viruses of all subtypes and lineages have been associated with acute respiratory episodes [20, 40-43]. In addition to viral dose, host factors such as immune status, age of pig at infection and coinfections with other respiratory pathogens also influence the course and severity of the disease [19]. Commonly in field situations, other respiratory pathogens act in concert with swIAV to cause respiratory disease in the pig host. Many studies have confirmed that secondary infections with bacteria, such as *Actinobacillus pleuroneumoniae*, *Pasteurella multocida*, *Mycoplasma hyopneumoniae*, *Haemophilus parasuis*, and *Streptococcus suis* type 2, enhance the severity and

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course of infections with swIAV [19, 44]. Bacteria can raise the pathogenicity of swIAVs because bacteria can supply proteases that may bring about cleavage beyond that which normally occurs in the respiratory epithelium thereby extending the cell tropisms of the influenza virus [45].

As with swIAV, other respiratory disease causing viruses such as PRCV and PRRSV frequently infect the older grower pigs (>16 weeks). This is also the time point when maternal antibodies they carry have waned sufficiently to make them vulnerable to infection [46]. Among these pathogens, PRRS-virus, PCV2, EP-virus and swIAV infect pigs frequently giving clinical signs of porcine respiratory disease complex (PRDC) [47, 48]. Several experimental infection studies in pigs with swIAV in combination with EP-virus, PRCV, or PRRS-virus have shown more severe disease under dual or more types of infections as compared to single virus infections [44].

Beyond the respiratory tract, there is little evidence that swIAVs can spread to the other tissues. Virus excretion and transmission are via the respiratory route exclusively. To assuage the initial fears of the public on the unknown risk with pork consumption, pig infection studies H1N1pdm09 under experimental conditions demonstrated no virus in pig muscle or that virus positive cells existed outside the respiratory tract [49, 50].

Immunity and diagnosis

Adaptive immune response to SI infection includes both the production of AB and cell-mediated immunity. Immune response to SI infection is rapid and efficient and commonly results in complete elimination of the virus form the respiratory tract within one to two weeks post inoculation [51]. Haemaglutination inhibiting AB will first appear by 7-10 days' post-inoculation and peak by 2-3 weeks post inoculation [36, 40,

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51-57]. Concurrent circulation of different subtypes of swIAVs would expose pigs to multiple, antigenically different swIAVs during their lifetime and produce cross-protective immunity against different influenza viruses [56, 58, 59].

Although maternally derived serum IgG AB to SI can protect young pigs against antigenically related viruses, they decline in the pig over a period of about 4-14 weeks making them susceptible to new infection by 16 weeks of age or earlier [60].

Clinical diagnosis is difficult because there are no pathognomonic signs for swIAV infection, especially when there are other respiratory pathogens on the differential diagnosis list. A definitive diagnosis is possible with IAV-specific laboratory tests such as isolation of virus, detection of viral antigens or nucleic acid, or the demonstration of virus specific antibodies [26, 61-63]. The sensitivity of these approaches will depend on the specialized reagents used within the assay and their "degree of match" to circulating field strains.

In virus isolation, the first step is to isolate the virus in chicken embryos or Madin-Darby canine kidney (MDCK) cells [64]. Next is the subtyping procedure by using HI test and neuraminidase inhibition tests [65]. Although the cell culture method is sensitive, it requires viable virus. The more common method is using the molecular technique of reverse transcription-polymerase chain reaction (RT-PCR) to detect virus antigens or nucleic acid in samples of mucus obtained by swabbing the nasal passages of pigs. The virus is usually present in nasal and pharyngeal secretions during the febrile phase of the illness. The World Health Organization (WHO) has published a protocol for the detection and characterization of H1N1pdm09 by RT-PCR [32]. RT-PCR assays are highly sensitive and specific in detecting viral nucleic acid.

As Norwegian pig farmers do not vaccinate their pigs against IAVs, diagnosis by serology is therefore possible by demonstrating the presence of IAV AB in pigs if the herd is infected. Norway's surveillance program screens as many as 500 to 700 pig herds (~ a quarter of pig herds in Norway) every year for a number of pathogens inter alia IAV infection by using enzyme-linked immunosorbent assays (ELISA). ELISA detects AB to a conserved core antigen of influenza resulting in a high test sensitivity [66]. For ELISA positive samples, the second step is to identify the subtype of the IAV by using HI assay test. Elsewhere, the existence of multiple IAV strains circulating geographically in the same place can complicate serological diagnosis. For example, in Western Europe where the swIAV of H1N1 subtype is endemic, H1N1pdm09 could escape detection by ELISA or HI assay testing because of cross-reactions [67].

At the herd level, the probability of a correct diagnosis of an infected herd depends on the test sensitivity, the animal prevalence and the number of pigs tested [68, 69]. The probability of detection increases with increasing animal prevalence and sample size. Serological interpretation of ELISA screening test could, however, be complicated by HI results because of cross-reactions against multiple antigens. Four antigens are routinely used for HI tests at NVI, namely H1N1pdm09, H1N1, H1N2 and H3N2, following a positive ELISA test. Careful interpretation of serological results at the herd level is therefore necessary not to miss an actual infection from a new subtype. In-depth discussion of serological results is beyond the scope of this thesis. Suffice to say that there is a high probability that H1N1pdm09 was the only IAVs infection circulating in the pig population during the period of study (2009 - 2014) for this thesis.

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Interspecies transmission and emergence of new strains

Interspecies transmission of IAVs occurs between pigs and other species including man [19, 70-73]. Termed as reverse zoonosis or human-to-animal transmission, this phenomenon has occurred with human strains of influenza such as H1N1 and H3N2 influenza viruses [42, 74-79]. There is a molecular explanation to the greater ease for human influenza viruses to infect pigs rather than the reverse. The respiratory epithelium of pigs possesses surface cell receptors for the widest range of influenza viruses, including the human influenza viruses and avian influenza viruses [80, 81]. With this ability to bind a diversity of viruses, pigs can harbour and potentially increasing the diversity of influenza viruses through genetic re-assortment [7, 27, 80, 82]. Although there is no proof that pigs had harbored the H1N1pdm09 first before transmitting it to humans, theoretically pigs is nature's most likely 'factory' for pandemic strains of influenza [83, 84]. A country conducive for studying the pig's role in increasing influenza diversity is China because it has many densely populated areas of humans living in close proximity with live poultry and pigs such as live markets and backyard-farming situations. It is therefore not surprising that many studies in China have demonstrated co-infection of avian and human IAVs in the pig host [85-90], which gives China the dubious honor of being the hypothetical epicenter for the genesis of influenza pandemics [91, 92].

In herd introduction, persistence, prevention and control

Outbreaks of influenza generally appears with the introduction of carrier pigs into a herd, through either the movement or mixing of infected pigs with susceptible animals and through human-to-pig transmission in situations when the subtypes have both humans and pigs as hosts (Figure 5) like with H1N1pdm09.
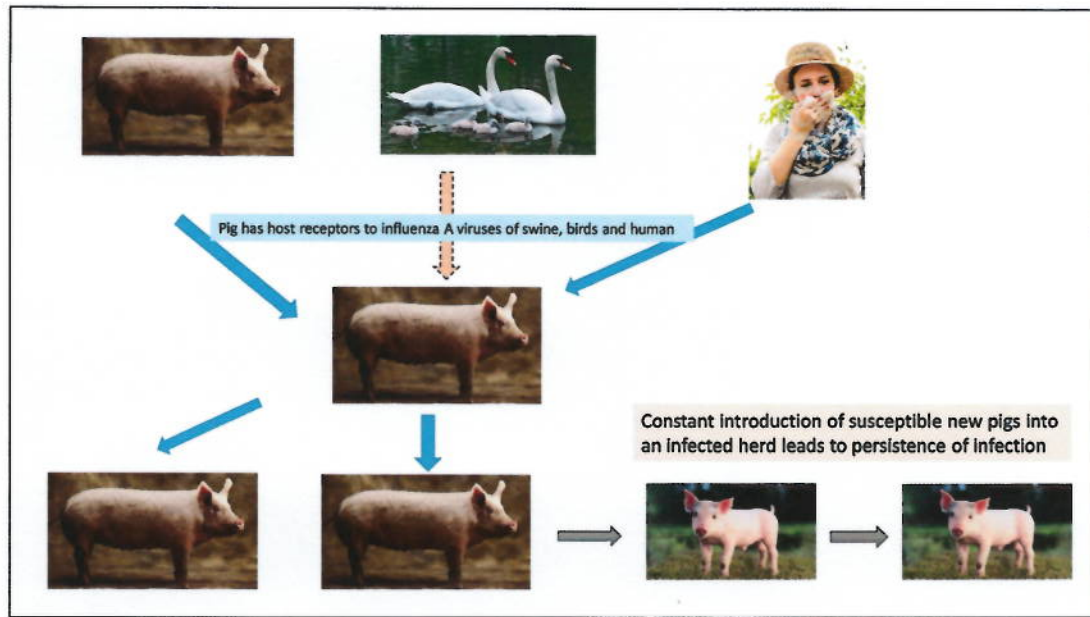
Figure 5 Origin and perpetuation of influenza A viruses in pig herds (original drawing).

Within the figure:

Pig has host receptors to influenza A viruses of swine, birds and human

Constant introduction of susceptible new pigs into an infected herd leads to persistence of infection

The primary route of virus transmission between pigs or between humans and pigs is direct contact via the nasopharyngeal route whereby the viruses are shed in nasal secretions, disseminated also through aerosols, and eventually inhaled by a susceptible host [19]. Close contact between pigs because of high stocking density typical of pig production, stressful situations, meteorological and environmental factors are conducive to increasing infection dose and the spread of influenza viruses. The persistence of the infectivity of influenza virus in aerosols is optimised under low humidity (15 - 40%) and have been demonstrated for 24 hours using infection in mice as detection method if the relative humidity was 17% - 24% [93]. Although there is no direct study on the long range aerosol spread between pig herds, a study in Queensland, Australia showed that equine influenza virus will routinely spread over 1 - 2 km via wind-borne aerosol [94]. Such studies have inferred that airborne transmission, given the right conditions (humidity, wind speed, temperature, dilution

factor), could contribute to the spread of swIAVs in areas densely populated with large herds of pigs, even with farms that have high biosecurity standards [19].

The virus can persist in an infected herd through the continued production of susceptible piglets and the introduction of new stock (Figure 5), often leading to the herd becoming infected endemically or recurrently [95, 96].

A recent study of H1N1pdm09 isolates from Norwegian pigs indicated that transmission from humans to pigs was still responsible for some new infections in 2014 [97].

For prevention and control of swIAV infection in pigs, some countries vaccinate the pigs, usually the sows, intramuscularly with inactivated vaccines so that piglets are born with protective maternal antibodies. As the virus does not survive very long outside the living hosts, the introduction of the virus into the herd is via a living host carrying the virus. Biosecurity measures preventing carrier pigs or infected humans having close access to the pigs can prevent the infection of a herd of susceptible pigs. Measures adopted by Norwegian farmers include getting timely flu vaccinations and to avoid working with pigs if they have ILI. Use of facemask and gloves are measures to minimise human-to-pig transmissions by aerosol spread or by fomite. Countries such as in Canada, pig farmers also received recommendations of similar precautionary measures especially just before the human flu season [98].

Impact on production

SwIAVs infections in naïve herds typically has a high morbidity (up to 100%) but the mortality is low (less than 1%) [19]. There is little information on the primary economic impact of influenza such as retarded growth rate and increase in days needed to reach market weight [99]. Published information on measurements of

negative effects caused by swIAVs as a single pathogen on a pig's growth performance is hard to come by. More often, the reports on negative effects of SI are in the presence of concurrent viral respiratory infections like PRRSv infection or secondary bacterial infections like EP or as part of the PRDC [19, 100-102]. The economic effects of swIAV infection on a pig herd would therefore vary between herds of different health profiles. Conversely, Norway given its freedom status of these respiratory pathogens, it was possible to study the production impact of H1N1pdm09 in the pig population unencumbered by coinfections.

European surveillance of influenza in pigs

Other than in Norway, there is no long-term active surveillance of influenza infection in the pig population elsewhere. Influenza surveillance in pig population, such as in Europe, is ad hoc or passive. A surveillance from 2011 to 2013 in 17 European countries detected IAVs in 31% of the 9000 herds examined. The dominating subtypes were the three European endemic swIAVs: avian-like swine H1N1 (53.6%), human-like re-assortant swine H1N2 (13%) and human-like re-assortant swine H3N2 (9.1%), as well as H1N1pdm09 (10.3%) [70]. The remaining 13.9% of the viruses represented re-assortant varieties of these four lineages, with prevalence of H1N1pdm09 and its re-assortant on the rise [70, 103]. These four lineages of viruses co-circulated in different relative levels in different countries. Areas free of H3N2 viruses exhibited highest frequencies of circulating H1N2 viruses. H1N1pdm09 has been isolated at an increasing frequency in some countries from 2010 to 2013, indicating that this subtype has become established in European pig population as a new enzootic lineage [15].

This early detection of IAV incursion in the Norwegian pig population was a result of ongoing disease-free-status national surveillance, the low occurrence of other

respiratory diseases and the awareness among veterinarians and farmers of influenza as a list B disease [2].

The incursion of H1N1pdm09 in Norway's naïve pig population with its high health and welfare status created a unique research opportunity to fill knowledge gaps and provide supporting evidence for decisions and management routines for the industry and the competent authority.

Aim and objectives

The overall aim of this thesis was to describe the epidemiology of H1N1pdm09 infection in pig herds, its impact on pig production and the ecology of the virus in the pig population by using a series of observational population studies conducted in the Norwegian pig population, a previously naïve population to all IAVs infections.

To achieve this, the following specific objectives were fulfilled by five observational studies:

1. **Clinical impact of infection.** Describe the clinical impact of introduction of H1N1pdm09 infection in the Norwegian pig population (Paper 1).

2. **Transmission route.** Identify the main transmission route for pig herds during the outbreak in 2009 (Paper 2).

3. **Adverse effects on growth performance.** Quantify the adverse effects on growth performance in fattening pigs (Paper 3).

4. **Production impact.** Estimate the production impact in a batch of 150 fattening pigs (Paper 4).

5. **Spatial and temporal patterns.** Describe the spatial and temporal patterns of herd incidence and prevalence for the H1N1pdm09 infection in the Norwegian pig population from 2010 to 2014 (Paper 5).

Materials and methods

Paper 1 – Clinical impact of infection

This was a case-control study for the one-year period after the incursion of virus in October 2009. With herd as the unit of analysis, the study sample was the entire source population of 115 nucleus (n=47) and multiplier herds (n=68). Surveillance data from the Norwegian Veterinary Institute allowed us to differentiate positive from negative herds by serology or molecularly. A positive herd was one with at least one rRT-PCR-positive sample, or had at least three of the blood samples tested positive for AB against influenza A virus. One researcher conducted all telephone interviews with the pig herd owners or managers between November 2010 and January 2011. Descriptive statistics and standard graphical methods presented the information from the interviews on observed clinical impact.

Paper 2 – Main transmission route

From the descriptive study **of Paper 1**, the case-control study proceeded to identify risk factors for transmission of the virus to a naïve herd. The telephone survey with pig farmers also collected information on herd characteristics, occurrence of influenza like illness in humans and pigs, time invariant and variant biosecurity measures, reception of human flu vaccination and chronological relationship between all the time variant variables. The focus of this research was to answer whether humans had transmitted the influenza virus to the 48 H1N1pdm09-positive breeding herds. The study also examined other variables that may have predisposed these herds to infection and the protective effects of existing or newly introduced biosecurity measures. With the collected data, we sought answers by using univariable and multivariable logistic regression by forward selection strategy guided by the plausible causal diagram [104]. After including the primary predictor (infected humans in

contact with pigs), we used the Wald statistics to test the significance of secondary predictors as they entered the model. We did not evaluate newly introduced biosecurity measures that could be effective against aerosol transmission and close contacts, such as facemasks and use of gloves, because very few farms (n=13) had adopted such time variant measures near the time outbreak.

Paper 3 – Adverse effects on growth performance

We investigated the negative effects of H1N1pdm09 infection on growth performance of grower pigs (33 kg – 100 kg bodyweight) by analysing the growth rates, feed intakes and laboratory data of 1955 pigs. The study sample were pigs from all active 43 nucleus herds in Norway, tested at a boar testing station between 2009 and 2012. Each pig had automated daily recordings of feed intake and body weight for its duration of stay at the station. We used serological tests and PCR testing of nasal swabs to ascertain the Infection status of each pig to classify the five groups of pigs. They were; seronegative pigs at 100 kg bodyweight (n = 887); seropositive pigs before 100 kg bodyweight (n = 874); positive for virus at bodyweight between 33 kg and 60 kg (n = 123); pigs positive for virus at bodyweight between 61 kg and 80 kg (n = 34); and pigs positive for virus at bodyweight between 81 kg and 100 kg (n = 37). The longitudinal data with clustering of data in the herd and pig allowed a multilevel statistical analysis. With herd ID and pig ID as random effects, infection status and birthdate, breed, and appetite as four fixed effects covariates, a mixed level regression analysis assessed the adverse effect of infection on growth performance. Multi-level linear regression estimated the marginal effects of the virus infection on the outcomes, which accounted for known fixed effects (breed, birthdate, average daily feed intake and growth phase) and random effects (cluster effects of pig and herd).

Paper 4 – Production impact

Based on the findings of Paper 3, the fourth paper projected the effects of reduced growth efficiency in individual pig to the batch level. In the model, a batch size of 150 fattening pigs was the representative of a typical batch in Norway. In this observational longitudinal study, analysis of growth performance data from 728 control pigs and 193 infected pigs with known viral shedding time points went into mixed linear regression models to give estimates of the marginal effects of infection. Normal distributions describing the variability of the estimates at the individual pig level were the fundamental inputs to the stochastic Monte Carlo models. The models simulated the summed negative effects of the infection at the batch level of 150 fattening pigs growing from 33 to 100 kg. Other inputs of variability and uncertainty were 1) batch transmission points, 2) pig infection points to reflect the disease transmission dynamics of the virus, and 3) final prevalence of infected pigs in the batch. Monte Carlo random sampling gave 5,000 estimates on the outputs of the marginal effects for each pig. Summing the individual effects of 150 pigs gave estimates for a batch size of 150 pigs. After model simulation, sensitivity analyses by Tornado charts ranked the variability inputs in terms of their impact on the conditional means and variance of the outputs.

Paper 5 – Spatial and temporal patterns

We used descriptive and trend analysis on the five years of active serosurveillance data collected from 1 January 2010 to 31 December 2014 study the spatial and temporal patterns of infection in the Norwegian pig population since its incursion in the previously naïve pig population. The study sample involved 1567 pig herds (~ 75% of the total 2000 pig herds in Norway) which had undergone 5643 herd tests involving 23026 blood samples from individual pigs. The pig herds in the sampled

population represented the five production classes (fattening; nucleus; multiplier; conventional sow herds and sow pools). Graphical, temporal and spatial analysis were undertaken using the lowess smoothing function in Stata to plot the running means of the herd infection status (seropositive=1, negative=0) with sampling day as time point. Stratifying herd seroprevalence over the 19 counties, with different pig farming densities illustrated the spatial variability in the distribution of infected herds. Further analysis included observation of variations amongst the five production classes. To find the potential effect of proximity to neighbouring farms, the lowess smoothing function plotted the running mean herd seroprevalence with the mean distance of each farm with the four nearest pig herds (proxy for pig density). For within herd seroprevalence investigation, the analysis of 1028 positive herd tests with at least five pigs involved enabled a cumulative probability plot for animal prevalence observed. Further investigation proceeded on the variations relative to the three fixed effects namely, year of test, production class and size of herd.

Main results

Paper 1 – Clinical impact of infection

The Norwegian pig population has been free from IAVs until 2009. The H1N1pdm09 outbreak during the autumn 2009 provided an opportunity to study the clinical impact of this infection in an entirely naïve pig population. This paper describes the results of a case-control study on the clinical impact of H1N1pdm09 infection in the nucleus and multiplier herds in Norway. The infection spread readily and led to seroconversion of 42% of the Norwegian nucleus and multiplier herds within a year. Forty percent of the positive herds reported clinical signs in all age groups with varying morbidity and duration of respiratory disease. Some also reported reduced reproductive performance in the affected sows.

Paper 2 – Main transmission route

Following a widespread infection in the human population in Norway, H1N1pdm09 was introduced to the influenza A immunologically naïve Norwegian pig population, and within a few months, pigs in more than one third of Norwegian pig herds had AB against the virus. This chronological pattern led to the suspicion that the infected humans had transmitted the virus to the pigs. The incidence of serologically positive pigs was similar in both multiplier herds (41%) and closed nucleus herds (43%). Multivariable logistic regression showed that presence of farm staff with influenza-like illness (ILI) (OR=4.15, CI 1.5-11.4, p=0.005) and herd size (OR=1.01, CI 1-1.02, p=0.009) were risk factors for infection. The rapid and widespread seroconversion for AB against H1N1pdm09 in the Norwegian pig population can be explained by the emergence of a novel virus that is readily transmitted between people and swine in a largely susceptible population of humans, and an entirely naïve population of pigs.

Paper 3 – Adverse effects on growth performance

The seropositive and virus positive pigs had decreased (p< 0.05) growth performance compared to seronegative pigs even though feed intake did not decrease. Reduced feed conversion efficiency led to lower average daily growth, additional feed requirement and longer time needed to reach the 100 kg bodyweight. A pig infected between 33 and 100 kg bodyweight would consume between 6 to 8 kg of additional feed compared to an uninfected pig to grow to 100 kg bodyweight. Despite increased feed intake observed in the virus positive pigs, their growth rates were lower and they took more time to reach 100 kg bodyweight compared to the seronegative pigs. An infected pig will have their production time extended by 1.6 to 2.4 pig days compared to an uninfected pig. The study rejected the view that predominantly subclinical infections with H1N1pdm09 have negligible adverse effects on growth performance of Norwegian pigs.

Paper 4 – Production impact

For a batch of 150 fattening pigs randomly selected from the population, the marginal effects of the infection were 1) 835 kg to 1,350 kg (5th-95th percentile) increased feed intake and 2) 194 to 334 (5th-95th percentile) days in excess of expected figures for an uninfected batch. A batch infected during growth phase 3 (81 to 100 kg BW) gave the worst results since the longitudinal study showed that a pig infected during growth phase 3 required more feed and a more protracted production time compared to pigs infected at a younger age. Sensitivity analysis showed that the final prevalence had the greatest impact on the conditional mean and variation of the marginal effects of infections. The batch transmission point was the next most influential factor. Lowering the final prevalence will have the greatest benefit in saving feed cost and reducing delay in getting the pigs to the market.

Paper 5 – Spatial and temporal patterns

Norway, with her long-standing active serosurveillance for swIAV since 1997, was efficacious in detecting the incursion of H1N1pdm09 of its pig population in 2009. Since then, surveillance data from 2010 to 2014 revealed that 54% of 5643 herd tests involving 1567 pig herds and 28% of 23036 blood samples were screened positive for AB against influenza A virus and confirmed to be H1N1pdm09 infection by HI. In 50% of positive herd tests, ≥ 60% of the sampled pigs in each herd had AB against H1N1pdm09. This within-herd animal seroprevalence did not vary amongst types of production, herd size or year of test. The overall running mean of national herd seroprevalence, and annual herd incidence risks fluctuated narrowly around the means of 45% and 32% respectively with the highest levels recorded in the three densest pig-producing counties. The probability of a herd being seropositive varied amongst the five production classes, which were sow pools, multiplier herds, conventional sow herds, nucleus, and fattening herds in descending order of likelihood. Large herds were more likely to be seropositive. Seropositive herds were highly likely to be seropositive the following year. The study shows that H1N1pdm09 is established in the Norwegian pig population with recurrent and new herd infections every year with the national herd seroprevalence in 2014 hovering at around 43% (95% CI 40-46%).

Discussion

The discussions with reference to methodological challenges presented here follow each paper respectively and sequentially.

Paper 1 – Clinical impact of infection

We observed high infection rates of H1N1pdm09 in the immunologically naïve Norwegian pigs and pig herds, but interestingly many newly infected herds did not have detectable clinical signs attributable to influenza. Studies in other European countries [105] have reported that H1N1pdm09 infection in pigs follows similar pathogenic course as the longer existing swIAVs [106, 107] where morbidity is close to 100% but mortality is negligible. A study in Australia also reported the subclinical picture or low morbidity in the infection of one naïve pig herd with a low disease burden [108]. A UK study in 2009 by Brookes et el. [49] investigated the clinical impact of H1N1pdm09 by a Randomized Clinical Trial (RCT) in which she infected eleven landrace-cross pigs through intranasal inoculation of specific doses of H1N1pdm09 and subsequently another eight pigs by contact with the eleven infected pigs. They achieved 100% incidence and morbidity with their 19 pigs. Our observations in the field clearly contrasted with the RCT because the viral doses that Norwegian pigs experienced in the field under Norwegian conditions may be lower. Even if we compare field infections in Norway to countries with more endemic strains of swIAV, the clinical picture would probably differ because of differing matrix of factors important in disease manifestation. These factors include the viral dose, the immune status of the host pig, co-infecting respiratory pathogens, animal welfare standards, and husbandry practices such as the pig's age of weaning (~5 weeks in Norway, 3 - 4 weeks in EU [109]).

Cytokines produced by the host during the acute stage of infection determine the difference between subclinical infection and disease [110-114]. Lower doses of viral infection producing milder or absence of clinical signs is a phenomenon seen also in pigs for non-influenza type viruses [115, 116]. It therefore follows logically that by altering conditions to lower infection doses of virus will also decrease the rate of viral increase in the lungs, and hence result in milder lung inflammation with less specific signs, i.e. nasal discharge, sneezing, a low to moderate fever, or subclinical infection [37, 39, 40]. Conditions that will interact with the viral dose include room ventilation, immunity, or sanitary measures to reduce infection pressure, are thus likely to dampen the severity of clinical signs because of the lower immune response such as the activation of a cytokines storm [117].

In Norway like our Scandinavian neighbours, the mild or absence of clinical illness in the pig herds could also be attributed to low levels of diseases and high animal welfare standards [118] [109]. Therefore, although H1N1pdm09 is widespread in Europe [70], the validity of our findings to the rest of Europe is limited.

We chose nucleus and multiplier herds because they kept better health records than the conventional sow herds or fattening herds. Being at the top of the pig production pyramid (Figure 2), they are high-priority herds that participate in the national serosurveillance at least once a year. During the outbreak in 2009, these high priority herds tested before others (conventional sow herds and fattening herds), so their infection statuses were available earliest.

To collect information on exposure factors from the farm, only one interviewer solicited information by telephone with personnel working directly on the farm, which allowed clarification during the interview if something was unclear. Confidentiality of

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identity encouraged willingness to share information. All interviewees received clear and simple definitions of influenza-like illness (fever, malaise, coughing and sore throat) and other technical terms. Although the interview was 1 year after the outbreak, we believe recall bias was minimal because this was a newly emerged disease in both pigs and humans concurrently with tremendous news coverage and involvement of the NFSA to heighten awareness. This was critical because timing of our main risk factor, humans with ILI, had to precede the pigs getting sick to make it a valid exposure factor.

To ascertain the infection status of each herd, the NFSA decided to double the number of pigs tested per herd to 20 pigs and thus reduced the misclassification bias. This was necessary because many farmers of positive herds did not detect any clinical signs. The probability of a correct diagnosis of a positive herd was very high at >95% probability based on an animal prevalence of at least 13%, a very conservative figure. Influenza virus transmits easily in a herd of pigs kept in close proximity to one another [19]. Our later studies in **Paper 3 and 5** supported that H1N1pdm09 prevalence in an infected herd is generally much higher where 50% of infected herds would have >60% of animal prevalence.

Our clinical impact findings in the nucleus and multiplier herds were valid for the target population given that all pig herds in Norway had uniform naïve status before the outbreak. They have similar husbandry, welfare, environment conditions and health profiles. A practical implication in H1N1pdm09 being a subclinical infection is that it makes movement restrictions of pigs or any biosecurity measures based on detection of clinical signs ineffective to control the spread of the H1N1pdm09 by animal contacts between herds.

Paper 2 – Main transmission route

Our case-control study with all 115 nucleus and multiplier pig herds gave strong evidence for human-to-pig transmission route during the early period of the outbreak in Norway. Not only was there strong statistical evidence, with a large study and OR >4, but also the simultaneous explosive detection of positive pig herds over great distances in a short period ruled out animal contacts and wind borne spread [19, 35]. As infected humans were the primary source of H1N1pdm09 in Norway, we believe that humans-infecting-pigs was the main route of infection for the target population of conventional sow herds, fattening herds and sow poos (~95% of pig herds) during the initial phase of the outbreak (October 2009 to October 2010). Successful genetic matching of the pig strain with the circulating human strain reinforced our findings [34]. An earlier publication from Canada had also suggested that human-to-pig transmission based on anecdotal evidence had also occurred on a Canadian farm [119, 120]. Subsequent to our study, more publications on human-to-pig transmission appeared [108, 119, 121, 122], confirming further the validity of our findings. This study was time sensitive in validity because humans were the only source of H1N1pdm09 before the outbreak occurred in the immunologically naïve pigs of Norway.

The extensive testing executed quickly and simultaneously by the NFSA allowed us to conclude that there was no spatial evidence of a point source type outbreak that is typical of disease spread by animal-to-animal contacts. Positive herds surfaced in Norway long distances apart with no history of animal contacts between the herds. Furthermore, 43% of closed nucleus and some multiplier farms that self-recruit their replacement stock also tested positive, rulling out animal-to-animal introduction of H1N1pdm09 to the first pig. The probability of infection was also similar in farms with

or without conventional biosecurity measures such as the practice of quarantine of new pigs, personnel changing overalls before entering the premises, physical barriers and scheme of clean movement of animals and personnel. In recent years, many reports have also documented that humans had transmitted H1N1pdm09 to pig herds [70, 123-125]. Nelson's review stated that IAV transmission from humans-to-pigs is actually far more frequent than the sporadically reported pigs-to-human zoonosis [62, 126-130]. The biological explanation to this phenomenon is that pigs have a wider range of receptors for IAVs on their respiratory epithelium. Whether it was humans-to-pigs, or pigs-to-humans or horizontal spread between humans or between pigs, it is well documented that that all IAVs are spread via contacts and aerosols [19], but perish quickly once outside the hosts. Soon after the outbreak, NFSA quickly advised pig farmers on the use of facemasks, receiving timely influenza vaccinations, and for pig farmers with ILI to refrain from working with pigs. Although farmers were generally compliant in taking advice, the spread of the highly contagious infection was too rapid for new biosecurity measures including human vaccination to be effective for the initial phase of the outbreak. Nevertheless, even though H1N1pdm09 is now endemic in Norway in both human and pig population, pig farmers should continue biosecurity measures to prevent recurrent or new herd infections by the current H1N1pdm09 and other future IAVs. This advice is useful not just for pig farmers but by other livestock farmers as well. In March 2016, Norway recorded the first subclinical infection of H1N1pdm09 in a turkey flock. Like pigs, humans-to-turkeys was concluded to be the likely route of infection [131].

Paper 3 – Adverse effects on growth performance

Our study proved convincingly that H1N1pdm09 infection could cause an overall drop in the growth rate of pigs even though they would consume more feed than their

uninfected counter parts. This is because the H1N1pdm09 infected pigs experience poorer feed conversion ratios. Although we did not find breed differences between Duroc and Landrace in the marginal effects of H1N1pdm09, the magnitudes varied depending on the age of infection of the pig. However, due to the sample sizes of the virus positive groups, the power was limited to ascertain which age group was most severely or least affected.

Our Norwegian field study was unique not just for H1N1pdm09, but for all swIAVs, because elsewhere in the world it is very difficult to replicate the conditions of our study. IAVs infection in pigs in other countries invariably exist as coinfections with different strains of IAVs or with other respiratory pathogens such as EP and PRRS or both [132]. Van Reeth did, in a RCT [133], demonstrated retardation in mean daily growth in four SIV inoculated pigs in comparison with eight control pigs. Our study was much larger with 193 naturally infected pigs and 887 control pigs to give greater precision to the results. In addition to comparing growth rate, our study went further by measuring daily feed intake and calculating the feed conversion ratio so that we could compare the feed efficiency between infected and control pigs.

The source population in our study were fattening pigs from all nucleus herds born between 2008 and 2012. There were forty-three active nucleus herds during this period that had sent grower boars to Norway's only boar testing station for performance testing. Although the study focused on a very specialised group of pigs at the boar testing station, we tried to achieve a high external validity with minimising confounding bias with the uniform conditions in both housing and pigs (matching), study design and use of mixed effects regression analysis. Furthermore the focus was on the marginal effects of H1N1pdm09 (coefficient) and not on outcome (growth performance), thus making the variance component analysis key in extending validity

of our results externally. The automated daily recording of growth performance data for each pig eliminated human error in data collection. Given the longitudinal nature of the data, there was clustering of observations in each pig and in each nucleus herd. The result was a hierarchical data structure where the assumption of statistical independence of observations was invalid because there were correlations of the multiple observations in the same pig and in the same herd. We used mixed effect regression model as the robust analytical approach to deal with the hierarchical data. To investigate if the virus acted differently between breeds (Duroc or Landrace) or if the effects of infection varied between time of infection related to body weight, interaction terms (virus*breed and virus*growth phase) were included in the model. Investigating these differential effects of the virus on breed has a biological basis since Landrace has a better feed-efficiency compared to the Duroc. Similarly as the pig grows, it undergoes anatomical and physiological changes, including digestive functions [134].

The birth date of the pig was an important fixed effect in the model because the growth performances of the pigs have improved with every generation. We also controlled the effects of changing feed efficiency and feed intake as the pig grows by including growth phase as another fixed effect in the model.

In comparing the feed efficiency of uninfected pigs with infected pigs, errors in classifying the pig's infection status could have biased the results particularly towards the null, especially if there was high enough proportion of misclassification, in our setting a low sensitivity, but a very high specificity. Misclassification could have occurred in three ways. Firstly, the less-than-perfect sensitivity of the ELISA test (93% by manufacturer's data). Secondly, some control pigs tested by serology before they reached 100kg (13% of control pigs). Thirdly, there is a lag period of 7 to 10 days

before antibodies appear from the point of infection [67, 135]. On hindsight, we should have been more stringent in selection of pigs for the control group by picking only pigs tested negative for antibodies at least 10 days after reaching the targeted bodyweight of 100 kg.

Paper 4 – Production impact

The stochastic models predicted that if the batch of 150 pigs was infected, it would consume extra pig feed ranging from 0.8 tons to 1.4 (5th - 95th percentile) tons. In proportion, that was an additional four to six percent of the normal average ~22 tons of feed requirements to grow the pigs to market weight. The farmer must also face additional two to three percent increase in production time because H1N1pdm09 infection depresses the overall growth rate of his infected pigs. This study was done to make the results of **Paper 3** more relevant and practical for the farmers. Using stochastic models, we derived predictions on the extra feed and the extended production time a pig farmer may face if his fattening herd was infected. We preferred stochastic models over deterministic ones because they gave a spectrum of the outcomes (additional feed and increased production time) based on probability distributions of the variables related to H1N1pdm09 infection in a pig herd.

The usual difficulty in building good stochastic models is in deriving and inputting the correct probability distributions. Fortunately, we were able to derive some of these probability distributions based on our actual field experiment in **Paper 3**; namely the individual variability between pigs, the variability of infection dynamics of H1N1pdm09 in a newly infected batch of pigs; and the final animal prevalence variability. We had one uncertainty variable; at which time point the virus infects the first pig, which we presented as three equal scenarios in the results. When comparing the cumulative probability plots for increased feed requirement and additional production time

between the three groups of viral positive pigs (Early, mid and late), one must be aware that the 5000 Monte Carlo sampling for each pig in the 150 pigs sized batch from the three Gaussian distributions for each group. The mid (n=34) and late (n=37) infected groups were smaller groups that had much larger SE than the early group (n=123). Correspondingly, the cumulative probability plots amplified the effects of greater variability of a greater SE.

We used the batch size of 150 fattening pigs to represent a typical fattening herd in Norway, which is small by world's standards. Another advantage of using stochastic model was we could perform sensitive analysis to see which variable had the greatest impact as their values change. This has an obvious advantage in cost benefit analysis of interventions to pick the right measure to reduce the most impact of H1N1pdm09. Our study provided key quantitative information for an economic evaluation that will help the farmer in deciding the best profitable approach to give him maximum producer surplus. For example, if NFSA changes its policy to allow vaccination in pigs, farmers may consider the expense of vaccination vis-à-vis avoidance of extra feed and shorter production time. Of course, introduction of vaccination will also render the national surveillance ineffective in detecting future incursions of new IAVs in its pig population.

Paper 5 – Spatial and temporal patterns

H1N1 has become endemic in Norwegian pig population as evidenced by the recurrent and new herd infections every year. Overall, the herd seroprevalence and herd incidence of H1N1pdm09 have stabilized at ~ 45% and ~ 32% respectively, without showing decline even at the end of 2014 [2]. In the rest of Western Europe, H1N1pdm09 has also become widespread in the pig population [70, 95, 125].

However, in contrast to Norway, Europe has other established swIAVs. Hence, it is not surprising that re-assortant subtypes have already appeared in Europe involving H1N1pdm09, most likely from coinfections with the existing swIAVs [70].

The seroprevalence trend for the fattening herds stood out in showing the greatest fluctuations amongst the five production classes, rising gradually from 2010, peaked in 2011 with subsequent declining before swinging up again in 2015, giving the appearance of a sine curve. The rise and fall paralleled with the rise and fall of the herd prevalence of conventional sow herds in Norway. As more sow herds acquired active immunity through infection, weaner pigs from them would increasingly benefit from protective maternal antibodies. This is critical as pigs move to fattening units, usually at 10 weeks of age which is before maternal antibodies wane below protective levels (16 weeks) [60]. The stress from moving and the mixing of pigs from different piglet producing herds increases the risk of infection if maternal antibodies are absent. Generally, the incidence rates of fattening herds were consistently lowest amongst the five production classes. This was partly due to the age bias as fattening pigs test at a younger age ~ 6 months of age at the slaughterhouse. Other herd types tested older sows with higher probability of successful infective exposure to the virus during their extended lifetime. In addition, fattening herds with fewer human-pig contacts by virtue of their operations will have less chance of reverse zoonosis taking place. Infections from humans as a route of infection for pig herds continued to be possible because H1N1pdm09 has remained to be the dominant IAV in the Norwegian human population since 2009 [136]. Virus comparisons of isolates from pigs and humans also proved that human-to-pig transmissions were still active in 2014 [97]. With H1N1pdm09 remaining as the dominant strain in the human population, it would make it even more difficult to control or eradicate the disease

from the pig population, even if public health or economic reasons become compelling.

The annual serosurveillance of swIAV in the Norwegian pig population that began in 1997 has since 2009 become an annual monitoring program for the endemic H1N1pdm09 infection in pig herds. The original aim of the national surveillance program was to ascertain disease freedom and for early detection when there is an incursion of exotic pig diseases into the Norwegian pig population. The aims remain the same. It is, however, not ideal for measuring herd prevalence given the uneven sampling of pigs per herd test and hence variation of sensitivity of herd tests between pig herds. In 2011, there was a change in sampling strategy such that many herd tests involved only one pig. This happened especially with conventional sow herds. Since herd sensitivity is equivalent to the apparent animal prevalence in an infected farm, the likelihood of wrongly classifying a positive herd as negative increases at lower animal prevalence and with fewer pigs sampled per herd test.

Despite the underestimation of the prevalence and incidence rates especially in conventional sow herds, **paper 5** presents a relevant picture of the factors that determine distribution and trend of H1N1pdm09 infection in pig herds vis-a-vis livestock environments and human activities. It investigated the persistence of IAVs by examining the variation in frequency of the disease in different strata of the population e.g. production type, regional difference and herd characteristics such as size. The variance patterns of the prevalence and incidence between the different production systems give important clues on the infection dynamics of the virus, and variations of biotic and abiotic between production types. Knowledge of biotic and abiotic factors for IAV survival in environmental matrices (e.g., air, soil, feces, water and fomites) are critical in forming effective control or preventive measures against

the infection [137]. These can be quarantine, downtime, setback distances, or eradication programs in livestock production systems [138]. In the absence of other strains of swIAVs, H1N1pdm09 would likely continue as a stable lineage of IAV in Norway. The incidence of H1N1pdm09 in humans would decrease when new strains of human influenza strains replace it as the next dominant strains. Although unknown when this will happen, we must review issues of control and eradication of H1N1pdm09 in the pig population in this new context when it happens.

Conclusions

H1N1pdm09 has adapted well in the Norwegian pig population. It will possibly persist as a relatively stable lineage in the absence of intervention measures and absence of other swIAVs exerting either selection pressures or competitive exclusion. The persistence in the pig population has appeared to parallel the situation in the human population. Since its incursion in 2009, the prevalence in pig herds has stabilized between 40 and 50 percent with new or recurrent herd infections from animal contacts and most probably also from human-to-pig transmission annually. The rapid turnover and introduction of new and susceptible pigs facilitate the persistence and propagation of the virus. While infected pig herds largely show subclinical patterns, they suffer reduced growth performances resulting in increased feed requirements and longer production time. IAVs infection is very contagious, and once established, is very difficult and expensive to eradicate. Most countries have voluntary vaccinations at the farm level to reduce severe effects of confections of swIAVs and other respiratory pathogens but none has attempted to eradicate it at the country level.

H1N1pdm09 represents a one-health situation involving humans and pigs. It is more likely that H1N1pdm09 remains in the pig population for a longer time than in the Norwegian human population. The average Norwegian travels quite frequently and may contract new strains of human influenza viruses overseas. Visitors to Norway may also bring new human IAVs to Norway. These new human IAVs could eventually replace H1N1pdm09 or make it less dominant in the Norwegian human population. With respect to the Norwegian pig population, the one-health challenge would be to prevent Norwegians from transmitting another human IAV strain to the pigs. A second strain of IAV to infect Norwegian pigs will most likely come from humans, if

Norway continues not to import live pigs. If a second strain of IAV does infect the Norwegian pig population or if the level of H1N1pdm09 in humans subsides, the question begging an answer would be should Norway then continue to adopt a 'to do nothing' approach' or consider control measures such as vaccination of pigs? In that scenario, a comprehensive economic evaluation and risk analysis would be necessary to guide decision makers on alternative options.

References

1. Rootwelt, V., et al., *Blood variables and body weight gain on the first day of life in crossbred pigs and importance for survival.* J Anim Sci, 2012. **90**(4): p. 1134-41.

2. Lium, B., C. Er, and A. Zerihun, *The surveillance and control programme for specific virus infections in swine herd in Norway 2013.* , in *Surv. & Contr. Prog.s for Terr. and Aquatic Ani. in Norway. Annual report 2012.*, S. Sviland and h. hellberg, Editors. 2014, Norwegian Veterinary Institute: Oslo, Norway. .

3. Sandberg, M., O. Alvseike, and E. Skjerve, *The prevalence and dynamics of Salmonella enterica IIIb 61:k:1,5,(7) in sheep flocks in Norway.* Prev Vet Med, 2002. **52**(3-4): p. 267-75.

4. WHO. *World Health Organisation Influenza fact sheet.* 2014; Available from: http://www.who.int/mediacentre/factsheets/fs211/en/.

5. Morens, D.M. and J.K. Taubenberger, *Historical thoughts on influenza viral ecosystems, or behold a pale horse, dead dogs, failing fowl, and sick swine.* Influenza Other Respir Viruses, 2010. **4**(6): p. 327-37.

6. Earn, D.J.D., J. Dushoff, and S.A. Levin, *Ecology and evolution of the flu.* Trends Eco Evo, 2002. **17**(7): p. 334-340.

7. Kida, H., et al., *Potential for transmission of avian influenza viruses to pigs.* J Gen Virol, 1994. **75 (Pt 9)**: p. 2183-8.

8. Mehle, A. and J.A. Doudna, *A host of factors regulating influenza virus replication.* Viruses, 2010. **2**(2): p. 566-73.

9. Zanin, M., et al., *Pandemic Swine H1N1 Influenza Viruses with Almost Undetectable Neuraminidase Activity Are Not Transmitted via Aerosols in Ferrets and Are Inhibited by Human Mucus but Not Swine Mucus.* J Virol, 2015. **89**(11): p. 5935-48.

10. Treanor , J., *Influenza Vaccine — Outmaneuvering Antigenic Shift and Drift.* N Engl J Med, 2004. **350**(3): p. 218-220.

11. Vincent, A., et al., *Review of Influenza A Virus in Swine Worldwide: A Call for Increased Surveillance and Research.* Zoonoses Public Health, 2014. **61**(1): p. 4-17.

12. Pensaert, M., et al., *Evidence for the natural transmission of influenza A virus from wild ducts to swine and its potential importance for man.* Bull World Health Organ, 1981. **59**(1): p. 75-8.

13. Marozin, S., et al., *Antigenic and genetic diversity among swine influenza A H1N1 and H1N2 viruses in Europe.* J Gen Virol, 2002. **83**(Pt 4): p. 735-45.

14. Van Reeth, K., et al., *Seroprevalence of H1N1, H3N2 and H1N2 influenza viruses in pigs in seven European countries in 2002-2003.* Influenza Other Respir Viruses, 2008. **2**(3): p. 99-105.

15. Brown, I.H., *History and epidemiology of Swine influenza in Europe.* Curr Top Microbiol Immunol, 2013. **370**: p. 133-46.

16. Campitelli, L., et al., *Continued evolution of H1N1 and H3N2 influenza viruses in pigs in Italy.* Virology, 1997. **232**(2): p. 310-318.

17.   Brown, I.H., *History and epidemiology of swine influenza in Europe.* Swine Influenza, 2013. **370**: p. 133-146.

18.   Van Reeth, K., et al., *Seroprevalence of H1N1, H3N2 and H1N2 influenza viruses in pigs in seven European countries in 2002-2003.* Infl & Other Resp Viruses, 2008. **2**(3): p. 99-105.

19.   Van Reeth, K., I.H. Brown, and C.W. Olsen, *Influenza Virus,* in *Diseases of Swine,* J. Zimmerman, et al., Editors. 2012, Wiley-Blackwell.

20.   Loeffen, W.L., et al., *Survey of infectious agents involved in acute respiratory disease in finishing pigs.* Vet Rec, 1999. **145**(5): p. 123-9.

21.   Kiss, I., et al., *Swine influenza viruses isolated in 1983, 2002 and 2009 in Sweden exemplify different lineages.* Acta Vet Scand, 2010. **52**.

22.   Harms, P.A., P.G. Halbur, and S.D. Sorden, *Three cases of porcine respiratory disease complex associated with porcine circovirus type 2 infection.* J Swine Health and Prod, 2002. **10**(1): p. 27-30.

23.   Loeffen, W.L.A., et al., *Survey of infectious agents involved in acute respiratory disease in finishing pigs.* Vet Rec, 1999. **145**(5): p. 123-129.

24.   Neumann, G., T. Noda, and Y. Kawaoka, *Emergence and pandemic potential of swine-origin H1N1 influenza virus.* Nature, 2009. **459**(7249): p. 931-939.

25.   Smith, G.J., et al., *Origins and evolutionary genomics of the 2009 swine-origin H1N1 influenza A epidemic.* Nature, 2009. **459**(7250): p. 1122-5.

26.   Garten, R.J., et al., *Antigenic and Genetic Characteristics of Swine-Origin 2009 A(H1N1) Influenza Viruses Circulating in Humans.* Science, 2009. **325**(5937): p. 197-201.

27.   Team, N.S.-O.I.A.V.I., *Emergence of a Novel Swine-Origin Influenza A (H1N1) Virus in Humans.* New Engl J Med, 2009. **360**(25): p. 2605-2615.

28.   Shortridge, K.F., et al., *Persistence of Hong Kong influenza virus variants in pigs.* Science, 1977. **196**(4297): p. 1454-5.

29.   Independent. *Controversial US scientist creates deadly new flu strain for pandemic research.* 2014; Available from: http://www.independent.co.uk/news/science/exclusive-controversial-us-scientist-creates-deadly-new-flu-strain-for-pandemic-research-9577088.html.

30.   Folkehelseinstituttet. *Tema: Sesongsinfluensa [In Norwegian].* 2009; Available from: www.fhi.no/influensa

31.   Hofshagen, M., et al., *Pandemic influenza A(H1N1)v: human to pig transmission in Norway?* Euro Surveill, 2009. **14**(45).

32.   WHO. *The WHO Collaborating Centre for influenza at CDC Atlanta, United States of America. CDC protocol of realtime RTPCR for influenza A(H1N1).* Available from: http://www.who.int/csr/resources/publications/swineflu/CDCRealtimeRTPCR_SwineH1Assay-2009_20090430.pdf. .

33. Tellier, R., *Aerosol transmission of influenza A virus: a review of new studies.* J Royal Soc Interface, 2009.

34. Gjerset, B., et al., *Experiences after Twenty Months with Pandemic Influenza A (H1N1) 2009 Infection in the Naive Norwegian Pig Population.* Influenza Res Treat, 2011. **2011**: p. 206975.

35. De Vleeschauwer, A., et al., *Efficient Transmission of Swine-Adapted but Not Wholly Avian Influenza Viruses among Pigs and from Pigs to Ferrets.* J Infection, 2009. **200**(12): p. 1884-1892.

36. Khatri, M., et al., *Swine Influenza H1N1 Virus Induces Acute Inflammatory Immune Responses in Pig Lungs: a Potential Animal Model for Human H1N1 Influenza Virus.* J Virol, 2010. **84**(21): p. 11210-11218.

37. Brown, I.H., et al., *Pathogenicity of a Swine Influenza H1n1 Virus Antigenically Distinguishable from Classical and European Strains.* Vet Rec, 1993. **132**(24): p. 598-602.

38. Larsen, D.L., et al., *Systemic and mucosal immune responses to H1N1 influenza virus infection in pigs.* Vet Microbiol, 2000. **74**(1-2): p. 117-131.

39. Richt, J.A., et al., *Pathogenic and antigenic properties of phylogenetically distinct reassortant H3N2 swine influenza viruses cocirculating in the United States.* J Clin Microbiol, 2003. **41**(7): p. 3198-205.

40. Larsen, D.L., et al., *Systemic and mucosal immune responses to H1N1 influenza virus infection in pigs.* Vet Microbiol, 2000. **74**(1-2): p. 117-31.

41. Karasin, A.I., et al., *Isolation and characterization of H4N6 avian influenza viruses from pigs with pneumonia in Canada.* J Virol, 2000. **74**(19): p. 9322-7.

42. Karasin, A.I., et al., *Genetic characterization of H3N2 influenza viruses isolated from pigs in North America, 1977-1999: evidence for wholly human and reassortant virus genotypes.* Virus Res, 2000. **68**(1): p. 71-85.

43. Zhou, N.N., et al., *Genetic reassortment of avian, swine, and human influenza A viruses in American pigs.* J Virol, 1999. **73**(10): p. 8851-6.

44. Loving, C.L., et al., *Influenza virus coinfection with Bordetella bronchiseptica enhances bacterial colonization and host responses exacerbating pulmonary lesions.* Microb Pathog, 2010. **49**(5): p. 237-245.

45. Steinhauer, D.A., *Role of hemagglutinin cleavage for the pathogenicity of influenza virus.* Virology, 1999. **258**(1): p. 1-20.

46. Van Reeth, K. and M. Pensaert, *Porcine respiratory coronavirus mediated interference against influenza virus replication in the respiratory tract of feeder pigs.* Am J Vet Res, 1994. **55**: p. 1275 - 1281.

47. Tico, G., J. Segales, and J. Martinez, *The blurred border between porcine circovirus type 2-systemic disease and porcine respiratory disease complex.* Vet Microbiol, 2013. **163**(3-4): p. 242-247.

48. Hansen, M.S., et al., *An Investigation of the Pathology and Pathogens Associated with Porcine Respiratory Disease Complex in Denmark.* J Comp Pathol, 2010. **143**(2-3): p. 120-131.

49. Brookes, S., et al., *Replication, pathogenesis and transmission of pandemic (H1N1) 2009 virus in non-immune pigs.* PLoS One, 2010. **5**: p. e9068.

50. Vincent, A.L., et al., *Absence of 2009 pandemic H1N1 influenza A virus in fresh pork.* PLoS One, 2009. **4**(12): p. e8367.

51. Brown, I.H., *The epidemiology and evolution of influenza viruses in pigs.* Vet Microbiol, 2000. **74**(1–2): p. 29-46.

52. Heinen, P., et al., *Systemic and mucosal isotype-specific antibody responses in pigs to experimental Influenza A virus infection.* Viral Immunol, 2000. **13**: p. 237 - 247.

53. Kitikoon, P., et al., *The immune response and maternal antibody interference to a heterologous H1N1 swine influenza virus infection following vaccination.* Vet Immunol Immunopathol, 2006. **112**(3-4): p. 117-28.

54. Kitikoon, P., et al., *Swine influenza matrix 2 (M2) protein contributes to protection against infection with different H1 swine influenza virus (SIV) isolates.* Vaccine, 2009. **28**(2): p. 523-531.

55. Kitikoon, P., et al., *Vaccine efficacy and immune response to swine influenza virus challenge in pigs infected with porcine reproductive and respiratory syndrome virus at the time of SIV vaccination.* Vet Microbiol, 2009. **139**(3-4): p. 235-244.

56. Vincent, A.L., et al., *Swine Influenza Viruses: A North American Perspective.* Adv Virus Res, Vol 72, 2008. **72**: p. 127-154.

57. Vincent, A.L., et al., *Failure of protection and enhanced pneumonia with a US H1N2 swine influenza virus in pigs vaccinated with an inactivated classical swine H1N1 vaccine.* Vet Microbiol, 2008. **126**(4): p. 310-23.

58. De Vleeschauwer, A.R., et al., *Cross-protection between antigenically distinct H1N1 swine influenza viruses from Europe and North America.* Influenza Other Respir Viruses, 2011. **5**(2): p. 115-122.

59. Van Reeth, K., et al., *Genetic relationships, serological cross-reaction and cross-protection between H1N2 and other influenza - A virus subtypes endemic in European pigs.* Virus Res, 2004. **103**(1-2): p. 115-124.

60. Loeffen, W., et al., *Effect of maternally derived antibodies on the clinical signs and immune response in pigs after primary and secondary infection with an influenza H1N1 virus.* Vet Immunol Immunopathol, 2003. **92**: p. 23 - 35.

61. Valheim, M., et al., *Pathological Findings and Distribution of Pandemic Influenza A (H1N1) 2009 Virus in Lungs from Naturally Infected Fattening Pigs in Norway.* Influenza Res Treat, 2011. **2011**: p. 565787.

62. Shu, B., et al., *Genetic analysis and antigenic characterization of swine origin influenza viruses isolated from humans in the United States, 1990-2010.* Virology, 2012. **422**(1): p. 151-160.

63. Swenson, S.L., et al., *A comparison of diagnostic assays for the detection of type A swine influenza virus from nasal swabs and lungs.* J Vet Diagn Invest, 2001. **13**(1): p. 36-42.

64. Choi, Y.K., et al., *Detection and subtyping of swine influenza H1N1, H1N2 and H3N2 viruses in clinical samples using two multiplex RT-PCR assays.* J Virol Method, 2002. **102**(1–2): p. 53-59.

65. Murphy, B.R. and R.G. Webster, *Orthomyxoviruses*, in *Virology*, N.B. Fields, D.M. Knipe, and P.M. Howley, Editors. 1996, Lippincott-Raven: Philadelphia. p. 1397-1446.

66. Ciacci-Zanella, J.R., et al., *Detection of anti-influenza A nucleoprotein antibodies in pigs using a commercial influenza epitope-blocking enzyme-linked immunosorbent assay developed for avian species.* J Vet Diagn Invest, 2010. **22**(1): p. 3-9.

67. Kyriakis, C.S., et al., *Serologic cross-reactivity with pandemic (H1N1) 2009 virus in pigs, Europe.* Emerg Infect Dis, 2010. **16**(1): p. 96-9.

68. AusVet. *Herd-level sensitivity and specificity for testing in an infinite population.* Available from: http://epitools.ausvet.com.au/content.php?page=HerdSens1.

69. Christensen, J. and I.A. Gardner, *Herd-level interpretation of test results for epidemiologic studies of animal diseases.* Prev Vet Med, 2000. **45**(1-2): p. 83-106.

70. Simon, G., et al., *European surveillance network for influenza in pigs: surveillance programs, diagnostic tools and Swine influenza virus subtypes identified in 14 European countries from 2010 to 2013.* PLoS One, 2014. **9**(12): p. e115815.

71. Zell, R., C. Scholtissek, and S. Ludwig, *Genetics, Evolution, and the Zoonotic Capacity of European Swine Influenza Viruses.* Swine Influenza, 2013. **370**: p. 29-55.

72. Jhung, M.A., et al., *Outbreak of variant influenza A(H3N2) virus in the United States.* Clin Infect Dis, 2013. **57**(12): p. 1703-12.

73. Wong, K.K., et al., *Outbreak of influenza A (H3N2) variant virus infection among attendees of an agricultural fair, Pennsylvania, USA, 2011.* Emerg Infect Dis, 2012. **18**(12): p. 1937-44.

74. Roberts, D.H., S.F. Cartwright, and G. Wibberley, *Outbreaks of classical swine influenza in pigs in England in 1986.* Vet Rec, 1987. **121**(3): p. 53-5.

75. Cappuccio, J.A., et al., *Outbreak of swine influenza in Argentina reveals a non-contemporary human H3N2 virus highly transmissible among pigs.* J Gen Virol, 2011. **92**: p. 2871-2878.

76. Ottis, K., et al., *Human influenza A viruses in pigs: isolation of a H3N2 strain antigenically related to A/England/42/72 and evidence for continuous circulation of human viruses in the pig population.* Arch Virol, 1982. **73**(2): p. 103-8.

77. Castrucci, M., et al., *Genetic reassortment between avian and human influenza A viruses in Italian pigs.* Virology, 1993. **193**: p. 503 - 506.

78. Karasin, A.I., S. Carman, and C.W. Olsen, *Identification of human H1N2 and human-swine reassortant H1N2 and H1N1 influenza A viruses among pigs in Ontario, Canada (2003 to 2005).* J Clin Microbiol, 2006. **44**(3): p. 1123-6.

79. Yu, H., et al., *Further evidence for infection of pigs with human-like H1N1 influenza viruses in China.* Virus Res, 2009. **140**(1-2): p. 85-90.

80. Ito, T., et al., *Molecular basis for the generation in pigs of influenza A viruses with pandemic potential.* J Virol, 1998. **72**(9): p. 7367-73.

81. Nelli, R.K., et al., *Comparative distribution of human and avian type sialic acid influenza receptors in the pig.* BMC Vet Res, 2010. **6**: p. 4.

82. Castrucci, M.R., et al., *Genetic Reassortment between Avian and Human Influenza A Viruses in Italian Pigs.* Virology, 1993. **193**(1): p. 503-506.

83. Ma, W., R.E. Kahn, and J.A. Richt, *The pig as a mixing vessel for influenza viruses: Human and veterinary implications.* J Mol Genet Med, 2009. **3**(1): p. 158-166.

84. Scholtissek, C., *Pigs as 'Mixing Vessels' for the Creation of New Pandemic Influenza A Viruses.* Med Princ Pract, 1990. **2**(2): p. 65-71.

85. Peiris, J.S., et al., *Cocirculation of avian H9N2 and contemporary "human" H3N2 influenza A viruses in pigs in southeastern China: potential for genetic reassortment?* J Virol, 2001. **75**(20): p. 9679-86.

86. Cao, N., et al., *Avian influenza A (H5N1) virus antibodies in pigs and residents of swine farms, southern China.* J Clin Virol, 2013. **58**(4): p. 647-51.

87. Zhang, G., et al., *Identification of an H6N6 swine influenza virus in southern China.* Infect Genet Evol, 2011. **11**(5): p. 1174-7.

88. Tu, J., et al., *Isolation and molecular characterization of equine H3N8 influenza viruses from pigs in China.* Arch Virol, 2009. **154**(5): p. 887-90.

89. Su, S., et al., *Complete genome sequence of an avian-like H4N8 swine influenza virus discovered in southern China.* J Virol, 2012. **86**(17): p. 9542.

90. Nfon, C., et al., *Molecular and antigenic characterization of triple-reassortant H3N2 swine influenza viruses isolated from pigs, turkey and quail in Canada.* Transbound Emerg Dis, 2011. **58**(5): p. 394-401.

91. Nakajima, K., et al., *Further genetic evidence for maintenance of early Hong Kong-like influenza A(H3N2) strains in swine until 1976.* Virology, 1982. **116**(2): p. 562-72.

92. Shortridge, K.F. and C.H. Stuart-Harris, *An influenza epicentre?* Lancet, 1982. **2**(8302): p. 812-3.

93. Tellier, R., *Review of aerosol transmission of influenza A virus.* Emerg Infect Dis, 2006. **12**(11): p. 1657-62.

94. Davis, J., M.G. Garner, and I.J. East, *Analysis of local spread of equine influenza in the Park Ridge region of Queensland.* Transbound Emerg Dis, 2009. **56**(1-2): p. 31-8.

95. Kyriakis, C.S., et al., *Influenza A virus infection dynamics in swine farms in Belgium, France, Italy and Spain, 2006-2008.* Vet Microbiol, 2013. **162**(2-4): p. 543-50.

96. Rose, N., et al., *Dynamics of influenza A virus infections in permanently infected pig farms: evidence of recurrent infections, circulation of several swine influenza viruses and reassortment events.* Vet Res, 2013. **44**: p. 72.

97.   Forberg, H., et al., *Swine influenza in Norway: a distinct lineage of influenza A(H1N1)pdm09 virus.* Influenza Other Respir Viruses, 2013. **7 Suppl 4**: p. 21-6.

98.   ThePigSite. *Pork Producers Encouraged to Prepare for Flu Season.* 2016; Available from: http://www.thepigsite.com/swinenews/42333/pork-producers-encouraged-to-prepare-for-flu-season/.

99.   Kothalawala, H., M.J.M. Toussaint, and E. Gruys, *An overview of swine influenza.* Vet Quart, 2006. **28**(2): p. 45-53.

100.   Beskow, P., M. Norqvist, and P. Wallgren, *Relationships between selected climatic factors in fattening units and their influence on the development of respiratory diseases in swine.* Acta Vet Scand, 1998. **39**(1): p. 49-60.

101.   Wallgren, P., et al., *Experimental infections with Actinobacillus pleuropneumoniae in pigs - II. Comparison of antibiotics for oral strategic treatment.* J Vet Med B Infect Dis Vet Public Health, 1999. **46**(4): p. 261-269.

102.   Wallgren, P., et al., *Incidence of Infections in Pigs Bred for Slaughter Revealed by Elevated Serum Levels of Interferon and Development of Antibodies to Mycoplasma-Hyopneumoniae and Actinobacillus-Pleuropneumoniae.* J Vet Med B Infect Dis Vet Public Health, 1993. **40**(1): p. 1-12.

103.   Kyriakis, C.S., et al., *Virological Surveillance and Preliminary Antigenic Characterization of Influenza Viruses in Pigs in Five European Countries from 2006 to 2008.* Zoonoses Public Health, 2011. **58**(2): p. 93-101.

104.   Dohoo, I.R., W. Martin, and H. Stryhn, *Veterinary Epidemiologic Research.* 2nd ed. 2010, Charlottetown. Prince Edward Island. Canada: VER Inc.

105.   Brookes, S.M., et al., *Replication, Pathogenesis and Transmission of Pandemic (H1N1) 2009 Virus in Non-Immune Pigs.* Plos One, 2010. **5**(2).

106.   Lange, E., et al., *Pathogenesis and transmission of the novel swine-origin influenza virus A/H1N1 after experimental infection of pigs.* J Gen Virol, 2009. **90**: p. 2119-2123.

107.   Brookes, S.M. and I.H. Brown, *A/H1N1/pdm09 virus: dynamics of infection in pigs and people.* Vet Rec, 2011. **169**(6): p. 151-152.

108.   Holyoake, P.K., et al., *The first identified case of pandemic H1N1 influenza in pigs in Australia.* Aust Vet J, 2011. **89**(11): p. 427-31.

109.   Schmid, O. and R. Kilchsperger. *Overview of animal welfare standards and initiatives in selected EU and third countries.* 2010; Available from: http://www.econwelfare.eu/publications/econwelfared1.2report_update_nov2010.pdf.

110.   Barbe, F., K. Atanasova, and K. Van Reeth, *Cytokines and acute phase proteins associated with acute swine influenza infection in pigs.* Vet J, 2011. **187**(1): p. 48-53.

111.   Kim, B., et al., *Association of tumor necrosis factor-alpha with fever and pulmonary lesion score in pigs experimentally infected with swine influenza virus subtype H1N2.* J Vet Med Sci, 2009. **71**(5): p. 611-6.

112.   Van Reeth, K., H. Nauwynck, and M. Pensaert, *Bronchoalveolar interferon-alpha, tumor necrosis factor-alpha, interleukin-1, and inflammation during acute influenza in pigs: A possible model for humans?* J Infect Dis, 1998. **177**(4): p. 1076-1079.

113. Van Reeth, K., S. Van Gucht, and M. Pensaert, *In vivo studies on cytokine involvement during acute viral respiratory disease of swine: troublesome but rewarding.* Vet Immunol Immunopathol 2002. **87**(3-4): p. 161-168.

114. Forberg, H., et al., *Early responses of natural killer cells in pigs experimentally infected with 2009 pandemic H1N1 influenza A virus.* PLoS One, 2014. **9**(6): p. e100619.

115. Billinis, C., et al., *Effect of challenge dose and age in experimental infection of pigs with encephalomyocarditis virus.* Vet Microbiol, 2004. **99**(3-4): p. 187-95.

116. Pietschmann, J., et al., *Course and transmission characteristics of oral low-dose infection of domestic pigs and European wild boar with a Caucasian African swine fever virus isolate.* Arch Virol, 2015. **160**(7): p. 1657-67.

117. Kowalczyk, A., et al., *Cytokine and chemokine mRNA expression profiles in BALF cells isolated from pigs single infected or co-infected with swine influenza virus and Bordetella bronchiseptica.* Vet Microbiol, 2014. **170**(3-4): p. 206-12.

118. Fiskum, S.E. *Experience from Norway on ensuring the welfare of pigs in the light of Council Directive 2008/120.* 2011; Available from: http://ec.europa.eu/dgs/health_food-safety/information_sources/docs/presentation_08032013_5_norway_foredrag_en.pdf.

119. Howden, K.J., et al., *An investigation into human pandemic influenza virus (H1N1) 2009 on an Alberta swine farm.* Can Vet J, 2009. **50**(11): p. 1153-61.

120. Forgie, S.E., et al., *Swine outbreak of pandemic influenza A virus on a Canadian research farm supports human-to-swine transmission.* Clin Infect Dis, 2011. **52**(1): p. 10-8.

121. Kyriakis, C.S., et al., *Serological Evidence of Pandemic H1N1 Influenza Virus Infections in Greek Swine.* Zoonoses Public Health, 2016. **63**(5): p. 370-3.

122. Watson, S.J., et al., *Molecular Epidemiology and Evolution of Influenza Viruses Circulating within European Swine between 2009 and 2013.* J Virol, 2015. **89**(19): p. 9920-31.

123. Nelson, M.I. and A.L. Vincent, *Reverse zoonosis of influenza to swine: new perspectives on the human-animal interface.* Trends Microbiol, 2015. **23**(3): p. 142-53.

124. Nelson, M.I., et al., *Global transmission of influenza viruses from humans to swine.* J Gen Virol, 2012. **93**(Pt 10): p. 2195-203.

125. Watson, S.J., et al., *Molecular Epidemiology and Evolution of Influenza Viruses Circulating within European Swine between 2009 and 2013.* J Virol, 2015.

126. Shinde, V., et al., *Triple-Reassortant Swine Influenza A (H1) in Humans in the United States, 2005-2009.* New Engl J Med, 2009. **360**(25): p. 2616-2625.

127. Claas, E.C.J., et al., *Infection of Children with Avian-Human Reassortant Influenza-Virus from Pigs in Europe.* Virology, 1994. **204**(1): p. 453-457.

128. Claas, E.C.J., *Pandemic influenza is a zoonosis, as it requires introduction of avian-like gene segments in the human population.* Vet Microbiol, 2000. **74**(1-2): p. 133-139.

129.   Komadina, N., et al., *Genetic analysis of two influenza A (H1) swine viruses isolated from humans in Thailand and the Philippines.* Virus Genes, 2007. **35**(2): p. 161-165.

130.   Myers, K.P., C.W. Olsen, and G.C. Gray, *Cases of swine influenza in humans: A review of the literature.* Clin Infect Dis, 2007. **44**(8): p. 1084-1088.

131.   Sjurseth, S.K. *Detection of pandemic H1N1 in a turkey breeder farm in Norway.* 2016; Available from: http://ec.europa.eu/food/animals/docs/ad_cm_20160420_22nd-annual-meetings-pres11.pdf.

132.   Kay, R.M., S.H. Done, and D.J. Paton, *Effect of sequential porcine reproductive and respiratory syndrome and swine influenza on the growth and performance of finishing pigs.* Vet Rec, 1994. **135**(9): p. 199-204.

133.   Van Reeth, K., H. Nauwynck, and M. Pensaert, *Dual infections of feeder pigs with porcine reproductive and respiratory syndrome virus followed by porcine respiratory coronavirus or swine influenza virus: a clinical and virological study.* Vet Microbiol, 1996. **48**(3-4): p. 325-35.

134.   Lindberg, J.E. and B. Ogle, *Digestive Physiology of Pigs.* Digestive Physiology of Pigs, ed. J.E. Lindberg and B. Ogle. 2001.

135.   Brookes, S.M., et al., *Influenza A (H1N1) infection in pigs.* Vet Rec, 2009. **164**(24): p. 760-1.

136.   Folkehelseinstituttet, *Norwegian Insitutute of Public Health. Influenza surveillance week 20, 2013-2014.* Folkehelseinstituttet webpage http://www.fhi.no/eway/?pid=240, 2014.

137.   Julian, T.R. and K.J. Schwab, *Challenges in environmental detection of human viral pathogens.* Curr Opin Microbiol, 2012. **2**(1): p. 78-83.

138.   Irwin, C.K., et al., *Using the systematic review methodology to evaluate factors that influence the persistence of influenza virus in environmental matrices.* Appl Environ Microbiol, 2011. **77**(3): p. 1049-60.

Enclosed papers 1- 5

Paper 1

Research Article

Clinical Impact of Infection with Pandemic Influenza (H1N1) 2009 Virus in Naïve Nucleus and Multiplier Pig Herds in Norway

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The Norwegian pig population has been free from influenza viruses until 2009. The pandemic influenza outbreak during the autumn 2009 provided an opportunity to study the clinical impact of this infection in an entirely naïve pig population. This paper describes the results of a case-control study on the clinical impact of pandemic influenza (H1N1) 2009 infection in the nucleus and multiplier herds in Norway. The infection spread readily and led to seroconversion of 42% of the Norwegian nucleus and multiplier herds within a year. Positive and negative herds were identified based on surveillance data from the Norwegian Veterinary Institute. Telephone interviews were conducted with pig herd owners or managers between November 2010 and January 2011. Pigs with clinical signs were reported from 40% of the case herds with varying morbidity and duration of respiratory disease and reduced reproductive performance. Clinical signs were reported in all age groups.

1. Introduction

Pandemic influenza A (H1N1) 2009 virus was first recorded in Norwegian pig herds in October 2009 [1]. Before that, documentation on freedom from several specific viral diseases in the pig population was provided by the surveillance and control program, where swine influenza (subtypes H1N1 and H3N2) has been included since 1997 [2]. All the nucleus and multiplying herds are included in this program.

The Norwegian pig population is also documented free from porcine reproductive and respiratory syndrome virus, Aujeszky's disease, porcine respiratory corona virus, and transmissible gastroenteritis [2]. In 2009 the pig population in Norway was declared free from enzootic pneumonia (*Mycoplasma hyopneumoniae*) [3]. Porcine circovirus type 2 is, however, presumed to be present in all Norwegian swine herds, including nucleus and multiplier herds.

In contrast to Norway, the pig populations in most other countries are endemically infected with different swine adapted subtypes of influenza A virus [4–6]. Typical clinical signs associated with influenza infection are characterized by an acute onset of fever of short duration, inappetence, lethargy, coughing, dyspnea, and nasal discharge. Morbidity within infected herds is high (approaching 100%), but mortality is typically low (less than 1%) [7]. In recent experimental studies with pandemic influenza (H1N1) 2009 virus, a similar clinical picture has been described [8, 9]. Influenza viruses can also act synergistically with other viral and bacterial pathogens to cause porcine respiratory disease complex [10–12]. The course and severity of an influenza virus infection in pigs are influenced by co-infecting agents, the pig's age, overall health and immune status, and potentially the strain of influenza virus involved [7]. It has been suggested that influenza infections may lead to reduced reproductive performance in affected animals [13]. However, there is insufficient data to conclude that influenza viruses have a specific and direct relationship to the occurrence of reproductive problems in pigs [7].

The favorable health situation provided a unique opportunity to study the clinical impact of infection with pandemic influenza (H1N1) 2009 virus, and this paper describes the results of a case-control study performed on a naïve Norwegian pig subpopulation consisting of all nucleus and multiplier herds.

2. Materials and Methods

2.1. Study Population and Laboratory Methods. The study population comprised all 118 Norwegian nucleus and multiplier herds, which were all farrow-to-finish herds. The herds were tested serologically for Influenza A specific NP antibodies by ELISA (ID Screen Influenza A Antibody Competition test, IDVET, according to manufacturer's instructions) and for hemagglutinating antibodies using hemagglutination-inhibition (HI) assays according to the method described in the OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals [14]. In addition some herds were tested for presence of viral RNA by real-time reverse transcription polymerase chain reaction (rRT-PCR) during the risk period (30th September 2009 until 31st October 2010) [15, 16].

2.2. Study Design and Data Collection. The study was designed as a case-control study. A case (positive herd) was defined as a herd with at least one rRT-PCR-positive sample, or if three or more of at least 20 blood samples were positive for antibodies against influenza A virus. If only one or two of the first 20 samples from a herd were positive with ELISA, the herd was retested with blood samples from 20 previously untested pigs and concluded positive if at least one of these samples were positive.

A questionnaire of 137 questions (123 were closed) was created to record demographics, husbandry information on the herds, and variables of interest. All farmers, irrespective of whether they represented case or control herds, were asked to report if they had observed signs of coughing, sneezing, depression, decrease in feed intake, or increase in reproductive disturbances in their pigs. Farmers who reported clinical signs were asked to estimate the proportion of affected pigs in different age groups. In Norway all nucleus and multiplier herds must keep written records (herd health cards) of all treatments irrespective of whether they were performed by a veterinarian or herd personnel. Farmers were asked to review the herd health cards for all veterinary or farmer treatments initiated during the study period.

The animals in the herds were grouped into four age groups: piglets (suckling piglets before weaning at approximately 5 weeks/10 kg), weaners (piglets after weaning, until approximately 30 kg), growers/finishers/recruit sows (from approximately 30 kg to slaughter weight or breeding age/weight), and sows. This age grouping was chosen because this is the way pigs are most commonly grouped and housed in the Norwegian herds. The transition from one group to the next is in most cases synonymous with a change in the pigs environment.

The interviewees were asked to indicate the occurrence of all observed clinical signs. It was emphasized by the interviewer that the occurrence of clinical signs should be reported as a deviation from the herds' normal clinical situation to lessen the risk of attributing regularly occurring clinical signs to the outbreak of pandemic influenza A (2009) virus. Farmers were asked to indicate the severity of observed clinical signs, but difficulties precisely defining degrees of severity between mild, moderate, or severe signs based on farmers subjective observation led to a simplified binomial classification where signs were classified as either present or absent. In addition, they were asked to estimate the duration of clinical signs in the different age groups of animals. The answer alternatives for duration of clinical signs were less than one week, one to two weeks, or more than two weeks.

The questionnaire was distributed by surface mail in the middle of November 2010. A letter was enclosed with the questionnaire encouraging the farmers to familiarize themselves with the questions and informing them that they would be asked to answer the questionnaire in a telephone interview within the following weeks. The interviews were performed by telephone over a period of 7 weeks between November 2010 and January 2011 by the first author. A paper copy of the questionnaire was used to register the answers for each interview. The data collected were later entered into a purpose-built form using Microsoft Excel 2010 (Microsoft Corporation, Redmond, WA, USA). Basic data analyses were performed in this database.

2.3. Statistical Methods. Inference statistics were done by calculating the 95% confidence interval (CI) of the binomial proportions, except when numbers were too small for statistical significance, in which case only descriptive statistics are presented.

3. Results

All 118 herds answered the questionnaire, giving a response rate of 100%. Three herds were later excluded on the basis of uncertain infection status at the time of the study, leaving the study with 115 herds comprising 47 nucleus herds and 68 multiplier herds. A total of 20 (43%) nucleus herds and 28 (41%) multiplier herds were classified as positive for pandemic influenza (H1N1) 2009 by the case definition. This gave 48 case herds and 67 control herds for the study, which gave a herd prevalence of 42% (95% CI of 33–51%). The distribution of herd categories in the study is shown in Figure 1. In the study population, 100 herds (87%) had batch farrowing, and the distribution of number of weeks between batches was 1 (1 herd), 2 (1 herd), 3 (32 herds), 5.5 (19 herds), and 7 (47 herds). The number of weeks between batches is the period of time elapsed between each time another group of sows is moved to the farrowing unit. The remaining 15 farms practiced continuous farrowing.

Nineteen (40%) (95% CI of 26–55%) of the 48 positive herds reported clinical signs of pig ILI (influenza-like illness) and/or increased reproductive disturbances in one or more age groups. The distributions and the type of observed clinical signs in the different age groups in clinically affected herds are shown in Table 1. Seventeen herds reported clinical

TABLE 1: Distribution of observed clinical signs in different age groups in clinically positive herds. The number in brackets refers to percentage of herds with these signs in different age groups of pigs.

Clinical signs	Sows (%), n = 17	Unweaned piglets (%), n = 8	Weaned piglets (%), n = 6	Growers, finishers, recruit sows (%), n = 8
Coughing	5 (29.4)	5 (62.5)	6 (100)	5 (62.5)
Sneezing	4 (23.5)	4 (50.0)	5 (83.3)	4 (50.0)
Lethargy	7 (41.2)	3 (37.5)	2 (33.3)	2 (25.0)
Fever	6 (35.3)	NR	2 (33.3)	2 (25.0)
Decreased feed intake	12 (70.6)	2 (25.0)	2 (33.4)	4 (50.0)
Abortions	8 (47.0)			
Stillbirths	5 (29.4)			
Reduced litter sizes	8 (47.0)			
Returns to estrus	9 (53.0)			
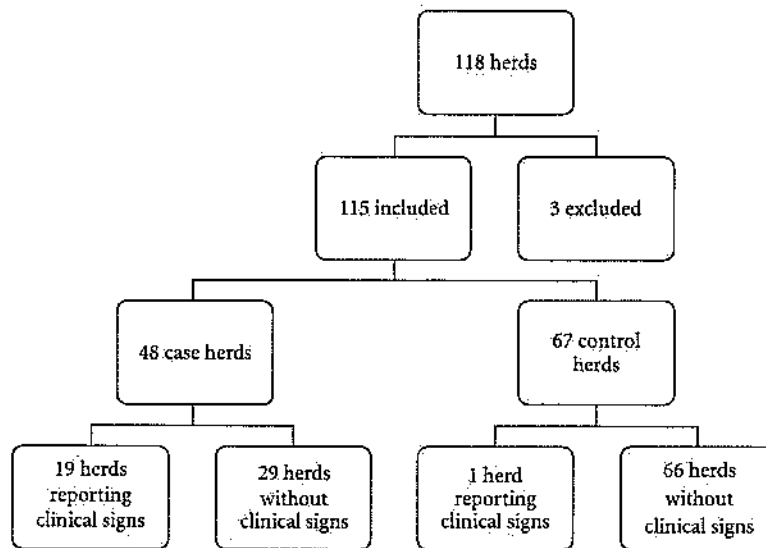
FIGURE 1: The distribution of the herds in the study population.

TABLE 2: Proportion of animals showing clinical signs of influenza like various age groups (n = number of herds, CI = confidence interval).

	Min. %	Max. %	Mean %	95% CI	
				Lower	Upper
Sows (n = 17)	10	100	43.3	28.4	58.2
Piglets (n = 8)	10	50	24.6	14.6	34.6
Weaners (n = 6)	10	80	41	18.1	63.9
Growers, finishers, recruit sows (n = 8)	4	65	27	12.3	41.7

signs in sows while 8, 6, and 8 herds reported signs in piglets, weaners, and/or growers/finishers/recruit sows, respectively. With the exception of six herds reporting clinical signs only in sows, and one herd reporting clinical signs only in growers/finishers/recruit sows the remaining 12 herds reported signs in two or more age groups of animals. Three herds reported clinical signs of ILI in all age groups of animals.

The proportions of affected animals in the respective age groups are shown in Table 2. Two interviewees were unable to estimate a proportion of affected animals by age group. One of the control herds reported clinical signs of pig ILI as a mild, transient sneezing in approximately 5% of the sows. The remaining 66 (98.5%) negative herds and 29 (60%) positive herds reported no typical disease signs of pig ILI in any age groups.

Clinical signs were reported in similar proportion from all age groups (13–17%), with the exception of decreased feed intake, which was reported with a higher number of observations (25%) in sows. Some farmers also reported fever in weaned piglets, growers/finishers/recruit sows, and sows. Twelve interviewees reported an increase in reproductive disturbances, specifically an increase in returns to estrus, abortions, and decreased litter sizes. Increased numbers of stillbirths were less frequently reported.

The duration of observed clinical signs varied between herds and between age groups. The results for sows are divided in two groups, one group for herds that reported

TABLE 3: Duration of clinical signs in herds with reported signs of pig-influenza-like illness.

	Total no. of herds with obs. clinical signs	One week or less	One to two weeks	Two weeks or more	Unsure about duration
Sows	17	6	0	8	3
(i) Reproductive signs	12	2	0	7	3
(ii) Only other clinical signs	5	4	0	1	0
Piglets	8	4	0	4	0
Weaners	6	2	0	2	2
Growers, finishers, recruit sows	8	3	2	2	1

reproductive disturbances and one group for herds that did not report reproductive disturbances. The results of the reported duration in different age groups are shown in Table 3.

4. Discussion

This study shows that 40% of positive nucleus and multiplier herds reported clinical signs of pig ILI and/or increased reproductive disturbances. The low morbidity is surprising as one might expect higher morbidity rate given the naïve population and the nature of the disease. The low morbidity, however, corresponds well with another study carried out by the Norwegian Pig Health Service (personal communication Anne Jørgensen) where 51% of infected herds (including non-breeding herds) reported clinical signs. The high health status of pigs in Norway could have resulted in the lower morbidity, as some herds might have experienced subclinical infection or mild disease that was not registered nor reported by the farmer. Farmers were chosen as respondents in this study because they are more likely to have the most complete observations of an influenza outbreak occurring in their farm. While veterinarians are undoubtedly more qualified to perform clinical examinations and evaluations, they normally spend a limited amount of time on each farm, and typically do not observe the animals in a given herd as frequently and regularly as the farmer.

Recall bias is a potential weakness in this retrospective study as the interviews took place approximately one year after the first incursion of pandemic influenza A (2009) virus. Given the Norwegian situation with an outbreak of a previously undiagnosed infectious disease, one would expect farmers to have a heightened awareness and, thus, be more likely to remember and report clinical signs beyond the normal situation. The awareness of pig farmers was also likely affected by the attention given to the outbreak of pandemic influenza A (2009) virus by the public and veterinary health authorities and the media. In addition, the nucleus and multiplier herds in Norway are obliged to keep written records of all treatments of animals, and farmers were encouraged to review these records in a letter enclosed with the questionnaire before the interview. Thus, the high proportion (60%) of positive herds reporting no clinical signs of ILI or increase in reproductive disturbances indicates a high proportion of subclinical infections in cases not

complicated by concurrent infections with other respiratory pathogens. The fact that the Norwegian pig population is free from many of the most severe infectious respiratory diseases might lead to a clinical picture less likely to be confounded or masked by concurrent infections. In the present study, only one of the control herds reported clinical signs of ILI.

The low morbidity emphasizes the need for a continued active surveillance program to monitor the status of infection in a naïve pig population. Passive surveillance based on reports of clinical disease would have a low sensitivity as many positive herds would be missed. It also poses challenges when trying to prevent herd-to-herd transmission of pandemic influenza A (2009) virus, as the risks of unintentionally introducing virus-shedding animals to seronegative recipient herds are likely to be increased when the animals are not displaying signs of disease. It also increases the potential risk of pig-to-human transmission. Low morbidity in positive herds indicates a limited economic impact of infection in these herds.

The proportion of infection was approximately the same in both closed, self-replacing nucleus herds and multiplier herds that buy replacement sows from nucleus herds. This supports that introduction of new pigs was unlikely to be the primary source of infection on farms, as previously described by Hofshagen et al. [1].

Information bias is a weakness when open questions are used, especially errors that result from a misunderstanding of questions by respondents. The risk of information bias is, however, reduced in an interview situation by the opportunity to clarify any misunderstandings and by having one person conducting all interviews.

Clinical signs typical of swine influenza were observed in all age groups of animals. Not all infected pigs showed signs even though all were susceptible in the initial phase of infection. Acute outbreaks of swine influenza are more likely to give signs of disease in fully susceptible, seronegative animals. In the present study, the interviews were focused on the alterations in observed signs of disease in the initial phase of the pandemic influenza A (2009) virus outbreak. As described, the Norwegian swine population was free from swine influenza (subtypes H1N1 and H2N3) before the outbreak in 2009 [2]. The observation of clinical signs in all age groups of animals might be the case only in the initial phase of infection in previously naïve herds. When a herd has experienced an infection and subsequent seroconversion, later

reintroductions or persistence of infections might lead to clinical signs being observed only in age groups of pigs previously unexposed to active or passive immunization against the specific virus. For instance, the morbidity and duration of clinical signs in piglets and weaners could potentially be affected by maternally derived immunity against influenza [17].

In more than half of the clinically affected herds, decreased feed intake and/or increased reproductive disturbances was reported in sows. These parameters are easily monitored, and farmers use them as reference parameters of performance. As a result, farmers could be more sensitive to alterations in these parameters. None of the control herds reported an increase in reproductive disturbances in sows. The direct role of swine influenza virus in abortions is unclear, and it is commonly believed that the reproductive problems caused by influenza viruses in pigs are due to high fever [18]. Fever was reported in sows, weaners, and growers/finishers/recruit sows, but in this study pyrexia was not emphasized as it was unclear how many farmers routinely checked the rectal temperature of the pigs.

The proportion of observed clinical signs varied between herds and age groups, although the numbers of observations were too small to show statistical significance. This difference could be explained by several factors, the most relevant being herd health status, concurrent infections, differences in sow management, true differences, or recall bias (sows in farrowing unit might "represent" the entire sow population). In addition, some clinical signs (e.g., coughing or sneezing) are more apparent and, therefore, more likely to be recorded and influence the proportion.

In contrast to the low herd morbidity seen in our study, influenza in nonimmune pigs is usually considered to be a disease with high morbidity, low mortality, and with a sudden and remarkable recovery that usually begins within 5–7 days after onset [7]. Experimental infection studies using pandemic influenza (H1N1) 2009 virus have shown a similar clinical picture [8, 9] and reports from natural infections also support this, but with varying morbidity [19, 20]. A recent Australian study in pigs naturally infected with pandemic influenza (H1N1) 2009 virus showed low morbidity and mainly mild clinical signs [21].

This present study show that nearly 50% (95% CI of 29–77%) of the respondents reported a duration of clinical signs of two weeks or more. The reported duration most likely reflects on presented clinical signs within a herd level or epidemiological unit, so one would expect there to be a prolongation because of pig-to-pig transmission after introduction of virus and incubation time. Reproductive disturbances subsequent to an outbreak of ILI will often be observed and recorded for some time after the acute signs have subsided. This was the case in the present study where 88% of recorded clinical signs in sows lasting two weeks or longer were reproductive disturbances. Concurrent or complicating infections, like bacterial infections, can also prolong the clinical manifestation of respiratory illness.

5. Conclusion

This study shows the clinical impact of acute infection with pandemic influenza (H1N1) 2009 virus in a naïve pig population. Typical signs of influenza-like illness and/or increased reproductive disturbances were reported from 40% of herds where infection with pandemic influenza (H1N1) 2009 has been documented. Clinical signs were reported from all age groups of animals. The proportion of animals affected, duration, and type of clinical signs varied between herds. Further studies are needed to investigate the reported reproductive disturbances in sows and to evaluate the economic impact of pandemic influenza A (2009) virus infection in the Norwegian pig population.

References

[1] M. Hofshagen, B. Gjerset, and C. Er, "Pandemic influenza A(H1N1)V: human to pig transmission in Norway," *Eurosurveillance*, vol. 14, pp. 15–17, 2009.

[2] B. Lium, B. Gjerset, C. Er, and P. Hopp, "The surveillance and control programme for specific virus infections in swine herds in Norway," in *Surveillance and Control Programmes for Terrestrial and Aquatic Animals in Norway. Annual Report 2009*, A. C. Karlsson, H. M. Jordsmyr, H. Hellberg, and S. Sviland, Eds., National Veterinary Institute, Oslo, Norway, 2010.

[3] B. Lium, A. Jorgensen, and M. Gjestvang, "The Norwegian program for eradication of Mycoplasma hyopneumoniae is successfully completed," in *Proceedings of the 21st International Pig Veterinary Society Congress (IPVS '10)*, p. 643, Vancouver, Canada, 2010.

[4] I. H. Brown, "The epidemiology and evolution of influenza viruses in pigs," *Veterinary Microbiology*, vol. 74, no. 1-2, pp. 29–46, 2000.

[5] C. S. Kyriakis, I. H. Brown, and E. Foni, "Virological surveillance and preliminary antigenic characterization of influenza viruses in pigs in five European countries from 2006 to 2008," *Zoonoses and Public Health*, vol. 58, pp. 93–101, 2011.

[6] M. Simon-Grife, G. E. Martin-Valls, and M. J. Vilar, "Seroprevalence and risk factors of swine influenza in Spain," *Veterinary Microbiology*, vol. 149, pp. 56–63, 2011.

[7] C. W. Olsen, I. H. Brown, B. C. Easterday, and K. Van Reeth, "Swine influenza," in *Diseases of Swine*, B. E. Straw, J. J. Zimmerman, S. D'Allaire, and D. J. Taylor, Eds., pp. 469–482, Blackwell Science, Oxford, UK, 9th edition, 2006.

[8] S. M. Brookes, A. Núñez, B. Choudhury et al., "Replication, pathogenesis and transmission of pandemic (H1N1) 2009 virus in non-immune pigs," *PLoS ONE*, vol. 5, no. 2, Article ID e9068, 2010.

[9] E. Lange, D. Kalthoff, U. Blohm et al., "Pathogenesis and transmission of the novel swine-origin influenza virus A/H1N1 after experimental infection of pigs," *Journal of General Virology*, vol. 90, no. 9, pp. 2119–2123, 2009.

[10] M. S. Hansen, S. E. Pors, H. E. Jensen et al., "An investigation of the pathology and pathogens associated with porcine respiratory disease complex in Denmark," *Journal of Comparative Pathology*, vol. 143, no. 2-3, pp. 120–131, 2010.

[11] J. Kim, H. K. Chung, and C. Chae, "Association of porcine circovirus 2 with porcine respiratory disease complex," *Veterinary Journal*, vol. 166, no. 3, pp. 251–256, 2003.

[12] E. L. Thacker, "Immunology of the porcine respiratory disease complex," *The Veterinary Clinics of North America—Food Animal Practice*, vol. 17, no. 3, pp. 551–565, 2001.

[13] P. Vannier, "Infectious causes of abortion in swine," *Reproduction in Domestic Animals*, vol. 34, no. 3-4, pp. 367–376, 1999.

[14] Office International des Epizooties, *Manual of Standards for Diagnostic Tests and Vaccines for Terrestrial Animals (Mammals, Birds and Bees)*, Office International des Epizooties, Paris, France, 6th edition, 2008.

[15] Robert Koch-Institut, *TagMan Real-Time PCR zur Detektion von Porcinen Influenza A/H1N1-Viren. Empfehlung für den Nachweis der Porcinen Influenza A/H1N1-Viren Mittels Real-Time PCR*, Robert Koch-Institut, Berlin, Germany, 2011.

[16] World Health Organization. The WHO Collaborating Centre for influenza at CDC Atlanta, *CDC Protocol of Realtime RT-PCR for Influenza A(H1N1)*, WHO, Atlanta, Ga, USA, 2009.

[17] W. L. A. Loeffen, P. P. Heinen, A. T. J. Bianchi, W. A. Hunneman, and J. H. M. Verheijden, "Effect of maternally derived antibodies on the clinical signs and immune response in pigs after primary and secondary infection with an influenza H1N1 virus," *Veterinary Immunology and Immunopathology*, vol. 92, no. 1–2, pp. 23–35, 2003.

[18] K. J. Yoon and B. H. Janke, "Swine influenza: etiology, epidemiology, and diagnosis," in *Trends in Emerging Viral Infections of Swine*, A. Morilla, K. J. Yoon, and J. J. Zimmerman, Eds., pp. 23–28, Iowa State Press, Ames, Iowa, USA, 2002.

[19] S. E. Forgie, J. Keenliside, and C. Wilkinson, "Swine outbreak of pandemic influenza A virus on a Canadian research farm supports human-to-swine transmission," *Clinical Infectious Diseases*, vol. 52, pp. 10–18, 2011.

[20] K. J. Howden, E. J. Brockhoff, F. D. Caya et al., "An investigation into human pandemic influenza virus (H1N1) 2009 on an Alberta swine farm," *Canadian Veterinary Journal*, vol. 50, no. 11, pp. 1153–1161, 2009.

[21] P. K. Holyoake, P. D. Kirkland, R. J. Davis et al., "The first identified case of pandemic H1N1 influenza in pigs in Australia," *Australian Veterinary Journal*, vol. 89, pp. 427–431, 2011.

Paper 2

Influenza A(H1N1)pdm09 virus infection in Norwegian swine herds 2009/10: The risk of human to swine transmission

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ABSTRACT

Influenza A viruses cause respiratory infection in humans and pigs, and some serotypes can be transmitted between these species. The emergence of influenza A(H1N1)pdm09 virus infections in the spring of 2009 quickly led to a worldwide pandemic in humans, with subsequent introduction of the virus to pig populations. Following a widespread infection in the human population in Norway, influenza A(H1N1)pdm09 virus was introduced to the influenza A naïve Norwegian pig population, and within a few months pigs in more than one third of Norwegian swine herds had antibodies against the virus. A cross-sectional study was performed on all swine nucleus and multiplier herds in Norway to analyze risk factors for introduction of infection, and the preventive effects of recommended biosecurity practices. A surveillance program provided information on infection status of the study herds, and a questionnaire was administered to all 118 nucleus and multiplier herds to collect information on herd variables. The surveillance program revealed that pigs in 42% of the herds had antibodies against influenza A(H1N1)pdm09 virus. The incidence of serologically positive pigs was similar in both multiplier herds (41%) and closed nucleus herds (43%). Multivariable logistic regression showed that presence of farm staff with influenza-like illness (ILI) (OR = 4.15, CI 1.5–11.4, p = 0.005) and herd size (OR = 1.01, CI 1–1.02, p = 0.009) were risk factors for infection. The rapid and widespread seroconversion for antibodies against influenza A(H1N1)pdm09 virus in the Norwegian pig population can be explained by the emergence of a novel virus that is readily transmitted between people and swine in a largely susceptible population of humans, and an entirely naïve population of pigs.

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1. Introduction

In April 2009, a novel subtype of influenza A(H1N1) virus was detected in people in Mexico and the United States (Dawood et al., 2009). The new virus contained gene segments from both American and Eurasian swine lineages of influenza virus (Garten et al., 2009; Smith et al., 2009).

The virus spread rapidly in the human population within the following months, and on 11 June 2009 the World Health Organization (WHO) declared the first pandemic of the 21st century (Chan, 2009).

The initial evidence on human-to-pig transmission of the novel influenza A subtype (H1N1)pdm09 virus was reported from Canada as early as April 2009 (Howden et al., 2009; Weingartl et al., 2010). During the next few months, spread of the new virus to pigs was described from countries all over the world, and several studies have shown the reverse zoonotic potential, i.e. transmission

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from infected humans to pigs, of influenza A(H1N1)pdm09 virus (Hofshagen et al., 2009; Lange et al., 2009; Brookes et al., 2010; Forgie et al., 2011). In a recent study, 49 different introductions of H1N1pdm09 virus from humans into swine were identified globally during 2009–2011 (Nelson et al., 2012). Furthermore, experimental studies have shown that pigs are highly susceptible to infection with influenza A(H1N1)pdm09 virus, and that the virus is transmitted readily between immune-naïve pigs (Lange et al., 2009; Brookes et al., 2010).

Influenza virus infection of pigs is associated with high morbidity within infected herds (up to 100%), but mortality is typically low (less than 1%) (Van Reeth et al., 2012). Experimental studies with influenza A(H1N1)pdm09 virus have shown the clinical signs of infection to include a sudden onset and short course of fever, inappetence, lethargy, coughing, dyspnea, and nasal discharge (Lange et al., 2009; Brookes et al., 2010). Recent reports of pigs naturally infected with influenza A(H1N1)pdm09 virus have shown more variation in morbidity and signs of disease (Howden et al., 2009; Forgie et al., 2011; Holyoake et al., 2011). In a Norwegian field study clinical signs of disease were generally mild, and in more than half of the infected herds no clinical signs were observed by the farmers (Grøntvedt et al., 2011). Influenza viruses can act synergistically with other viral and bacterial pathogens to cause porcine respiratory disease complex (PRDC) (Thacker, 2001; Kim et al., 2003; Hansen et al., 2010). The observed inter-herd variation of clinical signs of influenza-like illness (ILI) reported from Norway may have been influenced by synergistic co-infections, although the Norwegian pig population has documented freedom from important respiratory pathogens like Porcine Reproductive and Respiratory Syndrome Virus, Porcine Respiratory Coronavirus and *Mycoplasma hyopneumoniae* (Lium et al., 2012).

The first human cases of infection with influenza A(H1N1)pdm09 virus in Norway were recorded in early May 2009 among travelers returning from countries where the virus was circulating. A minor epidemic of infection in the human population occurred between July and August, and the peak of infection was reached during October and November the same year. From December onwards, the number of cases steadily decreased (Herrador et al., 2012). The first occurrence of influenza A(H1N1)pdm09 virus in a Norwegian swine herd was recorded on 10 October 2009 (Hofshagen et al., 2009). Prior to this the Norwegian pig population was documented free from swine influenza subtypes H1N1 and H3N2 (Lium et al., 2010). Within a few months the influenza A(H1N1)pdm09 virus had spread to more than one third of swine herds in the country, including closed nucleus herds with high levels of biosecurity (Gjerset et al., 2011). This indicated risk factors for infection other than the import of live pigs into the closed nucleus herds. The national surveillance programme for specific virus infections in swine for 2010 showed that 41% of herds tested had antibodies against influenza A(H1N1)pdm09 virus, while 48% were seropositive in 2011 (Lium et al., 2012). This strongly indicates that influenza A(H1N1)pdm09 virus is established as an endemic infection in the Norwegian swine population.

The aim of this study was to identify risk factors associated with the introduction of influenza A(H1N1)pdm09 virus into naïve Norwegian nucleus and multiplier pig herds during the outbreak in 2009/2010, and to evaluate the preventive effects of commonly practiced biosecurity measures in the initial phase of the outbreak.

2. Materials and methods

2.1. Study population

The study population included all 118 Norwegian nucleus and multiplier herds and was identified using a computerized data base from the Norwegian Pig Health Service. They were all farrow-to-finish herds.

2.2. Laboratory methods

All herds in the study population were tested serologically for influenza A specific NP antibodies by ELISA (ID Screen® Influenza A Antibody Competition test, IDVET, Montpellier, France, according to manufacturer's instructions) and samples tested positive in the ELISA were retested for hemagglutinating antibodies using hemagglutination-inhibition (HI) assays according to the method described in the OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals (Office International des Epizooties, 2008). In addition, where there was suspicion of an active infection due to reported clinical signs in pigs or humans with pig contact, herds were tested for presence of viral RNA by real-time reverse transcription polymerase chain reaction (rRT-PCR) (World Health Organization. The WHO Collaborating Centre for Influenza at CDC Atlanta, 2009; Robert Koch-Institut, 2011).

2.2.1. Study design and case definitions

The study was designed as a cross-sectional study, and the observation period (30 September 2009 until 31 October 2010) lasted from before the detection of the first case to the distribution of the questionnaires. The national surveillance program provided documentation on the historic freedom from swine influenza viruses (Lium et al., 2010). The outbreak of influenza A(H1N1)pdm09 virus prompted an extraordinary surveillance program, where a larger screening of the population was initiated using serological laboratory methods to monitor the virus exposure in the herds. All the nucleus and multiplier herds were included in this serological screening. In additions, herds with suspicion of an active infection were tested for the presence of viral antigens. The main diagnostic criterion for a positive herd was having at least one virus positive sample or three or more blood samples (out of 20 or more) positive for antibodies against influenza A virus. If only one or two of the first 20 samples from a herd were seropositive, the herd was retested with blood samples from 20 previously untested pigs, and concluded positive if at least one of these samples were positive. The number of samples collected from each herd was based on an expected within-herd prevalence of 20%. Data from the initial phase of the Norwegian outbreak showed within-herd prevalence ranging from 5 to 100% (mean 59%) (Gjerset et al., 2011). The

Table 1

Classification and description of variables in the questionnaire collected by telephone interview in a cross-sectional study from 118 Norwegian nucleus and multiplier farms.

Variable group	Variable subgroup	Variable description
Demographics	Farm details	Contact information of farmer, address, contact information of veterinarian.
Herd characteristics	Type of production	Nucleus herd (self-replacement), multiplier herd (self-replacement, purchase replacement).
	Size of production	Number of breeding animals, number of litters per herd and year, number of litters during study period of 18 months.
	Management	Farrowing system, type of batch farrowing, all in–all out practice in different stages of production.
	Trade of live animals	From nucleus herds to multipliers (replacement gilts) and commercial herds (growers or fatteners). From multipliers to commercial herds only (replacement gilts, growers or fatteners).
Health status	Pig health status	Disease status as perceived by the farmer; occurrence, severity, duration and proportion of coughing, sneezing, depression, fever; loss of appetite and/or increase in reproductive disturbances.
	Human health status	Date and occurrence of human influenza-like illness in farmer, close family, staff, veterinarian and/or visitors; confirmation on diagnosis by physician, confirmation on diagnosis by laboratory testing.
	Human vaccination	Date of vaccination against influenza A(H1N1)pdm09 in farmer, close family, staff, veterinarian and/or visitors; vaccination before or after observation of clinical signs in pigs.
Biosecurity	Live animal transport	Farmer owned vehicle, slaughterhouse owned vehicle, co-transport to/from different herds, routines of washing and disinfection of vehicles between transports (every time, occasionally, never), presence of live animals in transport vehicle on arrival at farm. Separate room for animals before loading onto transport. Separation of animals sold as live animals, or sold for slaughter. Ramp for loading animals onto transport vehicle, with at least one door closed to remaining animal housing.
	Prevention of pathogen introduction by farmer, staff, veterinarian and/or visitors	Extent and frequency of animal contact by farmer, staff, veterinarian and visitors. Design and use of physical hygiene barrier in pig house entrance; change of footwear and/or coveralls; handwashing and/or hand disinfection; disposable gloves; disposable facemask; use and duration of human quarantine after travel abroad. Alterations in any of these measures before and after influenza outbreak.
	Prevention of pathogen introduction by animals	Type, duration and extent of quarantine of introduced animals, multisite quarantine, separate ventilation, separate manure handling.

sample size selected provides a 94% confidence of detecting a within-herd prevalence of 0.2 by using a test with 95% sensitivity (Tse et al., 2012).

2.3. Herd data

A questionnaire of 137 questions (123 were closed) was created to record name and contact information for the farmer, husbandry information on the herds, the health status of humans and pigs, and biosecurity measures practiced. All variables collected by questionnaire are shown in Table 1.

The questionnaire was distributed by surface mail in mid-November 2010. Enclosed with the questionnaire was a letter encouraging the farmers to familiarize themselves with the questions, and informing them that they would be invited to answer the questionnaire in a telephone interview within the weeks that followed. The interviews were performed by the first author during a period of 7 weeks between November 2010 and January 2011. A paper copy of the questionnaire was used to register the answers for each interview.

2.4. Data analysis

2.4.1. Software

Unless otherwise specified, all data handling and statistical analysis were conducted using Stata Version 12 (Stata-Corp, College Station, TX, USA).

2.5. Statistical analysis

2.5.1. Definition of the outcome and explanatory variables

The herd was the unit of analysis and status of influenza A(H1N1)pdm09 virus infection was the binary outcome variable for the statistical analyses. A causal diagram was constructed, hypothesizing humans with influenza-like illness in close contact with pigs as the primary predictor, with secondary and potentially interactive predictors grouped under herd characteristics and biosecurity. Univariable analysis was performed for these predictors (Table 1) to measure the association with the outcome variable by calculating odds ratios (OR) and their statistical significance (Fisher exact test). Continuous variables were

tested by univariable logistic regression after their linearity with the logit of the outcome were confirmed using lowess curves, and normality was assessed using graphical methods.

2.5.2. Variables without values

Missing values occurred for some of the variables because they were not applicable to a particular herd. For example, questions on relief workers had no values recorded in herds where relief workers were not employed. Such a variable was combined with other similar variables to form a combined variable for the multivariable model to preserve maximum degrees of freedom and representation. For example, farmers were combined with workers and relief workers to form a new variable called "humans in frequent direct contact with pigs".

2.5.3. Building the multivariable logistic regression model

A multivariable logistic regression model was constructed to predict the likelihood that a herd would be infected (Dohoo et al., 2009). The parsimonious model was built by forward selection. Inclusion criteria for building the multivariable model were predictors that showed significant association (Wald statistics) with the binary outcome in the univariable analysis. Due to the large number of risk factors evaluated, in order to decrease the Type 1 error rate, the alpha level for significance was lowered to 0.01. Confounding was checked by observing whether there was a change of at least 30% to the ORs or coefficients of existing predictors when a new predictor was added. To assess the quality of the final model's fit with the data, the Hosmer–Lemeshow goodness of fit diagnostics and LROC curves were used.

3. Results

Farm personnel (mainly farm owners) from all 118 herds answered the questionnaire, giving a response rate of 100%. Three herds were excluded from the statistical analyses because of uncertain infection status at the time of the study, leaving the study with 115 herds comprising 47 nucleus herds and 68 multiplier herds. A total of 20 (43%) nucleus herds and 28 (41%) multiplier herds were classified as positive for influenza A(H1N1)pdm09 by the diagnostic criteria previously described. This gave 48 positive herds and 67 negative herds for the study. Chronological data on presence of clinical signs of ILI from both humans and pigs were reported from 14 of the 48 positive herds. Twelve (85.7%) of these 14 herds had ILI in humans occurring before the pigs began to show typical signs of influenza infection. The median time between occurrences of clinical signs in humans and in pigs was 21 days.

3.1. Multivariable analysis

In the final logistic regression model, the primary predictor of human ILI in farm staff (farmer, farm worker or relief farm worker) (OR = 4.15, CI 1.5–11.4, p = 0.005) was associated with positive herd status. In addition, risk of infection increased with herd size (OR = 1.01, CI 1–1.02, p = 0.009) represented by number of sows per year.

The area under the ROC curve was 0.71 and goodness of fit statistics such as Pearson (Chi-square = 49, p = 0.52) and Hosmer–Lemeshow (Chi-square = 6.72, p = 0.56) suggest an acceptable fit of the model. At the predicted probability of 0.5 as cut point, about 65% of the farms were correctly classified, which indicated that the model had limited predictive ability.

4. Discussion

Before the introduction of influenza A(H1N1)pdm09 virus the Norwegian pig population was documented naïve to influenza A viruses and pigs were not vaccinated against influenza infection (Lium et al., 2010). Thus no protective or cross-protective immunity was present at the time of introduction of the novel virus, and the pigs were assumed to be highly susceptible to the infection. The following routes of introduction of influenza A virus into pig herds have to be considered when identifying and assessing possible risk factors: direct or indirect contact with infected host (animal or human), animate or inanimate vector or potentially airborne spread of virus by aerosols (Tellier, 2006, 2009; Torremorell et al., 2012). There is no evidence that influenza virus in pigs is transmitted through semen (Torremorell et al., 2012).

The results of this study show that ILI among farm staff was identified as the most important risk factor associated with introduction of influenza A(H1N1)pdm09 virus to the swine herds in the initial phase of the outbreak. The hypothesis of people as the primary source of infection to the Norwegian nucleus and multiplier herds was strengthened by chronological information. In 14 herds with dates on occurrence of ILI in both humans and pigs, 12 herds reported human ILI preceding signs of infection in the pigs.

Size of production (as indicated by the number of sows) was the only other risk factor (OR 1.01, p < 0.01) remaining after multivariable analysis, indicating an increased risk of infection by increasing herd size. Typically, the number of contacts (people, vehicles, and animals) increases with herd size. In the reverse zoonotic situation, an increase in human to pig contacts might be one of the possible explanations on the effect of herd size.

The relative importance of humans as the main source of introduction is likely reduced as the circulation of influenza A(H1N1)pdm09 virus in the human population in Norway has been low in the two influenza seasons that have passed since 2009/10 (Folkehelseinstituttet, 2011, 2012). In addition, human immunity rapidly increased due to vaccination and natural infections. In herds where a positive diagnosis of infection with H1N1pdm09 was established by detection of virus or suspected by the presence of clinical signs of pig ILI, restrictions on animal movement were implemented by the Norwegian Food Safety Authority. This might also have contributed to reducing spread of disease between individual herds. However, the preventive effects of restrictions on animal movement was likely to be limited at the population level because in 60% of positive herds, farm personnel did not detect or report any signs of influenza in pigs, and thus were not subject to restrictions (Grøntvedt et al., 2011).

Biosecurity practices and pig vaccination are considered the primary means of preventing or minimizing transmission of influenza A virus in pigs and from pigs to other species (Torremorell et al., 2012). In the Norwegian pig population the documented freedom from influenza infection and the presence of an active serological surveillance program has led to a strict no-vaccination policy determined by the Norwegian Food Safety Authority. The multivariable analysis in this study failed to find any statistically significant protective effect of the recommended biosecurity measures. This apparent lack of protective effects may have several explanations in addition to the possibility of actual failure in protectiveness. The degree of compliance and implementation of recommended biosecurity measures varied, especially recommendations given in context with the disease outbreak like the use of disposable gloves and disposable facemasks. In addition, reliable information on the time of implementation of such biosecurity measures relative to the time of herd infection was difficult to ascertain (Grøntvedt et al., 2011).

The herd sizes in Norway are generally small and they have few farm personnel. This may in turn lead to difficulties having substitute farm workers available in cases of illness in the regular staff and thus forcing staff with ILI to work in close contact with susceptible animals despite their illness, challenging the biosecurity routines. This impression was verified by respondents' remarks during the telephone interviews in this study. Human vaccination in Norway started on 19 October 2009 with an AS03 adjuvanted monovalent vaccine against influenza A(H1N1)pdm09 (Pandemrix®, GlaxoSmithKline Biologicals s.a., Rixensart, Belgium). Vaccination of personnel in close contact with swine production was recommended by the Norwegian Institute of Public Health on 23 October (Folkehelseinstituttet, 2009; Herrador et al., 2012). By 26 October, a positive diagnosis for influenza A(H1N1)pdm09 had been verified for 23 out of 51 (45.1%) tested herds (Hofshagen et al., 2009). By the end of 2009, 35.8 per cent (91/254) of all herds tested in Norway were positive for A(H1N1)pdm09 virus (Gjerset et al., 2011). Although there was a high compliance of vaccination among farm personnel in the present study (range 70.4–91.2%), the possible protective effects on human-to-pig transmission was likely reduced due to late implementation. For preventive practices to have effect, they need to be established in advance of exposure.

The questionnaires were conducted by a single interviewer with personnel working directly on the farm to reduce information bias. Information bias was also reduced in the interview by the opportunity to clarify misunderstanding of questions. Confidentiality of results was guaranteed to reduce information bias, as information on relevant variables was reported by the respondents and not by direct observation. The respondents were given a pre-defined case definition of influenza-like illness (fever, malaise, coughing and sore throat) at the appropriate time during the telephone interview to reduce misclassification of human ILI. The outcome criteria as positive and negative were based mainly on serological data, where positive ELISA tests for influenza A virus antibodies were confirmed with a HI test for influenza A(H1N1)pdm09 virus specific

antibodies to optimize specificity. In addition, detection of viral antigens was used to strengthen the diagnosis in herd with clinical signs of ILI in pigs or humans with pig contact.

Recall bias is a potential challenge in retrospective studies, and the interviews in this study were performed approximately 1 year after the disease outbreak. However, the incursion of a previously undiagnosed infectious disease in the Norwegian pig population, combined with a concurrent pandemic outbreak in humans and the continued attention by public and veterinary health authorities and media would likely lead to a heightened awareness and recollection among farmers. In addition, the nucleus and multiplier herds are obliged to keep written records on all medical treatments of pigs.

5. Conclusion

The introduction of influenza A(H1N1)pdm09 virus to Norway led to a unique situation where highly susceptible human and naïve pig populations were exposed to a virus that readily transmits between humans and pigs. In conclusion, this study shows that the most important risk factor in the initial phase of the epidemic was farm personnel with ILI, with herd size as a secondary risk factor. Therefore, the critical preventive measure to avoid introduction of influenza A(H1N1)pdm09 virus was to enforce strict prohibition of access for people with ILI. The results of this study could indicate that transmission from infected humans in direct contact with susceptible pigs was likely to occur irrespective of the implementation of recommended biosecurity measures in the initial phases of the influenza A(H1N1)pdm09 outbreak.

Conflict of interest

None.

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References

Brookes, S.M., Nunez, A., Choudhury, B., Matrosovich, M., Essen, S.C., Clifford, D., Slomka, M.J., Kuntz-Simon, G., Garcon, F., Nash, B., Hanna, A., Heegaard, P.M.H., Quéguiner, S., Chiapponi, C., Bublot, M., Garcia, J.M., Gardner, R., Foni, E., Loeffen, W., Larsen, L., Van Reeth, K., Banks, J., Irvine, R.M., Brown, I.H., 2010. Replication, pathogenesis and transmission of pandemic (H1N1) 2009 virus in non-immune pigs. PloS ONE 5, e9068.
Chan, M., 2009. World now at the start of 2009 influenza pandemic: statement to the press by WHO Director-General Dr Margaret Chan 11.06.09, WHO.
Dawood, F.S., Jain, S., Finelli, L., Shaw, M.W., Lindstrom, S., Garten, R.J., Gubareva, L.V., Xu, X.Y., Bridges, C.B., Uyeki, T.M., 2009. Emergence of a novel swine-origin influenza A (H1N1) virus in humans. N. Engl. J. Med. 360, 2605–2615.

Dohoo, I., Martin, W., Stryhn, H., 2009. Logistic regression. In: Veterinary Epidemiologic Research, 2nd ed. VER inc., Charlottetown, Canada, pp. 395–466.

Folkehelseinstituttet: Influensaovervåking 2010–2011, uke 20 (In Norwegian). http://www.fhi.no/eway/default.aspx?pid=233&trg=MainLeft_5565&MainArea_5661=5565:0:15,3938:1:0:0:::0:0&MainLeft_5565=5544:90282::1:5694:32:::0:0&4468=5694:2 (accessed 02.08.12).

Folkehelseinstituttet: Influensaovervåking 2011–2012, uke 20 (In Norwegian). http://www.fhi.no/eway/default.aspx?pid=233&trg=MainLeft_5565&MainArea_5661=5565:0:15,3938:1:0:0:::0:0&MainLeft_5565=5544:97639::1:5569:1:::0:0 (accessed 02.08.12).

Folkehelseinstituttet: Rapport nummer 2 om vaksinasjonsrekkefølge, versjon 2, 2009 (In Norwegian) http://www.fhi.no/dokumenter/f332d0ae39.pdf (accessed 10.07.12).

Forgie, S.E., Keenliside, J., Wilkinson, C., Webby, R., Lu, P., Sorensen, O., Fonseca, K., Barman, S., Rubrum, A., Stigger, E., Marrie, T.J., Marshall, F., Spady, D.W., Hu, J., Loeb, M., Russell, M.L., Babiuk, L.A., 2011. Swine outbreak of pandemic influenza A virus on a canadian research farm supports human-to-swine transmission. Clin. Infect. Dis. 52, 10–18.

Garten, R.J., Davis, C.T., Russell, C.A., Shu, B., Lindstrom, S., Balish, A., Sessions, W.M., Xu, X., Skepner, E., Deyde, V., Okomo-Adhiambo, M., Gubareva, L., Barnes, J., Smith, C.B., Emery, S.L., Hillman, M.J., Rivailler, P., Smagala, J., de Graaf, M., Burke, D.F., Fouchier, R.A.M., Pappas, C., puche-Aranda, C.M., Lopez-Gatell, H., Olivera, H., Lopez, I., Myers, C.A., Faix, D., Blair, P.J., Yu, C., Keene, K.M., Dotson, P.D., Boxrud, D., Sambol, A.R., Abid, S.H., George, K.S., Bannerman, T., Moore, A.L., Stringer, D.J., Blevins, P., Demmler-Harrison, G.J., Ginsberg, M., Kriner, P., Waterman, S., Smole, S., Guevara, H.F., Belongia, E.A., Clark, P.A., Beatrice, S.T., Donis, R., Katz, J., Finelli, L., Bridges, C.B., Shaw, M., Jernigan, D.B., Uyeki, T.M., Smith, D.J., Klimov, A.I., Cox, N.J., 2009. Antigenic and genetic characteristics of swine-origin 2009 A(H1N1) influenza viruses circulating in humans. Science 325, 197–201.

Gjerset, B., Er, C., Løtvedt, S., Jørgensen, A., Hungnes, O., Lium, B., Germundsson, A., 2011. Experiences after twenty months with pandemic influenza A (H1N1) 2009 infection in the naïve Norwegian pig population. Influenza Res. Treat., 206975.

Grøntvedt, C.A., Er, C., Gjerset, B., Germundsson, A., Framstad, T., Brun, E., Jørgensen, A., Lium, B., 2011. Clinical impact of infection with pandemic influenza (H1N1) 2009 virus in naïve nucleus and multiplier pig herds in Norway. Influenza Res. Treat, 163745.

Hansén, M.S., Pors, S.E., Jensén, H.E., Bille-Hansen, V., Bisgaard, M., Flachs, E.M., Nielsen, O.L., 2010. An investigation of the pathology and pathogens associated with porcine respiratory disease complex in Denmark. J. Comp. Pathol. 143, 120–131.

Herrador, B.R.G., Aavitsland, P., Feiring, B., Bergsaker, M.A.R., Borgen, K., 2012. Usefulness of health registries when estimating vaccine effectiveness during the influenza A (H1N1)pdm09 pandemic in Norway. BMC Infect. Dis. 12.

Hofshagen, M., Gjerset, B., Er, C., Tarpai, A., Brun, E., Dannevig, B., Bruheim, T., Fostad, I.G., Iversen, B., Hungnes, O., Lium, B., 2009. Pandemic influenza A(H1N1)V: human to pig transmission in Norway? Euro Surveill. 14, 19406.

Holyoake, P.K., Kirkland, P.D., Davis, R.J., Arzey, K.E., Watson, J., Lunt, R.A., Wang, J., Wong, F., Moloney, B.J., Dunn, S.E., 2011. The first identified case of pandemic H1N1 influenza in pigs in Australia. Aust. Vet. J. 89, 427–431.

Howden, K.J., Brockhoff, E.J., Caya, F.D., Mcleod, L.J., Lavoie, M., Ing, J.D., Bystrom, J.M., Alexandersen, S., Pasick, J.M., Berhane, Y., Morrison, M.E., Keenliside, J.M., Laurendeau, S., Rohonczy, E.B., 2009. An

investigation into human pandemic influenza virus (H1N1) 2009 on an Alberta swine farm. Can. Vet. J. 50, 1153–1161.

Kim, J., Chung, H.K., Chae, C., 2003. Association of porcine circovirus 2 with porcine respiratory disease complex. Vet. J. 166, 251–256.

Lange, E., Kalthoff, D., Blohm, U., Teifke, J.P., Breithaupt, A., Maresch, C., Starick, E., Fereidouni, S., Hoffmann, B., Mettenleiter, T.C., Beer, M., Vahlenkamp, T.W., 2009. Pathogenesis and transmission of the novel swine-origin influenza virus A/H1N1 after experimental infection of pigs. J. Gen. Virol. 90, 2119–2123.

Lium, B., Gjerset, B., Er, C., Hopp, P., 2010. The surveillance and control programme for specific virus infections in swine herds in Norway. In: Karlsson A.C., Jordsmyr H.M., Hellberg H., Sviland S. (Eds.), Surveillance and Control Programmes for Terrestrial and Aquatic Animals in Norway Annual Report 2009. Norwegian Veterinary Institute, Oslo, Norway.

Lium, B., Zerihun, A., Tarpai, A., Hopp, P., 2012. The surveillance and control programme for specific virus infections in swine herds in Norway 2011. In: Sviland, S, Hellberg, H. (Eds.). Surveillance and Control Programmes for Terrestrial and Aquatic Animals in Norway. Annual report 2011. Norwegian Veterinary Institute, Oslo.

Nelson, M.I., Gramer, M.R., Vincent, A.L., Holmes, E.C., 2012. Global transmission of influenza viruses from humans to swine. J. Gen. Virol. 93, 195–203.

Office International des Epizooties, 2008. Manual of Standards for Diagnostic Tests and Vaccines for Terrestrial Animals (Mammals, Birds and Bees). 6th ed. Paris.

Robert Koch-Institut, 2011. TaqMan real-time PCR zur Detektion von porcinen Influenza A/H1N1-Viren. Empfehlung für den Nachweis der porcinen Influenza A/H1N1-Viren mittels real-time PCR. Robert Koch-Institut, Berlin.

Smith, G.J.D., Vijaykrishna, D., Bahl, J., Lycett, S.J., Worobey, M., Pybus, O.G., Ma, S.K., Cheung, C.L., Raghwani, J., Bhatt, S., Peiris, J.S.M., Guan, Y., Rambaut, A., 2009. Origins and evolutionary genomics of the 2009 swine-origin H1N1 influenza A epidemic. Nature 459, 1122–1125.

Tellier, R., 2006. Review of aerosol transmission of influenza A virus. Emerg. Infect. Dis. 12, 1657–1662.

Tellier, R., 2009. Aerosol transmission of influenza A virus: a review of new studies. J. Royal Soc. Interface 6, S783–S790.

Thacker, E.L., 2001. Immunology of the porcine respiratory disease complex. Vet. Clin. North Am. Food Anim. Prac. 17, 551–565.

Torremorell, M., Allerson, M., Corzo, C., Diaz, A., Gramer, M., 2012. Transmission of influenza A virus in pigs. Transbound. Emerg. Dis. 59, 68–84.

Tse, M., Kim, M., Chan, C.H., Ho, P.L., Ma, S.K., Guan, Y., Peiris, J.S.M., 2012. Evaluation of three commercially available influenza A type-specific blocking enzyme-linked immunosorbent assays for seroepidemiological studies of influenza A virus infection in pigs. Clin. Vaccine Immunol. 19, 334–337.

Van Reeth, K., Brown, I.H., Olsen, C.W., 2012. Influenza virus. In: Zimmerman, J.J., Karriker, L.A., Ramirez, A., Schwartz, K.J., Stevenson, G.W. (Eds.), Diseases of Swine., 10th ed. John Wiley, pp. 557–571 [chapter 40].

Weingartl, H.M., Berhane, Y., Hisanaga, T., Neufeld, J., Kehler, H., Emburry-Hyatt, C., Hooper-McGreevy, K., Kasloff, S., Dalman, B., Bystrom, J., Alexandersen, S., Li, Y., Pasick, J., 2010. Genetic and pathobiologic characterization of pandemic H1N1 2009 influenza viruses from a naturally infected swine herd. J. Virol. 84, 2245–2256.

World Health Organization. The WHO Collaborating Centre for Influenza at CDC Atlanta, 2009. CDC Protocol of Realtime RT-PCR for Influenza A(H1N1). WHO, Atlanta.

Paper 3

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Adverse effects of Influenza A(H1N1)pdm09 virus infection on growth performance of Norwegian pigs - a longitudinal study at a boar testing station

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Abstract

Background: Influenza A(H1N1)pdm09 virus infection in Norwegian pigs was largely subclinical. This study tested the hypothesis that the infection causes negligible impact on pigs' growth performance in terms of feed conversion efficiency, daily feed intake, daily growth, age on reaching 100 kg bodyweight and overall feed intake. A sample of 1955 pigs originating from 43 breeding herds was classified into five infection status groups; seronegative pigs (n = 887); seropositive pigs (n = 874); pigs positive for virus at bodyweight between 33 kg and 60 kg (n = 123); pigs positive for virus at bodyweight between 61 kg and 80 kg (n = 34) and pigs positive for virus at bodyweight between 81 kg and 100 kg (n = 37). Each pig had daily recordings of feed intake and bodyweight from 33 kg to 100 kg. Marginal effects of the virus infection on the outcomes were estimated by multi-level linear regression, which accounted for known fixed effects (breed, birthdate, average daily feed intake and growth phase) and random effects (cluster effects of pig and herd).

Results: The seropositive and virus positive pigs had decreased (P value<0.05) growth performance compared to seronegative pigs even though feed intake was not decreased. Reduced feed conversion efficiency led to lower average daily growth, additional feed requirement and longer time needed to reach the 100 kg bodyweight. The effects were more marked (P value<0.03) in pigs infected at a younger age and lasted a longer period. Despite increased feed intake observed, their growth rates were lower and they took more time to reach 100 kg bodyweight compared to the seronegative pigs.

Conclusion: Our study rejected the null hypothesis that the virus infection had negligible adverse effects on growth performance of Norwegian pigs.

Keywords: Growth performance, Daily growth, Feed conversion efficiency, Longitudinal study, Multi-level regression analysis, Random intercept model, Pigs, Feed intake, Influenza A(H1N1)pdm09 virus

Background

Respiratory diseases in pigs are serious concerns for pig producers worldwide because they cause substantial economic losses from increased mortality, reduced feed efficiency and growth rate, increased time to reach market weight, increased carcass condemnation at slaughter and costs of treatment and vaccination [1]. Among the many respiratory pathogens in pigs, swine influenza viruses (SIVs) are ubiquitous in intensive pig farming, and can

be primary agents in causing respiratory disease [2-4]. In April 2009, a new influenza A virus, named influenza A (H1N1)pdm09 emerged in North- and South-America. It spread rapidly in humans and pigs and soon became endemic in pig populations worldwide including Norway [5-7]. Like other SIVs, this A(H1N1)pdm09 virus spreads easily between pigs and can cause acute respiratory disease [8-10] characterized by high fever, depression, loss of appetite, tachypnoea, abdominal breathing and coughing [7]. Uncomplicated SIV infections cause low mortality (usually less than 1%), but morbidity can reach 100% [7]. Respiratory disease caused by SIVs can be exacerbated

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by concurrent infections of other respiratory pathogens of which Porcine Reproductive and Respiratory Syndrome virus (PRRSV), *Mycoplasma hyopneumoniae*, *Pasteurella multocida*, and *Actinobacillus pleuropnuemoniae* are the most common. Such respiratory diseases are most frequently detected in 10- to 22-week-old pigs and are termed the porcine respiratory disease complex [1,11].

As compared to other pig populations in the world, Norwegian pigs have the favourable condition of being free from many respiratory pathogens like PRRSV, porcine respiratory coronavirus and *M. hyopneumoniae*, which are serious respiratory pathogens in almost all pig producing countries. Up until 2009, the Norwegian pig population had also been free from SIVs. However in the autumn of 2009, the Norwegian pig population experienced the first outbreak of influenza virus infection [12]. Pig farmers and farm workers infected with influenza A(H1N1)pdm09 virus transmitted the virus to the pigs [13,14]. Within a few months, one third of Norwegian pig herds were positive for antibodies against the virus [15]. Subsequent annual national surveillance from 2010 to 2012 revealed that 41-50% of pig herds were seropositive, indicating that the virus had become endemic in the Norwegian pig population [12]. Farmers of positive herds reported mild or absence of clinical signs in their pigs [16] in Norway and in other parts of the world [7].

Although there have been studies investigating the clinical signs, pathology and immunology related to SIVs, including influenza A(H1N1)pdm09 virus infection in pigs [2,9-11,17,18], there is little information available on the adverse effects of influenza virus infection on growth performance of pigs in the field. We therefore aimed by using a field study to investigate the adverse effects of influenza A(H1N1)pdm09 virus on pig production performance with longitudinal growth performance data from a pig testing station between 2009 and 2012. Given the mild clinical picture presented in previous studies from Norway [14-16], the present study tested the hypothesis that influenza A(H1N1)pdm09 virus has little or no impact on growth performances in infected grower pigs. The study also investigated whether the virus infection had different impacts on Landrace and Duroc breeds, on pigs infected at different ages, and the duration of the adverse effects if present.

Methods

Boar testing station

During our period of investigation between 2009 and 2012, the boar testing station in Norway [19] had a capacity of testing 1152 pigs in 16 separate rooms at the same time. Each room housed a cohort of 72 pigs grouped by breed (Landrace or Duroc) into six groups

of 12 pigs and placed in pens of 14 m^2 in size. The station received weekly, 72 growing pigs (11–12 weeks old with a mean bodyweight of 33 kg) from 46 breeding herds in Norway to monitor their growth performances until they reached a bodyweight of 100 kg. Electronic feeding stations in all pig pens used FIRE (Feed Intake Recording Equipment, Osbourne Ltd, UK) to record daily feed intake and daily weight gain of each pig individually. Pigs fed one at a time *ad libitum* from one electronic feed dispenser in each pen on conventional concentrate containing 161 g and 136 g digestible protein, 9.68 MJ, and 9.50 MJ net energy/kg before and after 50 kg live weight, respectively, with 1 month of mixing the two feeds to facilitate the feed change.

Study sample

The study sample consisted of 1955 pigs (55% Landrace, 45% Duroc) from 43 breeding herds that were performance tested at the testing station between 2009 and 2012. All pigs were tested for antibodies against influenza A virus by cELISA before leaving the station. During a clinical outbreak of influenza at the boar station between 1st April 2011 and 31st July 2011, nasal swabs and blood samples were collected from a total of 375 of these pigs (three pigs per pen) to test for presence of virus and antibodies against the virus, respectively. The testing methods have been described previously [15]. Based on laboratory findings, the 1955 pigs were classified into five infection status groups (INFGP). The seronegative group (SERONEG) with 887 pigs was defined as pigs tested negative for antibodies against influenza A(H1N1)pdm09 virus at the end of their performance testing period. The seropositive group (SEROPOS) with 874 pigs was defined as pigs tested positive for antibodies against influenza A(H1N1)pdm09 virus but with unknown point of infection. Most (n = 859) of these pigs were tested at the end of the performance testing period when they were about 100 kg in weight thus ruling out maternal antibodies in cELISA test results. The virus positive group one (VIR1) with 123 pigs was defined as pigs tested positive for influenza A (H1N1)pdm09 virus by RT-PCR when their bodyweight was between 33 kg and 60 kg (growth phase one or GF1). Of these VIR1 pigs, twenty-two were in the upper weight range of between 51 kg and 60 kg when they were tested positive for the virus. The virus positive group two (VIR2), with 34 pigs, was defined as pigs tested positive for influenza A(H1N1)pdm09 virus by RT-PCR when their bodyweight was between 61 kg and 80 kg (growth phase two or GF2). The virus positive group three (VIR3), with 37 pigs was defined as pigs that tested positive for influenza A(H1N1) pdm09 virus by RT-PCR when their bodyweight was between 81 kg and 100 kg (growth phase three or

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GF3). Figure 1 is a histogram showing the distribution of the bodyweights of these 194 virus positive pigs when they were tested. These pigs were then classified into three groups to ensure that there were at least 30 pigs in each group.

During the period when the station experienced a clinical outbreak of influenza A(H1N1)09pdm virus, the staff intensified the clinical observation of the 2045 pigs that stayed at the station during this period. The source of the outbreak was not investigated as the infection has become widespread in the Norwegian pig population since its incursion into Norway in 2009 [15].

Statistical analysis

Growth and feeding performances were represented by five outcomes; average daily growth (ADG, weight gain in kg/day), feed conversion efficiency (FCE, kg feed/kg weight gain), average daily feed intake (ADFI, feed intake in kg/day), age of pig when they reached 100 kg (Age100kg) bodyweight; and the overall feed intake (OFI) of the pigs to grow from a mean starting weight of 33 kg to 100 kg. Besides the infection status groups (INFGP; SERONEG, SEROPOS, VIR1, VIR2 and VIR3) as the main predictor of interest, important control predictors were birthdate (BD), breed (Br), ADFI, and GF. For each of the three outcomes of ADG, FCE and ADFI, every pig had three measures aggregated for the three stipulated growth phases. The SERONEG pigs were the reference group for comparison with the SEROPOS group and the three virus positive groups. The data was structured longitudinally on daily measures of growth

and feed intake [19] and was hierarchical with three levels. The observations for each outcome were nested in the 1955 pigs, which in turn were nested in the 43 herds. To estimate the negative effects of the virus infection on growth performance, we used multi-level random-intercept regression models to control for other important predictors, while accounting for clustering or random effects at the pig and herd levels [20]. We used analysis of covariance to determine the statistical significance (P value<0.05 for level of inclusion) of predictors for each of the five outcomes. Predictors BD (proxy for improvement over time from 2009 to 2012) and ADFI were continuous variables while INFGP, Br, GF and two interaction terms of GF*INFGP and Br*INFGP for stratification were categorical variables. The INFGP*GF interaction term was included in the regression model to investigate the modifying effect of age of infection on the virus [21]. The INFGP*Br interaction term was tested for statistical significance to see if the impact of the virus infection was different between Landrace and Duroc. The two outcomes, Age100 kg and OFI, were single measures for each pig's overall performance when they reached 100 kg bodyweight. The predictors for them were the same as for FCE and ADG, but without GF since the measures were aggregated over all the 3 growth phases. To select the best multi-level models for the five outcomes with the selected predictors, we used Akaike Information Criterion (AIC). We selected the model with the lowest AIC value. To determine the significance of additional predictors for each of the five models (one outcome per model), a difference of ±2 of the AIC value was
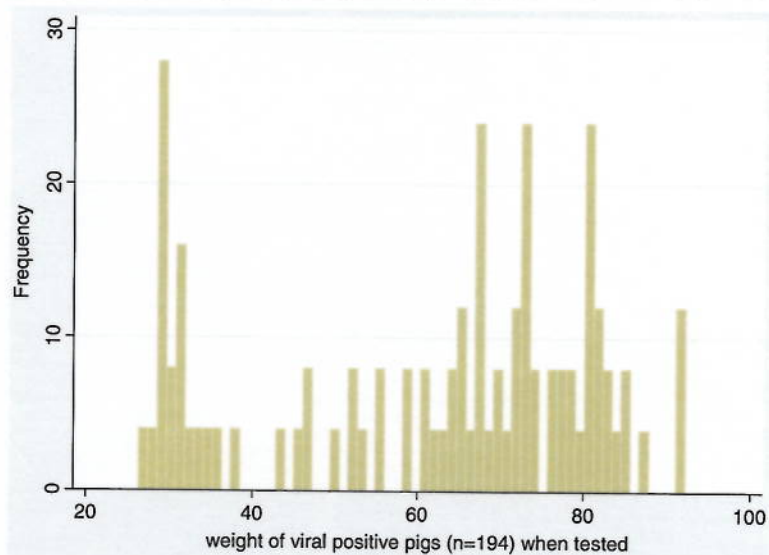

Figure 1 Histogram of weight of virus positive pigs (n= 194) when they were tested positive for virus shedding.

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regarded as non-significant and the most parsimonious model was then chosen [22].

Multi-level random-intercept regression models

$$Y_{[i,j,k]} = \beta_0 + \beta_1 X_{1[i,j,k]} + \ldots + \beta_h X_{h[i,j,k]} + u_{[j,k]} + v_{[k]} + \varepsilon_{[i,j,k]}.$$

Where:

Y is one of the five outcomes in this study (ADG, FCE, ADFI, Age100kg, or OFI,). Y_{ijk} is the value of the response for ith (i = 1,2,3) observation for jth pig (n_j = 1955) nested within the kth (n_k = 43) herd.

β is a vector of coefficients for predictors and their interactions, and $X_{[i,j,k]}$ is the vector of explanatory variables for the ith observation of the jth pig and kth herd.

u_{jk} is a vector of random intercepts unique to each pig in each herd, where $u_{jk} \sim N(0, \sigma^2_{pig})$, and v_k is a vector of random intercepts unique to each herd, where $v_k \sim N(0, \sigma^2_{herd})$.

ε_{ijk} is the vector of error terms where $\varepsilon_{ijk} \sim N(\mu, \sigma^2)$.

Predictors

Apart from BD and ADFI that are continuous predictors, the following are categorical predictors:

INFGP: SERONEG; SEROPOS; VIR1; VIR2; VIR3.
Br: Landrace; Duroc
GF: GF1 (33 kg to 60 kg); GF2 (61 kg to 80 kg); GF3 (81 kg to 100 kg).

Quantitative bias analysis by Episens [23] was used to estimate the magnitude of misclassification bias for adverse effects on FCE given that the cELISA test had a respective sensitivity and specificity of 93.7% and 99.1% (manufacturer's data sheet). A small number of misclassification of the 887 seronegative pigs and 874 seropositive pigs may have been possible. This required dichotomizing the continuous outcome FCE into high and low with median FCE value of 2.54 for the seronegative pigs chosen for the dichotomy.

We used software SAS Enterprise Guide 4.3 (SAS Institute Inc., Cary, NC, USA) and STATA version 12.0 (StataCorp LP, College Station, TX, USA) for data handling and statistical analysis. We plotted the predicted marginal effects of the five infection status groups of pigs (INFGP) for each of our five outcomes based on our regression models by keeping the other covariates at the sample mean values.

Ethics

This was a field study that involved pigs at a commercial pig testing station. No pigs were harmed during the collection of blood samples from the jugular vein or nasal swabs to ascertain their infection status.

Results

Statistical models

Based on AIC values and the parsimony principle, Table 1 shows the final multi-level models for the five outcomes. For outcomes ADG, FCE and ADFI, there were three levels (5,865 observations, 1955 pigs, and 43 herds) in the hierarchy. For the remaining two outcomes, Age100 kg and OFI, there were two levels (1955 observations and 43 herds) in the hierarchy. Only one interaction term of interest, between infection status and growth phase (INFGP*GF) was statistically significant indicating that the effect of the virus varied with the different growth phases. The second interaction of practical interest between infection status and breed (INFGP*Br), was found statistically insignificant during model selection indicating that that effects of the virus infection on Landrace and Duroc were similar or the power was insufficient to reject the null hypothesis.

ADG and FCE

Tables 2, 3 and 4 show that with SERONEG pigs as the reference, there were statistically significant marginal adverse effects on ADG and FCE in all infected groups (SEROPOS and VIRs 1–3). The marginal plots in Figures 2 and 3 show the predicted means of ADG and FCE while the covariates of BD, ADFI and Br were kept at the sample means. Only GF and INFGP were allowed to vary so that the effect of the virus infection on ADG and INFGP could be studied in the three strata of growth phases. The differences in the predicted FCE and ADG between the five groups of pigs in each of the three growth phases can then be attributed to infection status of the pig.

For SEROPOS pigs, the negative effect on growth performance was seen during GF3 where FCE was reduced by +0.029 kg feed/kg weight gain. Correspondingly, ADG also decreased by −0.015 kg/day.

For VIR1 pigs, adverse growth performance effects were not seen until GF2 even though these pigs were positive for virus during GF1. During GF2, FCE was reduced by +0.058 kg feed/kg weight gain, which led to a lower ADG by −0.033 kg/day. These negative effects extended into GF3 where FCE was reduced by +0.125 kg feed/kg weight gain and a corresponding lower ADG by −0.058 kg/day.

Removing twenty-two oldes pigs in VIR1 pigs to to leave 101 younger pigs (VIR1a) that were viral positive when they were 50 kg or less did not make any difference to the results we saw in Table 3 in that the delayed adverse effects were seen in GF2 and worsen in GF3 (Table 4).

For VIR2 and VIR3 pigs, the negative effects were confined to the same growth phase that they were positive for virus. For VIR2 pigs during GF2, FCE was reduced by +0.122 kg feed/kg weight and ADG was lower

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Table 1 Akaike information criterion (AIC) and the parsimony principle were used to select the best model for each outcome

Outcome	Infection status group (INFGP)	Breed (Br)	Growth Phase (GF)	Birthdate	Ave daily feed intake (ADFI)	Interaction term INFGP*GF	Interaction term INFGP*Br	Pig	Herd	AIC	ΔAIC
ADG	X	X	X	X	X	X	X	X	X	-7157.8	-1.7
	X	X	X	X	X	X	X		X	-7132.5	23.6
	X	X	X	X	X	X	X	X		-7147.8	8.3
→	X	X	X	X	X	X		X	X	**-7156.1**	0
	X	X	X	X	X		X	X	X	-7150	6.1
	X	X	X	X	X			X	X	-7149	7.1
FCE	X	X	X	X	X	X	X	X	X	-1889	3.7
	X	X	X	X	X	X	X		X	-1769	123.7
	X	X	X	X	X	X	X	X		-1878	14.7
→	X	X	X	X	X	X		X	X	**-1892.7**	0
	X	X	X	X	X		X	X	X	-1864	28.7
	X	X	X	X	X			X	X	-1867	25.7
ADFI	X	X	X	X	X	X	X	X	X	3299.8	2.8
	X	X	X	X	X	X	X		X	3564	267
	X	X	X	X	X	X	X	X		3307	10
→	X	X	X	X	X	X		X	X	**3297**	0
	X	X	X	X	X		X	X	X	3304	7
	X	X	X	X	X			X	X	3302	5
Age 100 kg	X	X		X	X		X		X	13111	5
	X	X		X	X		X			13205.5	99.5
→	X	X		X	X				X	**13106**	0
OFI	X	X		X	X		X		X	15281	3
	X	X		X	X		X			15362	84
→	X	X		X	X				X	**15278**	0

Abbreviations: ADG average daily growth in kg/day, FCE feed conversion efficiency in kg feed/kg weight gain, ADFI average daily feed intake in kg/day, Age 100 kg age of pig when they reached 100 kg, OFI overall feed intake in kg from 33 kg to 100 kg bodyweight.
Selected model is indicated by (→). Marking X indicates predictor was included in the model. ΔAIC = difference in AIC between the tested models and the selected model given in bold.

by -0.053 kg/day correspondingly. The FCE and ADG of VIR2 pigs returned to the same levels as seronegative pigs during GF3. For VIR3 pigs during GF3, the FCE was reduced by +0.091 kg feed/kg weight gain and ADG was lower by -0.045 kg/day correspondingly.

Average daily feed intake

Surprisingly we saw in Table 5 that there was no significant decrease in ADFI for the four infected groups (seropositive group and the three virus positive groups) since anorexia was listed as one of the clinical signs in pigs infected with SIVs. It was also interesting to observe that VIR1 pigs had increased feed intake during the post viral shedding period. Their average daily feed intake increased by 71 g/day and 0.104 kg/day during GF2 and GF3 respectively.

Age of pigs at 100 kg

Consequent to the reduced FCE and hence lower ADG, the virus positive pigs required longer time to reach the bodyweight of 100 kg (Figure 4 and Table 6). The VIR1, VIR2 and VIR3 pigs were slower by 1.6 days, 1.8 days and 2.4 days, respectively, in reaching 100 kg bodyweight. Although VIR2 was statistically insignificant (P value = 0.12), it was on similar trajectory as VIR1 and VIR3.

Overall feed intake

Consequent to the reduced FCE experienced by the four infected groups, Figure 5 and Table 7 show that all four infected groups needed additional feed to reach 100 kg bodyweight. The SEROPOS, VIR1, VIR2 and VIR3 pigs needed 2.3 kg, 8.0 kg, 5.9 kg and 7.2 kg additional feed, respectively.

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Table 2 Multilevel regression of average daily growth in pigs infected with influenza A(H1N1)pdm09 virus

Average Daily Growth (ADG)

Predictors	Coefficients	SE	P values	95% CI	
Breed					
Landrace	Reference				
Duroc	−0.028	0.006	<0.001	−0.039	−0.016
Growth phase					
GF1 (33_60 kg)	Reference				
GF2 (61_80 kg)	−0.062	0.0071	<0.001	−0.08	−0.05
GF3 (81_100 kg)	−0.134	0.0083	<0.001	−0.15	−0.12
Birthdate	0.00006	0.000006	<0.001	0.00005	0.00007
Average daily feed intake	0.31	0.0053	<0.001	0.30	0.32
Interaction term: INFGP*GF					
SERONEG*GF(1–3)	Reference				
SEROPOS*GF1	0.006	0.0064	0.37	−0.01	0.02
SEROPOS*GF2	−0.004	0.0064	0.54	−0.02	0.01
SEROPOS*GF3	−0.015	0.0064	0.02	−0.03	0.00
VIR1*GF1	0.008	0.0127	0.51	−0.02	0.03
VIR1*GF2	−0.033	0.0127	0.01	−0.06	−0.01
VIR1*GF3	−0.058	0.0127	<0.001	−0.08	−0.03
VIR2*GF1	0.002	0.0230	0.94	−0.04	0.05
VIR2*GF2	−0.053	0.0230	0.02	−0.10	−0.01
VIR2*GF3	−0.015	0.0230	0.51	−0.06	0.03
VIR3*GF1	0.014	0.0221	0.52	−0.03	0.06
VIR3*GF2	−0.020	0.0221	0.37	−0.06	0.02
VIR3*GF3	−0.045	0.0221	0.04	−0.09	0.00
Constant (β_0)	−0.746	0.1113	<0.001	−0.96	−0.53

Abbreviations: SERONEG Seronegative pigs; SEROPOS Seropositive pigs (not tested for virus), VIR1 pigs viral positive between bodyweight 33 kg and 60 kg, VIR2 pigs viral positive between bodyweight 61 kg and 80 kg, VIR3 pigs viral positive between bodyweight 81 kg and 100 kg.
The model is hierarchical with three levels; observations (n = 5865); pig (n = 1955) and herd (n = 43). *denotes interaction term.

Other predictors (covariates or control variables) in the models

The predictors birthdate, breed and growth phase were highly significant (P value<0.001) in our regression models for all five outcomes). For birthdate as a predictor, the outcomes of ADG, FCE, ADFI, Age100kg and OFI of pigs improved each year or every 365 days by 0.022 kg bodyweight/day, −0.055 kg feed/kg bodyweight, −0.007 kg feed/day, −2.2 days and −6.2 kg feed respectively.

On breed differences, Landrace pigs grew faster (ADG of +0.028 kg/day), had better efficiency in feed conversion (FCE was improved by −0.043 kg feed/kg bodyweight), reached the targeted weight seven days earlier, and required less feed (−5.9 kg feed) to reach 100 kg bodyweight.

Clinical observations between April and July 2011

During a clinical outbreak at the station from April 2011 to July 2011, a group 2045 pigs present at the station were monitored closely for clinical signs. These pigs entered the station at different time since the station received 72 new pigs on a weekly basis. Influenza-like illness was observed in 137 pigs, giving a crude morbidity of 7%. Clinical signs observed were transient anorexia or lethargy (45%), respiratory signs (coughing, laboured breathing or nasal discharge, 39%), and pyrexia (above 39°C, 27%). The prevalence of these clinical signs recorded within each of the 16 rooms ranged from 0% to 17%. For group specific morbidity, clinical signs were not detected in VIR1 pigs, while one (3%) out of 34 VIR2 pigs and five (14%) out of 37 VIR3 pigs had clinical signs reported. In the seropositive group, 39 (10%) out of 381 pigs had clinical signs of influenza-like illness during the testing period. No clinical signs were reported in the SERONEG pigs.

Discussion

The findings in our study support the alternate hypothesis that despite being a largely subclinical disease (<4%

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Table 3 Multilevel regression of feed conversion efficiency in pigs infected with influenza A(H1N1)pdm09 virus

Feed Conversion Efficiency (FCE)					
Predictors	Coefficients	SE	P value	95% CI	
Breed					
Landrace	Reference				
Duroc	0.0426	0.010	<0.001	0.0230	0.0622
Growth phase					
GF1(33_60 kg)	Reference				
GF2(61_80 kg)	0.2865	0.011	<0.001	0.26	0.31
GF3(81_100 kg)	0.5442	0.014	<0.001	0.52	0.57
Birthdate	-0.0002	0.00001	<0.001	-0.0002	-0.0001
Average daily feed intake	0.0238	0.010	0.019	0.004	0.044
Interaction term: INGP*GF					
SERONEG*GF(1-3)	Reference				
SEROPOS*GF1	-0.015	0.011	0.159	-0.037	0.006
SEROPOS*GF2	0.002	0.010	0.849	-0.018	0.022
SEROPOS*GF3	0.029	0.010	0.004	0.010	0.049
VIR1*GF1	-0.001	0.023	0.951	-0.046	0.043
VIR1*GF2	0.058	0.020	0.004	0.018	0.097
VIR1*GF3	0.125	0.020	<0.001	0.086	0.165
VIR2*GF1	-0.049	0.037	0.189	-0.121	0.024
VIR2*GF2	0.122	0.036	0.001	0.051	0.192
VIR2*GF3	0.033	0.036	0.361	-0.038	0.103
VIR3*GF1	-0.016	0.037	0.659	-0.089	0.056
VIR3*GF2	0.045	0.035	0.196	-0.023	0.112
VIR3*GF3	0.091	0.035	0.008	0.023	0.159
Constant (β_0)	4.6609	0.1934	<0.001	4.282	5.040

Abbreviations: SERONEG Seronegative pigs, SEROPOS Seropositive pigs (not tested for virus), VIR1 pigs viral positive between bodyweight 33 kg and 60 kg, VIR2 pigs viral positive between bodyweight 61 kg and 80 kg, VIR3 pigs viral positive between bodyweight 81 kg and 100 kg. The model is hierarchical with three levels; observations (n = 5865); pig (n = 1955) and herd (n = 43). *denotes interaction term.

morbidity in 194 viral positive pigs), influenza A(H1N1) pdm09 virus infection reduced the pigs' growth performance in terms of FCE and hence ADG. Consequently the infected pigs needed more time to reach 100 kg and additional feed was also consumed. The negative growth performance effects were most evident in the pigs that were infected at a young age, as shown by the VIR1 pigs. The negative effects of reduced FCE and ADG in VIR1 extended into growth phases two and three. That was beyond the viral shedding period of SIVs of about seven days [7]. We have no explanation why adverse effects in growth performance appeared only during the post viral period and lasted longer in these VIR1 pigs that were infected at bodyweight before 60 kg bodyweight. The duration of negative effects were shorter in the pigs infected

at a later age as represented by VIR2 pigs. Negative effects in these pigs were limited to GF2, the same growth phase that they were tested positive for the virus. Twenty two of the 123 VIR1 pigs were in the upper weight range of 50 kg and 60 kg. If these pigs were in the early viral shedding period of 7 days during testing, shedding of virus and presumably the manifestation of adverse effects on growth performance could cross over to the next growth phase (61-80 kg). Misclassication bias could then result in concluding the delayed and extended negative effects of the virus infection on FCE in VIR1 pigs (Table 3). However in our bias analysis (Table 4), the removal of these twenty-two heavier pigs, from VIR1 and leaving 101 younger pigs (VIR1a) that shed virus between 33 kg and 50 kg did not change the result. Just as in the original VIR1 group of 123 pigs, the adverse effects on growth performance of these 101 younger pigs (VIR1a) appeared during the post viral period of growth phase two (61 -80 kg) and deteriorated during growth phase three (81 -100 kg).

Interestingly, none of the infected groups had depressed average feed intake in any of the three growth phases as we expected because anorexia is listed as one of the clinical signs in pigs infected with the classical SIVs. Instead the pigs infected at a young age (VIR1) ate more during growth phases two and three, which were the post viral shedding period for VIR1 pigs. Even though they increased their feed intake, it was insufficient to compensate for the reduced FCE during growth phases 2 and 3 to raise their daily growth high enough to catch up with the seronegative pigs. Consequently the overall feed intake of these VIR1 pigs increased by 8 kg but were still slower by 1.6 days in getting to 100 kg bodyweight. Despite their lower FCE, the increased appetite of VIR1 pigs during the post viral shedding period of GF2 and GF3 was enough to allow them to reach the bodyweight of 100 kg earlier than VIR2 and VIR3 pigs which were infected at a later age.

Landrace and Duroc are the two major breeds in Norway. The proportions of both breeds were almost equal in our study. Even though there were intrinsic breed differences in growth profile with Landrace having a better feed conversion efficiency and higher daily feed intake and hence higher daily growth, our investigation found no differential adverse effects on growth performance caused by this virus infection on the two breeds.

Although the time period of infection for the 874 pigs in the SEROPOS group were unknown, these pigs could have been infected at any time during their growth phase before or after arriving at the boar station. Blood for cELISA tests were collected from these pigs when they were about 100 kg. With the exception of five pigs (67 days for the youngest) in the SEROPOS group, the remaining 869 pigs were older than 12 weeks when they were tested for antibodies. It was therefore unlikely that they harboured detectable maternal antibodies [24-26] at the time of testing.

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Table 4 Multilevel regression of feed conversion efficiency in pigs infected with influenza A(H1N1)pdm09 virus

Feed Conversion Efficiency (FCE)

Predictors	Coefficients	SE	P values	95% CI	
Breed					
Landrace	Reference				
Duroc	0.042	0.010	<0.001	0.023	0.062
Growth phase					
GF1(33_60 kg)	Reference				
GF2(61_80 kg)	0.287	0.011	<0.001	0.264	0.309
GF3(81_100 kg)	0.544	0.014	<0.001	0.517	0.571
Birthdate	-0.0002	0.00001	<0.001	0.000	0.000
Average daily feed intake	0.024	0.010	0.02	0.004	0.044
Interaction term: INFGP					
SERONEG*GF(1-3)	Reference				
SEROPOS*GF1	-0.015	0.011	0.1570	-0.037	0.006
SEROPOS*GF2	0.002	0.010	0.8560	-0.018	0.022
SEROPOS*GF3	0.029	0.010	0.0040	0.009	0.049
VIR1a*GF1	-0.008	0.025	0.7510	-0.057	0.041
VIR1a*GF2	0.050	0.022	0.0230	0.007	0.093
VIR1a*GF3	0.114	0.022	0.0000	0.071	0.157
VIR1b*GF1	0.026	0.049	0.5930	-0.070	0.122
VIR1b*GF2	0.092	0.044	0.0390	0.005	0.178
VIR1b*GF3	0.176	0.045	0.0000	0.087	0.265
VIR2*GF1	-0.049	0.037	0.1870	-0.122	0.024
VIR2*GF2	0.121	0.036	0.0010	0.051	0.192
VIR2*GF3	0.033	0.036	0.3640	-0.038	0.103
VIR3*GF1	-0.016	0.037	0.6560	-0.089	0.056
VIR3*GF2	0.045	0.035	0.1970	-0.023	0.112
VIR3*GF3	0.091	0.035	0.0090	0.023	0.159
Constant (β_0)	4.658	0.193	<0.001	4.279	5.037

Abbreviations: SERONEG Seronegative pigs, SEROPOS Seropositive pigs (not tested for virus), VIR1a pigs viral positive between bodyweight 33 kg and 50 kg, VIR1b pigs viral positive between bodyweight 51 kg and 60 kg, VIR2 pigs viral positive between bodyweight 61 kg and 80 kg, VIR3 pigs viral positive between bodyweight 81 kg and 100 kg. The model is hierarchical with three levels; observations (n = 5865); pig (n = 1955) and herd (n = 43). *denotes interaction term.

The observation that the SEROPOS pigs had similar results to the 37 virus positive pigs in the VIR3 group in that the adverse effect on FCE and ADG occurred during GF3 points to the possibility that the majority of seropositive pigs were infected at the older age like VIR3 pigs. Although these SEROPOS pigs reached 100 kg bodyweight at the same time as the SERONEG pigs, these SEROPOS pigs had reduced FCE during growth phase three which resulted in these pigs consuming an additional 2 kg feed to reach the bodyweight of 100 kg.

Bias analysis on misclassification

The cELISA test had a sensitivity and specificity of 93.7% and 99.1% (Manufacterers data sheets). Although these values are considered high for test performance, given the largely lack of clinical picture in this disease for corroboration, there could nevertheless be a small number of SERONEG and SEROPOS pigs that were misclassified and hence biased the adverse effects towards the null. Our quantitative bias analysis using Episens [23] for adverse effects on a dichotomized FCE showed that the odds ratio for a poorer FCE if the pig was SEROPOS versus SERONEG was 1.13. The odds ratio after adjusting for misclassification bias was 1.3, a change of 2 percent. The small bias of 2 percent in our study was towards the null (OR= 1).

The literature [7] states that SIVs in general, cause near if not 100% morbidity when they infect naïve pigs. An experimental study with influenza A(H1N1)pdm09 virus by Brookes et al. in 2010 [9] reported 100% morbidity involving 19 pigs in their experimental study, where 2 pigs were infected by contact. In contrast, we found in our field
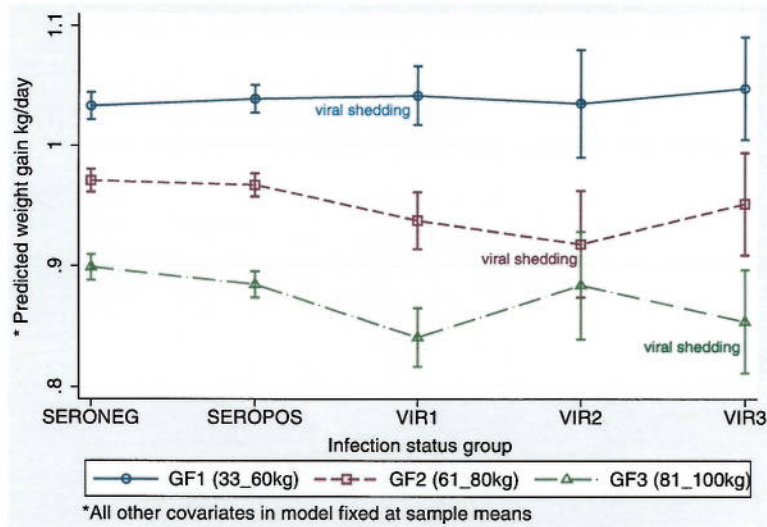
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Figure 2 Marginal plots for average daily growth (ADG) and adverse effects of influenza A(H1N1)pdm09 virus infection. Based on the model presented in Table 2, the mean ADG was predicted for each of the five infection status groups and the three growth phases (GF), while all other covariates of birthdate, feed intake and breed were fixed at the sample means. The effect of the virus infection is marked by comparing the four infected groups with the reference seronegative group on the same growth phase denoted by the line joining the groups. Comparisons between groups were made for each growth phase since ADG vary with age. As depicted in the graph, the younger pigs would hypothetically have a higher ADG because they have a better FCE (see Figure 2 and Table 3) if feed intake is fixed at the same level. The gradient of the lines joining the means of each group and the confidence intervals indicate whether there were differences between the groups. Abbreviations: INFGP = Infection status group; SEROPOS = seropositive pigs, SERONEG = seronegative pigs, VIR1 = PCR-positive pigs at bodyweight between 33 kg and 60 kg (GF1); VIR2 = PCR-positive pigs at bodyweight between 61 kg and 80 kg (GF2); VIR3 = PCR-positive pigs at bodyweight between 81 kg and 100 kg (GF3).
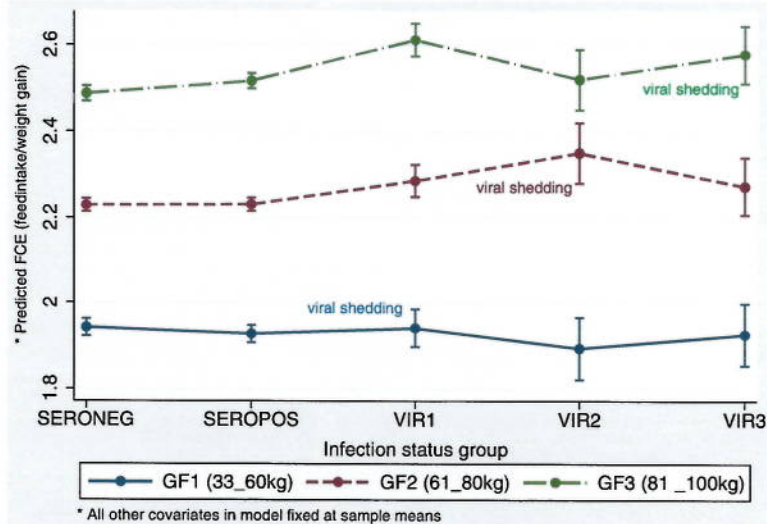

Figure 3 Marginal plots for feed conversion efficiency (FCE) and adverse effects of influenza A(H1N1)pdm09 virus infection. Based on the model presented in Table 3, the mean FCE was predicted for each of the five infection status groups and the three growth phases (GF) while all the other covariates of birthdate, feed intake and breed were fixed at the sample means. The effect of the virus infection is marked by comparing the four infected groups with the reference seronegative group on the same growth phase as denoted by the line joining the groups. Comparisons were made for each growth phase since feed efficiency decreases with age. The gradient of the lines joining the means of each group and the confidence intervals indicate whether there were differences between the groups. Abbreviations: INFGP = Infection status group; SEROPOS = seropositive pigs, SERONEG = seronegative pigs, VIR1 = PCR-positive pigs at bodyweight between 33 kg and 60 kg (GF1); VIR2 = PCR-positive pigs at bodyweight between 61 kg and 80 kg (GF2); VIR3 = PCR-positive pigs at bodyweight between 81 kg and 100 kg (GF3).

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Table 5 Multilevel regression of average daily feed intake in pigs infected with influenza A(H1N1)pdm09 virus

Average daily feed intake (ADFI)

Predictors	Coefficients	SE	P values	95% CI	
Breed					
Landrace	Reference				
Duroc	−0.10	0.0157	<0.001	−0.13	−0.07
Growth Phase					
GF1(33_60 kg)	Reference				
GF2(61_80 kg)	0.71	0.0136	<0.001	0.68	0.74
GF3(81_100 kg)	1.08	0.0136	<0.001	1.05	1.10
Birthdate	−0.00002	0.00002	0.257	−0.00005	0.00001
Interaction term: INFGP*GF					
SERONEG*GF(1-3)	Reference				
SEROPOS*GF1	−0.003	0.016	0.851	−0.034	0.028
SEROPOS*GF2	0.022	0.016	0.166	−0.009	0.053
SEROPOS*GF3	0.017	0.016	0.278	−0.014	0.049
VIR1*GF1	−0.024	0.032	0.444	−0.086	0.038
VIR1*GF2	0.071	0.032	0.025	0.009	0.133
VIR1*GF3	0.104	0.032	0.001	0.042	0.166
VIR2*GF1	0.077	0.057	0.176	−0.035	0.190
VIR2*GF2	0.032	0.057	0.574	−0.080	0.144
VIR2*GF3	0.070	0.057	0.223	−0.042	0.182
VIR3*GF1	0.059	0.055	0.283	−0.049	0.167
VIR3*GF2	0.077	0.055	0.164	−0.031	0.184
VIR3*GF3	−0.085	0.055	0.122	−0.193	0.023
Constant (β_0)	1.897	0.3107	<0.001	1.288	2.506

Abbreviations: SERONEG Seronegative pigs, SEROPOS Seropositive pigs (not tested for virus), VIR1 pigs viral positive between bodyweight 33 kg and 60 kg, VIR2 pigs viral positive between bodyweight 61 kg and 80 kg, VIR3 pigs viral positive between bodyweight 81 kg and 100 kg. The model is hierarchical with three levels; observations (n = 5865); pig (n = 1955) and herd (n = 43). *denotes interaction term.

study that influenza A(H1N1)pdm09 virus infection of Norwegian pigs was largely subclinical, with only six (<4%) in a sample of 194 virus positive pigs reported to have clinical signs. Apart from coughing, clinical signs, like nasal discharge, were so mild that only through closer observation or handling of the pig, for example nasal swabbing, could the signs be detected. The mild clinical signs and low morbidity recorded during the observation period were similar to the morbidity experienced by other Norwegian pig farms infected with influenza A(H1N1) pdm09 virus for the first time [15,16]. This shows that despite Norwegian pigs having no cross-protective immunity [27] to other strains of SIVs, the A(H1N1)pdm09 virus experienced in Norwegian pigs appeared to be of low pathogenicity that caused no or only mild clinical signs.

In recording the clinical signs at the pig testing station, possible bias of focusing on pigs with anorexia (shown on the computer records) may have led to underestimating the morbidity of the disease because we found no statistically significant decrease in appetite in the infected pigs in the stipulated three growth phases. A transient drop in appetite for one or two days would be masked by the three growth phases since the intervals in each growth phase were longer than two days and if there were compensatory increases in feed intake following one or two days of depressed feed intake.

Favourable conditions in our study

Pigs in our study did not have co-infections of other subtypes of influenza A viruses, M. hyopneumoniae, PRRSV, Aujesky's disease virus and porcine respiratory coronavirus, given Norway's disease free status for these pathogens [12]. Secondly, the daily recordings of feed intake and bodyweight for each pig were computerised without the presence of human interference. This ruled out human bias or error in making measurements to provide accurate calculations of the performance parameters. Thirdly, the repeated measures allowed the study of progressive effects of the virus in pigs infected at different ages and also the duration of the negative effects on growth performance.
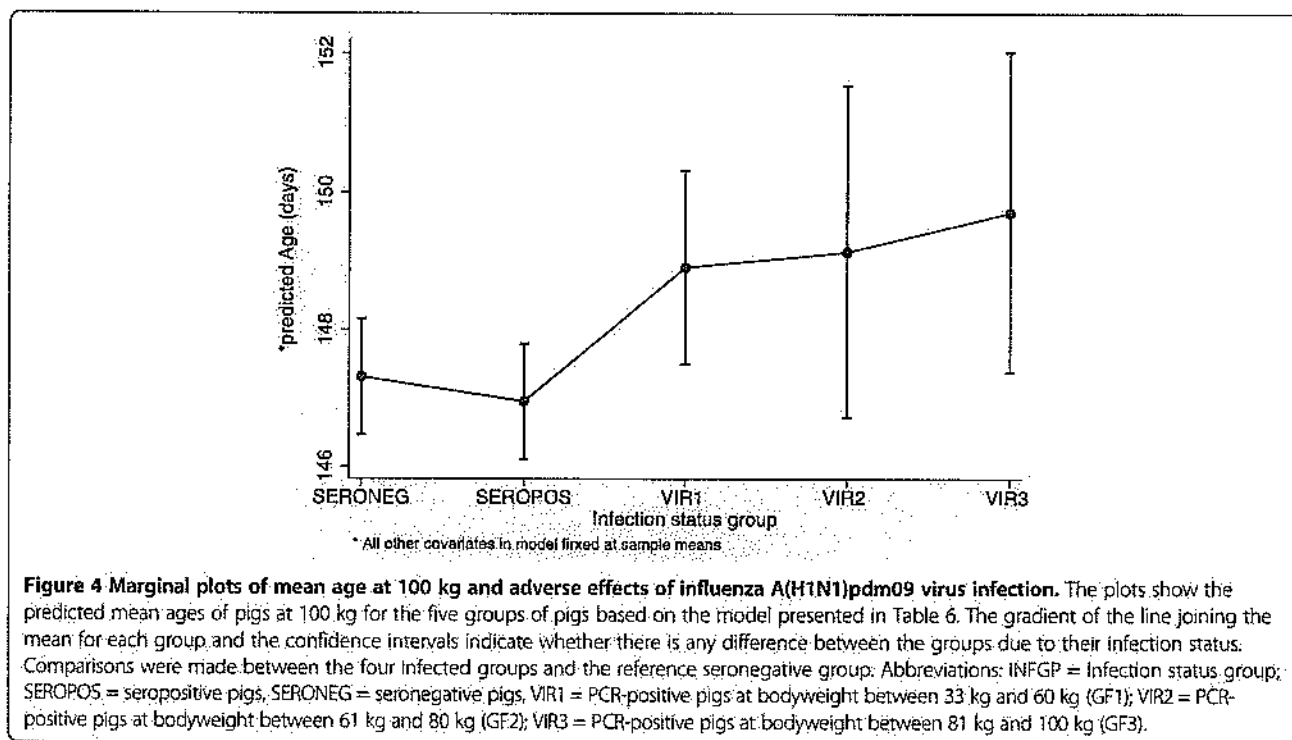
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Figure 4 Marginal plots of mean age at 100 kg and adverse effects of influenza A(H1N1)pdm09 virus infection. The plots show the predicted mean ages of pigs at 100 kg for the five groups of pigs based on the model presented in Table 6. The gradient of the line joining the mean for each group and the confidence intervals indicate whether there is any difference between the groups due to their infection status. Comparisons were made between the four infected groups and the reference seronegative group. Abbreviations: INFGP = infection status group; SEROPOS = seropositive pigs, SERONEG = seronegative pigs, VIR1 = PCR-positive pigs at bodyweight between 33 kg and 60 kg (GF1); VIR2 = PCR-positive pigs at bodyweight between 61 kg and 80 kg (GF2); VIR3 = PCR-positive pigs at bodyweight between 81 kg and 100 kg (GF3).

Validity of study design and statistical models

With 34 or more pigs in each of the five groups of pigs, the sample sizes are considered large for multi-level models [28]. The five statistical models were based on maximum likelihood in estimating the predictors that allow for inference to the Norwegian pig population.
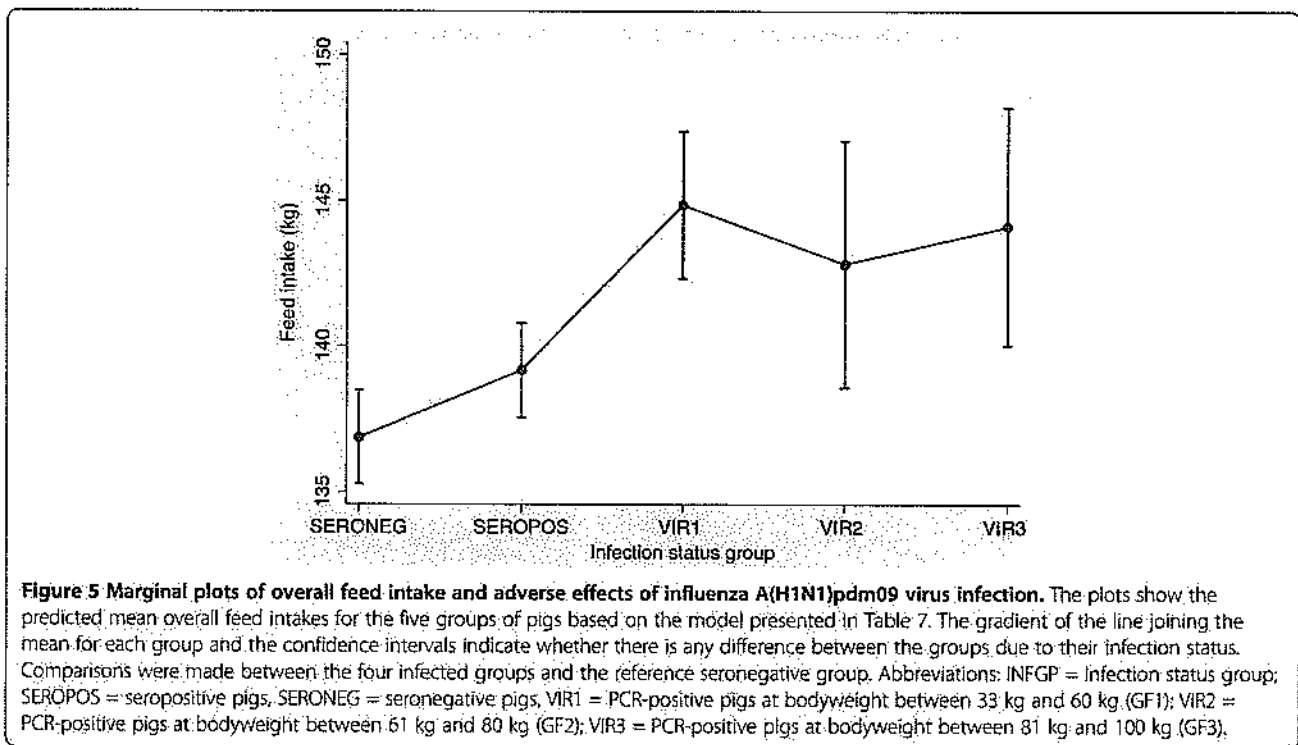
It was appropriate to use multi-level analysis because of the hierarchical nature of the data. The data, having 5865 observations nested in the 1955 pigs, which in turn were nested in the 43 herds, were handled to account for the variations between individual pigs and between the various herds including unmeasured confounders like other infections at the herd or individual level. Pigs from the study sample of 43 herds were represented in the reference group of 887 seronegative pigs. Consequently, the effects of virus infection (primary predictor of interest) and the known covariates (predictors we wanted to control) were more accurately estimated. Such

Table 6 Multilevel regression of pig's age at 100 kg if they are infected with influenza A(H1N1)pdm09 virus

Age of pig at 100 kg Predictors	Coefficients	SE	P values	95% CI	
Breed					
Landrace	Reference				
Duroc	7.3	0.83	<0.001	5.7	9.0
Birthdate	−0.006	0.0005	<0.001	−0.007	−0.005
Average daily feed intake	−28.5	0.94	<0.001	−30.3	−26.6
Infection status group					
SERONEG	Reference				
SEROPOS	−0.4	0.34	0.292	−1.0	0.3
VIR1	1.6	0.67	0.017	0.3	2.9
VIR2	1.8	1.20	0.129	−0.5	4.2
VIR3	2.4	1.15	0.038	0.1	4.6
Constant (β₀)	316.8	9.4	<0.001	298.3	335.3

Abbreviations: SERONEG Seronegative pigs, SEROPOS Seropositive pigs (not tested for virus), VIR1 pigs viral positive between bodyweight 33 kg and 60 kg; VIR2= pigs viral positive between bodyweight 61 kg and 80 kg, VIR3 pigs viral positive between bodyweight 81 kg and 100 kg. The model is hierarchical with two levels; pig (n = 1955) and herd (n = 43).

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Figure 5 Marginal plots of overall feed intake and adverse effects of influenza A(H1N1)pdm09 virus infection. The plots show the predicted mean overall feed intakes for the five groups of pigs based on the model presented in Table 7. The gradient of the line joining the mean for each group and the confidence intervals indicate whether there is any difference between the groups due to their infection status. Comparisons were made between the four infected groups and the reference seronegative group. Abbreviations: INFGP = Infection status group; SEROPOS = seropositive pigs, SERONEG = seronegative pigs, VIR1 = PCR-positive pigs at bodyweight between 33 kg and 60 kg (GF1); VIR2 = PCR-positive pigs at bodyweight between 61 kg and 80 kg (GF2); VIR3 = PCR-positive pigs at bodyweight between 81 kg and 100 kg (GF3).

hierarchical models solved some of the problems mentioned in a similar study by Straw et al. [18], in that this study was designed to control heterogeneities due to the environment, herd health status, host characteristics and management conditions inherent at the pig and herd level to reduce if not eliminate confounding [29,30]. Furthermore, keeping the pigs at one location in a uniform environment and husbandry eliminated these factors as

potential confounders in our model. Our models also proved to have a relatively high explanatory ability on the variance as the achieved adjusted R^2 were 51% (ADG), 59% (FCE), 51% (Age100 kg), 66% (ADFI) and 20% (OFI), which were proportions of variation that were explained by the predictors in the models. The longitudinal nature of the data for each pig allowed the statistical models to account for changes to FCE, ADFI and

Table 7 Multilevel regression for overall feed intake of pig infected with influenza A(H1N1)pdm09 virus

Overall feed intake of pig growing from 33 kg to 100 kg (OFI)					
Predictors	Coefficients	SE	P values	95% CI	
Breed					
Landrace	Reference				
Duroc	5.89	1.6	<0.001	2.8	9.0
Birthdate	−0.02	0.0009	<0.001	−0.020	−0.016
Average daily feed intake	11.74	1.6	<0.001	8.5	14.9
Infection group					
SERONEG	Reference				
SEROPOS	2.3	0.6	<0.001	1.1	3.5
VIR1	8.0	1.2	<0.001	5.7	10.3
VIR2	5.9	2.1	0.005	1.8	10.0
VIR3	7.2	2.0	<0.001	3.3	11.2
Constant (β_0)	445.0	16.5	<0.001	412.7	477.2

Abbreviations: SERONEG Seronegative pigs, SEROPOS Seropositive pigs (not tested for virus), VIR1 pigs viral positive between bodyweight 33 kg and 60 kg, VIR2 pigs viral positive between bodyweight 61 kg and 80 kg, VIR3 pigs viral positive between bodyweight 81 kg and 100 kg.
Growth phase was from 33 kg to 100 kg bodyweight. The model is hierarchical with two levels: pig (n = 1955) and herd (n = 43).

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hence ADG with respect to the stage in the pig's growth phase by including growth phase (GF) as a dummy variable in the statistical models thus controlling for confounding due to normal variation of feed conversion efficiency and daily feed intake with stage in growth phase.

Our samples of 1955 pigs included pigs tested at the station over four years from 2009 and 2012. We saw in our models that birthdate was a significant covariate because pigs born later had better growth performance as a result of improvement over time due to genetic selection, improved feed, and management improvement. Pigs belonging to the five infection status groups were disproportionately distributed over these four years since all 194 virus positive pigs were sampled in a single year (2011) while 560 pigs from seronegative and seropositive group were sampled in 2009 and 2010. Despite these disproportions, we were able to account for the marginal effects attributed to improvement over time by including birthdate as a covariate in our multi-level regression models. This allowed us to increase the study sample and hence the power of our study.

As normal occurrence and also seen in our study, the feed intake increases and FCE declines as a pig grows. Despite the reduced FCE in an older pig, its ADG is still higher because it consumes a higher amount of feed than the younger pig. Hypothetically, if older pigs ate the same amount of feed as the younger pigs, their ADG would be lower because of a reduced FCE as depicted by the coefficients of GF for outcome ADG and FCE in Tables 2, 3 and 4. This is also depicted in our marginal plots at Figures 2 and 3. This again underlines the importance of having growth phase as a dummy variable in our models to ensure comparisons of our outcomes between the 5 infection-status groups were valid because comparisons between groups were made in the same growth phase.

The coefficients of covariates breed, birthdate, growth phase and average daily feed intake in all five models were useful for the validation of our statistical models by comparing their values with other sources of pig performance data. An improbable coefficient would have raised a red flag on the models.

All five outcomes in our models were correlated. The calculation of feed conversion efficiency was based on average daily feed intake and average daily growth recordings. They in turn determined the remaining 2 outcomes on overall growth performance, which were age of pig at 100 kg bodyweight and overall feed intake. These latter 2 outcomes on overall growth performance are especially useful in evaluating economic consequences of the infection for farmers. Cost of extra feed and a delay in getting the pig to the market will lead to higher overheads (feed and veterinary costs) and lower income for the farmer since fewer pigs are sold in the fixed time period. We found that pigs infected when they were young (33 kg - 60 kg) required an additional 8 kg feed and were 1.6 days slower in reaching 100 kg bodyweight. Farmers can estimate the added operational costs if they know that their pigs were infected at a young or older age.

In other parts of the world, SIVs seldom act alone, but with concurrent infections to cause porcine respiratory disease complex where SIVs are the most common primary pathogens [1,2,7,31,32]. Hypothetically, the severity of influenza A(H1N1)pdm09 virus infection in terms of growth performance would be aggravated by concurrent infection of these other respiratory pathogens [7]. On the other hand, these pigs could also be protected from influenza A(H1N1)pdm09 virus infection because of presence of protective immunity against other strains of SIVs [27].

Conclusions

Our study shows that influenza A(H1N1)pdm09 virus infection in Norwegian pigs differs from the classical swine influenza experienced in other parts of the world because of the low morbidity and mild clinical signs. Although largely subclinical, the infection in Norwegian pigs did experience adverse effects on growth performance primarily because of reduced FCE. This is an important consideration for farmers because it directly influences the profitability of the pig production in terms higher overheads in terms of feed costs and additional time needed for infected pigs to reach market weight leading to lower income. The adverse effects were more severe and lasted longer in pigs infected at a younger age.

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References

1. Van Alstine WG: **Respiratory System**. In *Disease of Swine*. 10th edition. Edited by Zimmerman J, Karriker L, Ramirez A, Schwartz KJ. Wiley-Blackwell; 2012.

2. Loeffen WLA, Heinen PP, Bianchi ATJ, Hunneman WA, Verheijden JHM: **Effect of maternally derived antibodies on the clinical signs and immune response in pigs after primary and secondary infection with an influenza H1N1 virus**. *Vet Immunol Immunopathol* 2003, **92**(1–2):23–35.

3. Khatri M, Goyal SM, Saif YM: **Oct4+ stem/progenitor swine lung epithelial cells are targets for influenza virus replication**. *J Virol* 2012, **86**(12):6427–6433.

4. Crisci E, Mussá T, Fraile L, Montoya M: **Review: influenza virus in pigs**. *Mol Immunol* 2013, **55**(3–4):200–211.

5. Torremorell M, Allerson M, Corzo C, Diaz A, Gramer M: **Transmission of influenza a virus in pigs**. *Transbound Emerg Dis* 2012, **59**:68–84.

6. Garten RJ, Davis CT, Russell CA, Shu B, Lindstrom S, Balish A, Sessions WM, Xu XY, Skepner E, Deyde V, Okomo-Adhiambo M, Gubareva L, Barnes J, Smith CB, Emery SL, Hillman MJ, Rivailler P, Smagala J, de Graaf M, Burke DF, Fouchier RA, Pappas C, Alpuche-Aranda CM, López-Gatell H, Olivera H, López I, Myers CA, Faix D, Blair PJ, Yu C, Keene KM *et al.*: **Antigenic and genetic characteristics of swine-origin 2009 a(H1N1) influenza viruses circulating in humans**. *Science* 2009, **325**(5937):197–201.

7. Van Reeth K, Brown IH, Olsen CW: **Influenza Virus**. In *Diseases of Swine*. 10th edition. Edited by Zimmerman J, Karriker LA, Ramirez A, Schwartz K, Stevenson G. Wiley-Blackwell; 2012.

8. Brookes SM, Brown IH: **A/H1N1/pdm09 virus: dynamics of infection in pigs and people**. *Vet Rec* 2011, **169**(6):151–152.

9. Brookes SM, Núñez A, Choudhury B, Matrosovich M, Essen SC, Clifford D, Slomka MJ, Kuntz-Simon G, Garcon F, Nash B, Hanna A, Heegaard PMH, Quéguiner S, Chiapponi C, Bublot M, Garcia JM, Gardner R, Foni E, Loeffen W, Larsen L, Van Reeth K, Banks J, Irvine RM, Brown IH: **Replication, pathogenesis and transmission of pandemic (H1N1) 2009 virus in Non-immune pigs**. *PLoS One* 2010, **5**(2):e9068.

10. Lange E, Kalthoff D, Blohm U, Teifke JP, Breithaupt A, Maresch C, Starick E, Fereidouni S, Hoffmann B, Mettenleiter TC, Mettenleiter TC, Beer M, Vahlenkamp TW: **Pathogenesis and transmission of the novel swine-origin influenza virus A/H1N1 after experimental infection of pigs**. *J Gen Virol* 2009, **90**(Pt 9):2119–2123.

11. Thacker EL, Thacker BJ, Janke BH: **Interaction between Mycoplasma hyopneumoniae and swine influenza virus**. *J Clin Microbiol* 2001, **39**(7):2525–2530.

12. Lium B, Er C, Zerihun A: **The Surveillance and Control Programme for Specific Virus Infections in Swine Herd in Norway 2013**. In *Surveillance and Control Programmes for Terrestrial and Aquatic Animals in Norway Annual Report 2012*. Edited by Sviland S, Hellberg H. Oslo, Norway: Norwegian Veterinary Institute; 2014.

13. Grontvedt CA, Er C, Gjerset B, Hauge AG, Brun E, Jorgensen A, Lium B, Framstad T: **Influenza A(H1N1)pdm09 virus infection in Norwegian swine herds 2009/10: the risk of human to swine transmission**. *Prev Vet Med* 2013, **110**(3–4):429–434.

14. Hofshagen M, Gjerset B, Er C, Tarpai A, Brun E, Dannevig B, Bruheim T, Fostad IG, Iversen B, Hungnes O, Lium B: **Pandemic influenza A(H1N1)v: human to pig transmission in Norway?** *Euro Surveill* 2009, **14**(45):

15. Gjerset B, Er C, Lotvedt S, Jorgensen A, Hungnes O, Lium B, Germundsson A: **Experiences after twenty months with pandemic influenza a (H1N1) 2009 infection in the naive Norwegian Pig population**. *Influenza Res Treat* 2011, **2011**:206975.

16. Grontvedt CA, Er C, Gjerset B, Germundsson A, Framstad T, Brun E, Jorgensen A, Lium B: **Clinical impact of infection with pandemic influenza (H1N1) 2009 virus in naive nucleus and multiplier Pig herds in Norway**. *Influenza Res Treat* 2011, **2011**:163745.

17. Kitikoon P, Nilubol D, Erickson BJ, Janke BH, Hoover TC, Sornsen SA, Thacker EL: **The immune response and maternal antibody interference to a heterologous H1N1 swine influenza virus infection following vaccination**. *Vet Immunol Immunopathol* 2006, **112**(3–4):117–128.

18. Straw BE, Shin SJ, Yeager AE: **Effect of pneumonia on growth-rate and feed-efficiency of minimal disease pigs exposed to actinobacillus-pleuropneumoniae and mycoplasma-hyopneumoniae**. *Prev Vet Med* 1990, **9**(4):287–294.

19. Wetten M, Odegard J, Vangen O, Meuwissen TH: **Simultaneous estimation of daily weight and feed intake curves for growing pigs by random regression**. *Animal* 2012, **6**(3):433–439.

20. Schnyder U, Hofer A, Labroue F, Kunzi N: **Genetic parameters of a random regression model for daily feed intake of performance tested French landrace and large white growing pigs**. *Genet Sel Evol* 2001, **33**(6):635–658.

21. USCGf: **What Happens if you Omit the Main Effect in a Regression Model With an Interaction?** In 2012. http://www.ats.ucla.edu/stat/stata/faq/no_main_effect.htm.

22. Burnham KP, Anderson DR: *Model Selection and Multi-Model Inference: A Practical Information - Theoretic Apprach*. 2nd edition. USA: Springer-Verlag; 2002.

23. Orsini N, Bellocco R, Greenland S: *EPISENS: Stata Module for Basic Sensitivity Analysis of Epidemiological Results*. Statistical Software Components; 2006.

24. Wellenberg GJ, Bouwkamp FT, Wolf PJ, Swart WAJM, Mombarg MJ, de Gee ALW: **A study on the severity and relevance of porcine circovirus type 2 infections in Dutch fattening pigs with respiratory diseases**. *Vet Microbiol* 2010, **142**(3–4):217–224.

25. Wesley RD, Lager KM: **Overcoming maternal antibody interference by vaccination with human adenovirus 5 recombinant viruses expressing the hemagglutinin and the nucleoprotein of swine influenza virus**. *Vet Microbiol* 2006, **118**(1–2):67–75.

26. Markowska-Daniel I, Pomorska-Mól M, Pejsak Z: **The influence of age and maternal antibodies on the postvaccinal response against swine influenza viruses in pigs**. *Vet Immunol Immunopathol* 2011, **142**(1–2):81–86.

27. Busquets N, Segalés J, Córdoba L, Mussá T, Crisci E, Martín-Valls GE, Simon-Grifé M, Pérez-Simó M, Pérez-Maíllo M, Núñez JI, Abad FX, Fraile L, Pina S, Majó N, Bensaid A, Domingo M, Montoya M: **Experimental infection with H1N1 European swine influenza virus protects pigs from an infection with the 2009 pandemic H1N1 human influenza virus**. *Vet Res* 2010, **41**(5):74.

28. Snijders TAB, Bosker RJ: *Multilevel Analysis: An Introduction to Basic and Advanced Multilevel Modeling*. 2nd edition. London: Sage Publishers; 2012.

29. Dohoo IR, Martin W, Stryhn H: *Veterinary Epidemiologic Research*. 2nd edition. Charlottetown. Prince Edward Island. Canada: VER Inc; 2010.

30. Diggle P, Liang K, Zeger S: **Analysis of Longitudinal Data**. In Edited by Atkinson AC, Copas JB, Pierce DA, Schervish MJ, Titterington DM. Oxford: Clarendon Press; 1996:64–68.

31. Loving CL, Brockmeier SL, Vincent AL, Palmer MV, Sacco RE, Nicholson TL: **Influenza virus coinfection with Bordetella bronchiseptica enhances bacterial colonization and host responses exacerbating pulmonary lesions**. *Microb Pathog* 2010, **49**(5):237–245.

32. Done SH: **Environmental factors affecting the severity of pneumonia in pigs**. *Vet Rec* 1991, **128**(25):582–586.

Paper 4

Production impact of influenza A(H1N1)pdm09 virus infection on fattening pigs in Norway[1]

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ABSTRACT: Newly emerged influenza A(H1N1) pdm09 virus infection in Norwegian pigs, although often observed in a subclinical form, can lower the pig's growth performance by reducing feed efficiency in terms of a poorer feed conversion ratio. Infected pigs would consume more feed and require protracted production time to reach market weight. In our observational longitudinal study, growth performance data from 728 control pigs and 193 infected pigs with known viral shedding time points were analyzed using mixed linear regression models to give estimates of the marginal effects of infection. Gaussian curves describing the variability of the estimates at the individual pig level formed the fundamental inputs to our stochastic models. The models were constructed to simulate the summed negative effects of the infection at the batch level of 150 fattening pigs growing from 33 to 100 kg. Other inputs of variability and uncertainty were 1) batch transmission points, 2) pig infection points to reflect the disease transmission dynamics of the virus, and 3) final prevalence of infected pigs in the batch. Monte Carlo random sampling gave 5,000 estimates on the outputs of the marginal effects for each pig. These results were summed up to provide estimates for a batch size of 150 pigs. This figure was adjusted by our final prevalence distribution function, which was also derived from the longitudinal study with 12 cohorts of infected pigs. For a 150-fattening-pig herd randomly selected from the population, the marginal effects of the infection were 1) 835 kg (fifth percentile) to 1,350 kg (95th percentile) increased feed intake and 2) 194 (fifth percentile) to 334 (95th percentile) pig days in excess of expected figures for an uninfected batch. A batch infected during growth phase 3 (81 to 100 kg BW) gave the worst results since the longitudinal study showed that a pig infected during growth phase 3 required more feed and a greater protracted production time compared to younger infected pigs. Sensitivity analysis showed that final prevalence had the greatest impact on the conditional mean and variation of the marginal effects of infections. Batch transmission point was the next most influential factor. Lowering the final prevalence and preventing older fattening pigs from being infected will have the greatest benefit in saving feed cost and reducing delay in getting the pigs to the market.

Key words: feed conversion ratio, feed efficiency, influenza, mixed linear regression model, stochastic

INTRODUCTION

In April 2009, influenza A(H1N1)pdm09 virus (pH1N1v) emerged in North and South America as human infections (Centers for Disease Control and Prevention, 2009) before it became established in pig populations worldwide, including in Norway (Hofshagen et al., 2009; Torremorell et al., 2012; Van

Reeth et al., 2012). It was Norway's first influenza A virus infection in its pig population since the active national serosurveillance started in 1997 (Lium et al., 2014). A case control study (Grontvedt et al., 2013) revealed that the outbreak in pigs was likely caused by cross-species transmission from human to pigs. Although pH1N1v appeared highly contagious in naïve pigs like other swine influenza viruses (SIV; Brookes et al., 2010; Er et al., 2014), the virus appeared to be of low virulence since infection manifested itself mostly in the subclinical form in naïve Norwegian pig herds (Grontvedt et al., 2011; Lium et al., 2014). Despite the subclinical state, a longitudinal study at the Norwegian boar testing station (Er et al., 2014) showed that infected growing pigs had reduced feed efficiency because of a poorer feed conversion ratio. Consequently, infected pigs required additional feed and protracted production time compared to their uninfected counterparts. These findings, when considered at the batch level, are useful for economic analysis of a farm's profitability and for decision making with regard to disease control and biosecurity measures. However, to consider the sum total effects at the batch level, an understanding of factors that will vary the outcome is important for realistic estimations. It is therefore the aim of this paper to present a stochastic model for assessment of the production impact of pH1N1v infection in Norwegian pigs at the batch level. The stochastic model, based on a field observational study, will account for the variabilities and uncertainties that influence the individual impact of each pig for the summation at the batch level.

MATERIALS AND METHODS

The field study at the commercial boar testing station was purely observational. No pig was harmed during the process of taking blood samples from the jugular vein or taking nasal swabs.

Data

We conducted an observational longitudinal study on pigs that were performance tested at the Norwegian boar testing station between 2009 and 2012. The boar testing station (Wetten et al., 2012) had a capacity of testing 1,152 pigs in 16 separate rooms at the same time. Each room housed a cohort of 72 pigs grouped by breed (Landrace or Duroc) into 6 pens (14 m^2 in size) of 12 pigs each. Weekly, the station received 72 growing pigs (12 to 14 wk old with a mean BW of 33 kg) from 46 breeding herds in Norway to monitor their growth performances until they reached a BW of 100 kg. Electronic feeding stations in all pig pens used FIRE (Feed Intake Recording Equipment, Osbourne

Ltd., Newcastle, UK) to record daily feed intake and daily weight gain for each pig individually. Pigs fed 1 at a time ad libitum from 1 electronic feed dispenser in each pen on conventional concentrate containing 161 and 136 g digestible protein, 9.68 and 9.50 MJ net energy/kg, before and after 50-kg live weight, respectively, with 1 mo of mixing of the 2 feeds to facilitate the feed change. Blood samples for pH1N1v antibodies testing were routinely taken from pigs at the end of their stay at the boar testing station. Most of these pigs were at least 100 kg BW or more.

Study Sample

The study sample consisted of 921 pigs (53% Landrace, 47% Duroc) from 43 breeding herds that were performance tested at the testing station between 2009 and 2012. The control group of seronegative pigs ($n = 728$) included pigs tested by cELISA to be negative for antibodies against pH1N1v when they were at 100 kg or greater BW before leaving the station.

During an acute onset of clinical influenza pH1N1v infection at the station in April 2011, we investigated the pattern of disease occurrence in 12 cohorts of pigs housed in separate rooms at the station by using serology and PCR testing of nasal swabs taken from 375 pigs over a period of 4 mo. Real-time PCR (RT-PCR) identified 193 virus-positive pigs of varying ages (or BW). This group of viruspositive pigs ($n = 193$) was stratified into 3 subgroups according to their age (or BW) at moment of infection. Virus-positive group 1 (VIR 1, $n = 122$) included pigs that tested positive for pH1N1v by RT-PCR when they weighed between 33 and 60 kg (growth phase 1 [GF 1]). Virus-positive group 2 (VIR 2, $n = 34$) included pigs that tested positive for pH1N1v by RT-PCR when they weighed between 61 and 80 kg (growth phase 2 [GF 2]). Virus-positive group 3 (VIR 3, $n = 37$) included pigs that tested positive for pH1N1v by RT-PCR when they weighed between 81 and 100 kg (growth phase 3 [GF 3]).

Impact Measures for Reduced Feed Efficiency in Infected Pigs

Our impact measures for reduced feed efficiency due to the infection were the increased feed requirement and increased production time of an infected pig to grow from 33 to 100 kg BW compared to an uninfected pig. We used STATA version 14.0 (StataCorp LP, College Station, TX) to execute the mixed linear regression analysis on the hierarchical data ($N_{herds} = 43$, $N_{pigs} = 921$). The marginal effects attributed to the infection were represented by the coefficients of virus

infection status in the mixed-model regression analysis. Besides the infection status being the predictor of interest, variance component analysis identified breed, birthdate (**BD**), and ADFI for the regression model. Birthdate was an important covariate in the model because a pig born in 2012 performed better than a pig born in 2009 because of production improvement over time. Average feed intake was also an important covariate because it accounted for the effects of appetite on the outcomes.

Likelihood ratio tests and the Akaike information criterion (**AIC**) were used for model selection. We selected the model with the lowest AIC value and with the greatest likelihood in fitting the data. To determine the significance of additional predictors for the 2 models, a difference of ±2 of the AIC value was regarded as nonsignificant, and the most parsimonious model was chosen (Burnham and Anderson, 2002). Scatterplots, postmodel residual analysis, and graphical methods were used to check the continuous variables for linearity and for influential outliers. One very influential outlier was detected and removed from the final analyses. The continuous variables BD and ADFI were centered to focus on the average pigs in the study sample. Modifying effects by covariates on the predictor of interest (infection status group) were investigated by testing interactions between them in the regression analyses.

Mixed Random-Intercept Regression Models.
Our mixed linear regression was represented by the following equation:

$$Y_{[ijk]} = \beta_0 + \beta_1 X_{1[ij]} + \ldots$$

$$+ \beta_h X_{h[ij]} + u_{[j]} + \varepsilon_{[ij]}$$

where Y_{ijk} is the observation for the ith pig ($n_i = 921$) nested within the jth ($n_j = 43$) herd for the kth outcome ($n_k = 2$, where 1 = age of pig at 100 kg and 2 = overall feed intake of pig (33 to 100 kg BW), β is a vector of coefficients for predictors and their interactions, $X_{[ij]}$ is the vector of explanatory variables for the ith observation of the ith pig and jth herd, u_j is a vector of random intercepts unique to each herd, where $u_j \sim N$ (0, σ^2_{herd}), and ε_{ij} is the vector of error terms, where $\varepsilon_{ij} \sim N(\mu, \sigma^2)$.

Predictors. Apart from BD and ADFI, which are continuous predictors, the following are categorical predictors: 1) Infection status group includes seronegative, VIR 1, VIR 2, and VIR 3. Seronegative pigs are negative for antibodies against pH1N1v at 100-kg BW, VIR 1 pigs are positive for pH1N1v between 33 and 60 kg BW, VIR 2 pigs are positive for pH1N1v

between 61 and 80 kg BW, and VIR 3 pigs are positive for pH1N1v between BW 81 and 100 kg. 2) Breed includes Landrace and Duroc.

Stochastic Modeling

Variability and Uncertainty Inputs. To construct our stochastic models, we considered the variabilities and uncertainty of 1) uncertainty point of transmission to batch (batch transmission point) with respect to the growth phase, 2) age-dependent variability between pigs at moment of infection (pig infection point) revealed by the regression analysis, 3) transmission dynamics of a contagious pH1N1v with a short infective cycle (5 d) in a batch of immunologically naive pigs, and 4) the final animal prevalence in the batch when pigs have reached 100 kg in BW. Variabilities 3 and 4 were based on observations during the clinical outbreak at the testing station from April 2012 to July 2012.

Batch level stochastic summation. To give the batch level production effects caused by the infection, 150 infected pigs were sampled and summed up by a Monte Carlo sampling of 5,000 times per pig in relation to the variabilities and uncertainty mentioned. ModelRisk (version 5.3, Vosesoftware, Gent, Belgium) was used to perform the stochastic simulations.

Infection dynamics determine the patterns of infection within the batch. Given that the regression analyses (Tables 1 and 2) revealed that the marginal effects of the infection varied with the pig infection point (GF 1, GF 2, or GF 3), it is important to include variabilities related to the infectious disease dynamics in the stochastic model. Our observational data based on serology and PCR testing of nasal swabs taken from 375 pigs over a period of 4 mo revealed a steep spike in the epidemic curve near the beginning of the outbreak of pH1N1v infection, indicating a very contagious infection with a very short incubation period. This result was consistent with influenza A virus infections in a batch of immunologically naive pigs (Rose et al., 2013). One hundred and twenty-two pigs, or 63% of our 193 virus-positive pigs belonged to VIR 1. The remaining virus-positive pigs belonged to VIR 2 ($n = 34$, or 18%) and VIR 3 ($n = 37$, or 19%). Reflecting these 3 proportions of each of the pig infection points, ModelRisk fitted a discrete probability distribution for the pig's infection point to be ZTPoly(41563.8, 0.000027). This discrete probability distribution was input into our stochastic model to describe the infection dynamics of an infection such that when a virus enters a batch of susceptible pigs, the bulk (63%) of the pigs would infected within a short time (<d).

Table 1. Mixed linear regression of overall feed intake (kg) in pigs infected with influenza A(H1N1)pdm09 virus[1]

Overall feed intake of a pig growing from 33 to 100 kg				95% Confidence interval	
Predictors	Coefficients	SE	P-value		
Infection groups					
Seronegative	0	—	—	—	—
Virus positive (33–60 kg)	8.98	1.15	<0.001	6.72	11.23
Virus positive (61–80 kg)	7.63	2.02	<0.001	3.66	11.59
Virus positive (81–100 kg)	9.38	1.95	<0.001	5.57	13.19
Breed					
Landrace	0	—	—	—	—
Duroc	5.42	1.35	<0.001	2.77	8.06
ADFI (centered)	6.81	2.20	0.002	2.49	11.13
Birthdate (centered)	−0.02	0.00	<0.001	−00.023	−00.019
Constant β_0[2]	134.41	0.80	<0.001	132.85	135.97

[1]The coefficients and standard errors of the virus positive pigs were the parameters for Gaussian curves describing the variability between pigs on the marginal effects of infection at each of the three pig infection points: Virus positive during growth phase 1 (33 kg to 60 kg), growth phase 2 (61 kg to 80 kg) and growth phase 3 (81 kg to 100 kg).

[2]Constant represents the overall feed intake of a seronegative Landrace pig born on 3 October 2008 with an ADFI of 2.04 kg/d.

Table 2. Mixed linear regression for age (days) of a 100-kg pig infected with influenza A(H1N1)pdm09 virus[1]

Age of pig at 100 kg				95% Confidence interval	
Predictors	Coefficients	SE	P-value		
Infection groups					
Seronegative	0	—	—	—	—
Virus positive (33–60 kg)	1.65	0.70	0.02	0.27	3.02
Virus positive (61–80 kg)	1.89	1.23	0.13	−0.52	4.30
Virus positive (81–100 kg)	2.49	1.19	0.04	0.17	4.82
Breed					
Landrace	0	—	—	—	—
Duroc	6.15	0.98	<0.001	4.24	8.07
ADFI (centered)	−31.81	1.34	<0.001	−34.44	−29.18
Birthdate (centered)	−0.006	0.0006	<0.001	−0.008	−0.005
Constant β_0[2]	144.54	0.55	<0.001	143.47	145.61

[1]The coefficients and standard errors of virus positive pigs were the parameters for Gaussian curves describing the variability between pigs on the marginal effects of infection at each of the three pig infection points: Virus positive during growth phase 1 (33 kg to 60 kg), growth phase 2 (61 kg to 80 kg) and growth phase 3(81 kg to 100 kg).

[2]Constant represents the age (days) at 100 kg of a seronegative Landrace pig born on 3 October 2008 with an ADFI of 2.04 kg/d.

Comparisons between Batches with 3 Different Time Points of Virus Transmission

With the simulated batch level results from 5,000 iterations for each of the 150 pigs sampled, we used ModelRisk to plot cumulative probability plots to compare the batch level production impacts for the three possible batch transmission points at GFs1, 2 or 3). An additional plot was constructed for a batch infected during GF 3 with 100% animal prevalence to investigate the extent of overestimation if variability of animal prevalence was excluded.

Sensitivity Analysis to Rank Influence of the Variabilities

Tornado charts generated by ModelRisk were used to rank the variability inputs for the stochastic models in terms of their impact on the conditional means and variance of the outputs.

RESULTS

Batch Transmission Point

The time point at which the virus came into contact with a susceptible batch of pigs was treated as an uncertainty since we had no information on the prob-

abilities of batch transmission points on a national basis. We assumed all three batch transmission points of GFs 1, 2 and 3 had equal probabilities of taking place and therefore used the discrete probability distribution function ZTBinomial (3, 0.42) generated by ModelRisk to match our assumption.

Individual Pig Variability

The linear regression analysis in Tables 1 and 2 showed that besides infection status as the main predictor, covariates breed, BD, and daily feed intake were significant in predicting the outcomes of total feed intake and age of pig at 100 kg BW. The absence of significant interactions between the covariates and the main predictor suggested that the covariates did not modify the effects of the virus infection. For example, the negative effects of the infection were equal between the Landrace and Duroc given that interaction between breed and infection was absent. The coefficients and SE of the 3 infection groups of pigs (VIR 1, VIR 3, VIR 3) were the parameters for Gaussian distributions describing the variability between individual pigs.
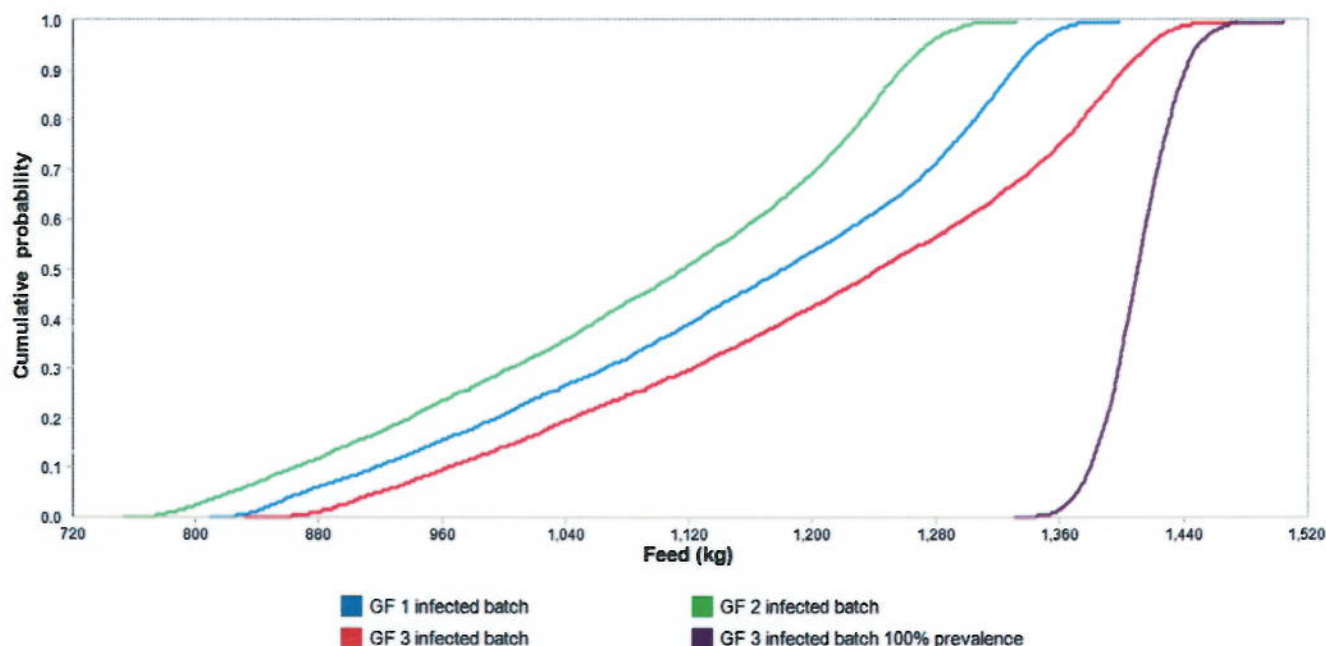
Figure 1. Cumulative probability plots of the additional feed requirement (kg) for a batch ($n = 150$) of fattening pigs infected with influenza A(H1N1) pdm09 virus growing from 33 to 100 kg. Based on the stochastic model, Monte Carlo of 5,000 sampling for each pig summed up to 150 pigs for batch level effects were executed for each of the 3 batch transmission points (GF 1, 2, and 3). In addition, variabilities in the stochastic model were 1) individual pig variability, 2) infection dynamics of a short cycled contagious pathogen, and 3) final animal prevalence. To show the extent of overestimation of the effects if variability of final animal prevalence was ignored, the right most s-curve (purple s-curve) represents a cumulative probability plot of batches infected during GF 3 with 100% animal prevalence.

Increased Feed Requirement: Batch Level Stochastic Simulations

Cumulative probability plots based on the stochastic model in Fig. 1 show that an infected batch of 150 pigs required additional feed ranging from 770 to 1,470 kg. A batch infected during GF 3 required the greatest amount of additional feed, whereas a batch infected during GF 2 required the least. The plots corresponded with the regression models in Tables 1 and 2. A majority of pigs infected during GF 2 would give the lowest marginal effect for the infection. A batch infected during GF 3 would cause only VIR 3 pigs to be infected, which would give the worst marginal effects of the infection compared with having younger infected pigs. Assuming a 100% prevalence of infection in the batch of pigs could overestimate the increase in feed requirement by as much as half a ton of feed in the case of batches infected during GF 3.

Increased Production Time: Batch Level

Infection Dynamics of Virus in a Cohort of Naïve Pigs. Cumulative probability plots in Fig. 2 show that infection by pH1N1v in a batch of 150 fattening pigs can prolong production time by as much as 430 d in a batch of pigs with transmission point at GF 3. This was no surprise since a VIR 3 pig performed worst in prolonging production time by 2.5 d (Table 2). Assuming a 100% prevalence of infection in the batch

of 150 pigs could overestimate the protracted production time by as much as 120 d in the case of batches infected during GF 3.

Final Prevalence. The longitudinal study at the boar testing station revealed a highly contagious infection with the final animal prevalence of 12 cohorts of pigs housed in close proximity in 12 separate rooms ranged from 62% to 100% (Table 3). ModelRisk fit-

Table 3. Final prevalence based on infectious status of 12 cohorts of pigs housed in 12 separate rooms when the pigs reached a BW of 100 kg[1]

Room	Seronegative at 100 kg	Virus positive	Seropositive at 100 kg	Infected	Total	Final prevalence
1	2	21	24	45	47	96%
2	4	11	12	23	27	85%
3	1	21	8	29	30	97%
4	1	11	15	26	27	96%
5	2	28	50	78	80	98%
6	6	25	45	70	76	92%
7	2	18	48	66	68	97%
8	25	10	31	41	66	62%
9	20	12	27	39	59	66%
10	10	15	33	48	58	83%
11	0	14	7	21	21	100%
12	9	6	14	20	29	69%

[1]By using ModelRisk (version 5.3, Vosesoftware, Gent, Belgium), a probability distribution of Beta4 (0.96,0.56,0.62,1) was fitted to the twelve final animal prevalence figures for the 12 cohorts of pigs.
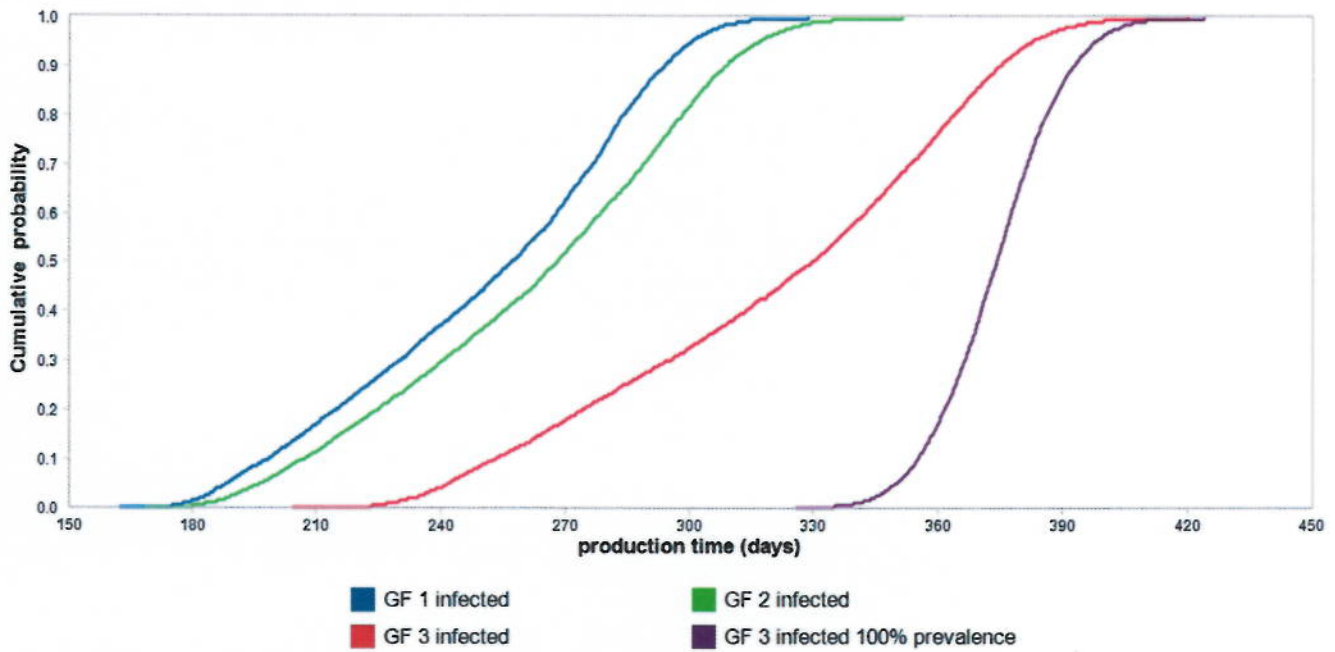
Figure 2. Cumulative probability plots of the increased production time (days) for a batch ($n = 150$) of fattening pigs infected with influenza A(H1N1) pdm09 virus growing from 33 to 100 kg. Based on the stochastic model, Monte Carlo of 5,000 samplings for each pig summed up to 150 pigs for batch level effects were executed for each of the 3 batch transmission points (GF 1, 2, and 3). In addition, variabilities in the stochastic model were 1) individual pig variability, 2) infection dynamics of a short cycled contagious pathogen, and 3) final animal prevalence. To show the extent of overestimation of the effects if variability of final animal prevalence was ignored, the right most s-curve (purple s-curve) represents a cumulative probability plot of batches infected during GF 3 with 100% animal prevalence.
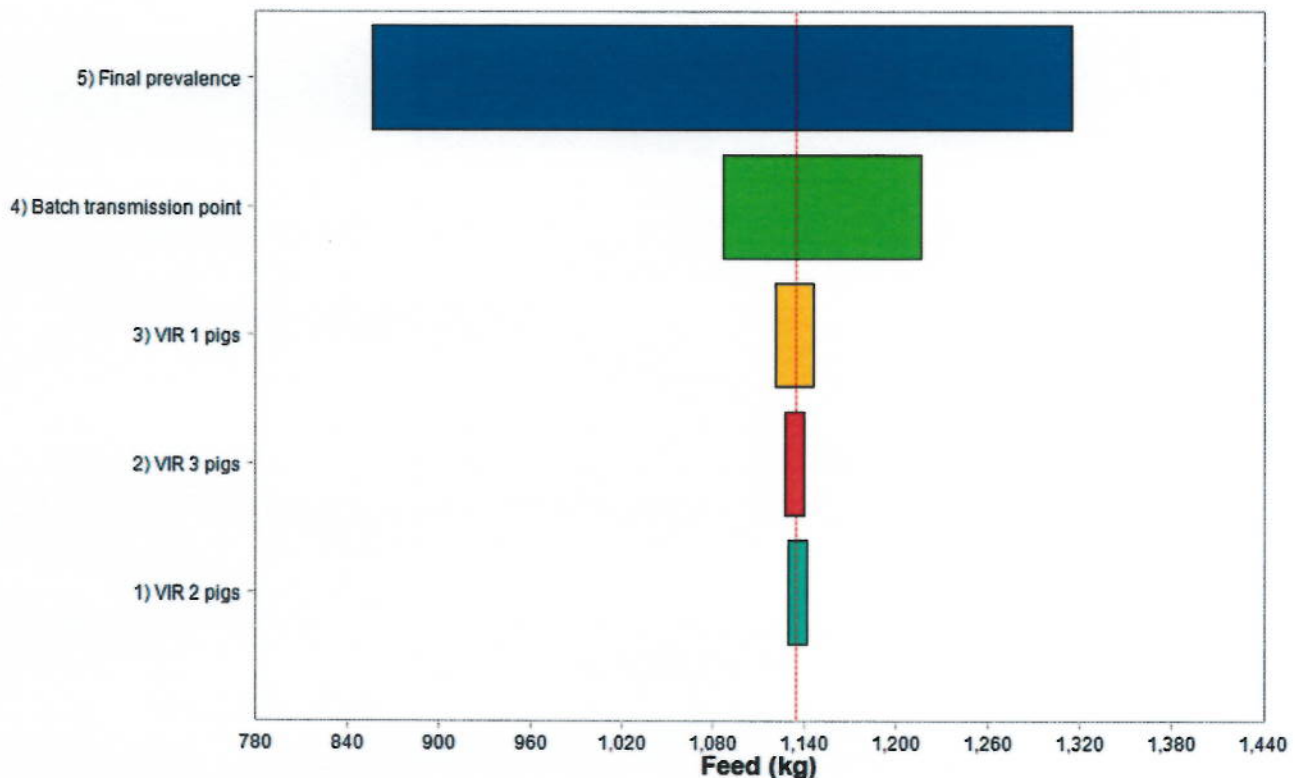

Figure 3. Sensitivity analysis ranking the variability and uncertainty contribution to the conditional means of additional feed intake in infected batches of 150 fattening pigs growing from 33 to 100 kg. For final prevalence in infected batch, the prevalence of infection when a batch of pigs has reached 100 kg BW is a variability input in the stochastic model and is described by a discrete probability distribution of Beta(0.96, 0.56, 0.62, 1) derived from data in Table 1. For batch infection point, 3 batch infection points (GF 1: 33 to 60 kg; GF 2: 61 to 80 kg; and GF 3: 81 to 100 kg) were assumed to have equal probability and were described by the discrete probability function ZTBinomial(3, 0.42). VIR 1 pigs are pigs infected when BW were between 33 and 60 kg, VIR 2 pigs are pigs infected when BW were between 61 and 80 kg, and VIR 3 pigs are pigs infected when BW were between 81 and 100 kg.
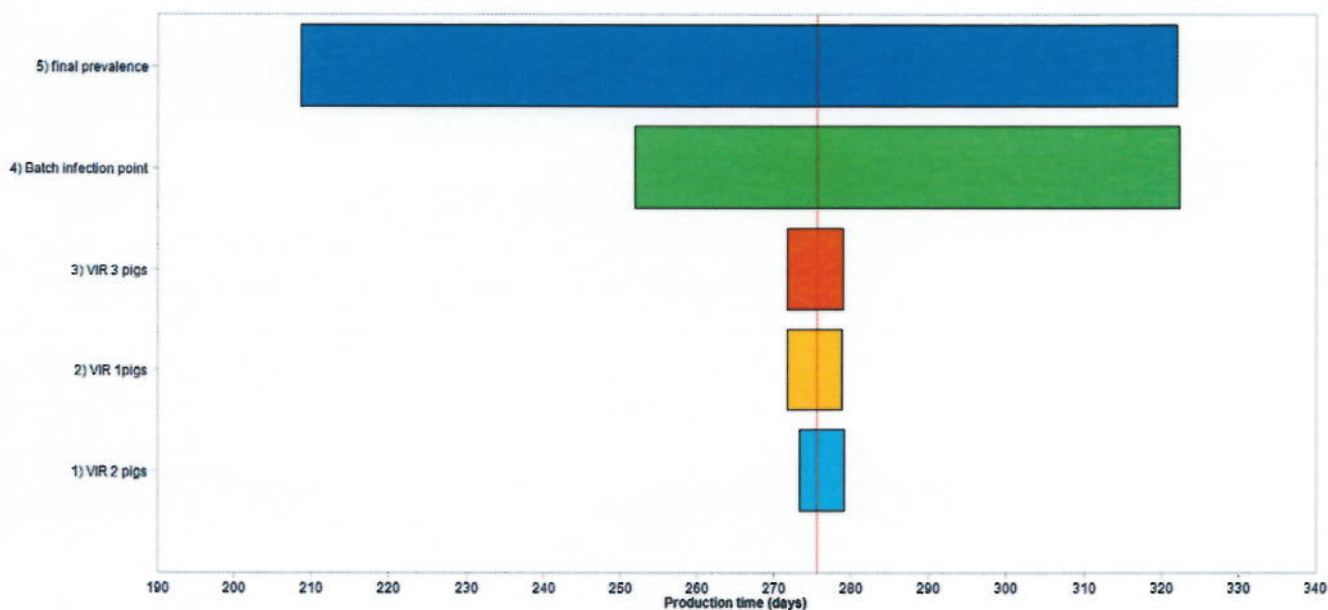
Figure 4. Sensitivity analysis ranking of the variability and uncertainty contribution to the conditional mean of the increase in production time in an infected batch of 150 fattening pigs growing from 33 to 100 kg. For final prevalence in infected batch, the prevalence of infection when batch of pigs has reached 100 kg BW is a variability input in the stochastic model. It is described by a discrete probability distribution of Beta(0.96, 0.56, 0.62, 1) derived from data in Table 1. For batch infection point, 3 batch infection points (GF 1: 33 to 60 kg; GF 2: 61 to 80 kg; and GF 3: 81 to 100 kg) were assumed to have equal probability and were described by the discrete probability function ZTBinomial(3, 0.42). VIR 1 pigs are pigs infected when BW were between 33 and 60 kg, VIR 2 pigs are pigs infected when BW were between 61 and 80 kg, and VIR 3 pigs are pigs infected when BW were between 81 and 100 kg.

ted the bounded continuous probability distribution to these 12 values with Beta4(0.96, 0.56, 0.62, 1) to described the likelihood of the final animal prevalence in an infected batch of pigs.

Sensitivity Analysis of Infected Batches Randomly Sampled from the Population

The Tornado charts in Fig. 3 and 4 show that the final prevalence contributed most to the conditional mean and variance. Batch transmission point with a lower magnitude was the other important uncertainty input that had a considerable impact on the conditional mean and variance.

DISCUSSION

Growth performance indicators such as feed conversion ratio and feed intake are important parameters when studying the economic effects of endemic disease in finishing pig production (Losinger, 1998; Jensen et al., 2008). Keeping pigs longer than necessary also requires additional costs for fuel, electricity, labor, etc., and can affect the number of fattening rounds in a year. Although many papers have stated that respiratory disease infection in pigs can reduce feed efficiency and lead to lower growth rates and hence delays in the pigs getting to market weight (Kothalawala et al., 2006; Van Alstine, 2012; Van Reeth et al., 2012), the negative impacts on growth performance have not

been properly quantified and published. Conversely, by using stochastic models in our study, we have quantitatively estimated the impact of the newly emerged influenza A(H1N1)pdm09 virus infection on growth performance in pigs in terms of increased feed and protracted production time. The models accounted for the heterogeneities between pigs and between batches to predict the likely variation between herds in Norway. The production impact of a subclinical disease like influenza A(H1N1)pdm09 virus for a batch of 150 naïve Norwegian pigs growing from 33 to 100 kg can be as much as 1.5 t of extra feed and 420 pig days of longer production time if most of the pigs are infected as older pigs during GF 3. This would occur if the batch transmission point was at GF 3, which explains why sensitivity analysis in Figs. 3 and 4 identified batch transmission point as an influential uncertainty. Overall, the upshot of protracted production time of infected pigs means that the number of possible cycles of fattening pigs would be reduced if farmers choose to keep their pigs until they reach the desired market weight. However, in all-in–all-out operations like fattening pig herds, farmers do not have the option to keep pigs longer than the designated market day and must sell their pigs at lighter BW than desired to clear the farm for the next batch.

Our stochastic models were built to reflect the influenza A(H1N1)pdm09 virus as a short-cycle pathogen with a short incubation period. This is consistent with other studies that have showed that the influenza

A(H1N1)pdm09 virus is transmitted efficiently between pigs, including subclinical pigs, by aerosol or direct contact with secretions of infected individuals or contaminated fomites (Brookes and Brown, 2011). It is highly possible that all pigs within a batch could eventually be infected in the worst-case scenario. This is also true for other SIV (H1N1, H1N2, and H3N2) currently circulating in most pig-producing countries, where all susceptible pigs in a herd are likely able to become infected and transmit the virus (Tellier, 2006, 2009; Torremorell et al., 2012). The sensitivity analysis of our stochastic models showed that reducing the final prevalence and keeping the proportion of pigs infected during GF 3 the smallest (or avoiding batch transmission point at GF 3) would have the greatest impact in reducing the negative effects of the infection.

The final prevalence and infection dynamics probability distributions in our model were dependent on and reflected the indoor environmental conditions such as temperature, humidity, air quality, and stocking density at the boar testing station. Although such environmental conditions may not vary much for fattening herds in Norway given that most, if not all, pigs are kept indoors under the Nordic conditions, they could be different in other pig production countries. Hence, it would be interesting for further studies to correlate the effect of housing and husbandry conditions on the final prevalence of this disease within a batch of pigs. Such studies could elucidate nonvaccine types of interventions such as altering the pig's environment with the aim of reducing the final prevalence of the infection or helping older pigs avoid infection. The ultimate goal is clearly to reduce the negative impact of the virus infection on production without resorting to vaccination.

We have chosen a batch size of 150 pigs to reflect the average batch size of fattening pig herds in Norway, which is small by international standards. However, extrapolation to other batch sizes is possible because the production impact is directly proportional to batch size assuming identical epidemiological patterns and similar production conditions in extrapolated herds.

Although influenza A viruses are ubiquitous in animals and endemic in most pig populations worldwide (Brown, 2000, 2013; Kothalawala et al., 2006; Van Reeth et al., 2012; Valls and Luque, 2015), a production impact such as the one we have presented from less virulent diseases like influenza A(H1N1)pdm09 virus infection in pigs could easily be overlooked, especially in countries where swine influenzas are classed under passive surveillance systems (Bowman et al., 2012). Norway is a rare exception in that it has ongoing active national serological surveillance for influenza A virus infection in its population of unvaccinated pigs. In the

last 5 yr from 2010 to 2014, Norway's herd prevalence for influenza A(H1N1)pdm09 virus infection has stabilized in the range of 41% to 50%, thus indicating that the infection has established itself in the pig population. Even though infection would confer long-term active immunity to a pig that recovered from infection, the quick turnover of fattening pigs whose lifespan is less than 7 mo ensures that large populations of immunologically naïve pigs are constantly produced, making the continuous propagation of influenza infection possible (Van Reeth et al., 2012). In addition, any maternal antibodies a fattening pig may have would decrease by the time the pig reaches 12 to 14 wk, or about 33 kg in BW (Loeffen et al., 2003), making them susceptible to infection. Hence, fattening pig herds in Norway constantly present themselves as susceptible for influenza virus infection and reduced growth performance from lower feed efficiency.

The Norwegian model may be too simplistic to estimate the impact of swine influenza on growth performance in pigs for other countries because it reflects Norway's production system and unique pig disease profile (Lium et al., 2014). The situation in other countries is different because they have various strains of SIV, including the influenza A(H1N1)pdm09 virus, that exist as coinfections (Maes et al., 2000; Song et al., 2010; Simon et al., 2014) and respiratory pathogens, such as the porcine reproductive and respiratory syndrome virus, the porcine respiratory coronavirus, and *Mycoplasma hyopneumoniae* (Crisci et al., 2013), which would undoubtedly complicate the picture.

In any case, given that the influenza A(H1N1) pdm09 virus is established in the Norwegian pig population, a Norwegian farmer or the national food safety authority may find our production impact model useful in estimating the burden of the infection at the farm and at the national level for economic analyses. Economic analyses to estimate producer surplus can help the farmer in making decisions on whether to implement biosecurity measures with regard to keeping a herd from getting infected during the next production cycle or keeping the prevalence in the batch as low as possible if infection occurs, assuming an all-in and all-out production system. It would make sense to implement effective biosecurity measures if the costs of these measures do not exceed the cost of the negative effects of the disease, thus giving a motivating producer surplus.

LITERATURE CITED

Bowman, A. S., J. M. Nolting, S. W. Nelson, and R. D. Slemons. 2012. Subclinical influenza virus A infections in pigs exhibited at agricultural fairs, Ohio, USA, 2009–2011. Emerg. Infect. Dis. 18:1945–1950. doi:10.3201/eid1812.121116

Brookes, S. M., and I. H. Brown. 2011. A/H1N1/pdm09 virus: Dynamics of infection in pigs and people. Vet. Rec. 169(6):151–152. doi:10.1136/vr.d4945

Brookes, S. M., A. Núñez, B. Choudhury, M. Matrosovich, S. C. Essen, D. Clifford, M. J. Slomka, G. Kuntz-Simon, F. Garcon, B. Nash, A. Hanna, P. M. H. Heegaard, S. Quéguiner, C. Chiapponi, M. Bublot, J. Maldonado Garcia, R. Gardner, E. Foni, W. Loeffen, L. Larsen, K. Van Reeth, J. Banks, R. M. Irvine, and I. H. Brown. 2010. Replication, pathogenesis and transmission of pandemic (H1N1) 2009 virus in non-immune pigs. PLoS ONE 5(2):e9068. doi:10.1371/journal.pone.0009068

Brown, I. H. 2000. The epidemiology and evolution of influenza viruses in pigs. Vet. Microbiol. 74 (1–2):29–46.

Brown, I. H. 2013. History and epidemiology of swine influenza in Europe. Swine Influenza 370:133–146. doi:10.1007/82_2011_194

Burnham, K. P., and D. R. Anderson. 2002. Model selection and multi-model inference: A Practical information-theoretic approach. 2nd ed. Springer, New York.

Centers for Disease Control and Prevention. 2009. Outbreak of swine-origin influenza A (H1N1) virus infection—Mexico, March–April 2009. JAMA 301(22):2321–2322.

Crisci, E., T. Mussá, L. Fraile, and M. Montoya. 2013. Review: Influenza virus in pigs. Mol. Immunol. 55(3–4):200–211. doi:10.1016/j.molimm.2013.02.008

Er, C., B. Lium, S. Tavornpanich, P. Hofmo, H. Forberg, A. Hauge, C. Grøntvedt, T. Framstad, and E. Brun. 2014. Adverse effects of Influenza A(H1N1)pdm09 virus infection on growth performance of Norwegian pigs—A longitudinal study at a boar testing station. BMC Vet. Res. 10:284. doi:10.1186/s12917-014-0284-6

Grøntvedt, C. A., C. Er, B. Gjerset, A. Germundsson, T. Framstad, E. Brun, A. Jorgensen, and B. Lium. 2011. Clinical impact of infection with pandemic influenza (H1N1) 2009 virus in naive nucleus and multiplier pig herds in Norway. Influenza Res. Treat. 2011:163745. doi:10.1155/2011/163745

Grøntvedt, C. A., C. Er, B. Gjerset, A. G. Hauge, E. Brun, A. Jorgensen, B. Lium, and T. Framstad. 2013. Influenza A(H1N1) pdm09 virus infection in Norwegian swine herds 2009/10: The risk of human to swine transmission. Prev. Vet. Med. 110(3–4):429–434. doi:10.1016/j.prevetmed.2013.02.016

Hofshagen, M., B. Gjerset, C. Er, A. Tarpai, E. Brun, B. Dannevig, T. Bruheim, I. G. Fostad, B. Iversen, O. Hungnes, and B. Lium. 2009. Pandemic influenza A(H1N1)v: Human to pig transmission in Norway? Euro Surveill. 14(45).

Jensen, T. B., N. P. Baadsgaard, H. Houe, N. Toft, and S. Ostergaard. 2008. The association between disease and profitability in individual finishing boars at a test station. Livest. Sci. 117:101–108. doi:10.1016/j.livsci.2007.12.003

Kothalawala, H., M. J. M. Toussaint, and E. Gruys. 2006. An overview of swine influenza. Vet. Q. 28:45–53.

Lium, B., C. Er, and A. Zerihun. 2014. The surveillance and control programme for specific virus infections in swine herd in Norway 2013. In: S. Sviland and H. Hellberg, editors, Surveillance and control programmes for terrestrial and aquatic animals in Norway. Annual Report 2013. Norwegian Vet. Inst., Oslo.

Loeffen, W. L. A., P. P. Heinen, A. T. J. Bianchi, W. A. Hunneman, and J. H. M. Verheijden. 2003. Effect of maternally derived antibodies on the clinical signs and immune response in pigs after primary and secondary infection with an influenza H1N1 virus. Vet. Immunol. Immunopathol. 92:23–35. doi:10.1016/S0165-2427(03)00019-9

Losinger, W. C. 1998. Feed-conversion ratio of finisher pigs in the USA. Prev. Vet. Med. 36:287–305. doi:10.1016/S0167-5877(98)00094-4

Maes, D., H. Deluyker, M. Verdonck, F. Castryck, C. Miry, B. Vrijens, and A. de Kruif. 2000. Herd factors associated with the seroprevalences of four major respiratory pathogens in slaughter pigs from farrow-to-finish pig herds. Vet. Res. 31:313–327.

Rose, N., S. Herve, E. Eveno, N. Barbier, F. Eono, V. Dorenlor, M. Andraud, C. Camsusou, F. Madec, and G. Simon. 2013. Dynamics of influenza A virus infections in permanently infected pig farms: Evidence of recurrent infections, circulation of several swine influenza viruses and reassortment events. Vet. Res. 44:72. doi:10.1186/1297-9716-44-72

Simon, G., L. E. Larsen, R. Durrwald, E. Foni, T. Harder, K. Van Reeth, I. Markowska-Daniel, S. M. Reid, A. Dan, J. Maldonado, A. Huovilainen, C. Billinis, I. Davidson, M. Aguero, T. Vila, S. Herve, S. O. Breum, C. Chiapponi, K. Urbaniak, C. S. Kyriakis, Esnip Consortium, I. H. Brown, and W. Loeffen. 2014. European surveillance network for influenza in pigs: Surveillance programs, diagnostic tools and swine influenza virus subtypes identified in 14 European countries from 2010 to 2013. PLoS One 9(12):e115815. doi:10.1371/journal.pone.0115815

Song, X. H., H. Xiao, Y. Huang, G. Fu, B. Jiang, Y. Kitamura, W. Liu, D. Liu, and G. F. Gao. 2010. Serological surveillance of influenza A virus infection in swine populations in Fujian Province, China: No evidence of naturally occurring H5N1 infection in pigs. Zoonoses Public Health 57:291–298. doi:10.1111/j.1863-2378.2009.01270.x

Tellier, R. 2006. Review of aerosol transmission of influenza A virus. Emerg. Infect. Dis. 12:1657–1662.

Tellier, R. 2009. Aerosol transmission of influenza A virus: A review of new studies. J. R. Soc. Interface 6:S783–S790. doi:10.1098/rsif.2009.0302.focus

Torremorell, M., M. Allerson, C. Corzo, A. Diaz, and M. Gramer. 2012. Transmission of influenza A virus in pigs. Transbound. Emerg. Dis. 59:68–84. doi:10.1111/j.1865-1682.2011.01300.x

Valls, G., and I. Luque. 2015. Prevalence and diversity of swine influenza virus in Europe.

Van Alstine, W. G. 2012. Respiratory system. In: J. Zimmerman, L. Karriker, A. Ramirez, and K. J. Schwartz, editors, Diseases of swine. Wiley-Blackwell.

Van Reeth, K., I. H. Brown, and C. W. Olsen. 2012. Influenza virus. In: J. Zimmerman, L. Karriker, A. Ramirez, and K. J. Schwartz, editors, Diseases of swine. Wiley-Blackwell.

Wetten, M., J. Odegard, O. Vangen, and T. H. E. Meuwissen. 2012. Simultaneous estimation of daily weight and feed intake curves for growing pigs by random regression. Animal 6:433–439. doi:10.1017/S1751731111001832

Paper 5

Occurrence and spread of influenza A(H1N1)pdm09 virus infection in Norwegian pig herds based on active serosurveillance from 2010 to 2014

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SUMMARY

The incursion of influenza A(H1N1)pdm09 virus was detected by Norway's active serosurveillance of its pig population in 2009. Since then, surveillance data from 2010 to 2014 revealed that 54% of 5643 herd tests involving 1567 pig herds and 28% of 23 036 blood samples screened positive for antibodies against influenza A virus. Positive herds were confirmed to have influenza A(H1N1)pdm09 virus infection by haemagglutination inhibition test. In 50% of positive herd tests, $\geqslant 60\%$ of the sampled pigs in each herd had antibodies against influenza A(H1N1) pdm09 virus. This within-herd animal seroprevalence did not vary for type of production, herd size or year of test. The overall running mean of national herd seroprevalence, and annual herd incidence risks fluctuated narrowly around the means of 45% and 32%, respectively, with the highest levels recorded in the three densest pig-producing counties. The probability of a herd being seropositive varied in the five production classes, which were sow pools, multiplier herds, conventional sow herds, nucleus herds, and fattening herds in descending order of likelihood. Large herds were more likely to be seropositive. Seropositive herds were highly likely to be seropositive the following year. The study shows that influenza A(H1N1)pdm09 virus is established in the Norwegian pig population with recurrent and new herd infections every year with the national herd seroprevalence in 2014 hovering at around 43% (95% confidence interval 40–46%).

Key words: Active serosurveillance, influenza A, pandemic H1N1, pig, temporal and spatial.

INTRODUCTION

Influenza A viruses (IAVs) are ubiquitous in both humans and animals, and are endemic in most pig populations worldwide [1–6]. Several short-term influenza virus surveillance systems in the last two decades [7–13] revealed that the dominant circulating swine influenza A viruses (swIAVs) in European pigs were: the Eurasian avian-like H1N1 [14], human-like H3N2 [15], and triple assortant (swine, human, avian) H1N2 [4]. The most recent virus being influenza A(H1N1)pdm09 virus (H1N1pdm09), which joined the ranks of the preceding three subtypes with increasing incidence from 2010 [13, 16]. Subtype H1N1pdm09 was first reported in humans in April 2009, in North and South America [17]. Following outbreaks in humans, pig-producing countries worldwide increased their surveillance activities and also reported the detection of H1N1pdm09 in their pig populations [1, 18–20].

However, such coordinated surveillance activities in pig populations were short term and on an *ad hoc*

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basis, usually undertaken when funding was available. While prominent organizations like the Centers for Disease Control and Prevention (CDC), the European Influenza Surveillance Network (EISN) and the World Health Organization (WHO) have well-developed and continuous human influenza surveillance systems [21], sustained influenza virus surveillance in pigs is absent in most countries because swine influenza typically is neither a reportable nor a regulated pig disease. Although influenza surveillance in pigs since the emergence of H1N1pdm09 has improved around the world, including Europe [16], surveillance of IAV in pigs remains passive for the most part [22, 23]. The major shortcoming of a passive surveillance system is that infections like H1N1pdm09 in pigs can pose a problem because subclinical cases are often missed. A case-control study involving 118 nucleus and multiplier herds in Norway showed that only 19 (40%) of 48 seropositive herds had detectable clinical signs [24]. As such, the study of prevalence, incidence risks and temporal trends for a largely subclinical infection like H1N1pdm09 is difficult under passive surveillance systems. Herd prevalence, incidence and temporal trends of H1N1pdm09 infection in pigs, could, however, be studied in depth in Norway because swine influenza is a reportable disease and vaccination of pigs against swIAV is not practised. From the ~2000 pig herds in Norway (Fig. 1), about one third (500–750) of the herds are selected every year for screening against IAVs and other reportable diseases [25].

The ongoing annual active serosurveillance of swIAVs in Norway that began in 1997 [26], more than a decade before the outbreak of H1N1pdm09 infection in pigs, ascertained that its pig population had been free from all IAVs prior to the incursion of H1N1pdm09 in October 2009 [18]. This occurred a few months after the first human cases caused by the same strain of influenza virus were diagnosed in Norway [18, 27]. The same case-control study on clinical impact of the infection mentioned earlier also revealed that infected humans had transmitted the virus to the pigs by reverse zoonosis while working in close proximity with the pigs [28]. A ramped-up risk-based surveillance, following diagnosis of the index case herd, discovered that the infection had quickly become widespread in pig herds throughout Norway [27]. Ninety-one out of 215 herds tested positive serologically or by PCR testing within a 3- month period. The simultaneous detection in so many pig herds dispersed across Norway in a short time

suggested that the incursion was not a point-source pattern that is typical of diseases spread by animal movements and animal contacts. The initial planned eradication of the virus from the pig population by depopulation was aborted because it was deemed ineffective and cost-prohibitive. In addition, the prospect of humans as continued potential sources of infection to pigs also discouraged eradication procedures. The common view at the time was that the infection was expected to burn out with time given its highly contagious nature, short incubation, quick infective phase and recovery, especially in the relatively small pig herds typical in Norway [29]. Five years after the incursion in 2009, this has not happened. In 2014, the national herd seroprevalence remained high at >40% [25].

The accumulated data collected from Norway's ongoing active national serosurveillance of H1N1pdm09 virus gave us the opportunity to study the ecology of the virus, and the natural progression and epidemiology of this infection in the Norwegian pig population, which was a formerly naive population for all IAVs.

MATERIALS AND METHODS

The 5-year surveillance data from 1 January 2010 to 31 December 2014 involved 1567 pig herds (~75% of the 2000 pig herds in Norway based on the National Registry of Pig Herds, 2014) with a total of 5643 herd tests and a total of 23 026 individual blood samples (Fig. 1). Pig herds in the sampled population were classified into five production classes: (1) fattening; (2) nucleus herds; (3) multiplier herds; (4) conventional sow herds, and (5) sow pools. These five classes of pig herds form the breeding and health pyramid that creates a unidirectional animal flow in the production of pig meat (Fig. 2). At the top are the closed nucleus herds ($n \approx 40$) where pure genetic lines are constantly improved. Expanding in the next level are the multiplier herds ($n \approx 60$) where some multiplier herds are closed and most are associated with one nucleus herd. They produce maternal lines of Landrace-Yorkshire (LY) cross and supply gilts to conventional sow herds, which include both integrated and piglet-producing herds. Nevertheless, some commercial sow herds do replenish their sow numbers with gilts from their own production. Unique to Scandinavian countries with their small sow herds, the sow pool system in Norway involves a cooperation between 10–20 pig producers where
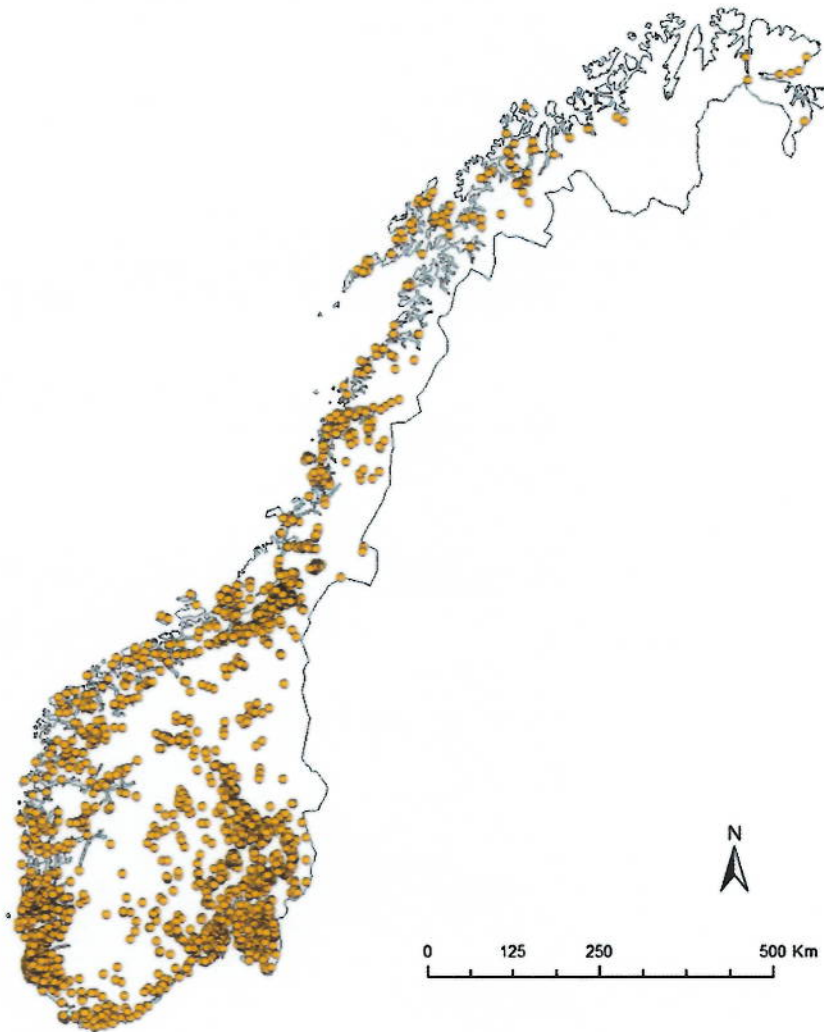
Fig. 1. Spatial distribution of pig herds ($n \approx 2000$) registered in Norway in 2014 [25].

one central gestation herd supplies the cooperating producers (satellite units) with pregnant sows in a leasing system [30, 31]. Tables 1 and 2 give a breakdown of the number of herd tests by the five production classes in the 19 counties of Norway.

Herd sampling

The Food Safety Authority carries out the sampling based on herds selected by the Norwegian Veterinary Institute every year. All nucleus, multiplier and sow pool herds are tested every year because they are high priority herds. Blood samples of ten pigs from all nucleus and multiplier herds ($n = 97$ in 2014) as well as 30 blood samples from the gestation units of every sow pool ($n = 14$ in 2014) are sampled

annually from each herd. Prior to 2011, conventional sow herds were proportionally selected annually from each of the 19 counties according to the number of herds registered with the National Registry of Pig Herds. In each of these herds, blood samples were taken from ten sows. However, in 2011 there was a change in the sampling strategy for conventional sow herds in that blood samples are now collected from slaughtered sows and boars at the 12 largest abattoirs where more than 97% of the pigs in Norway are slaughtered. The number of blood samples collected at each slaughterhouse per year is proportional to the total number of adult pigs slaughtered per year. Sampling days are distributed evenly throughout the year. Blood samples are collected from one to five sows for each selected herd and the same herd could
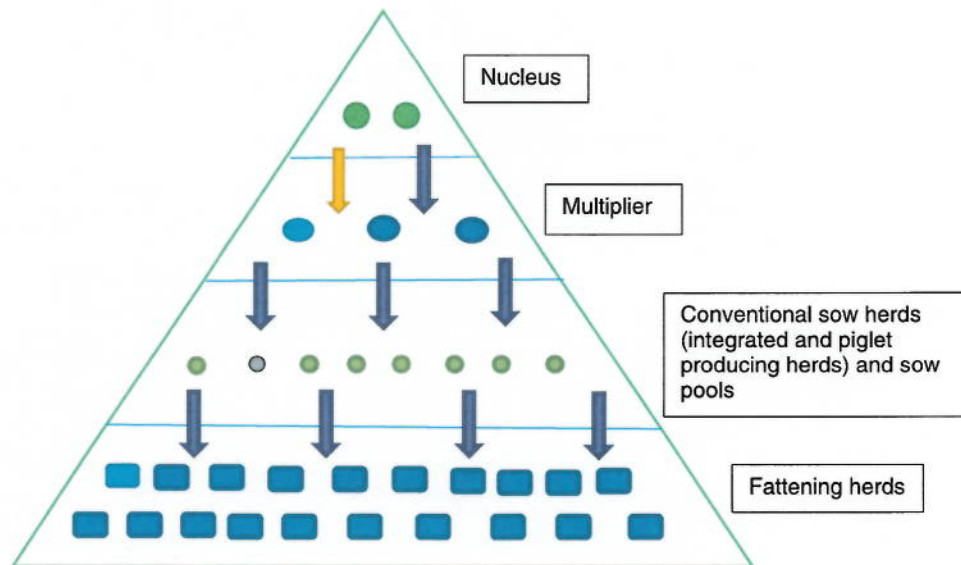
Fig. 2. Pyramid system of Norway's pig production system showing a unidirectional flow to optimize health and performance of genetic lines and heterosis.

Table 1. *Active serosurveillance for influenza A virus infection in the Norwegian pig population. Number of herd tests by county (n = 19) from 2010 to 2014*

County	2010	2011*	2012	2013	2014	Total
Østfold	44	89	95	95	50	373
Akershus/Oslo	27	79	59	47	36	248
Hedmark	41	126	152	131	120	570
Oppland	31	109	94	102	69	405
Buskerud	8	37	44	41	24	154
Vestfold	37	84	91	79	66	357
Telemark	8	28	29	25	28	118
Aust-Agder	2	5	1	6	6	20
Vest-Agder	4	17	12	9	6	48
Rogaland	145	289	338	424	317	1513
Hordaland	15	24	26	34	20	119
Sogn og Fjordane	14	6	27	27	23	97
Møre og Romsdal	4	11	21	20	20	76
Sør-Trøndelag	14	52	44	57	50	217
Nord-Trøndelag	69	291	281	212	196	1049
Nordland	22	40	44	72	61	239
Troms/Finnmark	5	1	3	20	11	40
Total	490	1288	1361	1401	1103	5643

* A change in sampling strategy beginning in 2011 where the same herd could be tested more than once in the same year. Sampling took place at the slaughterhouses.

be sampled several times a year. Regarding fattening herds, ten blood samples are collected every year from 40–60 selected herds.

With such a mixed sampling design, the number of pigs sampled per herd test is non-uniform across the

Table 2. *Number of herd tests involving serosurveillance of influenza A virus infection classified by the five production classes from 2010 to 2014*

Production class	2010	2011*	2012	2013	2014	Total
Fattening herd	50	79	64	65	47	305
Nucleus herd	52	79	78	82	71	362
Multiplier herd	73	117	152	148	137	627
Conv. sow herd	302	950	996	1047	798	4093
Sow pool	13	63	71	59	50	256
Total	490	1288	1361	1401	1103	5643

* A change in sampling strategy beginning in 2011 where the same herd could be tested more than once in the same year. Sampling took place at the slaughterhouses.

five production classes. Overall in our study, there were 3562 (63%) herd tests with <5 pigs sampled. In proportion to each production class with such small size samples, conventional sow herds had the highest with 77%, followed by multiplier herds with 38%, and nucleus herds with 30%. Of the 19 pig-producing counties of Norway, Rogaland, Nord-Trøndelag, and Hedmark with the largest number of pig herds, respectively, had the highest number of herd tests every year (Table 1).

Laboratory analyses and herd diagnosis

All serological analyses were performed at the Norwegian Veterinary Institute in Oslo. A commercial

competitive ELISA (ID Screen® Influenza A Antibody Competition multi-species kit; ID VET, France) with a reported sensitivity of 93% and specificity of 99% (manufacturer's data) was the screening test for serum antibodies against IAV. The ELISA test can detect IAV antibodies in any species including pigs. Titres ⩾40 were considered positive for IAV antibodies. In cases of positive or inconclusive results, the serum samples were re-tested using the haemagglutination inhibition test (HI), to detect antibodies against the four antigens, namely H1N1pdm09 (A/California/07/2009), European H1N1 [A/Sw/Belgium/1/98 (H1N1)], H1N2 [A/Sw/Gent/7623/99 (H1N2)] and H3N2 [A/Sw/Flanders/1/98(H3N2)]. CDC identified and described the first antigen [32], while the latter three antigens were identified and described in Belgium [33]. Testing of these serotypes have been described in the *OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals*. A herd was considered positive if at least one blood sample serially tested positive with ELISA first, followed by an HI test using antigens produced at the Norwegian Veterinary Institute. Pigs with an antibody titre ⩾10 in the HI test for a given subtype were regarded as positive or considered a cross-reaction if more than one type of antigen reacted positively. The herd-level diagnosis was based on which subtype had the highest mean titre, and the highest prevalence in a single herd test. Antigen reactions other than H1N1pdm09 were considered cross-reactions because they were either lower in titre, fewer in proportion in positive reactions, and unlike H1N1pdm09, they did not exist as single antigen reactions in any of the blood samples examined.

Test sensitivity and specificity at herd level

Based upon the individual test sensitivity, we calculated herd sensitivity by two formulae:

Probability of false-negative herd

$$= [(1 - \text{animal prevalence})$$
$$+ (1 - \text{sensitivity of ELISA})]^{\text{sample size per herd}}, \quad (1)$$

Herd sensitivity

$$= 1 - \text{probability of false-negative herd.} \quad (2)$$

The calculations show that a sample size of ten pigs per herd was sufficient to achieve at least 95% confidence of identifying a positive herd based on a within-herd prevalence of 26%. As the number of pigs

sampled per herd test varied considerably (range 1–40 pigs) and the animal prevalence also varied between herds, some herd tests were lower in sensitivity, and some higher. The probability of falsely classifying a positive herd as negative therefore is higher in herds with few pigs sampled and lower animal prevalence. Conversely, it is harder to classify a negative herd as falsely positive because all positive tests were followed by a HI test. Serial testing raised the specificity to almost 100% at the herd level.

Temporal and spatial analysis

Temporal trends of herd seroprevalence were investigated using Stata's lowess smoothing function (Stata v. 14.0, StataCorp LP, USA) to plot the running means of the herd infection status (seropositive = 1, negative = 0) against the sampling dates from 2010 to 2014. Stratifying herd seroprevalence by the 19 counties enabled the investigation of spatial variations across Norway with varying pig-farming densities in different counties. Stratifying the seroprevalence by the five production classes allowed the investigations of variations in probability of infection between the five different types of farm operation. The uniform distribution of herd sampling over 12 months enabled us to use day as the time unit to plot the temporal trends. The lowess smoothing function examined the spatial correlations between the probabilities of herds being seropositive with pig-farming density by plotting the mean herd seroprevalence with the mean distance of the four nearest pig herds. The mean distance of the four nearest pig herds was a proxy indicator of pig herd density.

Environmental and production conditions

To identify production factors associated with a seropositive pig herd, we used Stata to execute a mixed logistic regression analysis on the hierarchical data ($N_{\text{county}} = 19$, $N_{\text{herds}} = 1567$, $N_{\text{herd tests}} = 5643$) for the binary outcome of a herd testing positive.

With the existing sampling plan, each herd could have been sampled multiple times (between 1 and 12 times per year and up to 29 times during the 5-year study period). The data were nested in herd identity and county and were thus included as random effects in our logistic regression model. The random effects account for all variances of non-fixed effects related to the county and the individual herd to give as accurate as possible the estimates on the fixed effects.

Herd size, a continuous variable, was based on the live pigs on each farm as reported by pig farmers to the National Registry of Pig Herds twice a year on 31 July and 31 December. Given that herd size had a nonlinear relationship with outcome, the herd size data were transformed into an ordinal variable with three quantiles using specific cut-off points of pig numbers to give small (<350 pigs, $n = 698$), medium (351–665 pigs, $n = 450$), and large (666–4075 pigs, $n = 419$) herds.

Presence of sows and being a closed or open herd were collinear with production class and hence were excluded from the model. Similarly, the mean distance from the four nearest herds was highly correlated with the 19 counties and was therefore excluded as a predictor from the final mixed regression model.

The three categorical fixed effects in the mixed logistic regression model were:

Year of test (n = 5): included five years (2010, 2011, 2012, 2013, and 2014).
Herd size (n = 3): included three categories of small, medium and large.
Production class (n = 5): consisted of fattening herds, nucleus herds, multiplier herds, conventional sow herds and sow pools.

The mixed random-intercept logistic regression models were formulated as follows:

$$\ln\left((Y_{[ijk]})/(1 - Y_{[ijk]})\right) = \beta_0 + \beta_{\text{year}} X_{\text{year}[ijk]}$$
$$+ \beta_{\text{production}} X_{\text{production}[ijk]} + \beta_{\text{herd size}} X_{\text{herd size}[ijk]}$$
$$+ u_{[jk]} + v_{[k]} + \varepsilon_{[ijk]},$$

where Y_{ijk} is the binary outcome, where 0 = negative, 1 = positive of a herd test for the ith observation ($i = 1, 2, \ldots, 5643$), jth herd ($n_j = 1567$) nested within the kth ($n_k = 19$) county; β is a vector of coefficients for the three categorical fixed effects: (1) year of test, (2) herd size, and (3) production class; $X_{[ijk]}$ is the vector of for the three predictors in our two models: (1) year of test, (2) herd size, and (3) production class for the ith observation of the jth herd and kth county; u_{jk} is a vector of random intercepts unique to each herd, where $u_{jk} \sim N(0, \sigma^2_{\text{herd}})$, and v_k is a vector of random intercepts unique to each county, where $v_k \sim N(0, \sigma^2_{\text{county}})$; and ε_{ijk} is the vector of error terms where $\varepsilon_{ijk} \sim N(\mu, \sigma^2)$.

The likelihood ratio test aided model selection. To decide on the significance of additional predictors

for the two models, a difference of <2 of the log likelihood score was regarded as non-significant and the most parsimonious model was chosen [34]. We tested the models by assessing fit and residual patterns.

There were 1816 herd tests where herds were tested consecutively for at least 2 years. To examine recurrent herd infection rates, univariable logistic regressions stratified on production class estimated the probability that a seropositive herd would be seropositive again the following year. Similarly, we investigated new herd infection rates by estimating the probability that a seronegative herd would test seropositive the following year by using mixed logistic regression.

We also plotted the incidence risks stratified by the four production classes of fattening, nucleus, multiplier and conventional sow herds. We excluded the sow pools from this analysis given their small numbers of only 14 herds and also that there was only one or no uninfected sow pool herds to calculate incidence risk for the following year.

Within-herd seroprevalence

We investigated the within-herd animal seroprevalence by observing the proportion of pigs testing positive in 1028 positive herd tests that had at least five pigs tested. A cumulative probability on animal prevalence plot of these positive herds revealed the infectiveness of the disease in pigs kept in close proximity, typical of pig production. Factors causing variations to animal prevalence were investigated with scatter plots for the three categorical fixed effects of interest, i.e. year of test, production class and herd size. Graphical analyses were followed up with multivariable regression using general linear regression for categorical variables to investigate whether within-herd prevalence varied with the same three fixed effects.

Confidence intervals (CIs) using Stata for binomial outcomes gave inferential statistics on binomial probabilities of prevalence and incidence [35].

RESULTS

Herd seroprevalence, temporal trends

Surveillance data of 5643 herd tests on 23 039 samples from 2010 to 2014 showed that 6513 (28%) of the samples screened ELISA positive for antibodies against IAV in 2470 herd tests. Of these blood samples

positive for antibodies against IAV, 5857 were confirmed by the HI test to be antibodies against H1N1pdm09 with 23·6% showing reactions to sole antigen H1N1pdm09. Seventy-six per cent of the samples with reactions to multiple antigens in addition to H1N1pdm09 were all deemed cross-reactions by our criteria for herd diagnosis.

Figure 3 shows the spatial distributions of seropositive herds in 2010, 2014 and cumulatively from 2010 to 2014. Of the 1567 herds involved in the surveillance, 842 tested positive at least once thus giving a national cumulative herd seroprevalence of 54% (95% CI 51–56) by the end of 2014. There were no unique clustering patterns for positive herds. The heavy pig-farming-area counties correspondingly also had higher herd prevalence. As depicted by the temporal and spatial trends in Figure 4, the running mean herd seroprevalence for the top three major pig-producing counties, Rogaland, Nord-Trøndelag, and Hedmark fluctuated between 20% and 70% with no signs of decreasing at the end of 5 years after incursion of the virus in 2009. Nationally, the trajectory of the running mean herd prevalence was flat and hovered at around 42%. Comparison of the temporal trends of herd seroprevalence in the five production classes seen in Figure 5 revealed that fattening herds had the lowest running herd mean seroprevalence, which rose from ~20% in 2010 to 30% in 2011 before gradually decreasing to 9% in 2014. The three production classes of nucleus, multiplier and conventional sow herds had similar trajectories of running mean herd seroprevalence that fluctuated between 40% and 50%. In contrast to the fattening herds, the small group of sow pool herds had the highest levels of mean herd seroprevalence. Depicted by a wide U-shaped trajectory in Figure 5, sow pool herds began with nearly 100% seroprevalence in 2010, which fell to ~62% in 2011 before gradually rising to ~90% in 2014.

Recurrent herd infections

Looking at herds that were consecutively tested, there were 293 sow herds (nucleus, $n = 41$; multiplier, $n = 51$; conventional, $n = 188$; sow pools, n = 13) that were tested for multiple 4 or 5 years. The proportion of these herds by production class that were repeatedly seropositive for 4 or 5 years, were 11/41 (27%) for nucleus herds, 20/51 (40%) for multiplier herds, 54/188 (29%) for conventional sow herds and 11/13 (85%) for sow pools. Conversely, the proportion of herds

that tested negative for all the years they were tested were 11/41 (27%) for nucleus herds, 7/51 (14%) for multiplier herds, 23/188 (12%) for conventional sow herds and 0/13 (0%) for sow pools.

Herd incidence risk, temporal trends

Figure 6 shows herd incidence risks or the proportion of new infections by production classes plotted over the 5 years. Temporal trends of incidence risks combined with recurrent infection trends (not shown) would give our seroprevalence trends in Figure 4. Incidence risks of fattening herds rose from ~23% in 2010 to 29% in 2011 before trending downwards towards 9%. Multiplier herds had a sharp drop from ~39% in 2010 to 15% in 2011, where it remained in the range between 14% and 21%. Nucleus herds had a V-shaped pattern where their incidence risk dropped from ~25% in 2010 to 15% in 2012 before rising to 22% in 2014. Conventional sow herds fluctuated between 26% and 36%, which were potentially the most underestimated in the production classes because of the low sample sizes associated with these herds.

Herd prevalence and pig-farming density

The lowess smoothing plots in Figure 7 show that the mean running herd seroprevalence of the four production classes (fattening, nucleus, multiplier and conventional herds) were inversely proportional to the mean distance of the four nearest pig herds. We omitted sow pools because of the low numbers involved.

Within-herd prevalence

Figure 8 shows a cumulative probability plot of the proportion of samples that tested positive in herds with at least five animals sampled ($n = 1028$ herd tests in 488 herds). The 10th percentile was 20%, 25th percentile, 30%, 50th percentile or median, 60%, 75th percentile 81%, 90th percentile 100%. We did not find any variations in animal prevalence in the five production classes or in the three quantiles of herd sizes using graphical comparisons (not shown) or multivariable regression analysis (not shown).

Sensitivity of herd test

Given the possible variations in animal prevalence as shown in Figure 8 and the varying number of pigs per
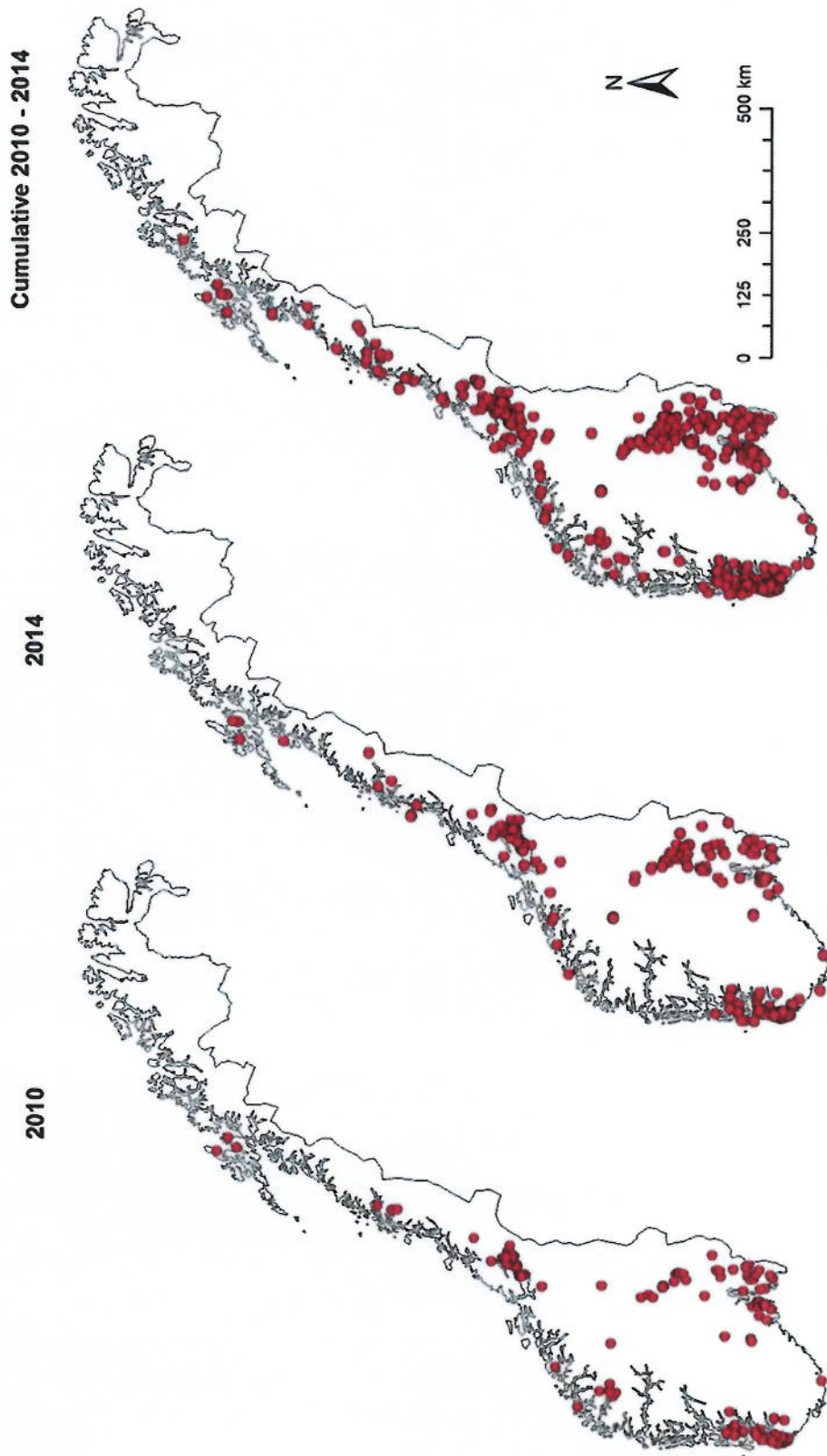
Fig. 3. Spatial distribution of Norwegian pig herds testing positive for antibodies against influenza A(H1N1)pdm09 virus in 2010 [41%, 95% confidence interval (CI) 37–45 seroprevalence]; 2014 (48%, 95% CI 45–51 herd seroprevalence); cumulative 2010–2014 (53%, 95% CI 50–56 herd seroprevalence).
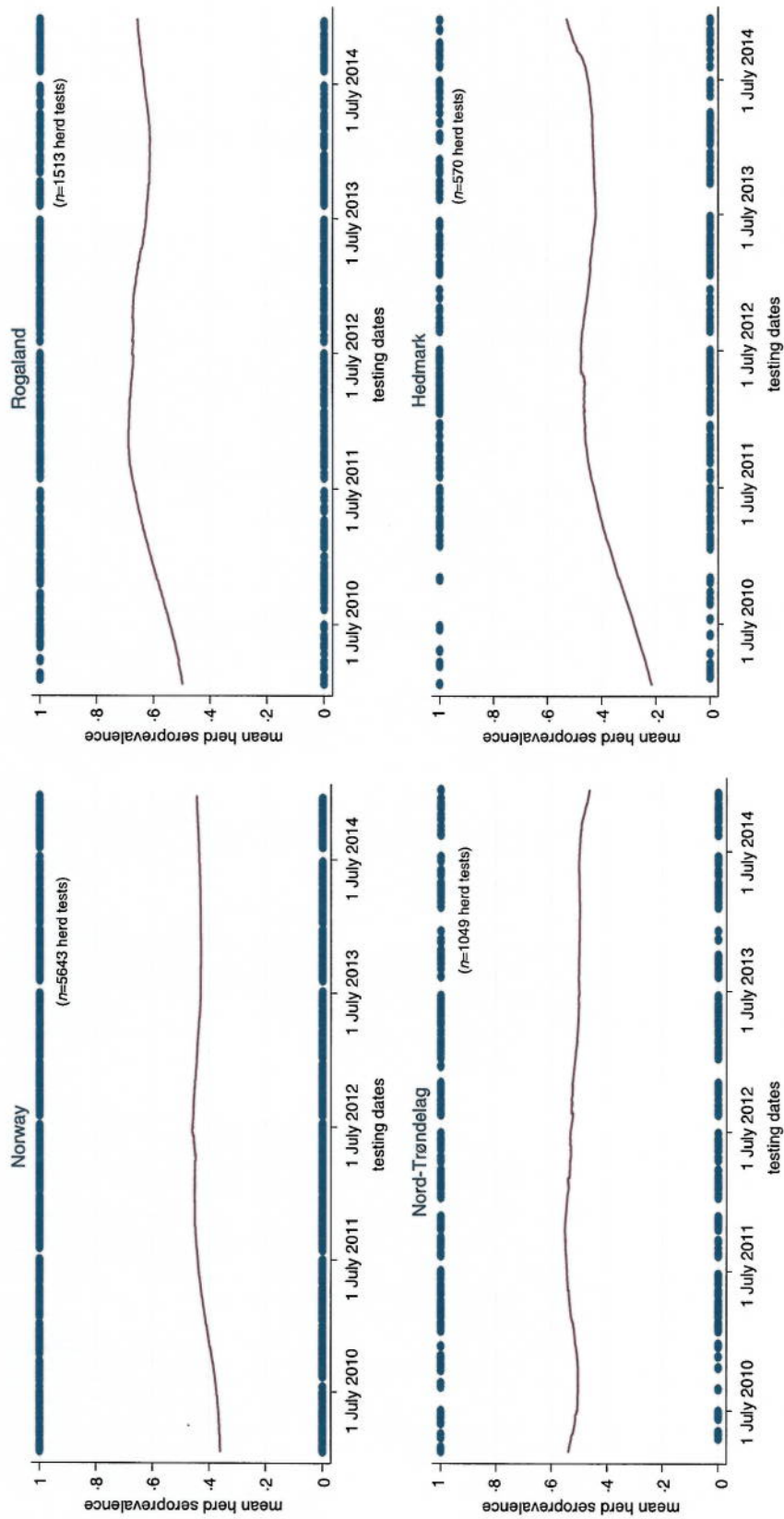
Fig. 4. Temporal trends* of pig herd seroprevalence for influenza A(H1N1)pdm09 virus infection in Norway, stratified by top three pig-farming counties from 1 January 2010 to 31 December 2014. (* Using Stata's lowess smoothing plots to show running mean between positive herds and negative herds. Date is the unit measure for cross-section of proportion of positive herds.)
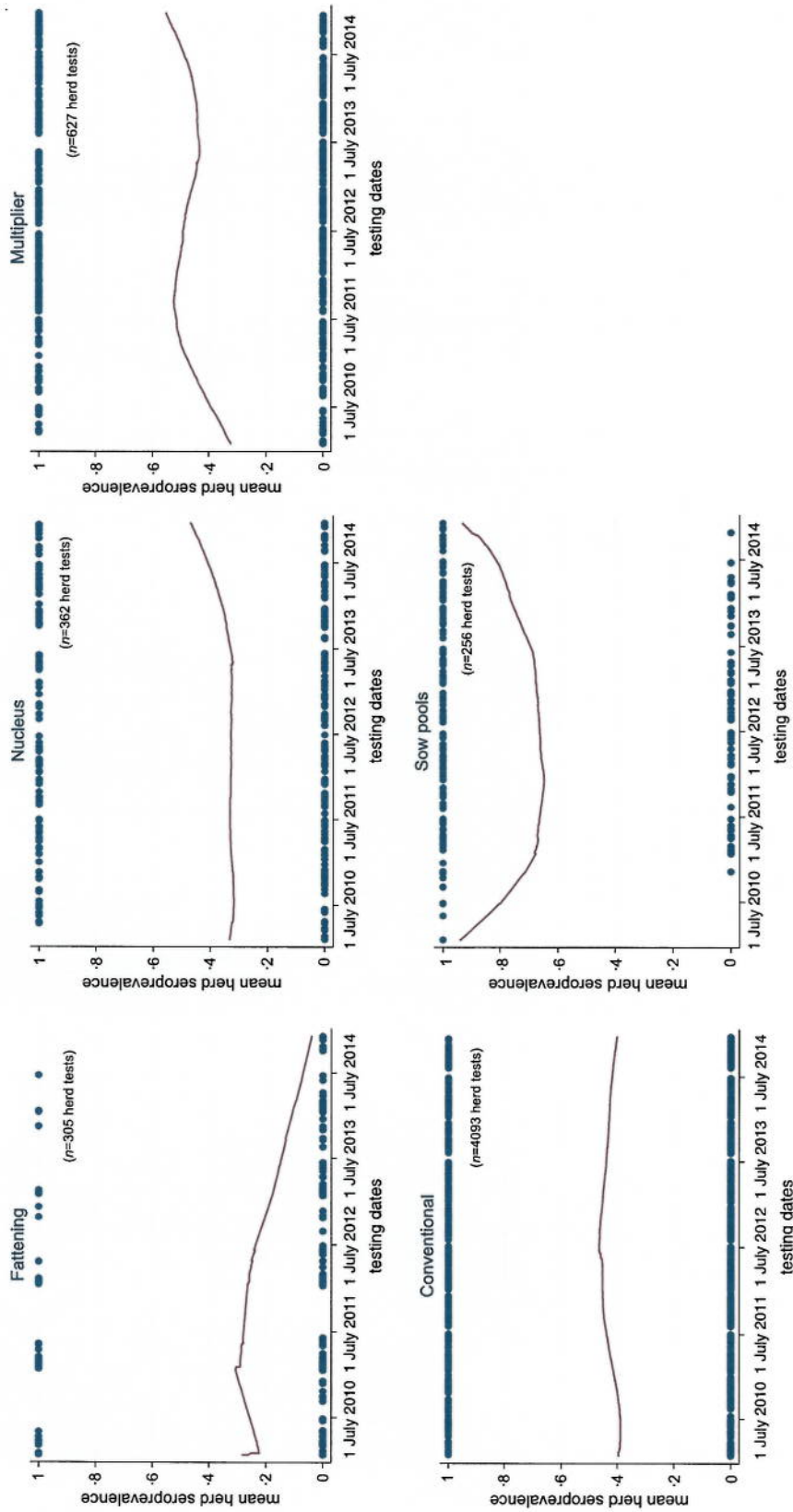
Fig. 5. Temporal trends* pig herds seroprevalence of influenza A(H1N1)pdm09 virus infection in Norway, stratified by five production classes from 1 January 2010 to 31 December 2014. (* Using Stata's lowess smoothing plots to show running mean between positive herds and negative herds with date as the unit measure for cross-section of proportion of positive herds.)
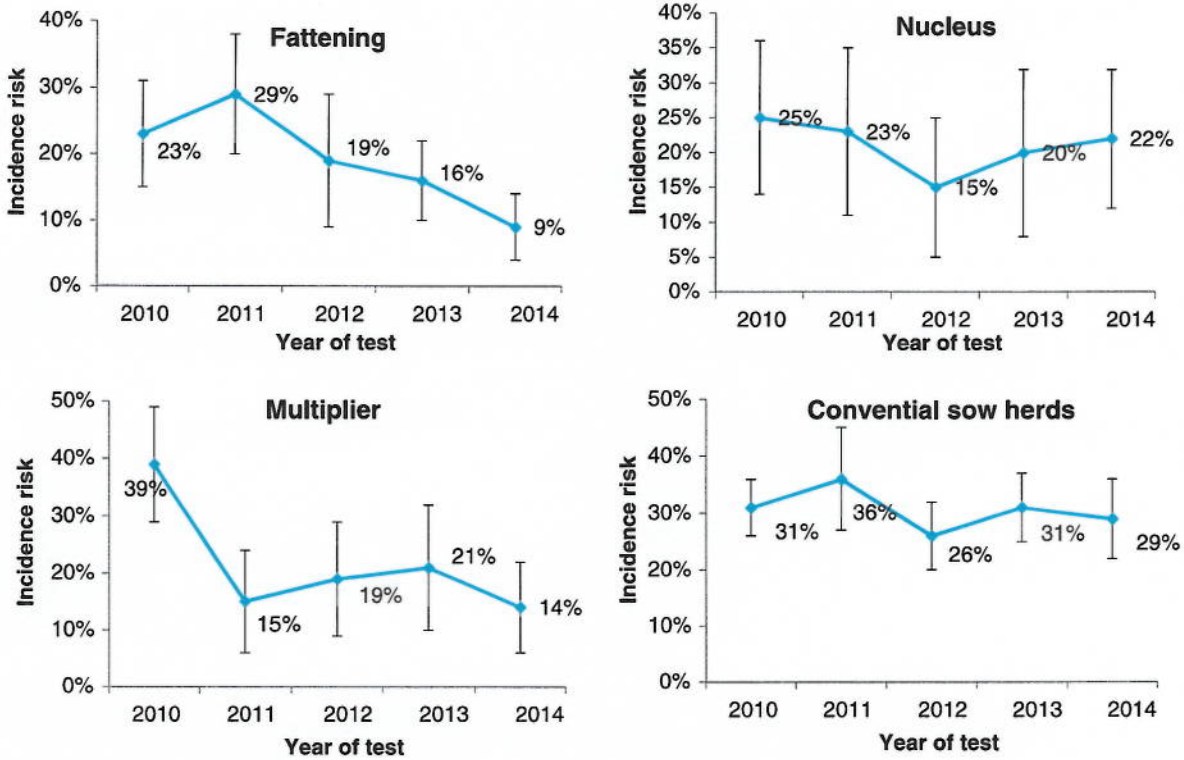
Fig. 6. Estimates of incidence risks with 95% confidence intervals of new pig herd infections of influenza A(H1N1)pdm09 virus infection in Norway stratified by four production classes from 1 January 2010 to 31 December 2014

herd test, Table 3 shows how the sensitivity of the herd test varied with the number of pigs sampled for a herd test. Given the bulk (72%) of the herd tests came from conventional sow herds and the bulk of these tests involved only one pig, the chance of misclassifying a positive herd as negative is >44% likely in 50% of true positive cases (based on median in Table 3).

Unconditional mixed logistic regression model

Mixed logistic regression analysis (Table 4) shows the herd was equally likely to be tested positive in any of the 5 years. Positive herds were either recurrent cases or new herd cases depicted by our incidence plots in Figure 6. The medium and large herds were more likely than the small herds to be seropositive, while the difference between medium and large herds were not significant. In terms of production class, fattening herds had the lowest probability while sow pools had the highest with an odds ratio (OR) of 24. The other three production classes of nucleus, multiplier and conventional sow herds had ORs of 2·78, 4·72 and 2·63, respectively.

For herds ($n = 1327$) that were negative and tested again the following year, Table 5 shows the results of a mixed logistic regression analysis of the probability of new infection (incidence risk). The risk of a new herd infection progressively increased from 2010 to 2014. With an OR of 48, multiplier herds were most likely to be newly infected. The risk in conventional sow herds (OR 9·75) was closer to the nucleus herds (OR 6·26). The risk of being infected were also stepwise higher with medium-sized herds having an OR of 6·3 and large herds having an OR of 9·2. Sow pool herds were dropped from the analysis because of small numbers ($n = 14$) and that they were either all positive and therefore had predicted the following year perfectly or there was one negative sow pool herd that was predicted perfectly to be negative next year.

DISCUSSION

What began in October 2009 as Norway's first IAV infection in pigs, H1N1pdm09, spread rapidly to naive pig herds throughout the country [18, 27]. The initial
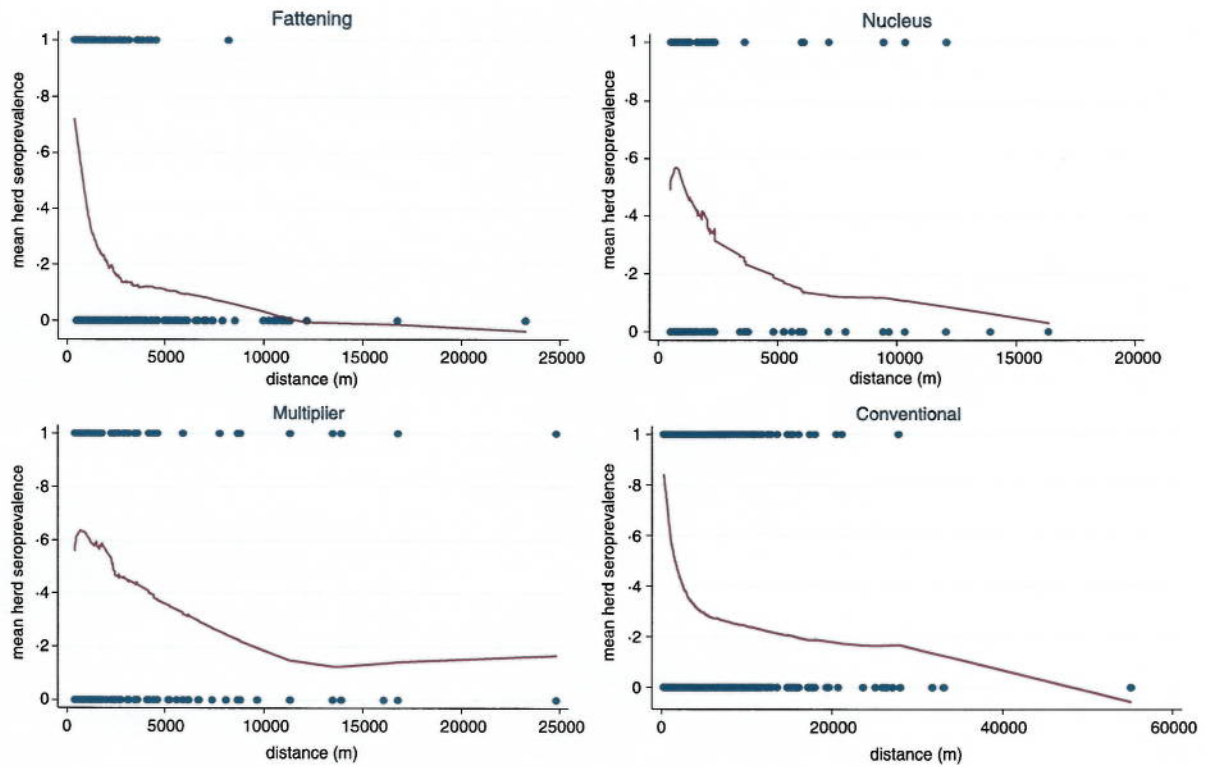
Fig. 7. Lowess smoothing curves showing spatial relationship of running mean herd seroprevalence with mean distance of four nearest pig herds.
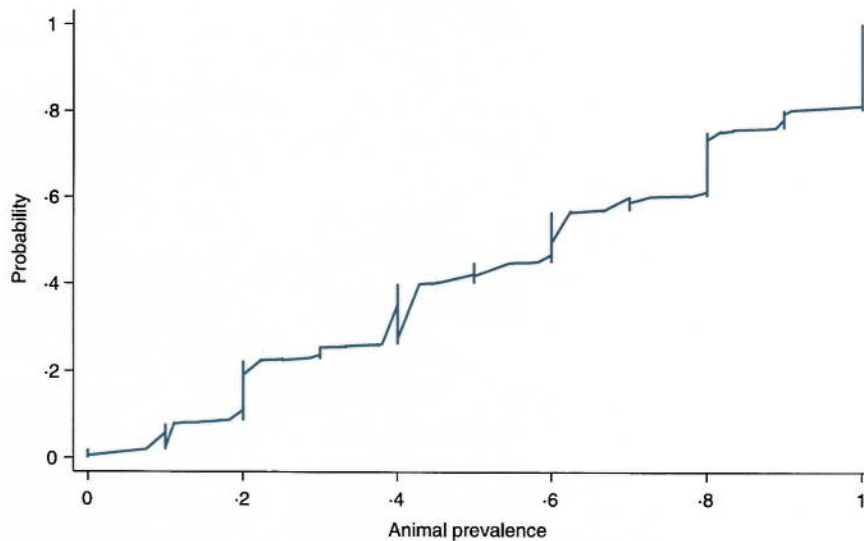
Fig. 8. Cumulative probability of the proportion of pigs screened ELISA-positive for influenza A antibodies in herds ($n = 1028$) diagnosed positive for influenza A(H1N1)pdm09 with at least five pigs sampled.

herd seroprevalence at the end of 2009 was 18%. In the absence of any vaccination practice, intervention measures from the food safety authorities, and the absence of other swIAVs, the seroprevalence had climbed to >40% in 2010 and has remained >40% ever since. The consistently high herd seroprevalence of the virus over the years was due partly to sustained recurrent infections in positive herds with sows

Table 3. *Sensitivity of herd test with respect to animal prevalence and number of pigs sampled per herd test*

No. of pigs sampled	Animal prevalence (percentile)				
	20% (10th)	30% (25th)	60% (median)	81% (75th)	100% (90th)
1	13	23	53	74	93
2	24	41	78	93	100
3	34	54	90	98	100
4	43	65	95	100	100
5	50	73	98	100	100
6	57	79	99	100	100
7	62	84	99	100	100
8	67	88	100	100	100
9	71	90	100	100	100
10	75	93	100	100	100

Values given are percentages.

(ranging from the lowest 27% in nucleus herds to the highest of 85% in sow pool system) and partly to new herd infections as evidenced by the 9–39% incidence risks in the four productions classes (Fig. 6).

Considering the rapid turnover of sows in Norwegian sow herds (culling at average three parities or ~2 years old), positive sow herds that were still positive after 2 years were the result of recurrent infections. Recurrent infections in positive herds occur frequently in nucleus, multiplier and conventional sow herds but with highest probability in the smallest group, the sow pools. This is not surprising because of this group's unique pattern of frequent contacts between multiple satellite herds.

Spatial analysis revealed that the counties with the three densest pig populations also correspondingly had the highest proportion of positive herds (Fig. 4). This is unsurprising as pig production is characterized by animals kept in close proximity and high turnover rate leading to susceptible new hosts being produced rapidly as required by highly contagious pathogens like IAV to propagate and maintain itself in the population. Higher density pig-farming counties also mean larger quantities of virus shed into the environment which increases the probability of transmission to susceptible hosts. Other studies have also shown that higher pig-density areas also have higher rates of respiratory diseases [36, 37].

In Norway, the persistence of H1N1pdm09 in the pig population can be attributed to several production factors that favour the infection dynamics of the influenza virus. Although our study has not ascertained the sources of the virus in these recurrent and new infections, the varying production class-specific probabilities revealed in our regression analysis and graphical plots suggest transmission patterns are related to their production operations. Elsewhere, since the outbreak of H1N1pdm09 in pigs, there have been studies on pigs such as those by the EISN, investigating the dominant swIAV subtypes circulating in European pig populations [13], and also *ad hoc* surveillance studies conducted to investigate the persistence and transmission dynamics of influenza viruses circulating in some European pig herds (Belgium, France, Italy, Spain) [38, 39]. These studies indicated that although there were various swIAVs circulating, some pig farms continually tested positive for the same swIAV subtypes over the six sampling periods from 2006–2009. Persistence of infection from horizontal transfer between animal contacts within these herds or re-introduction due to poor biosecurity was put forward as possibilities for these herds repeatedly testing positive. Although the scale of these studies was much smaller (3–80 herds) and the scope was restricted to only farrow-to-finish herds, the results on the dynamics of pig-to-pig transmission are partly helpful in elucidating the patterns of recurrent infections and new herd infections seen in our study.

Previous studies have shown that people working with pig herds may have transmitted H1N1pdm09 to pigs [7, 12, 16, 18, 20, 28]. Here in Norway, reverse zoonosis of humans carrying the virus and infecting the pigs they are in contact with remained highly probable during the study period. National influenza virus surveillance in humans by the Norwegian public health authorities during the previous two influenza seasons from 2012 to 2014 shows that more than 50% of all human influenza cases in Norway were still caused by H1N1pdm09 [40]. Hence, right up to the end of 2014, spillovers from human infections could have been an important source of virus for recurrent or new herd infections, especially so for nucleus herds which are closed to the introduction of pigs from other herds. Fattening herds had the lowest levels of herd seroprevalence consistently for all 5 years compared to the other production classes. This would be surprising if animal contacts were the sole mode of transmission because the majority of fattening herds buy piglets from many herds without requiring documentation of freedom from H1N1pdm09. Many fattening herds are all-in/all-out operations, at least at room level, with no contact between batches

Table 4. *Mixed logistic regression of the binomial outcome that a herd test was positive for antibodies against influenza A(H1N1)pdm09 virus infection based on haemagglutination inhibition test*

No. of observations (positives)	Fixed effects*	OR	95% CI	P value
	Year of test (n = 5)			
490 (204)	2010	1		
1288 (563)	2011	1·02	0·78–1·33	0·89
1361 (641)	2012	1·07	0·82–1·39	0·62
1401 (587)	2013	0·82	0·64–1·07	0·14
1103 (478)	2014	0·88	0·67–1·15	0·35
	Production class (n = 5)			
305 (69)	Fattening herd	1		
362 (123)	Nucleus herd	2·78	1·67–4·62	<0·001
627 (297)	Multiplier herd	4·72	3·05–7·30	<0·001
4093 (1826)	Conventional herd	2·63	1·87–3·70	<0·001
256 (155)	Sow pool	24·04	10·98–52·66	<0·001
	Herd size in three quantiles (pigs)			
1679 (601)	Small (<350 pigs)	1		
1909 (919)	Medium (350–665 pigs)	1·85	1·52–2·26	
1851 (881)	Large (>665 pigs)	1·63	1·33–1·99	
	Constant	0·50	0·33–0·75	
	Random effects†			
5643 (2473)	County (n = 19)			
	Var(const.)	4·45	2·16–9·15	
5643 (2473)	County>herd id (n = 1567)			
	Var(const.)	1·66	1·29–2·15	

OR, Odds ratio; CI, confidence interval.
* Three categorical fixed effects were: (1) year of test, (2) production class, and (3) herd size (based on national registry for subsidy).
† County and herd ID were included as random effects to account for non-fixed effects associated with county and the individual herd.

of pigs. Even if the young growers (~30 kg) came from positive herds, maternal antibodies may have protected them from infection and kept them virus free for transfer to the fattening herd. In fattening herds, fewer close human–pig interactions may have also contributed to a lower herd seroprevalence/incidence compared to the other four production classes. We see in Figures 5 and 6 that the running herd prevalence and incidence risks of fattening herds showed a marked decline after peaking in 2011. An explanation for the decline could be that more piglet-producing sow herds had developed active immunity with time and consequently fortified fattening pigs with protective maternal antibodies crucial for protection during the vulnerable transition to the grower phase where mixing between new pigs occurs. Nevertheless, new herd infections in fattening herds every year were still occurring as evidenced by the non-zero incidence risks. It was also highly likely that carrier piglets in the batch of fattening pigs could become a source of infection to other pigs that would become susceptible when

maternal antibodies waned sufficiently [39, 41, 42]. Age-related factors could play a role in causing the differential patterns in seroprevalence in the five production systems, especially between fattening herds and the other four production systems. Pigs sampled from fattening herds at the slaughterhouse were aged ~6 months whereas for other four types of sow herds (nucleus, multiplier, sow pools, conventional sow herds), older pigs like sows are sampled. Older animals, by virtue of their longer existence also means that their probabilities of exposure to the virus during their slightly extended lives before being sampled are higher.

The small group of sow pool herds (n = 14) had the highest levels of seroprevalence because they had the highest rates of recurrent infections. This was expected given their special operating mode that allows mixing of sows from various satellite pig herds (n = 10–20), thereby increasing the risk of horizontal spread between herds. Human–pig contact frequency and the accumulated duration with different people are also

Table 5. *Mixed logistic regression on the binomial outcome that a negative herd would test positive the following year for antibodies against influenza A(H1N1)pdm09 using the haemagglutination inhibition test*

Number of observations (positives)	Fixed effects*	OR	95% CI	P value
	Year of test ($n = 5$)			
174 (46)	2010	1		
406 (104)	2011	1·30	0·55–3·05	0·553
358 (121)	2012	5·72	2·31–14·17	<0·001
389 (128)	2013	6·40	2·43–16·83	<0·001
	Production class ($n = 4$)			
258 (64)	Fattening herd	1		
157 (43)	Nucleus herd	6·26	1·12–34·86	0·036
155 (56)	Multiplier herd	47·95	7·75–296·83	<0·001
757 (236)	Conventional herd	9·75	2·85–33·41	<0·001
	Herd size in three quantiles (pigs)			
399 (89)	Small (<350)	1		
405 (130)	Medium (350–665)	6·31	2·00–19·87	0·002
469 (165)	Large (>665)	9·24	2·78–30·72	<0·001
	Constant	0·0004	0·00003–0·00612	<0·001
	Random effects†			
1327 (399)	County ($n = 17$)			
	Var(constant)	2·72	1·01–7·35	
1327 (399)	County>Herd id ($n = 621$)			
	Var (constant)	19·72	10·34–37·61	

OR, Odds ratio; CI, confidence interval.
* The three categorical fixed effects were (1) year of test, (2) production class, and (3) herd size.
† County and herd ID were included as random effects to account for non-fixed efects associated with county and the individual herd.

higher in the sow pool system thus increasing the sources and risk of reverse zoonosis.

Regarding the nucleus, multiplier and conventional sow herds, their temporal trends in mean herd seroprevalence did not differ much. These three types of production classes fluctuated within a narrow range between 40% and 50%, with no signs of abating towards the end of 2014. Multiplier herds had a higher probability (OR 4·72, 95% CI 3·1–7·3) of testing seropositive compared to fattening herds. Nucleus herds, closed to pigs from other sources, had a lower probability of being positive (OR 2·78, 95% CI 1·7–4·6). Seroprevalence and incidence risks of conventional sow herds (OR 2·63, 95% CI 1·8–3·7) were unexpectedly similar to nucleus herds. The anomaly is accounted for by the lower sensitivity in herd tests for conventional herds because fewer pigs are sampled per herd test in these herds. As shown in Table 3, the likelihood of misclassification increases with decreasing within-herd prevalence and sample size. Herd tests involving conventional sow herds having ≤4 pigs were disproportionately high (77%). Many of these herd tests involved only one pig. Sampling

only one pig and given within-herd prevalences of 20%, 30%, 60%, 81%, and 100% (following the percentiles in Table 3) would respectively give herd test sensitivities of 13%, 23%, 53%, 74% and 93%. Therefore, the gap between the conventional sow herds and nucleus herds could be much wider when we factor in misclassification bias. In the same light, the seroprevalence trends depicted in Figures 4 and 5 (conventional sow herds in particular) reflect an underestimation of true herd prevalence since conventional sow herds made up the majority (72%) of the 5643 herd tests.

With regard to the closed nucleus herds with highest biosecurity, the recurrent infections in positive nucleus herds were likely caused by circulation of the virus within the herd or caused by continual spillovers from human infections as mentioned earlier. Nucleus herds generally have higher gilt replacement rates (60–70% are first-parity sows) in overlapping batches. The shorter cycle and higher rates of replacements also means that more newly susceptible hosts become available faster for new infections and the propagation of the virus.

As to animal prevalence in positive herds, ~60% of positive herds with at least 5 pigs sampled had ⩾50% of the animals testing seropositive (Fig. 8). The high within-herd seroprevalence is consistent with other swine influenza strains in that infections in pig herds are highly contagious with a short incubation period that could reach 100% infection rate within a short time [1, 39]. The animal prevalence did not differ between the production classes which is unsurprising given that the stocking density for all five production classes are similar and therefore would experience similar transmission dynamics for contagious diseases transmitted through contact and aerosols between animals in close proximity [1, 29].

The temporal trends of seroprevalence, recurrent and new herd infection rates observed in our surveillance data from 2010 to 2014 suggest that H1N1pdm09 will remain in the Norwegian pig population for as long as humans or pigs, or both, act as reservoirs and continue to transmit the virus to susceptible pigs. The persistence of the virus in the pig population has economic consequences even though the infection in the naive Norwegian pigs was mostly subclinical [24, 27]. A longitudinal study showed that the infection reduced the growth performance of growing pigs by reducing their feed efficiency. Infected grower pigs consumed more feed and had a protracted production time to reach the same market weight compared to their uninfected counterparts [26, 43].

H1N1pdm09 in pigs is not only widespread across Western Europe, reassortant subtypes have already appeared, probably due to co-infections of H1N1 pdm09 with established swIAVs [13]. It follows in the presence of established swIAVs, the prevalence and infection rates of H1N1pdm09 in pig herds in Western Europe were considerably lower [12, 16] compared to Norway. Conversely, H1N1pdm09 is likely to remain geographically a stable lineage in the Norwegian pig population given its continued sole existence as the only subtype circulating and coupled with Norway's continued closed-door policy on movement of live pigs across its borders. Nonetheless, the prospect of reassortment with human influenza could potentially occur if farmers with human IAVs introduce them to the pigs again, especially for subtypes like human H3N2 virus, for which antibodies to H1N1pdm09 do not offer cross-protection against (based on unpublished serology data at the Norwegian Veterinary Institute). To minimize such possibilities, it seems appropriate to continue with the recommendation that people working with pig herds should be immunized regularly with human influenza vaccines, and refrain from contacts with pigs if they have influenza-like symptoms.

In conclusion, although sampling for herd tests had varied across the pig herds and therefore made direct prevalence inferences somewhat challenging, we think that Norway's active surveillance gives a fairly representative picture of the natural infection dynamics of the virus in the Norwegian pig population given the absence of any intervention measures.

Five years after the incursion of the new influenza virus, H1N1pdm09, the prevalence of seropositive herds has not fallen below 40%. This strongly indicates that the virus has adapted well and established itself in the Norwegian pig population. To mount measures against the spread of influenza virus to new pig herds, and to break the chain of infection in infected herds, further research on the transmission dynamics of the H1N1pdm09 virus in the Norwegian pig population and economic analyses would give farmers and the food safety authorities guidance on feasible approaches.

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DECLARATION OF INTEREST

None

REFERENCES

1. **Van Reeth K, Brown IH, Olsen CW.** Influenza virus. In: Zimmerman J, *et al.*, eds. *Diseases of Swine*, 10th edn. Wiley-Blackwell, 2012.
2. **Kothalawala H, Toussaint MJM, Gruys E.** An overview of swine influenza. *Veterinary Quarterly* 2006; **28**: 45–53.
3. **Brown IH.** History and epidemiology of swine influenza in Europe. *Swine Influenza* 2013; **370**: 133–146.
4. **Marozin S, *et al.*** Antigenic and genetic diversity among swine influenza A H1N1 and H1N2 viruses in Europe. *Journal of General Virology* 2002; **83**: 735–745.
5. **Brown IH.** The epidemiology and evolution of influenza viruses in pigs. *Veterinary Microbiology* 2000; **74**: 29–46.
6. **Vincent A, *et al.*** Review of influenza A virus in swine worldwide: a call for increased surveillance and research. *Zoonoses and Public Health* 2014; **61**: 4–17.
7. **Van Reeth K, *et al.*** Seroprevalence of H1N1, H3N2 and H1N2 influenza viruses in pigs in seven European countries in 2002–2003. *Influenza and Other Respiratory Viruses* 2008; **2**: 99–105.

8. **Van Reeth K, Brown IH, Pensaert M.** Isolations of H1N2 influenza A virus from pigs in Belgium. *Veterinary Record* 2000; **146**: 588–589.

9. **Maldonado J, et al.** Evidence of the concurrent circulation of H1N2, H1N1 and H3N2 influenza A viruses in densely populated pig areas in Spain. *Veterinary Journal* 2006; **172**: 377–381.

10. **Brown IH, et al.** Multiple genetic reassortment of avian and human influenza A viruses in European pigs, resulting in the emergence of an H1N2 virus of novel genotype. *Journal of General Virology* 1998; **79**: 2947–2955.

11. **Brown IH, Harris PA, Alexander DJ.** Serological Studies of influenza viruses in pigs in Great Britain 1991–2. *Epidemiology and Infection* 1995; **114**: 511–520.

12. **Kyriakis CS, et al.** Serological evidence of pandemic H1N1 influenza virus infections in Greek swine. *Zoonoses and Public Health*. doi:10.1111/zph.12235.

13. **Simon G, et al.** European surveillance network for influenza in pigs: surveillance programs, diagnostic tools and Swine influenza virus subtypes identified in 14 European countries from 2010 to 2013. *PLoS ONE* 2014; **9**: e115815.

14. **Pensaert M, et al.** Evidence for the natural transmission of influenza A virus from wild ducts to swine and its potential importance for man. *Bulletin of the World Health Organization* 1981; **59**: 75–78.

15. **Castrucci M, et al.** Genetic reassortment between avian and human influenza A viruses in Italian pigs. *Virology* 1993; **193**: 503–506.

16. **Watson SJ, et al.** Molecular epidemiology and evolution of influenza viruses circulating within European swine between 2009 and 2013. *Journal of Virology* 2015; **89**: 9920–9931.

17. **Centers for Disease, C, Prevention.** Outbreak of swine-origin influenza A (H1N1) virus infection – Mexico, March-April 2009. *Morbidity and Mortality Weekly Report* 2009; **58**: 467–470.

18. **Hofshagen M, et al.** Pandemic influenza A(H1N1)v: human to pig transmission in Norway? *Eurosurveillance* 2009; **14**.

19. **Torremorell M, et al.** Transmission of influenza A virus in pigs. *Transboundary and Emerging Diseases* 2012; **59**: 68–84.

20. **Howden KJ, et al.** An investigation into human pandemic influenza virus (H1N1) 2009 on an Alberta swine farm. *Canadian Veterinary Journal* 2009; **50**: 1153–1161.

21. **Layne SP.** Human influenza surveillance: the demand to expand. *Emerging Infectious Diseases* 2006; **12**: 562–568.

22. **Itoh Y, et al.** In vitro and in vivo characterization of new swine-origin H1N1 influenza viruses. *Nature* 2009; **460**: 1021–U1110.

23. **Neumann GNoda T, Kawaoka Y.** Emergence and pandemic potential of swine-origin H1N1 influenza virus. *Nature* 2009; **459**: 931–939.

24. **Grontvedt CA, et al.** Clinical impact of infection with pandemic influenza (H1N1) 2009 virus in naive nucleus and multiplier pig herds in Norway. *Influenza Research and Treatment* 2011; **2011**: 163745.

25. **Lium B, Er C, Zerihun A.** The surveillance and control programme for specific virus infections in swine herd in Norway 2013. Oslo, Norway: Norwegian Veterinary Institute, 2014. (Surveillance and Control Programmes for Terrestrial and Aquatic Animals in Norway, Annual Report).

26. **Er C, et al.** Adverse effects of Influenza A(H1N1)pdm09 virus infection on growth performance of Norwegian pigs – a longitudinal study at a boar testing station. *BMC Veterinary Research* 2014; **10**: 284.

27. **Gjerset B, et al.** Experiences after twenty months with pandemic Influenza A (H1N1) 2009 infection in the naive Norwegian pig population. *Influenza Research and Treatment* 2011; **2011**: 206975.

28. **Grontvedt CA, et al.** Influenza A(H1N1)pdm09 virus infection in Norwegian swine herds 2009/10: the risk of human to swine transmission. *Preventive Veterinary Medicine* 2013; **110**: 429–434.

29. **Brookes SM, et al.** Replication, pathogenesis and transmission of pandemic (H1N1) 2009 virus in non-immune Pigs. *PLoS ONE* 2010; **5**: e9068.

30. **Engblom L, et al.** Factors affecting length of productive life in Swedish commercial sows. *Journal of Animal Science* 2008; **86**: 432–441.

31. **Dalin AM, Gidlund K, Eliasson-Selling L.** Post-mortem examination of genital organs from sows with reproductive disturbances in a sow-pool. *Acta Veterinaria Scandinavica* 1997; **38**: 253–262.

32. **Novel Swine-Origin Influenza A(H1N1) Virus Investigation Team.** Emergence of a novel swine-origin influenza A (H1N1) virus in humans. *New England Journal of Medicine* 2009; **360**: 2605–2615.

33. **Van Reeth K, et al.** Protection against a European H1N2 swine influenza virus in pigs previously infected with H1N1 and/or H3N2 subtypes. *Vaccine* 2003; **21**: 1375–1381.

34. **Burnham KP, Anderson DR.** Information an likelihood theory: a basis for model selection and inference. In: *Model Selection and Multimodel inference*, 2nd edn. USA: Springer-Verlag, 2002, pp. 49–96.

35. **Stata.** ci – Confidence intervals for means, proportions, and counts. STATA online manual (http://wwwstatacom/manuals13/rcipdf).

36. **Rose N, Madec F.** Occurrence of respiratory disease outbreaks in fattening pigs: relation with the features of a densely and a sparsely populated pig area in France. *Veterinary Research* 2002; **33**: 179–190.

37. **Maes D, et al.** Herd factors associated with the seroprevalences of four major respiratory pathogens in slaughter pigs from farrow-to-finish pig herds. *Veterinary Research* 2000; **31**: 313–327.

38. **Kyriakis CS, et al.** Influenza A virus infection dynamics in swine farms in Belgium, France, Italy and Spain, 2006–2008. *Veterinary Microbiology* 2013; **162**: 543–550.

39. **Rose N, et al.** Dynamics of influenza A virus infections in permanently infected pig farms: evidence of recurrent infections, circulation of several swine influenza viruses and reassortment events. *Veterinary Research* 2013; **44**: 72.

40. **Folkehelseinstituttet.** Norwegian Insitutute of Public Health. Influenza surveillance week 20, 2013–2014 (http://wwwfhino/eway/?pid=240), 2014.

41. **Loeffen WLA,** *et al.* Effect of maternally derived antibodies on the clinical signs and immune response in pigs after primary and secondary infection with an influenza H1N1 virus. *Veterinary Immunology and Immunopathology* 2003; **92**: 23–35.

42. **Loeffen WLA,** *et al.* Estimating the incidence of influenza-virus infections in Dutch weaned piglets using blood samples from a cross-sectional study. *Veterinary Microbiology* 2003; **91**: 295–308.

43. **Er C,** *et al.* Production impact of influenza A(H1N1)pdm09 virus infection on fattening pigs in Norway. *Journal of Animal Science* 2016; **94**: 751–759.