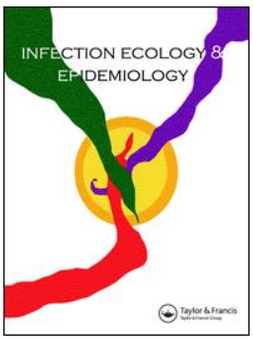

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RESEARCH ARTICLE

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Human to animal transmission of influenza A(H1N1)pdm09 in a turkey breeder flock in Norway

Siri Kulberg Sjurseth[a], Britt Gjerset[b], Karoline Bragstad[c], Olav Hungnes[c], Helene Wisløff[b], Chiek Er[b], Mette Valheim[b], Siri M. Løtvedt[d], Bruce David[e], Skjalg A. Hanssen[e], Siri H. Hauge[c] and Merete Hofshagen[a]

[a]Department of Animal Health and Food Safety, Norwegian Veterinary Institute, Oslo, Norway; [b]Department of Analysis and Diagnostics, Norwegian Veterinary Institute, Oslo, Norway; [c]Department of Influenza, Norwegian Institute of Public Health, Oslo, Norway; [d]Plant and Animal Department, Norwegian Food Safety Authority, Brumunddal, Norway; [e]Nortura SA, Oslo, Norway

ABSTRACT

Introduction: Routine surveillance samples disclosed seropositivity to influenza A virus (IAV) in a Norwegian turkey breeder flock. Simultaneous reports of influenza-like symptoms in farm workers and a laboratory confirmed influenza A(H1N1)pdm09 (H1N1pdm09) infection in one person led to the suspicion of a H1N1pdm09 infection in the turkeys. **Animals and methods**: H1N1pdm09 infection was confirmed by a positive haemaggutinin inhibition test using H1N1pdm09 antigens, and detection of H1N1pdm09 nucleic acid in reproductive organs of turkey hens. The flock showed no clinical signs except for a temporary drop in egg production. Previous reports of H1N1pdm09 infection in turkeys suggested human-to-turkey transmission (anthroponosis) during artificial insemination. **Results and discussion**: The flock remained seropositive to IAV and the homologous H1N1pdm09 antigen throughout the following 106 days, with decreasing seroprevalence over time. IAV was not detected in fertilised eggs or in turkey poults from the farm, however, maternally derived antibodies against H1N1pdm09 were found in egg yolks and in day-old poults. Genetic analyses of haemagglutinin gene sequences from one of the infected farm workers and turkeys revealed a close phylogenetic relationship, and confirmed human-to-turkey virus transmission.

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Influenza A(H1N1)pdm09; turkey; anthroponosis; artificial insemination; egg production; pandemic influenza

Introduction

Wild birds are the natural reservoir for all subtypes of influenza A viruses (IAVs), and thought to be the primary source of influenza A in other animals and humans [1]. Circulating avian influenza viruses (AIVs) evolve continuously due to antigenic drift and shift. Examples of human infections with avian and zoonotic influenza viruses include H5N1 and H7N9, which are causing sporadic infections with high mortality in Asia and Egypt [2].

Although anthroponoses are less debated and documented, IAVs may also be transmitted from humans to animals. Suspected cases of human to animal transmission of influenza A(H1N1)pdm09 (H1N1pdm09) virus have been reported in several mammalian species, including pigs, dogs, cats, and ferrets [3]. Although poultry has been regarded as less susceptible to the H1N1pdm09 virus [4], several international case reports have described H1N1pdm09 infection of suspected human origin in turkeys in the wake of the 2009 pandemic [5–8]. The transmission has occurred solely in turkey breeders, most likely during artificial insemination [9]. This paper describes an outbreak of H1N1pdm09 virus infection in a turkey breeder flock in Norway due to suspected human-to-turkey transmission.

Materials and methods

All turkey samples were analysed at the Norwegian Veterinary Institute. Blood samples were tested for antibodies against IAV using a blocking ELISA from IDvet (ID Screen influenza A antibody competition multi-species), and an indirect ELISA from IDEXX (IDEXX AI Ab test). For confirmation, positive samples were followed up with subtype specific haemagglutination inhibition (HI) tests [10]. Inactivated virus antigens and positive control sera for AI subtypes were obtained from the EU Reference Laboratory for Avian Influenza, Animal and Plant Health Agency, UK, and were as follows: H5N1 A/chicken/Scotland/59; H5N2 A/Ostrich/Denmark/72420/96; H5N3 A/teal/England/7394-2805/06; H5N7A/Mallard/Denmark/64650/03; H7N1 A/African starling/England/983/79; H7N7 A/turkey/England/647/77 [11]. Inactivated virus antigens and positive control sera for H1N1pdm09 were purchased from the University of Gent, Belgium (H1N1 A/Swine/California/07/09). Antibodies from egg yolk were extracted with chloroform, as described by Mohammed [12]. Turkey cloacal and oropharyngeal swabs and tissue samples were tested with a pan-influenza A real-time RT-PCR (rRT-PCR) [13], and subtype specific rRT-PCRs for H5, H7 and

CONTACT Siri Kulberg Sjurseth siri.sjurseth@vetinst.no Norwegian Veterinary Institute, P.O. Box 750, Oslo 0106, Norway

H1N1pdm09 [14–16]. HA gene sequences from positive samples were based on PCR fragments generated as previously described [17].

Human samples were tested at the Norwegian Institute for Public Health. Nucleic acid from human nasopharyngeal swabs were typed [18] and H1 subtyped [14] by rRT-PCR. The HA gene was sequenced by dye-terminator Sanger sequencing (PCR and sequencing primers available upon request). Nucleotide sequences were further analysed by BioNumerics (AppliedMaths, Belgium), BioEdit [19], and MEGA 6 software [20]. Phylogenetic trees were constructed with the neighbour-joining method, using Kimura2-parametre pairwise distances. Viral nucleotide sequences from the turkey sample (A/Turkey/Norway/1051/2016) and the human sample (A/Norway/1728/2016) were submitted to GISAID with the accession numbers: 11,046,376 and 1,046,371, respectively.

Outbreak history

Sera were collected on the 3rd of March 2016 at a turkey breeder farm as part of the national surveillance program for notifiable avian influenza (AI) in poultry, which investigates serological evidence for infection due to the H5 and H7 AIV subtypes [11]. Initially, antibodies against a highly conserved epitope of the IAV nucleoprotein was detected in 10 out of 10 sera using a blocking ELISA. Further analysis for H5/H7 subtype-specific antibodies using the HI test showed a low-titre reaction against H5 in12 out of 16 sera (titres of 1:8 or 1:16), which prompted immediate infection control measures to be taken at the farm [21].

With the exception of a transient decrease in egg production in February (Figure 1) the flock showed no clinical signs. House 1 and 3 at the farm contained 1000 turkey hens each, while 200 stags were kept in house 2. The entrances to all houses had contaminated and clean zones separated by step-over barriers. All surveillance sera were taken from house 3.

Further testing showed that animals in house 1 and 2 were negative for antibodies against IAV. Also, 20 + 20 (cloacal and oropharyngeal) swabs taken from each of the three houses and cloacal and oropharyngeal swabs and tissue samples from 13 recently dead birds recovered from a freezer at the farm were negative for IAV by rRT-PCR. The absence of clinical signs and pathological changes except for the drop in egg production, which was noted retrospectively, led to the suspicion of a past infection with a low pathogenicity (LP) AIV virus H5 strain.

Concurrent epidemiological investigations revealed that six out of nine (67%) workers at the farm had been sick with flu-like symptoms during the period from 24/12/2015 to late February 2016. Artificial insemination of turkey hens had been performed on a weekly basis since start of lay in January, but on different weekdays in the two hen houses. Antibodies against H1N1pdm09 virus were only confirmed in two of the workers, reporting respiratory illness from early January and February, respectively; H1N1pdm09 infection had been laboratory-confirmed on the 11th of February in one of these, raising suspicion of a human-to-turkey transmission of H1N1pdm09 . An HI test using H1N1pdm09 antigen was performed on IAV positive turkey sera, and

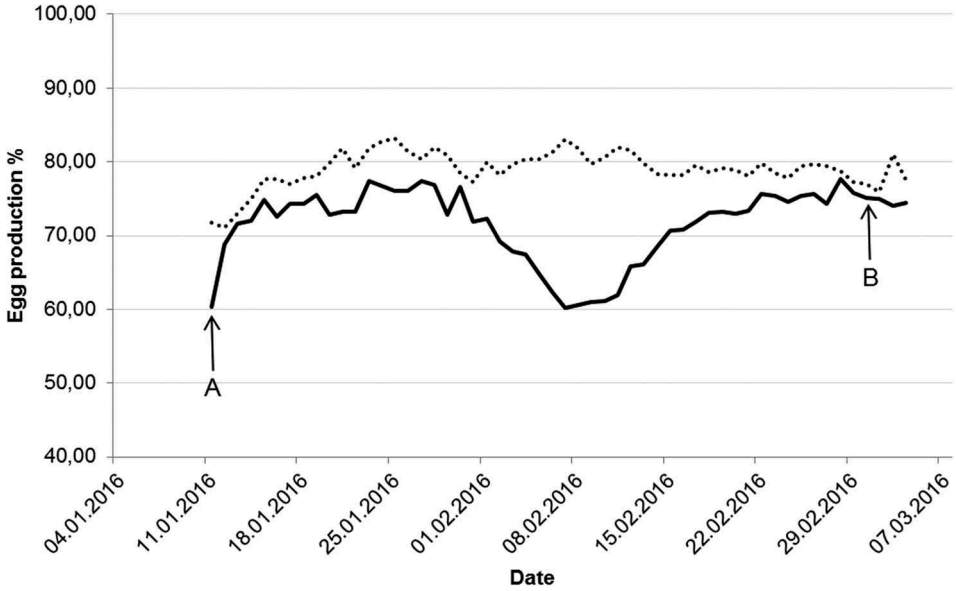


Figure 1. The solid line displays average egg production in the influenza A(H1N1)pdm09 infected turkey flock from start of lay (A) until blood samples seropositive for influenza A were detected in the flock (B). The dotted line shows average egg production in a previous turkey flock at the same farm.Average egg production was calculated as total daily egg-laying rate (%) for both houses at the farm, indicating that the actual reduction in the infected house was greater.

revealed that 95% of the sera were seropositive to H1N1pdm09, and that the majority (>70%) of positive sera had a titre of 1:1024 or higher. Subsequent rRT-PCR analyses showed that two dead turkey hens that had previously tested negative for IAV in cloacal and oropharyngeal swabs and other tissue samples tested positive for H1N1pdm09 in reproductive tissue (ovary and oviduct). Phylogenetic analysis of the human and turkey (partial) H1N1pdm09 sequences provided further evidence for a human-to-turkey transmission (Figure 2) as the virus sequence from one of the farmers and the virus sample from the turkey grouped together phylogenetically. Still, these two samples were not more similar to each other than many other H1N1pdm09 samples from Norway in the influenza season 2015/2016. The turkey sample did possess one unique amino acid substitution in the HA1 gene: N129S, not seen in any other Norwegian samples. This genetic polymorphism does not appear to be associated with any known phenotypic change.

Since infection with an AIV H1 strain is not a reportable disease in poultry, all governmental infection control measures were lifted, and the animals were released for continued egg production.

Results from subsequent testing in the flock

Affected turkey hens

The infected flock was monitored over the next few months with serology, rRT-PCR analyses and post mortem examinations. New blood samples were taken 45, 80 and 106 days post detection (dpd) and analysed with ELISA and HI test. Turkey hens in house 3 remained seropositive throughout the sampling period, but showed a statistically significant decline ($p \leq 0.05$) in seroprevalence for all tests between initial detection and 106 dpd (Figure 3). The homologous HI antigen seems to be more sensitive in detecting the humoral response for longer time intervals than the generic IAV ELISAs.

Post mortem examination of ten euthanized, non-productive hens at 45 and 80 dpd showed no pathological changes. H1N1pdm09 virus was detected in reproductive tissue of 3 out of 10 hens at 45 dpd, but not at 80 dpd. Post mortem examination and rRT-PCR was not performed on day 106.

Poults and hatching eggs

Eggs from the affected farm were collected at the hatchery at different time points in the hatching process. Egg white and embryo tissue from 20 eggs collected at the time of candling, 20 eggs with dead embryos collected at the time of transfer from the setter to the hatcher, and 20 non-hatched eggs tested negative for H1N1pdm09 virus by rRT-PCR. Yolks from 4 out of 10 eggs from house 3 collected at candling were positive for antibodies against H1N1pdm09, whereas eggs from house 1 were negative.

IAV was not detected in turkey poults from the affected farm at any age. In day-old poults, maternally derived antibodies against H1N1pdm09 were detected in 8 out of 10 poults from house 3, whereas poults from house 1 were negative. Blood samples taken from 8 weeks old turkey poults were all negative for antibodies against H1N1pdm09.

Discussion

The H1N1pdm09 virus infection in the Norwegian turkey flock was caused by a human-to-animal transmission of IAV. The virus was only found in the reproductive organs of turkey hens, which has previously been suggested to be a likely route of infection with H1N1pdm09 in turkeys in experimental settings [9]. The absence of virus in other tissue, including cloacal and oropharyngeal swabs, supports clinically that turkeys are refractory to H1N1pdm09 infection through aerosol and oral transmission routes. The biosecurity measures on the farm can be characterized as good, but the farm with its three houses should be considered as one epizootological unit. Given this, house-to-house contamination by fomites, faecal-oral transmission and aerosol transmission would be difficult to prevent since workers with influenza-like illness had access to all houses. The findings that only a single house was infected indicate that the aerosol and faecal-oral routes were not important for transmission. It is therefore likely that the process of artificial insemination is the mode of viral transfer and subsequent infection.

Although flu-like symptoms were reported in several farm workers, H1N1pdm09 infection was confirmed in only one case. Genetic analysis revealed that the turkeys were infected by human H1N1pdm09 virus. The sample from the infected farm worker was not more similar to the turkey sample than other human samples from the same region or other parts of Norway. Thus, the virus transmitter was not identified. Unfortunately, detailed work lists for each of the houses during the insemination process were not available. However, since turkey hens in different houses are inseminated at different weekdays, it is possible that a farm worker shedding virus worked only in one house, but not the other. The absence of systemic and respiratory disease in the flock and the drop in egg production during the period of suspected infection also support a virus transmission during the process of artificial insemination.

While sera from the affected turkey hens were negative for antibodies against the primary H5 antigen recommended by the EURL for the AI HI test (H5N3 A/teal/England/7394-2805/06), other H5 antigens showed a low-grade cross-reactivity against

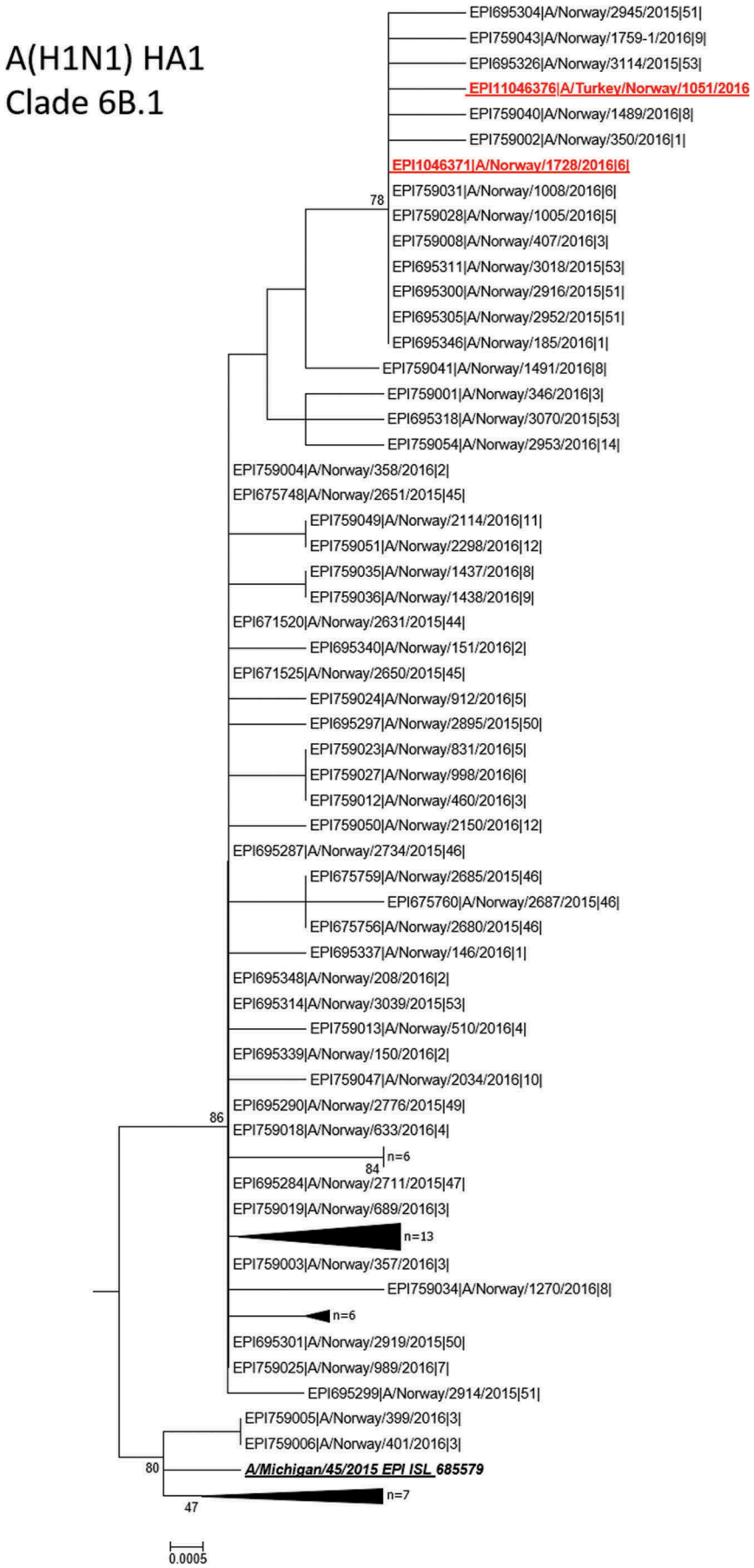
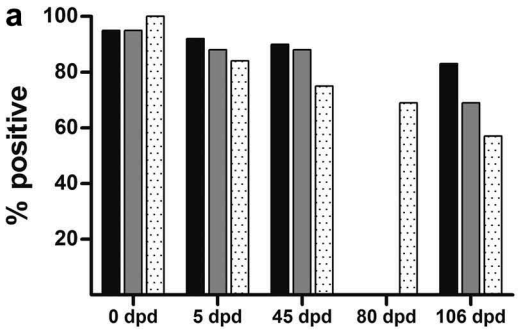


Figure 2. Phylogenetic reconstruction of Norwegian A/H1N1 genetic clade 6B.1 HA genes. (Subtree of all H1N1 viruses from Norway season 2015–16 with different clades representing reference viruses). Clade 6B.1 reference virus is in ***italic bold font***. Aligned partial HA1 gene sequences (856 bases) were subjected to phylogenetic analysis using neighbour-joining of Kimura-corrected genetic distances. The genetic distance between two strains is represented as the sum of the length of horizontal branches connecting them. Bootstrap values above 70% out of 500 resamplings are shown. Norwegian viruses from this season are named as 'GISAID accession number |Isolate ID |week'. The two virus sequences from this study are marked in **red bold font.**



	0 dpd	5 dpd	45 dpd	80 dpd	106 dpd
HI test	19	64	40	nd	42
ELISA A	19	64	40	nd	42
ELISA B	10	64	40	40	42

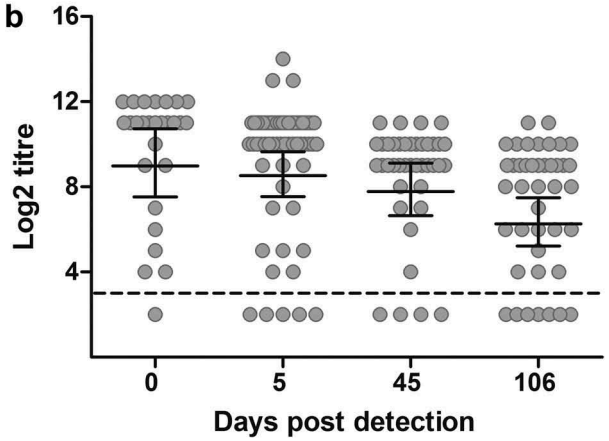


Figure 3. (a) Comparison of percentage positive results between the HI test (antigen A/Swine/California/07/09; H1N1) and two different influenza A ELISAs at different time-points after detection of the seropositive flock (days post detection = dpd). The number of sera analysed with each test at different time-points are given in the table below the figure. (b) Geometric mean (GM) haemagglutination inhibition (HI) test titres (antigen A/Swine/California/07/09; H1N1) at different time points after detection of the seropositive flock. The error bars indicate 95% confidence interval of the GM. The dotted line indicates the test cut-off at titre 1:8 (log2 = 3).

H1N1pdm09. Although for H5N1 a cross-reactivity towards the neuraminidase of H1N1pdm09 was expected, the cross reactivity towards subtypes with different H- and N-antigens (H5N2 and H5N7) was surprising. The same observations have been made in the USA during H1N1pdm09 infection in turkeys (Mia Kim Torchetti, David L Suarez, personal communications). Cross-reactivity was no longer observed in blood samples taken 45, 80 and 106 dpd, and in blood samples that had been frozen for some time. This might indicate that cross-reactive antibodies are not sustained in the same way as specific antibodies; however, further investigations into this matter were beyond the scope of this study.

Our work indicates that the H1N1pdm09 virus is not transferred vertically from parent stock to offspring. On seroconversion, antibodies are present for at least 15 weeks, with a declining prevalence, as expected. Antibodies detected in hatching eggs and day-old poults illustrate transmission of maternally derived antibodies, although their protective capacity in poults has not been investigated.

Although turkeys are not normally regarded as potential 'mixing vessels' for influenza viruses, they are susceptible to a wide variety of IAVs, including those from wild birds, swine and humans, providing the opportunity for influenza virus reassortants [22,23]. In that context, this paper adds to the discussions regarding the usefulness of vaccination in poultry farm workers.

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Disclosure statement

No potential conflict of interest was reported by the authors.

Notes on contributors

Siri Kulberg Sjurseth is currently working as a poultry health expert at the Norwegian Veterinary Institute. She is project leader for several surveillance programs for infectious diseases in poultry, and has long experience with serological diagnostics in production and companion animals. She received her DVM from the Ludwig-Maximillian University in Munich in 1997, and a PhD in immunology from the Norwegian School of Veterinary Science in 2004. Her research interests are immunological responses to infectious diseases in production animals, including immunogenetics.

Britt Gjerset has a research position at the Norwegian Veterinary Institute, Section for Immunology & Virology. The responsibilities includes diagnostics, research, preparedness and surveillance programmes for viral agents, national reference laboratory functions and containment laboratory for notifiable viral diseases in animals. Her research interest includes molecular epidemiology and characterization of viral diseases. She holds a PhD in virology from the Norwegian School of Veterinary Science.

Karoline Bragstad is currently senior scientist at the Norwegian Institute of Public Health, Department for Influenza, WHO National Influenza Centre Norway. Phd degree from the University of Copenhagen, Denmark and former director for the National Influenza Centre Denmark. She has since many years been working within several fields of influenza; surveillance, preparedness, influenza DNA vaccine development and immunology.

Olav Hungnes is a senior scientist in the Influenza Department at the Norwegian Institute of Public Health, where he directs the WHO National Influenza Centre for Norway/National reference laboratory for human influenza. He has longstanding experience in national and international work on influenza surveillance, preparedness, virus evolution and seroepidemiology. He has a Dr.scient. degree in molecular cell biology from the University of Oslo.

Helene Wisløff is currently working as a senior scientist at the Pathology section, Norwegian Veterinary Institute. She got her DVM in 2002 and her PhD in pathology in 2008, both at the Norwegian School of Veterinary Science. She has long experience in diagnostic pathology, research and preparedness concerning mammals and poultry.

Chiek Er has been a veterinary epidemiologist at the Norwegian Veterinary Institute since 2007. He routinely works with national disease surveillance data, and conducts risk analysis. His research work is focused on epidemiology aspects of influenza A(H1N1)pdm09virus infections in the Norwegian pig population. He has a PhD in Epidemiology from the Norwegian University of Life Sciences.

Mette Valheim is working at the Section of pathology at the Norwegian Veterinary Institute. She is working with diagnostic pathology on poultry and domestic mammals. She received her DVM and PhD at The Norwegian School of Veterinary Science. Her research interests are influenza A infections and mycobacterial infections.

Siri M. Løtvedt is a veterinarian currently working as a senior adviser at the Section for Animal Health at the Head Office of the Norwegian Food Safety Authority. She is mainly working with contingency planning and animal disease outbreak management.

Bruce David is currently employed as the chief poultry veterinarian at Nortura SA. He also teaches poultry medicine at the Norwegian School of Veterinary Science. He has previously been employed as the poultry health expert at the Norwegian Veterinary Institute.

Skjalg A. Hanssen is currently employed as a poultry veterinarian at Nortura SA. His main work is on diagnostics and national welfare programs, but he is also involved in major projects concerning poultry health and diseases. Previously he has been working at the Norwegian Food Safety Authority, mainly on poultry health and welfare issues, and poultry meat hygiene. He received his degree in veterinary medicine (cand. med. vet.) at The Norwegian School of Veterinary Science in Oslo.

Siri H. Hauge is Department Director for the Influenza Department at the Norwegian Institute of Public Health. She is a medical doctor trained at the Vrije Universiteit in Amsterdam, and has completed the EPIET training in epidemiology. The Department of Influenza is responsible for both virological and epidemiological surveillance of human influenza in Norway, and runs the influenza vaccination program in Norway.

Merete Hofshagen is currently working as Head of Department for Animal Health and Food Safety at the Norwegian Veterinary Institute. She has been involved in national and international poultry related research projects, and in surveillance activities in poultry and other animals. She received her DVM and her PhD in microbiology from the Norwegian School of Veterinary Science.

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