Influenza on Dutch mink farms in 2011

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Abstract

In the middle of May 2011 three farms in the Netherlands claimed increase of mortality of the young pups round the age of 15 days old. The three farms were scattered over The Netherlands. Employees of the diseased farms even as the farmer and his family were suffering from influenza like symptoms. The pups at the 3 farms feel bad and show pumping breath like the acute form of plasmacytosis. The mortality of the pups at the 3 farms run from 11% to 30%. The lungs of the pups at the obduction were very red and were sinking in water. Histological research prove that the pups had an acute interstitial pneumonitis, perhaps caused by a viral infection. Bacteria were not found. Immunohistochemical stains for influenza type A were positive in the lungs of these pups. The PCR influenza A H1N1 was positive in all three cases. In the ELISA test for influenza antibodies from blood samples of ad random chosen adult females at the diseased farms run from 21.8% to 97.3% positives. The ELISA test demonstrated that 72% of 25 selected farms scored positive in this test. The positive females run from 2.7% to 97.3%. Sixty percent of these farms had no clinical symptoms with the pups. Retesting in August of 25 females who lost one or more pups at the diseased farms, demonstrated that mothers were 64% to 96% positive reactors in the ELISA test. Positive reacting pups in the ELISA test were running from 6% to 12%. Till this moment it is not yet clear where the infection at the stroked farm came from and why the farms with minks with positive Elisa for influenza had no clinical problems.

Keywords: mortality, plasmacytosis, pneumonitis, serology, zoonosis

Introduction

Influenza A viruses are highly contagious pathogens that have been isolated from a wide variety of animals, including humans, birds, pigs, horses, minks, seals, whales, cats, dogs and most recently from skunks (Yassine *et al.*, 2009; Britton *et al.*, 2010). The segmented nature of the influenza A virus genome (8 individual segments of single stranded RNA) enables genetic reassortment which can lead to the generation of novel strains of the virus (Yassine *et al.*, 2009).

Different strains can induce respiratory problems in minks (*Neovison vison*). In Japanese experiments (Matsuura *et al.*, 1979; Okazaki *et al.*, 1983a,b) it was possible to infect minks with influenza A strains H3N8, H3N2, H11N4, H7N7, H8N4 and H5N3 (1,2,7). During an outbreak in 1990 of respiratory disease in mink in the south east of Sweden (Berg *et al.*, 1990; Englund, 2000) was influenza A H10N4 and H10N7strain isolated.

During the outbreak in 2009 at 11 mink farms in North-West Jutland of Denmark with respiratory problems was the morbidity 0.2-1.3% and the mortality 0.1-2.35%. Registered respiratory problems 2 days before dying were sneezing and coughing. The dead animals were

in good condition. On necropsy the lungs showed acute pneumonitis sometimes related with *E. coli* bacteria. At 9 farms the PCR influenza A H3N2 was positive (Criél *et al.*, 2010). A recent publication on a 2010 H3N2 influenza outbreak on a 15,000 head mink farm in the Midwest United States describes a severe respiratory distress and nose and/or mouth bleeding, with losses averaging 10 animals per day. Both adults and kids were affected (Yoon *et al.*, 2012).

Outbreak influenza in the Netherlands

In the middle of May of 2011 when the mink pups were between 10-15 days old suddenly the mortality raised at 3 farms in the Netherlands. Two farms were located in the south and 1 farm in the west of the Netherlands. Two farms got food from one feed producer and 1 farm from another feed producer. The employees of the 3 farms claimed respiratory problems. One employee had severe respiratory problems and spend 2 days in hospital.

Before the pups died they showed a severe pumping, costo-abdominal, breath. Two farms had a history of plasmacytosis (Aleutian disease). One farm was free of plasmacytosis and got new animals in 2011. The farmers claimed an amount of empty females between 20%-30%. The females showed no clinical respiratory symptoms. The problems at the 3 farms lasted round about 21 days with an total loss of the kids respectively 11%, 23% and 30%. All the kids with clinical signs died. The first impression was acute plasmacytosis because the first pathological histological findings showed acute pneumonitis, caused by a virus infection. The immunohistochemical staining for distemper was negative.

The farmer of one of the plasmacytose positive farms told that the problems were started in one corner of the farm and spread from that corner over the farm. In that corner were many wild birds. After these information was further research started with influenza as one of the possible causes of the problems at these farms. In the Netherlands influenza was not diagnosed in minks with respiratory problems before 2011.

Pathological examination

Submitted mink kids were examined grossly. On macroscopy lungs were heavy, oedemateus and red. Other organs were not affected. Diverse tissues were stored in 10% buffered formalin and in -70 °C storage. Formalin-fixed tissues were embedded in paraffin and stained with heamatoxylin and eosin stain (HE-stain) for histopathological examination. Histopathology showed severe interstitial pneumonia with mild necrotizing bronchiolitis. An immunohistochemical staining for pan-influenza A virus was performed (M-x-INF A; clone 1331).These showed positivity in alveolar type 1 cells, alveolar macrophages, and scarcely in bronchiolar epithelial cells. No bacteria were cultured from these lungs.

Material serological screening

One heparin micro tubes blood was sampled per animal by cutting of a toe nail for the serological screening June 2011. After centrifugation was the serum re-set on Elisa test paper. At the 3 hidden farms from 25 females who lost the most of their pups and her remaining pups, 2 cc blood was

sampled out the vena jugularis in August 2011. More blood was necessary if it should be wishfully, if serum samples were positive in the Elisa test, to type the influenza antibodies in the HAR test.

Tests

The Elisa test gave only information about the H antigen of Type A influenza. It could be every influenza type who belonged to A type. Differensation of Influenza was not possible with this test. With PCR it was possible to find the influenza virus type. For typing of influenza antibodies in serum is the HAR (Haeam Agglutination Reaction test) used. The Counter Immuno Electro Phoresis (CIEP test) test is used to detect antibodies against plasmacytosis.

Screening results

Twenty five farms were in June 2011 examined with the Elisa test on antibodies against influenza. Per farm were 100 samples collected. In the Elisa were in 72% (18 farms) positive on antibodies against influenza. 28% (7 farms) of the examined farms had not antibodies against influenza. It was not possible for all the farms to find back the empty females and the females who lost all their kids and the females who lost a more than 50% of their litter (Table 1). There was a great difference between the percentages of positive females between the farms. Farm W, X and Y were the farms with outbreak of influenza.

In August 2011 was blood screened in the Elisa test from the thee affected farms. Twenty five females per farm, who lost a great part of their pups, and her remaining pups (Table 2). In October 2011, 99 selected females at the 3 hidden farms were tested in the Elisa screening.

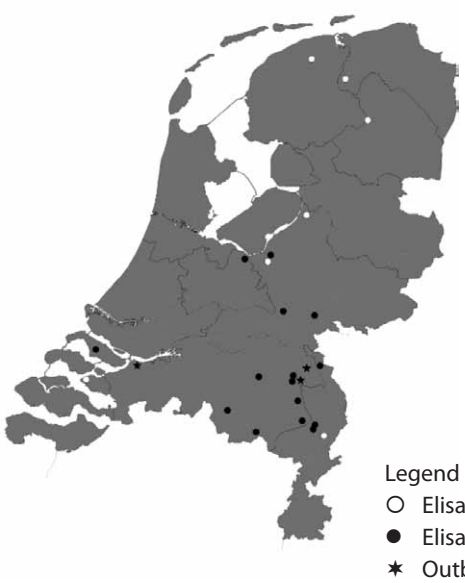

Figure 1. Localisation screened farms June 2011 in the Netherlands.

Table 1. Percentage positive Elisa blood samples at 22 selected farms and 3 affected farms.

Farm	Total	Empty females and zero litters	Females with pups
A	21.2	24.0	12.5
B	29.0		
C	19.0	14.7	32.0
D	8.3		
E	5.0		5.0
F	11.8		
G	0.0		
H	0.0	0.0	0.0
I	0.0		
J	2.6		
K	23.0	18.7	36.0
L	0.0	0.0	0.0
M	0.0	0.0	0.0
N	35.6	35.5	36.0
O	35.8	6.3	16.7
P	11.1		
Q	0.0	0.0	0.0
R	11.0		
S	10.1		
T	0.0	0.0	0.0
U	8.0		8.0
V	2.7	1.3	5.4
W	21.8		
X	45.0	45.0	
Y	97.3		

Table 2. Percentage positive females and kids in the Elisa test at the affected farms August 2011.

Farm	Females (n=25) + pups (n=100)	Females (n=25)	Pups (n=100)
W	19	64	6
X	22	48	6
Y	30	96	12

Table 3 shows a review about the results after 3 times screening research in June, August and October 2011 at the affected farms.

Table 3. Percentage positive females in the Elisa test at the affected farms.

Farm	June: females (n=100)	August: females (n=25)	October: selected (n=99)
W	22	64	54
X	45	48	9
Y	97	96	30

Conclusions and discussion

The first farm visit and the first microscopically results gave the impression of acute viral inflammation perhaps according to plasmacytosis. Plasmacytosis could not be likely because two farms were positive on plasmacytosis and one farm not. Females with sick kids at the plasmacytosis free farm scored negative the CIEP test for antibodies against plasmacytosis. There must be another acute viral infection. To exclude distemper, in relation to the acute pneumonitis, was the immunohistochemical staining for distemper done. This staining was negative.

After the information from the farmer of one of the affected farms, that he had problems with many birds in the corner where the respiratory problems started, new fresh lungs from all the tree hidden farms were positive tested on influenza virus type A in the Immunohistochemical staining. In the PCR were the lungs of the kids from the three affected farms positive of H1N1, the humane variant agree to Mexican flu.

Influenza was an zoonosis and employees of three farms had clinical respiratory problems. It was not excluded that mild Influenza virus types in animals can transform in more aggressive types for human people (Gonzales *et al.*, 2012).

Reason to start a blood screening programs at 22 a-selected mink farms in the Netherlands. The results were important to react adequate at questions and rules formulated by the Dutch Government for the mink production in the Netherlands. In 2012 the Elisa blood screening program will be continued and blood of positive reacting minks tested on influenza virus type in the HAR test.

To control the possible clinical influenza symptoms of human people at and round all the Dutch minks farms were the farmer, his family and his employees advised to vaccinate themselves yearly against influenza.

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