

# The surveillance programme for avian influenza (AI) in wild birds in Norway in 2016



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ISSN 1894-5678

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Design Cover: Reine Linjer  
Photo front page: Colourbox

## Summary

Surveillance in 2016 did not detect infection with highly pathogenic avian influenza virus in wild birds.

## Introduction

The Norwegian Food Safety Authority is responsible for the implementation of the active surveillance programme for avian influenza (AI) in wild birds. The programme, which started in 2005, is based on virological investigations in healthy, live or hunted birds. The Norwegian Veterinary Institute is responsible for planning, laboratory investigations and reporting components of the programme. The programme has been running from 2005-2007, from 2009-2010, and in 2016.

AI viruses are highly contagious, extremely variable viruses that are widespread in birds. Wild waterfowl such as ducks, geese, swans, waders and gulls are the natural reservoirs for all low pathogenic influenza A virus subtypes. These virus carriers do not usually develop clinical disease, but shed large amounts of virus in their faeces (1). The highly pathogenic avian influenza virus H5N1 is primarily shed via the airways (2).

In domestic poultry infection with influenza A viruses causes two main forms of disease, distinguished by high and low virulence. Most of the low pathogenic AI (LPAI) viruses cause only mild disease in poultry, however LPAI strains of H5 or H7 subtype have the potential to mutate to highly pathogenic AI (HPAI) viruses following introduction to poultry populations. HPAI viruses induce a serious, highly contagious disease of poultry and other captive birds. All HPAI epidemics so far have been of hemagglutinin subtype H5 or H7.

Wild migratory birds have been suggested to play a major role in the spread of HPAI viruses over long distances (3, 4). Virus detections in wild birds mainly occur in migratory duck species, swans, sea gulls and birds of prey. HPAI has never been reported in wild birds in Norway.

## Aims

The aim of the national surveillance programme for AI in wild birds is to study and understand the threats posed by wild birds in relation to influenza viruses of avian origin, with special emphasis on H5 and H7 viruses. In 2016, surveillance for Newcastle disease (avian paramyxovirus type 1) and *Salmonella* infection was implemented in the surveillance programme for avian influenza in wild birds.

## Materials and methods

In 2016 the programme for wild birds consisted of molecular (PCR) screening of cloacal and tracheal swabs from healthy birds shot during the 2016 hunting season. Sampling equipment consisted of flocked swabs and tubes containing virus transport medium for viral sampling, and cotton swabs and tubes with charcoal medium for bacterial sampling. Sampling equipment was sent to hunters in the county of Rogaland, Østfold, Hedmark and Trøndelag. Choice of region was based on relative density of poultry farms in the area, and their overlap with the flyways and resting areas of many species of waterfowl (5). Choice of hunters was based on their proficiency during previous hunting seasons. The hunters were given written instructions on how to collect samples, and were requested to fill in registration forms for individual birds. Swabs were taken from shot birds, placed in transport medium and sent by overnight mail to the Norwegian Veterinary Institute in Oslo. The samples were frozen at -70 °C upon arrival. The sampling comprised nine types of species as shown in Table 1.

## Avian influenza

The samples were registered upon arrival and screened using a real-time reverse transcriptase polymerase chain reaction (rRT-PCR). The screening rRT-PCR used was a pan-influenza A virus rRT-PCR recommended by the European Union reference laboratory for AI (APHA, Weybridge), that reveals the presence of all

subtypes of influenza type A virus. The method does not, however, give information as to which hemagglutinin (HA) or neuraminidase (NA) subtype is present in influenza positive samples. Therefore, the samples found to be positive in the initial pan-influenza A virus rRT-PCR were further tested, using H5, H7 and N1 specific PCRs (6). Hemagglutinin (HA) and neuraminidase (NA) sequence analysis was performed on some of the positive samples (7, 8).

### Newcastle disease

Samples were tested for avian paramyxovirus type 1 (APMV-1) by using the rRT-PCR recommended by the European Union reference laboratory for Newcastle disease (APHA, Weybridge) (9).

### Salmonella

Testing for the presence of *Salmonella* was done according to ISO 6579:2002/Amd.1:2007(E): Annex D: Detection of *Salmonella* spp. in animal faeces and in environmental samples from the primary production stage. In the initial stage, each cotton swab was incubated in 5 mL phosphate buffered peptone water. Serotyping was done by slide glass agglutination according to the Kauffmann-White scheme.

## Results and Discussion

In total, samples from 358 birds were received (Table 1, Figure 1). Of these, 27 (7.5 %) were positive for influenza A virus. None of the samples were positive for HPAI viruses.

Proportions of influenza A virus detected in different species of waterfowl during surveillance were common teal (*Anas crecca*) 14.7 % (5/34), mallard (*Anas platyrhynchos*) 8.5 % (14/164), European herring gull (*Larus argentatus*) 5.7 % (4/70) and Eurasian widgeon (*Anas penelope*) 3.8 % (2/52).

All influenza A positive samples were further tested for the presence of subtype H5 and H7. Out of the 27 influenza A positive samples, two samples were H5 positive, whereas one sample was H7 positive. All H5/H7 positive samples were from mallards. None of these were positive for neuraminidase subtype N1. Sequencing of the HA genes identified all three viruses as low pathogenic (LPAI). Preliminary sequence results indicate findings of HA subtypes H4, H6, H12 and H16 among the non-H5/-H7 positive samples.

Table 1. Number and species of birds tested in the surveillance programme for avian influenza in wild birds in 2016.

Species	No. tested	InfA negative	InfA positive	LPAI H5	HPAI H5	LPAI H7	HPAI H7
Common goldeneye ( <i>Bucephala clangula</i> )	4	4	0	-	-	-	-
Common merganser ( <i>Mergus merganser</i> )	1	1	0	-	-	-	-
Common teal ( <i>Anas crecca</i> )	34	29	5	0	0	0	0
Eurasian widgeon ( <i>Anas penelope</i> )	52	50	2	0	0	0	0
Mallard ( <i>Anas platyrhynchos</i> )	164	150	14	2	0	1	0
Tufted Duck ( <i>Aythya fuligula</i> )	4	4	0	-	-	-	-
Ducks (species unknown) ( <i>Anatidae</i> )	10	8	2	0	0	0	0
Common gull ( <i>Larus canus</i> )	15	15	0	-	-	-	-
European herring gull ( <i>Larus argentatus</i> )	70	66	4	0	0	0	0
Great black-backed gull ( <i>Larus marinus</i> )	4	4	0	-	-	-	-
<b>Total</b>	<b>358</b>	<b>331</b>	<b>27</b>	<b>2</b>	<b>0</b>	<b>1</b>	<b>0</b>

Sixty-five samples from randomly selected gulls were tested for *Salmonella* (Table 2). *Salmonella* Typhimurium was found in one sample from a European herring gull. The other samples were negative.

None of the 358 samples were positive for avian paramyxovirus type 1.

From 2009 the total number of samples collected in the surveillance programme for avian influenza in wild birds was greatly reduced. In addition, suspension of the programme from 2010 to 2015 impedes the study of temporal trends in AI prevalence in wild birds at annual intervals. However, the prevalence of AI infection amongst wild birds tested in 2016 was lower than in 2007, 2009 and 2010 (Figure 2) (10, 11, 12).

Table 2. Number and species of gulls tested for Salmonella in the surveillance programme for avian influenza in wild birds in 2016.

Species	No. examined	Salmonella negative	Salmonella positive	Serotyping
Common gull ( <i>Larus canus</i> )	15	15	0	-
European herring gull ( <i>Larus argentatus</i> )	46	45	1	<i>S. Typhimurium</i>
Great black-backed gull ( <i>Larus marinus</i> )	4	4	0	-
<b>Total</b>	<b>65</b>	<b>64</b>	<b>1</b>	

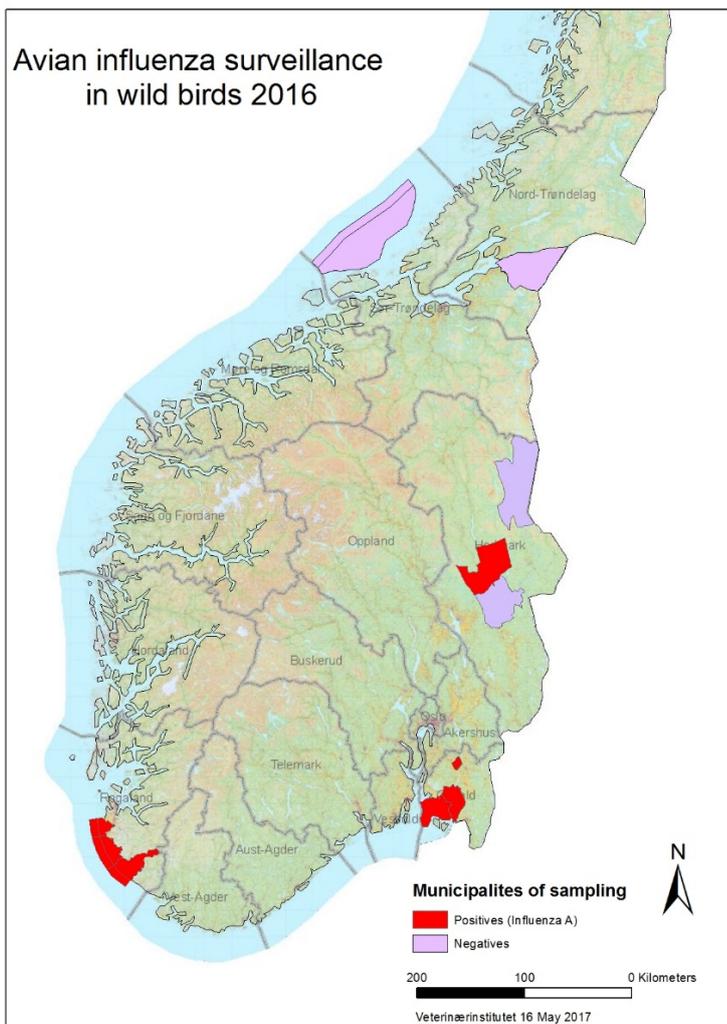


Figure 1. Map showing regions of wild bird sampling during the 2016 surveillance programme for avian influenza in wild birds. Red colour marks regions where birds positive for influenza A were sampled, whereas pink colour marks regions with only negative results.

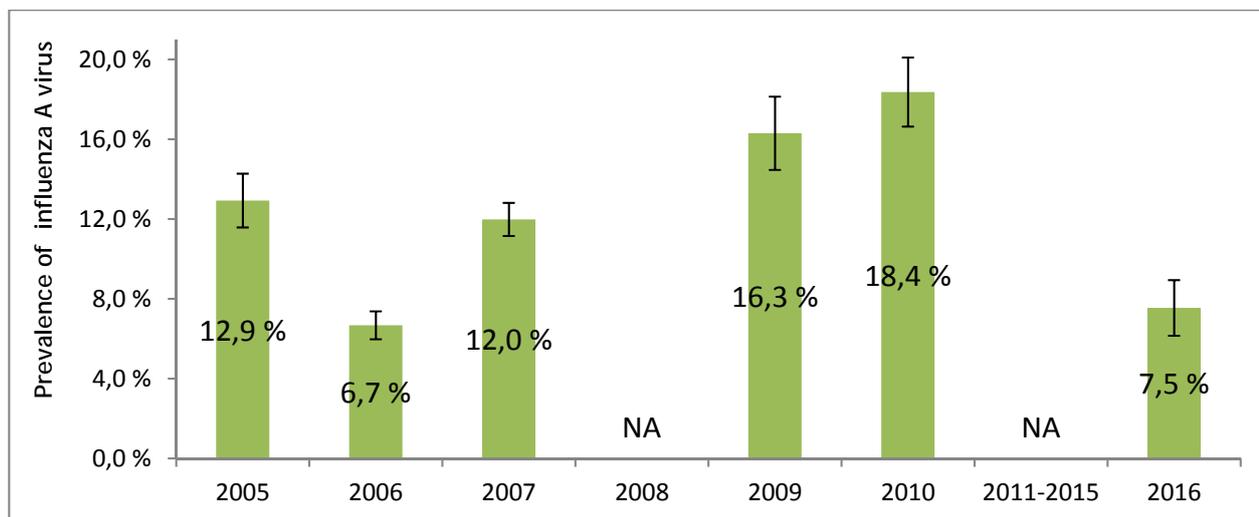


Figure 2. Prevalence of influenza A virus in ducks and gulls in the surveillance programme for avian influenza in wild birds from 2005-2016.

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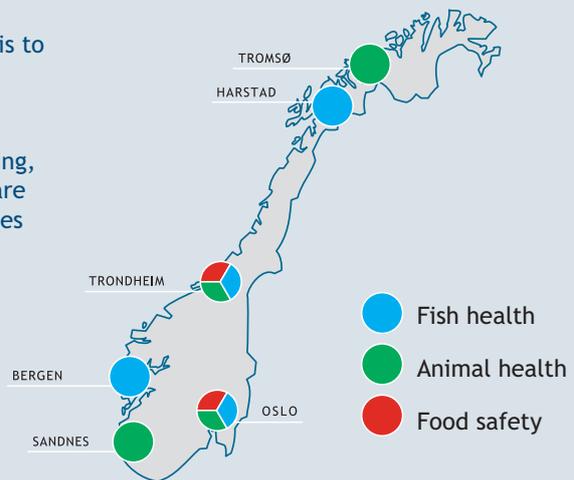
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