

**The avian influenza virus H5N1 -
possible consequences for the NTNU animal
facilities**

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The avian influenza virus H5N1

Pandemics is an epidemic that is geographically widespread; occurring throughout a region or even throughout the world. Influenza experts have predicted the next pandemic flu for many years [1]. Novel viruses that are able to bring about pandemics are not a new issue to the world. In the 20th century the world experienced three severe outbreaks of influenza that killed millions of people world wide [2]. The most severe of these pandemics were “The Spanish flu” which alone killed 50 - 100 million people, whom 13 000 - 15 000 in Norway [3]. Less severe outbreaks occurred in 1957 by the “Asian flu” and in 1968 by the “Hong Kong flu”[2]. All three of these pandemics were caused by Avian influenza type A viruses and at present we might be facing the threat of another pandemic caused by a new virus, namely the H5N1 avian influenza virus [1]. The H5N1 virus is mainly an avian virus however, incidents of transfection from bird to human and other mammals has been reported in some rare cases. To this date WHO has reported 122 cases of confirmed infection, of which caused 62 deaths [4]. It is now a concern that the virus will go through the mutational alterations necessary for fast and easy transfer between humans.

In this assignment we want to look into the possible risk of H5N1 avian flu infection in the animal facilities at NTNU, we will suggest methods of increased biosecurity, and give advice to the competent person and the staff working with the animals.

Virology of H5N1

Influenza viruses are small packets of single stranded RNA enclosed in a lipid capsule. The genome consist of eight segments encoding 10 proteins [5]. The eight segments have common nucleotide sequences at the 5' and 3' ends which are necessary for replication of the genome [4]. These sequences are complementary to one another and the ends of the genome segments are held together by base pairing, forming hairpin structures, which are involved in replication. The RNA genome segments are packed in association with the gene 5 product, the nucleoprotein are visible in electron microscope as helical structures, see figure 1.

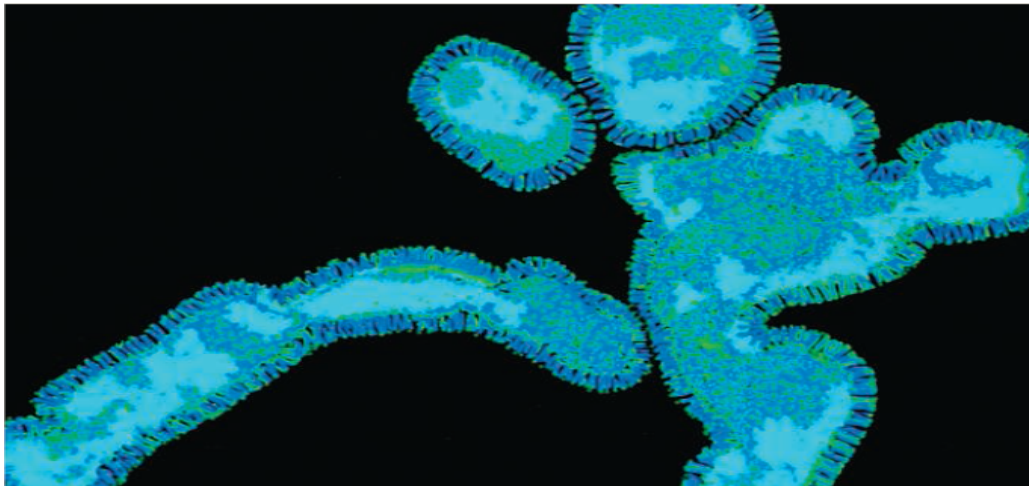


Figure 1 : RNA genome sequences of the influenza virus are packed in association with a nucleoprotein and is therefore visible by electron microscopy.

The lipid layer of the virus is perforated with two different glycoproteins, hemagglutinin (HA) and neuraminidase (NA) and one transport protein (M2) (see figure 2). The glycoproteins make it possible for the virus to detect and enter the host cell and use the cell's protein machinery to produce new viruses. Immunity to the virus is obtained if the infected organism is able to recognise HA and NA proteins on the virus surface. M2 is protein channels which regulate the pH inside the lipid capsule, the antiviral medicine has the ability to interfere with these channels causing unfavourable conditions inside the viral capsule [4].

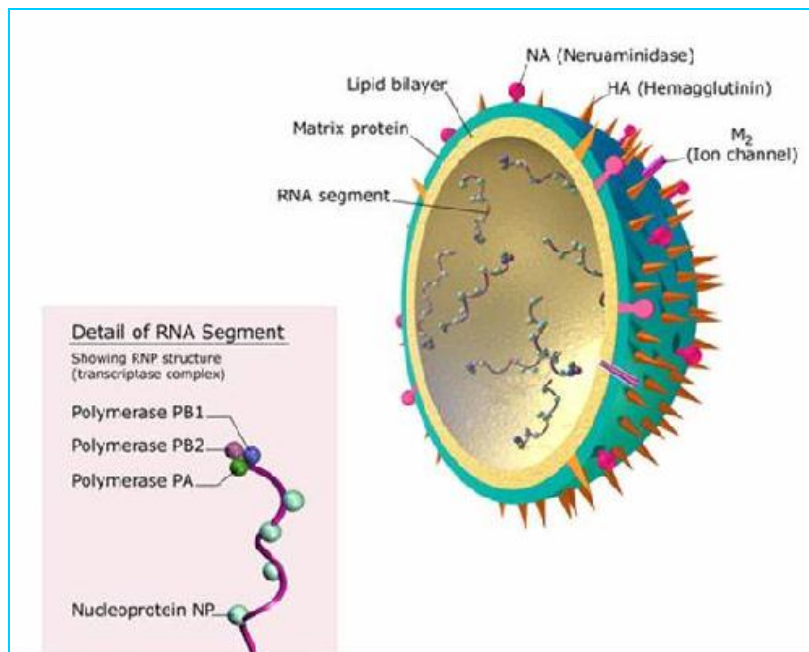
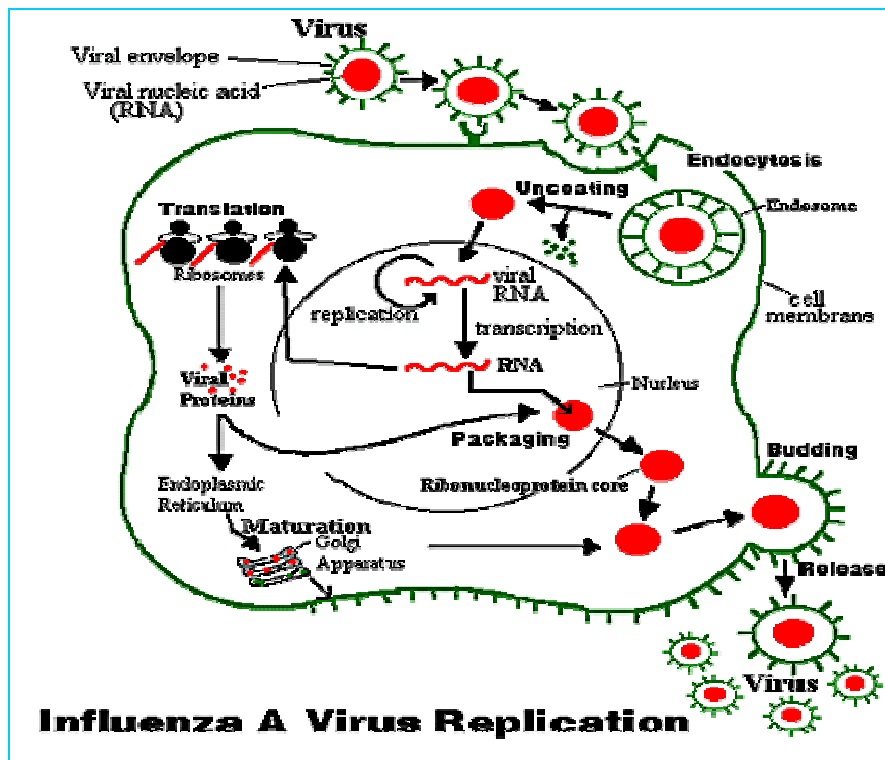


Figure 2: The influenza A virus with its segmented genome, lipid capsule and proteins.
(www.niaid.nih.gov/newsroom/focuson/flu04/)

How does the virus infect?

The viral attack starts with binding of hemagglutinin molecule to carbohydrate anchored on the host cells glycoproteins . This mediates endocytosis by forming an endosome (endocytic vesicle). A drop in pH of the endosome produces a change in the structure of the viral hemagglutinin. This enables fusion between the viral membrane and the vesicle membrane and release of the virus content. The viral RNA enters the nucleus of the cell where the replication of the viral genome will occur. Replicated RNAs returns to the cytosol where they serve as mRNA molecules in the protein synthesis, producing viral proteins. Finally fresh viruses buds off from the plasma membrane of the cell (aided by the neuraminidase) thus spreading the infection to new cells, see figure 3 [6].



Figur3 : General infection and replication of influenza viruses.

Characteristics of avian influenza A viruses that increase the chance of a pandemic

In addition to humans, they are known to infect pigs, horses sea mammals and birds

The different subtypes make up a diverse genetic collection which gives higher potential for negative mutations and makes it difficult to fight.

They regularly cause seasonal epidemics in humans, some times with heavy tolls in morbidity and mortality

They undergo antigenic drift which serves as a survival tactic because it alter their antigens making it harder for the host organisms immune system to detect the virus [7]

Bird migration transports the virus to new and uninfected locations

Frequent mutations

Normally viruses go through a genetic drift where one sees gradual accumulation of minor mutations in the virus genome (see figure 4). This leads to altered coding potential and therefore altered antigenicity [8]. The immunesystem of the host organism constantly adapts to novel antigenic structures. A sudden and dramatic change in the antigenicity of the virus due to reassortment of the segmented virus genome with another genome of a different antigenic type are called antigenic shift (see figure 4). This will cause a failure of the immunesystem to recognize a new antigenic type and an infection is established [8].

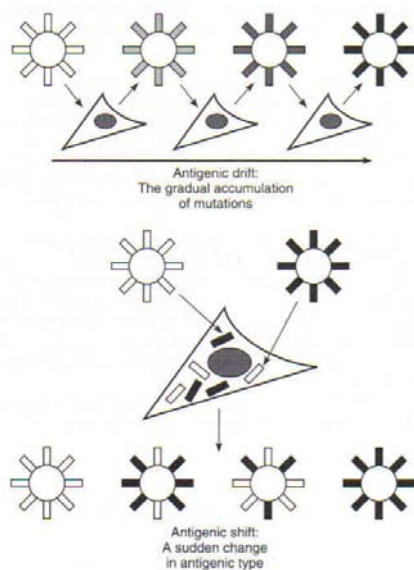


Figure 4: Genetic drift is a slow natural viral evolution and adaptation and genetic shift is rapid alterations of the viral genome due to multiple infections in a host cell.

15 different avian influenza A subtypes are known to infect birds. The different subtypes make up a diverse genetic collection which gives higher potential for negative mutations and makes it difficult to fight. The virus subtypes are named after the influenza type, host of origin, the place of isolation, the strain number, the year of isolation and composition of their HA and NA glycoproteins. The full name of H5N1: A/Duck/Vietnam/11/048(H5N1). Avian Influenza A is known to have 16 H subtypes and 9 N subtypes.

Reservoir hosts

The avian influenza viruses circulates in aquatic bird populations, were the virus seem to have found optimal conditions, making up a complex reservoir of potential harmful viruses [7]. Bird migration transports the virus to new and uninfected locations. The avian influenza virus can in addition to transfer from birds to birds, be transferred from birds to human, pigs, horses and sea mammals [9].

Transmission and symptoms

Birds

Transmission between birds occurs directly or indirectly through feces or aerosols, water and food contaminated with the virus. The incubation time differs among different viruses, ranging from 24h to a couple of weeks depending on bird species and virus subtype [10]. Exposure of the H5N1 virus in different species showed a broad spectrum of reactions from mild respiratory illness, depression and anorexy to the most severe fatal systemic diseases [11]. Viruses capable of inducing severe epidemic in bird population (fowl plague) are categorized as highly pathogenic and are currently restricted to two different subtypes, the H7 and H5. Symptoms of poultry infected with highly pathogenic viruses includes, decrease in egg production, respiratory signs, edema of the head, diarrhea, neurological symptoms and death [4].

Mammals

Through genetic modification viruses can be able to transfer from birds to other animals, this interspecies transfer has been detected in seals [12], horses, pigs, cats, tigers and leopards. The danger of infection rises when closely related species are kept in the same environment, but distant related species are also capable of infecting each other. Hens are known to have the ability to infect pigs and also humans with the virus [10].

Humans

Humans are mainly infected through direct contact with birds or objects contaminated with feces from birds. To this date infected people usually live in rural areas were families having their own poultry flocks, which often roam freely. An infected bird disperses large quantities of virus in its feces making it a risk for humans, especially kids playing in the area. Human exposure is also likely to occur during slaughtering, defeathering, butchering and in preparing the bird for cooking.

Human to human dispersal of the virus has been reported in some rare incidents between family members, but it seems that today's virus does not infect through aerosols. A pandemic outbreak seems therefore not likely unless the virus mutates into a more contagious form [6].

The result of a normal non lethal influenza infection usually gives the victim soar throat, fever and in worst case pneumonia. H5N1 and other pathogenic subtypes gives the same symptoms but evolves into severe infection in the respiratory system, and in some cases diarrhea, coma, renal failure and immune-mediated pathology killing 50% of the infected people [4]. Autopsy of deceased patients have revealed high concentrations of the virus mRNA in both respiratory, gastrointestinal tract an nervous system, indicating replication at these locations [10].

How can a viral infection be detected?

When it is suspicion of an animal or human infection of an avian virus it is important to run tests to confirm or disprove presence of highly pathological types of the virus, so that these individuals can be isolated from the rest of the population. The analyses must therefore be able to distinguish between dangerous subtypes and normal less virulent viruses.

Detection of influenza A viral antigens by methods using antibodies (immunofluorescence, immunochromatographic and ELISA) are widely used in diagnosis of human influenza. However, in patients with avian influenza these assays seems limited because they are mostly not able to distinguish A, B and C viruses, and none of these kits can detect different subtypes of influenza A [4].

RT-PCR (Reverse transcriptase polymerase chain reaction) methods allow for sensitive and quantitative measurements of influenza A subtype nucleic acid. During an early outbreak in

Hong Kong and southeast Asia, RT-PCR for specific detection of H5N1 viral RNAs have proven valuable and seem the diagnostic method of choice in case of an outbreak of highly pathogenic avian influenza [4].

How can we protect ourself from the virus?

Profylactic

Although a vaccine against H5N1 are under development, no efficient vaccines protecting from a pandemic variant of avian viruses are available at this moment [6].

Drugs

Currently, two classes of drugs with antiviral activity against influenza viruses are available on the market. Amantadine and rimantadine inhibit the ion-channel activity of the M2 protein located in the plasma membrane resulting in decrease in fever and illness in adults and children. Major disadvantages of these drugs include neurotoxicity and rapid development of drug resistance during treatment. Some types of the H5N1 virus seem to be resistant to these drugs [4].

Oseltamivir and zanamivir are known to inhibit the neuraminidase (NA) glykoproteins in the viral capsule. Both drugs have proven effective when administered early during the course of illness. The ability to form resistance during treatment has been reported for both drugs. Some patients infected by H5N1 have been given these drugs, but no statistics of treatment are available at the time [4].

Domestic animals

Most cases of avian bird flu in humans have been in people with close encounter with poultry [13]. Eliminating the source of infection has been the most effective way of controlling the earlier outbreaks in countries like Hong Kong, Netherlands and Canada. However, considering the large geographical area, unreported incidents and established knowledge of infectious migrating birds it seems that culling and destruction of domestic animals is not enough to control a widespread dispersal of avian virus [4].

Biosecurity

The concept of prevention of disease entry (or escape) is called biosecurity and a break within the sanitary barrier of biosecurity measures will increase the risk of infection entry [14].

The H5N1 virus is excreted in large concentration through faeces, and the main route of transmission is via direct and indirect contact with infected birds [13]. There is a possibility of people bringing the virus into the facilities after handling domestic poultry like ducks. Ducks and Geese are able to excrete large quantities of lethal virus without showing signs of visible disease [15].

There are ways to increase Biosecurity and general advices to avoid outbreaks in poultry farms can be transferred into an animal research facility [14].

- Prevent access of strangers to areas where animals are housed

- Provide protective clothing, including “shoe-bags”, to anyone visiting

- Ensure that workers do not own birds of their own

- Ensure that all animal health officials visiting premises are aware that they could be responsible for spread of infection and disease

- Be aware of the origin of food and water and check their quality regularly

- Clean and disinfect any equipment and instrumentation to be used

- Ensure that all animals introduced to the facilities are healthy, obtain health certification if possible

- Establish quarantine area for housing new animals and handle and feed new animals last

- Use separate workers to handle the different animals if possible

- A period of quarantine should be maintained for staff, researchers and students after working with birds in other facilities or in countries that have outbreaks of the avian influenza

The risk of infections in the animal facilities of the Department of Biology

Is there a risk of avian flu infection H5N1 in the animal facilities of the Department of Biology at NTNU? At the moment Zebra finches are kept at the facilities, and plans of maintaining a stock of Ptarmigans have been made.

The introduction of the virus into our facilities will possibly occur through:

introduction of infected animals to the facilities

through infected food

contaminated material brought by people (clothes, shoes and soiled hands)

No incidents of the avian influenza H5N1 has been identified in Norway. In the case of an outbreak in Norway, the government will inform the public, give advice and introduce rules and regulations necessary to minimize the damage through Folkehelseinstituttet, Sosial- og helsedirektoratet, Mattilsynet and Veterinærinstituttet. Also, an outbreak in our neighbour country Sweden will result in the same alert since birds do not respect the borders. The table shows the status of the European countries at the 7th of November 2005.

Table 1: Confirmed and suspected instances of the avian influenza virus H5N1 in poultry and wild birds of Europe by November 7th, 2005 [16]

Land	Provins/ region	Art	Mistanke dato	Bekreftet dato	Type
Tyrkia	Balikesir	Kalkun	01.10.2005	13.10.2005	H5N1
Romania	Tulcea	Ender og høns	07.10.2005	16.10.2005	H5N1
Russland	Tula	Ender, moskusender, høns, gjess og kalkun	14.10.2005	19.10.2005	H5N1
Kroatia	Virovitico-Podravaska	Svaner	19.10.2005	26.10.2005	H5N1
Kroatia	Osjecko-Baranjska	Svaner	21.10.2005		
Romania	Vaslui	Hegre	21.10.2005	27.10.2005	H5N1
UK	I karantene	Papegøye	21.10.2005	23.10.2005	H5N1
Russland	Stavropol		23.10.2005		
Russland	Tambow		23.10.2005		
Romania	Constanta	Svaner, gjess	01.11.2005		

Consequences of a possible infection

A possible outbreak of H5N1-virus in the animal facilities will be a tragedy for the animals and the researchers conducting experiments in the facilities. However, the possible infection of humans and the development of a “bird-flu epidemic” among staff and their families is the biggest threat.

Zebra finches

The zebra finches kept at the facilities today originated from birds that were brought in during year 2000, and there are no plans of introducing new individuals in the near future (personal communication with B.Moe). In a study by Perkins and Swayne the pathogenicity of a Hong Kong origin H5N1 avian influenza virus was investigated in four passerine species and budgerigars. The virus was administered intranasally. In this study Zebra finches was the most severely affected species demonstrating anorexia, depression and a 100% mortality within 5 days of inoculation [11]. The route of transmission will influence the degree of susceptibility to the H5N1 however, it is certain that the zebra finches are at a potential risk of infection if the virus should enter the facilities.



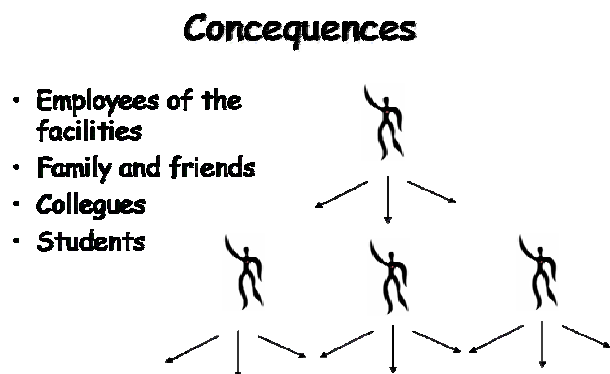
Ptarmigans

“Poultry” means live chickens, doves, ducks, geese, grouse. Poultry are susceptible to avian influenza. Ptarmigans (*Lagopus sp.*) live wild in alpine and arctic tundra throughout the northern hemisphere [17]. There are plans of introducing ptarmigans to the animal facilities in the near future. The introduction of wild birds into the facilities will be safe as long as the new

birds are uninfected by diseases that possibly could harm the other animals of the facility. As described earlier some species of wild birds can be infected by lethal avian influenza virus without showing signs of visible disease [15]. There has not to our knowledge been any findings of the H5N1 virus in Ptarmigans or any of their close wild relatives [18], in addition to that the virus is not yet found in Norway [16] indicates that import of wild Ptarmigans to the facilities is not a violation of the biosecurity of the facilities. However, one should be aware of changes in the situation, especially as birds start to migrate when seasons change [16].

Human

So far the H5N1 virus has not demonstrated easy transmissibility between animals and people or between persons. Investigations in Vietnam and Thailand indicate limited risk of human to human transmission [19]. Risk of this particular virus a possible infection will affect the staff and their close relations like families and colleagues see figure 5. Transfer of the H5N1 influenza form bird to human could start an epidemic or in the worse case scenario give rise to a pandemic.



Possible epidemic?

Figure 5: If the H5N1 virus enters our animal facilities, it may have severe consequences for the staff and their close relations.

Quarantine

In a scenario where an outbreak of an H5N1 strain able to infect humans is detected, the facilities will most likely be closed down. The facilities will be in quarantine for a long time and all activity concerning birds will have to end. This will of course harm the department of Biology since many employees and students will have to end their projects.

Suggestions

Today the situation of the animal facilities is that there are only a limited number of people with access to the facilities, this reduces the chance of infection. Also the staff members are trained in handling of animals and keeping a sanitary environment. However, there is always the possibility of improvement and one should increase the biosecurity to minimize the risk of introducing the virus into our facilities in the future.

All employees and students of the department involved in animal experiments and research must be aware of the development of possible transmission between species and emergence of H5N1 in Scandinavia. The person in charge should at all time be updated and he or she must make sure all users of the facilities are informed. However, routines that ensure biosecurity at the facilities will help prevent access of a possible infection. It is important to compare the cost of restricted use to the actual danger of an outbreak.

At present there are no indications that there is a danger of H5N1 entering our animal research facilities.

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