

Articles

Transmission of H7N7 avian influenza A virus to human beings during a large outbreak in commercial poultry farms in the Netherlands

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Summary

Background An outbreak of highly pathogenic avian influenza A virus subtype H7N7 started at the end of February, 2003, in commercial poultry farms in the Netherlands. Although the risk of transmission of these viruses to humans was initially thought to be low, an outbreak investigation was launched to assess the extent of transmission of influenza A virus subtype H7N7 from chickens to humans.

Methods All workers in poultry farms, poultry farmers, and their families were asked to report signs of conjunctivitis or influenza-like illness. People with complaints were tested for influenza virus type A subtype H7 (A/H7) infection and completed a health questionnaire about type of symptoms, duration of illness, and possible exposures to infected poultry.

Findings 453 people had health complaints—349 reported conjunctivitis, 90 had influenza-like illness, and 67 had other complaints. We detected A/H7 in conjunctival samples from 78 (26.4%) people with conjunctivitis only, in five (9.4%) with influenza-like illness and conjunctivitis, in two (5.4%) with influenza-like illness only, and in four (6%) who reported other symptoms. Most positive samples had been collected within 5 days of symptom onset. A/H7 infection was confirmed in three contacts (of 83 tested), one of whom developed influenza-like illness. Six people had influenza A/H3N2 infection. After 19 people had been diagnosed with the infection, all workers received mandatory influenza virus vaccination and prophylactic treatment with oseltamivir. More than half (56%) of A/H7 infections reported here arose before the vaccination and treatment programme.

Interpretation We noted an unexpectedly high number of transmissions of avian influenza A virus subtype H7N7 to people directly involved in handling infected poultry, and we noted evidence for person-to-person transmission. Our data emphasise the importance of adequate surveillance, outbreak preparedness, and pandemic planning.

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See Commentary page 582

Introduction

On March 1, 2003, the Dutch Ministry of Agriculture announced a ban on the export of all poultry and poultry-related products. This measure was taken in response to outbreaks of a disease highly lethal to chickens on six farms in the province of Gelderland, an area with a high density of poultry farms. The infection spread to 255 farms, and the Ministry's order for the culling of all infected flocks led to the killing of around 30 million chickens—about 28% of the total chicken population in the Netherlands. The annual export value of poultry and eggs contributes €284 million to the Dutch economy every year.

The pathogen was identified as a highly pathogenic avian influenza A virus (HPAI) subtype H7N7, and was related to viruses detected in 2000 during routine surveillance of avian influenza in ducks in the Netherlands. All internal genes of the viruses were of avian origin.¹ Epizootics and solitary infections of A/H7N7 avian influenza virus in poultry have been reported in surveillance studies, and humans were thought to be at low risk of infection, although there have been occasional reports of H7N7-associated conjunctivitis.^{2–4} In 1996, influenza A/H7N7 virus (A/England/268/96) was isolated from a 43-year-old duck owner with mild one-sided conjunctivitis.^{4,5}

In the week following the announcement of the avian influenza outbreak, four independent anecdotal reports suggested an increased incidence of health complaints, particularly conjunctivitis, in people involved in the control of the epizootic. Coincidentally, data from routine influenza virus surveillance suggested a late seasonal increase in the rate of human influenza viruses. With the almost simultaneous confirmation of an influenza virus A/H7N7-associated conjunctivitis and human influenza virus A/H3N2 in two different veterinarians involved in control measures for the HPAI epizootic, physical prevention measures were reinforced and we began vaccination and actively seeking out people with symptoms—ie, cases.

Here, we describe the epidemiological and virological results of our case finding and the preventive measures taken to control the outbreak in human beings.

Methods

Study organisation

After the first confirmation of chicken-to-human transmission of influenza A/H7N7, an outbreak investigation team was assembled at the RIVM (Rijksinstituut

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voor Volksgezondheid and Milieu [National Institute of Public Health and the Environment]). The population at risk was defined as the group of people living or working in the Netherlands after February 28, 2003, who had direct contact with poultry or poultry products that could have been infected with H7, or who had close contact with an H7-infected person. We set up a case register using Microsoft Excel 97 to record and follow up all reports of health complaints from people within the population at risk. We designed a health questionnaire with questions about symptoms, possible exposures, and background demographic data to allow us to generate hypotheses on risk factors for infection. Public-health nurses or doctors from the Municipal Health Service (MHS) administered questionnaires to all people in the case register. Workers from the MHS offered to take eye swabs and nose/throat swabs for diagnostic testing. Results from laboratory testing and the trawling questionnaires were added to the individual's information in the case register.

Furthermore, the MHS was asked to participate in active case finding by visiting families and workers on all poultry farms in the region. A medical post was set up at the regional crisis centre in the most severely affected area, to do the mandatory vaccination for human influenza and to have close access to all workers involved in the culling of poultry. People with confirmed A/H7 infection were contacted by telephone for interviews on possible symptoms in close contacts; symptomatic contacts were then visited by staff from the MHS, or referred to the medical post or their primary care physician for samples to be taken. Rumours of possible illness in contacts (eg, from discussions in the crews of poultry workers) were followed up by one of the team members.

Patients gave written consent for nose and throat swabs to be done.

Case register and case definition

Cases who were in close contact with poultry were designated as primary cases. Cases who had had contact with a poultry worker or farmer were designated as secondary cases. Completed health questionnaires were entered in the case register and linked by unique case identifier to the laboratory information management system of the RIVM virology laboratory. Test results were uploaded daily at 1600 h to allow inclusion of the latest laboratory results in the daily updates.

For conjunctivitis and influenza-like illness, probable cases which tested positive for influenza A virus but could not be subtyped were classified as probable cases.

Conjunctivitis—we defined a probable case of A/H7 conjunctivitis as a person who had possible close contact with HPAI A/H7 (in poultry or human beings) in the Netherlands on or after Feb 28, 2003, and who had two or more of: red eyes, tearful eyes, itching eyes, painful eyes, burning eyes, purulent fluid in eyes, or sensitivity to light. A confirmed case of A/H7 conjunctivitis met

criteria as defined for a probable case of A/H7 conjunctivitis but also had at least one positive laboratory result for their eye swab, or their nose/throat swab. Laboratory confirmation was by either RT-PCR for influenza A virus followed by subtype H7 specific RT-PCR, or by isolation of influenza virus in cell culture and typing of the virus by haemagglutination inhibition assay.

Influenza—we defined a probable case of A/H7 influenza as a person who had the opportunity of close contact with HPAI A/H7 (in poultry or human beings) in the Netherlands on or after Feb 28, 2003, with acute onset of symptoms (prodromal phase maximum 4 days) and fever (if measured, then $\geq 38.5^{\circ}$) and who had at least one of: cough, rinorrhoea, sore throat, myalgia, or headache. A confirmed case of A/H7 influenza was defined by the same criteria as those for a probable case of A/H7 influenza, but also with at least one positive laboratory result for influenza A/H7 virus.

Sampling collection

We distributed prepacked and labelled sampling kits and instructions to the medical post at the regional crisis centre and to all 39 MHS. Every kit contained two tubes with virus transport medium, four cotton swabs, and a trawling questionnaire. Swabs were collected from both eyes (by protracting the lower eye-lid and rubbing the conjunctiva with the swab), and from the oropharynx and nasopharynx. Tubes and questionnaires were packed and labelled for every patient with a unique LIMS number for matching of epidemiological data and results from virus tests. After sampling, the packages were stored at room temperature and collected every day by courier or sent with the Dutch postal service.

Virological analysis

We first tested swabs for the presence of influenza virus using cell culture and RT-PCR. Influenza virus grown in cell culture was typed and subtyped antigenically by haemagglutination inhibition assays. After the first 25 cases confirmed by cell culture, RT-PCR was used as the initial screening method. Samples of the untreated fluid, and RNA extracts of each specimen were packed in ice and sent to the Erasmus Medical Centre for independent confirmation of RT-PCR and for subtyping (H3, H7) by molecular methods.

We unpacked and prepared samples for RNA extraction and cell culture under BSL-2 conditions. Swabs were vortexed in the virus transport medium, the fluid was collected, and swabs were discarded. Samples were divided into three aliquots of 750 μ L for testing by RT-PCR, cell-culture methods, and confirmation at the second laboratory.

For RT-PCR, a negative control (virus transport medium) was included for every four clinical samples. A positive control sample (influenza A/Net/287/00, subtype H3N2) was included in every RNA extraction and PCR run. RNA was isolated with a high pure RNA isolation kit (Roche Molecular Biochemicals) in accordance with

	Conjunctivitis	Conjunctivitis+ILI	Conjunctivitis total	ILI only	Other	Total
Final laboratory results						
Negative	198 (63.1%/66.9%)	39 (12.4%/73.6%)	237 (75.5%/67.9%)	27 (8.6%/72.9%)	50 (15.9%/74.6%)	314 (100%/69.3%)
A/H3 positive	2 (33.3%/0.7%)	3 (50.0%/5.7%)	5 (83.3%/1.4%)	1 (16.7%/2.7%)	0	6 (100%/1.3%)
A/H7 positive	78 (87.6%/26.4%)	5 (5.6%/9.4%)	83 (93.3%/23.8%)	2 (2.2%/5.4%)	4 (4.5%/6.0%)	89 (100%/19.6%)
Influenza A positive, no subtyping data	8 (57.2%/2.7%)	2 (14.3%/3.8%)	10 (71.4%/2.9%)	2 (14.3%/5.4%)	2 (14.3%/3.0%)	14 (100%/3.1%)
Not tested	10 (33.3%/3.4%)	4 (13.3%/7.5%)	14 (46.7%/4.0%)	5 (16.7%/13.5%)	11 (36.7%/16.4%)	30 (100%/6.6%)
Total	296 (65.3%/100%)	53 (11.7%/100%)	349 (77.0%/100%)	37 (8.2%/100%)	67 (14.8%/100%)	453 (100%/100%)*

ILI=influenza-like illness. Data are n (% of row total/% of column total). *322 men, 128 women, data missing for 3.

Table 1: Results of laboratory testing for influenza in people possibly exposed to HPAI A/H7 in the Netherlands, grouped by presenting symptoms

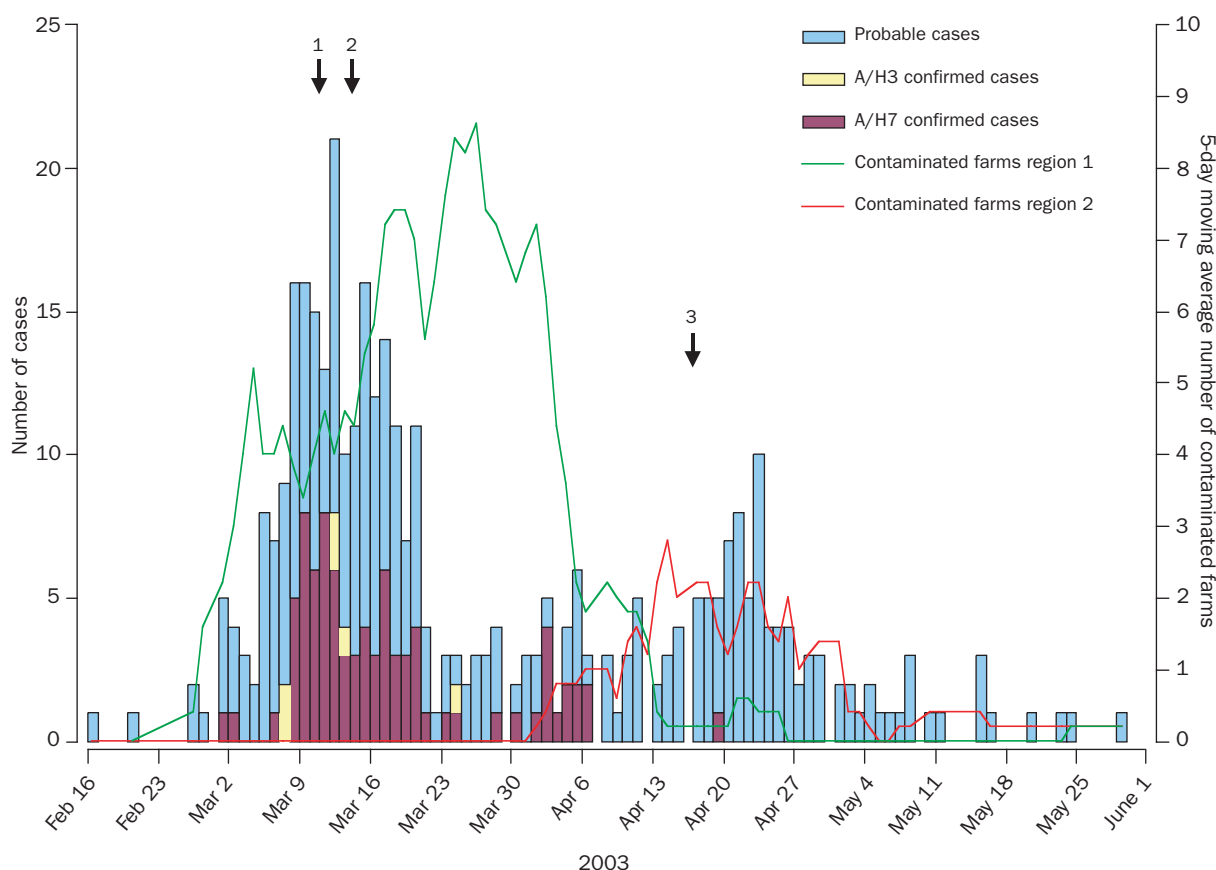


Figure 1: Probable and confirmed cases of human conjunctivitis and influenza-like illness associated with HPAI H3 and H7 infection during the avian influenza epizootic in the Netherlands, 2003

Green and red lines show 5-day moving average of newly diagnosed A/H7 contaminated farms in two regions. Arrows show start of active case finding (1), start of prophylactic treatment with oseltamivir (2) and death of case with HPAI infection (3).

manufacturer's instructions, and with the addition of poly A RNA as carrier. The RT-PCR screening for influenza A virus was done essentially as described elsewhere.⁶ RNA extraction, preparation of PCR mixtures, and addition of RNA were done in a biosafety hood equipped with ultraviolet germicidal lamps which were in separate, dedicated, positive-pressure laboratories (>10 Pa) with a negative-pressure-lock gate. Dedicated pipettes with disposable filter tips, disposable gloves, disposable laboratory coats, and non-reusable waste containers were used in these rooms. Thermo cycling and amplicon detection were done in a separate laboratory on a different floor.

H3 genetic subtyping of samples positive for influenza A virus was done at the Erasmus Medical Centre as described.⁷ A real-time RT-PCR specific for the H7 virus circulating in the Netherlands was developed at Erasmus Medical Centre.¹ At the RIVM, real-time RT-PCR specific for H7 was developed with primers based on the haemagglutinin gene of A/H7N7 avian isolates, provided by G Koch (Central Institute for Animal Disease Control, Lelystad, Netherlands) (forward H7-4 5'TTTGT AATCTGCAGCAGTTC3', reverse and RT primer H7-7 5'AGCAGGGC AGTAGGAAAATG3'). A more detailed protocol is available from M Koopman.

A positive control sample (influenza A/Parrot/Northern Ireland/VF-73-67/73 H7N1 provided by C van Maanen [Animal Health Service, Deventer, Netherlands]) was included in each PCR run. Since H1 molecular typing was not operational at the EMC, samples that could not be typed were assayed by cell culture and subtyped by haemagglutination inhibition test.

For cell culture, transport medium was centrifuged (10 min at 3000 g) and the supernatant was used for virus isolation in accordance with standard protocols.⁷ We typed and subtyped influenza viruses in haemagglutination inhibition assays using turkey erythrocytes with standard antisera against influenza A virus subtypes H1 and H3, and influenza B virus, provided by the European Influenza virus Surveillance Scheme.^{8,9} The antiserum used for H7 typing was provided by C van Maanen; it had been obtained from pathogen-free chickens injected with low pathogenic influenza virus A/Parrot/Northern Ireland/VF-73-67/73 (H7N1).

Susceptibility testing

The susceptibility of the virus isolated from the clinical sample of the first reported case was assessed, in anticipation of the possible use of antivirals for the prophylaxis and treatment of A/H7N7 in humans. This stock virus was subtyped as H7 by haemagglutination inhibition assays. The susceptibility of the virus for the neuraminidase inhibitors oseltamivir (Roche Diagnostics, Mannheim, Germany) and zanamivir (GlaxoSmithKline, Zeist, Netherlands) was tested with a miniaturised format of the fetuin based biochemical assay.¹⁰ We used a known sensitive influenza virus (A/Chicken/Pennsylvania/21525/83 H5N2) as positive control.

Statistical analysis

Data analysis for descriptive epidemiology was done with MS-Excel 97. We used χ^2 test (with continuity correction) to compare proportions of persons with symptoms in A/H7 positive and negative people.

	Cases	Negative*	H3	H7	Positive; no subtype	Unknown	Estimate of number at risk	% cases/% H7 positive (estimate)
Poultry farmer/family	109	77 (70.6%)	1 (0.9%)	16 (14.7%)	3 (2.8%)	12 (11.0%)	1400	7.8%/1.1%
Cullers	131	60 (45.8%)	1 (0.7%)	54 (41.2%)	8 (6.1%)	8 (6.1%)	1800	7.2%/2.9%
Veterinarians	19	12 (63.2%)	1 (5.3%)	5 (26.3%)	1 (5.3%)	0	180	10.6%/2.8%
Medical personnel†	12	12 (100%)	0	0	0	0	60	20%/0%
Others	182	153 (84.1%)	3 (1.6%)	14 (7.7%)	2 (1.1%)	10 (5.5%)	NK	NA
Total	453	314 (69.3%)	6 (1.3%)	89 (19.6%)	14 (3.1%)	30 (6.6%)	>3410	NA

NK=not known; NA=not applicable. Data are n or n (%). *% are proportion of cases in risk group. †Staff from hospitals involved in care of patient who died.

Table 2: Reported cases by risk group and laboratory result

Role of the funding source

The Dutch Ministry of Health was informed of all activities but had no decisive role in study design, data collection, data analysis, data interpretation, writing of the report, or the decision to submit the paper for publication.

Results

Case finding

On March 5, 2003, a veterinarian who visited several farms with HPAI-infected poultry flocks developed acute conjunctivitis. The symptoms in the first eye started 30 h after his last farm visit; within the next 24 h, similar problems arose in the other eye. Eye swabs collected about 60 h after the onset of symptoms were positive for influenza A/H7 by RT-PCR and tissue culture. On the basis of these findings, active case finding was started from March 10, 2003.

By June 9, 2003, 453 people who might have been exposed to avian influenza virus A/H7 reported illness of some kind. Of these, 349 (77%) met the probable case definition for conjunctivitis (279 primary and 70 secondary cases) and 90 (20%) the probable case definition for influenza-like illness (77 primary and 13 secondary cases) (table 1). A/H7 was detected by RT-PCR, virus isolation, or both in 82 primary cases (two with influenza-like illness only, 75 with conjunctivitis only, and five who had both), three secondary cases (all with conjunctivitis, of whom one also had influenza-like illness), and in two people with "red eyes" only (ie, they did not fit any case definition). A/H7 was also detected in two people for whom we did not receive a trawling questionnaire and, therefore, who could not be categorised by symptoms.

Of the two primary A/H7-cases with influenza-like illness only, one had a previous eye injury, precluding evaluation for conjunctivitis, the other was a veterinarian with chronic blepharitis who developed a respiratory distress syndrome and died.

The veterinarian, who had previously been healthy, developed high fever and severe headache without signs of respiratory or ocular diseases 2 days after visiting a farm with infected chickens. He had not used antiviral prophylactic treatment. He consulted a general practitioner for persisting fever and headache, and 1 week after his farm visit, samples were taken to test for avian influenza. Results of RT-PCR tests done in two laboratories were negative for avian influenza virus and for a range of other respiratory pathogens. 9 days after exposure, he was admitted with pneumonia. His condition deteriorated despite treatment with antibiotics, and on day 12, he developed multi-organ failure. On day 15, he died of respiratory insufficiency. A

bronchio-alveolar lavage sample collected on day 11, and lung tissue taken during autopsy tested positive for A/H7 with RT-PCR and cell culture. Histopathology of the lung tissue showed extensive diffuse alveolar damage.

We estimate that about 4500 people were exposed to A/H7-infected poultry in the Netherlands. The 553 people reporting health complaints represent 12.3% of this group. The attack rate of conjunctivitis was 7.8%, whereas influenza-like illness was reported in 2%.

The mean age of people included in the case register was 32.8 years (SD 16.4, [range 0–103]). In confirmed A/H7 cases the average age was 30.4 years (12.3, [13–59]). The peak incidence was between March 8, and March 20, 2003 (figure 1). Most H7 cases were detected in workers who were culling chickens (table 2). The attack rate (proportion of persons at risk who developed symptoms) of conjunctivitis was highest in veterinarians, and cullers and veterinarians had the highest estimated attack rate of confirmed A/H7 infections.

Symptoms in people who had been in contact with people with confirmed A/H7 infection were assessed via the health questionnaires. Of those exposed to 83 primary cases of confirmed A/H7 infection, 70 people reported conjunctivitis, 13 influenza-like illness, and 14 other illness. Three exposed contacts had confirmed A/H7 infection (table 3). All of these secondary cases shared a household with a poultry worker or farmer. The first contact was the 13-year-old daughter of a poultry worker, who developed conjunctivitis about 10 days after the onset of symptoms in her father. Eye swabs from the child tested positive for A/H7. 2 days later, she also developed moderate influenza-like illness, and A/H7 was noted in one of the eye swab samples submitted for diagnostic testing. 4 days after the onset of illness in her daughter, the 37-year-old mother developed conjunctivitis, and RT-PCR showed samples taken from eyes and throat swabs were positive for A/H7. Both patients were given a therapeutic course of neuraminidase inhibitor, and recovered uneventfully.

The third contact was the 44-year-old father of an infected poultry worker with conjunctivitis, who developed conjunctivitis 1 day after onset of symptoms in his son. Contacts of the deceased veterinarian with symptoms were subjected to a broader panel of diagnostic tests but A/H7 was not detected. Two of the 19 people with symptoms were positive for *Chlamydia pneumoniae* infection, and one person was positive for rhinovirus.

Symptoms of influenza-like illness were reported less often by A/H7-positive cases than by people who tested negative for the viruses (table 4).

	Conjunctivitis	Conjunctivitis + ILI	Conjunctivitis total	ILI	Other	Total
A/H3 positive	1 (33.3%/1.7%)	2 (66.7%/20%)	3 (100%/4.3%)	0	0	3 (100%/3.4%)
A/H7 positive	2 (66.7%/3.3%)	1 (33.3%/10%)	3 (100%/4.3%)	0	0	3 (100%/3.4%)
Negative	57 (74.0%/95.0%)	5 (6.5%/50%)	62 (80.5%/88.6%)	3 (3.9%/100%)	12 (15.6%/85.7%)	77 (100%/88.5%)
Not tested	0	2 (50%/20%)	2 (50%/2.9%)	0	2 (50%/14.3%)	4 (100%/4.6%)
Total	60 (69%/100%)	10 (11.5%/100%)	70 (80.5%/100%)	3 (3.4%/100%)	14 (16.1%/100%)	87 (100%/100%)

ILI=influenza-like illness. Data are n (% of row total/% of column total).

Table 3: Results of laboratory testing by category of symptoms in contacts from cases with confirmed A/H7 infection

	H7 positive (%) (n=89)	H7 and H3 negative (%) (n=314)	p
Ocular symptoms			
Red eyes	82 (92%)	184 (59%)	<0.001
Tearful eyes	67 (75%)	165 (53%)	<0.001
Burning eyes	55 (62%)	192 (61%)	1.000
Painful eyes	27 (30%)	133 (42%)	0.055
Itching eyes	50 (56%)	148 (47%)	0.166
Pus in eyes	41 (46%)	109 (35%)	0.067
Photophobia	28 (31%)	97 (31%)	1.000
Influenza-like symptoms			
Fever	8 (9%)	75 (24%)	0.004
Cough	13 (15%)	125 (40%)	<0.001
Rhinorrhoea	25 (28%)	129 (41%)	0.036
Sore throat	9 (10%)	110 (35%)	<0.001
Headache	13 (15%)	114 (36%)	<0.001
Myalgia	7 (8%)	79 (25%)	<0.001

Table 4: Symptoms in A/H7 positive, and A/H7 and A/H3-negative cases

Virological analysis

The proportion of samples positive for A/H7 was highest in the first 4 days from the onset of illness, and eye swabs were more frequently positive than were throat swabs. Maximum detection rates were 44% of eye swabs collected on the second day of illness, and 12% for throat swabs taken on the second day of illness (figure 2). 39 eye swabs shown to be positive by RT-PCR were cultured. 31 (79%) of these yielded infectious A/H7 virus. 13 RT-PCR-negative eye swabs were all culture negative. Characterisation of the viruses found in the index case, the fatal case, and the three contact cases was done to exclude the possibility of spread of a reassorted influenza virus variant. All viruses characterised were completely of avian origin.¹

Susceptibility to antivirals

The 50% inhibitory concentration of oseltamivir for the H7N7 virus was 1.29 nmol/L (95%CI 1.19–1.40 nmol/L) and of zanamivir 3.94 nmol/L (3.61–4.29). A known

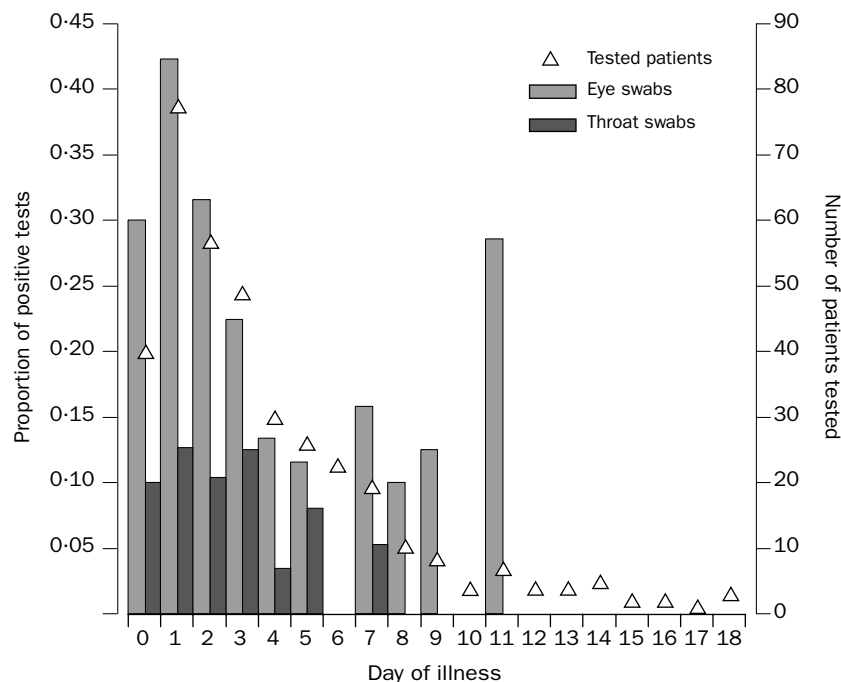


Figure 2: Proportion of eye swabs and nose/throat swabs positive for influenza by RT-PCR

13 patients were tested between days 19 and 61 of illness, but no eye swabs or nose/throat swabs were positive for influenza.

sensitive control virus (A/Chicken/Pennsylvania/21525/83 H5N2) had a 50% inhibitory concentration of 0.33 nmol/L (0.30–0.36) for oseltamivir, results which are similar to previous findings in our laboratories. These values were well within the range of 50% inhibitory concentrations for sensitive H1N1 and H3N2 clinical isolates (0.2–6.8 nmol/L for oseltamivir and 0.3–13.1 nmol/L for zanamivir, dependent on isolate and assay).¹¹ 90 people in the case registry reported that they had had prophylactic treatment. Avian influenza virus infection was detected in one of 38 (2.6%) people who used oseltamivir, compared with five of 52 (9.6%) who reported that they had not taken prophylactic medication (p=0.38).

Overview of control measures taken

On March 3, 2003, following confirmation that A/H7N7 was the cause of the avian influenza outbreak, the outbreak management team advised all workers who screen and cull poultry to wear protective eye glasses and mouth and nose masks to reduce contact with avian influenza virus. People with symptoms of influenza-like illness were exempted from work. When the first case of A/H7-confirmed conjunctivitis was diagnosed on March 7, 2003, and in view of the concomitant increase in the number of influenza virus cases, on March 9, 2003, the outbreak management team recommended mandatory vaccination with inactivated influenza virus vaccine be offered to all poultry workers who handle, screen, or cull potentially infected chickens. This policy aimed to reduce the risk of a possible genetic mixing or reassortment of avian and human influenza virus in one person through prevention of infection with human influenza virus. As of March 10, 2003, all workers had agreed to be vaccinated. Based on the virology update of March 14, 2003, when 19 confirmed cases of A/H7 were discussed, as well as the first confirmed contact transmission, preventive measures were stepped up. The need for personal protection was emphasised, and the importance of washing hands after leaving the workplace and personal hygiene at home was stressed.

Immediate treatment with oseltamivir was recommended for all new conjunctivitis cases, and a prophylactic regimen of oseltamivir (75 mg daily) was started for all people handling potentially infected poultry, to be continued for 2 days after last exposure. The recommendation for vaccination was extended to all poultry farmers and their families in a 3 km radius of infected poultry farms, and those suspected of having the infection.

Discussion

We describe a large outbreak of avian influenza A/H7 in human beings, with 89 infected people, of whom 85 fitted case definitions of conjunctivitis or influenza-like illness. Conjunctivitis was noted as the prevailing symptom in three secondary cases, confirming the predilection of these viruses for the eye.

That influenza-like symptoms were reported less often by A/H7-positive cases than other cases suggests that the influenza A/H7 viruses do not cause influenza-like illness. Alternatively, the detection methods we used could have been less suitable for virus detection in infected people without conjunctivitis—

a suggestion lent support by the negative results for swabs collected 5 days after symptom onset from the veterinarian who subsequently died. However, such differences in rate of detection have not been noted for human influenza A viruses, and, therefore, are not likely.

It has been postulated that the detection of influenza A virus in eye swabs by RT-PCR may be the result from mechanical contamination by virus-containing dust. However, virus was detected more frequently in people who had recently developed symptoms. Since all poultry workers were still working at the time samples were taken, simple contamination would be expected to occur more randomly. The association of positive virus tests with recent onset of illness, and the finding that contacts had ocular shedding, led us to conclude that the conjunctivitis was caused by replicating avian influenza A viruses.

Three household contacts in our survey had avian influenza virus A/H7, which may have been attributable to person-to-person transmission. This finding raised concerns of possible viral adaptation or reassortment. Birds, and especially waterfowl, can serve as a reservoir for a wide range of genes of influenza A viruses, thus contributing to the potential risk of generation of a novel pandemic influenza virus strain.¹² A prerequisite for reassortment is simultaneous infection of a susceptible host (such as a pig or human being) with both avian and human influenza viruses, resulting in viral offspring that has a mixture of the genome segments derived from both parental strains. Essential contributing factors, such as the transmission of influenza viruses from birds to pigs, and from pigs to humans, have been noted on several occasions.¹²⁻¹⁸

Full details of the virological characterisation in this outbreak will be published elsewhere, but to date, all viruses examined had internal gene segments from avian influenza A viruses and not from circulating human influenza A viruses. This work builds on previous findings that avian influenza viruses have pathogenic potential for humans.¹⁹⁻²²

Whether the behaviour of the A/H7/N7 viruses in this outbreak was very different from that of other avian influenza viruses in humans is unclear. During a recent A/H7N1 outbreak in chickens in Italy, virological analysis and serologic tests did not yield evidence of infection in human beings. However, during the Italian outbreak disease reporting was passive, and the absence of a serological response has been documented for people with H7N7 infection.^{2,3,23} By contrast, the avian influenza virus A subtype H5N1 has caused illnesses ranging from mild respiratory disease to influenza-like illness with pneumonia and a high case-fatality rate. Yet, follow-up investigations after the 1997 outbreak of the H5N1 virus in Hong Kong showed anti-H5 antibodies in poultry workers and health-care workers, which suggests that transmission is much more widespread and that the case-fatality rate is lower than previously supposed.²⁴⁻²⁷

In 1999, direct transmission of avian influenza A virus subtype H9N2 occurred, again in Hong Kong, and the two identified patients did seroconvert. Furthermore, screening for antibodies to H9 showed evidence of widespread exposure to the avian viruses in blood donors but not health-care workers.^{21,28} These data illustrate the need for a better understanding of the extent of transmission of the avian viruses to humans, to help assess the potential risk of emerging variants in times of outbreaks. Data suggesting a common background to some of the genes found in both viruses (H9N2, H5N1) that have caused significant human disease are intriguing in this respect.^{29,30}

Based on our data, veterinarians and people who cull infected poultry have had the highest risk of A/H7

infection. The veterinarian who died had spent a few hours screening flocks that were later confirmed to be positive for HPAI. Yet, poultry farmers did not have high rates of infection. Cohort studies are underway to explain these differences in infection rates between workers. Comparisons of transmission rates will be done after serological tests are complete.

On Friday April 4, 2003, the outbreak of HPAI expanded to two different regions of the Netherlands (North Brabant and Limburg), where there were about 62 million chickens (figure 1). The data in the case register suggest that before April 4, 2003, at least eight people reported health complaints during the culling in Gelderland, but lived in North Brabant and Limburg. Two had confirmed A/H7 infection, and it is possible that humans have contributed to the spread of A/H7 outside of Gelderland. Veterinary control of the outbreak highlighted the importance of movement restrictions for animals, vehicles, and human beings, but several breaches of practice were detected. Also, the size of the outbreak meant that there was a shortage of experienced poultry workers. In addition, we have at least four reports of confirmed A/H7 illness in poultry workers from other countries, who travelled back to their country of origin during the time when they were likely to be shedding the virus. The need for coordination of international responses during outbreak control is an important lesson for pandemic preparedness planning: at present, all control measures in Europe stop at national borders.

A challenging aspect of any outbreak of an emerging disease is the translation of findings into control measures, since there may be very few data available on which to base decisions. During the course of the HPAI outbreak, measures were gradually increased in stringency, because the initial assessment had been that, although A/H7N7 might be a threat to human health, the risk was thought to be low. Treatment of all people involved in handling infected poultry was only started in the middle of March, after confirmation of several cases of A/H7 and the first secondary case. The rationale was to reduce the probability of coinfection of individuals by the avian virus and any fortuitously circulating human influenza virus.

A difficulty faced by health planners was the paucity of data about widespread and lengthy use of oseltamivir for prophylaxis. Arguments against widespread use of oseltamivir were: (1) the ethical dilemma of prescribing a drug with possible side-effects to healthy people so as to protect others; (2) mass prescription of drugs without individual medical guidance could negatively affect the national policy of restricted drug use; (3) potential for development of resistance; (4) implementation and improvement of personal protection measures might be as effective as drug treatment; and (5) the rate of non-adherence to oseltamivir might be as high as that for personal protection. At first, people were slow to accept antiviral medication; however, the uptake rate increased after the fatal case.

Our experience with the largest documented outbreak of avian influenza A virus of subtype H7N7 in human beings provided important new data on the potential for transmission of these viruses to humans. Attack rates far exceeded those reported previously, but it remains unclear whether this finding was due to unique properties of the viruses, the type of poultry work taking place during this outbreak, or a consequence of active case finding including assessment of people with mild illness. A follow-up cohort study is underway to assess the extent of transmission to poultry workers (as measured by testing for anti H7 antibodies) and potential risk factors.

The size of the outbreak, which coincided with the peak activity of human influenza virus, reinforced the message that emergence of new pandemic influenza viruses might arise via the mixing of genes from avian and human viruses in humans, and that tracking and containment of these viruses might be very difficult. Although we launched a large and costly outbreak investigation (using a combination of pandemic and bioterrorism preparedness protocols), and despite decisions being made very quickly, a sobering conclusion is that by the time full prophylactic measures were reinforced (1 week after the first confirmation of human infection), more than 1000 people from all over the Netherlands and from abroad had been exposed. Therefore, if a variant with more effective spreading capabilities had arisen, containment would have been very difficult. We see this outbreak as providing strong support for the need for pretested pandemic preparedness plans, including the stockpiling of essential control components such as vaccines and antivirals.

Contributors

M Koopmans, A Bosman, and M Conyn designed the outbreak investigation and the case registry; designed questionnaires; organised logistics, data aggregation and transfer, and daily updates for all groups. M Koopmans and A Bosman prepared the body of the manuscript. B Wilbrink, H van der Nat, and A Meijer ran the lab logistics at RIVM, including preparation of sampling kits, virological analyses, and coordination of shipments and data for subtyping. H Vennema developed and implemented H7 realtime typing in the course of the investigation; J van Steenberg coordinated activities between the outbreak team and other parties such as the municipal health services, medical microbiology laboratories, and the ministries. G Natrop did the medical examination of possible cases at the regional crisis centre; R Fouchier and A Osterhaus confirmed avian influenza virus infections as National Reference laboratory for Influenza viruses. M Koopmans, M Conyn, J van Steenberg, and A Osterhaus served as members of the (National) outbreak management team, and helped formulate guidelines for control of the outbreak.

Conflict of interest statement

None declared.

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