# Outbreak of avian influenza H7N3 on a turkey farm in the Netherlands

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**This case report describes the course of an outbreak of avian influenza on a Dutch turkey farm. When clinical signs were observed their cause remained unclear. However, serum samples taken for the monitoring campaign launched during the epidemic of highly pathogenic avian influenza in 2003, showed that all the remaining turkeys were seropositive against an H7 strain of avian influenza virus, and the virus was subsequently isolated from stored carcases. The results of a reverse-transcriptase PCR showed that a H7N3 strain was involved, and it was characterised as of low pathogenicity. However, its intravenous pathogenicity index was 2·4, characterising it as of high pathogenicity, suggesting that a mixture of strains of low and high pathogenicity may have been present in the isolate. The outbreak remained limited to three farms.**

IN the last few years severe outbreaks of highly pathogenic avian influenza have occurred, for example in Italy in 1997 to 1998 (Capua and others 2000, 2003), in Hong Kong in 1997, 2001 and 2002 (Sims and others 2003), in the Netherlands in 2003 (Elbers and others 2004, Stegeman and others 2004), and the ongoing Eurasian outbreaks. These outbreaks not only caused great economic damage and resulted in the destruction of millions of birds, but, as has become clear over the past few years, may also have public health implications (Alexander and Brown 2000, Katz 2003).

The outbreaks are assumed to have originated from circulating strains of low pathogenicity in wild aquatic birds (Alexander 2000, Alexander and Brown 2000), which can transmit the virus to poultry. These strains may circulate within poultry flocks, causing mild clinical signs, and often not being recognised as avian influenza. Random mutations in their nucleotides may result in a highly pathogenic strain, and consequently in an outbreak of highly pathogenic avian influenza (Banks and others 2001). Because wild birds are endemically infected (Alexander 2000, Alexander and Brown 2000), new outbreaks can be expected at any time, and it is therefore necessary to detect circulating strains of avian influenza early to reduce that risk. Serological monitoring of birds with mild clinical signs might help to detect a strain of low pathogenicity before it mutates into a highly pathogenic strain, and help to prevent serious epidemics like those that occurred in Italy, the Netherlands and south east Asia.

During the outbreak of the highly pathogenic H7N7 strain in the Netherlands in 2003, the Dutch Ministry of Agriculture tried to trace the source of the outbreak and determine whether more strains of the virus might have been circulating unnoticed by implementing a serological monitoring programme. Three flocks of turkeys in the south west of the Netherlands, were seropositive for antibodies against avian influenza virus, but because no serious clinical signs had been observed or reported officially, it was assumed that the infection was caused by a strain of low pathogenicity. The question arose as to whether no clinical signs had been observed on these farms, or whether any signs had not been recognised as due to avian influenza. Detailed reports were available from one farm about the clinical signs, postmortem findings, and the isolation and characterisation of the virus. This paper describes the course of the infection and the virological findings systematically, to help practitioners to recognise avian influenza in turkeys, and to develop surveillance programmes based on the clinical signs.

## CASE REPORT

A meat-turkey farm in the south west of the Netherlands kept turkeys in two houses connected by a common changing room. Initially 9085 male and 8445 female turkeys (Big 6; British United Turkeys) hatched on July 19, 2002, were housed, separated by a fence, in one house; and at six weeks of age the male turkeys were transferred to the second house. All the birds were vaccinated against turkey rhinotracheitis on the day they hatched, and against Newcastle disease on day 14 and in the fifth and ninth week; the males were additionally vaccinated at 16 weeks of age. The females were slaughtered on November 7, and the males remained at the farm until December 10.

A second flock of 9267 male and 8870 female turkeys of the same breed, hatched on November 22, 2002, were brought into one of the houses on the day they hatched, and vaccinated according to the same protocol as the first flock.

### Clinical signs, postmortem results and treatments

At 19 weeks of age, on November 30, the food intake of the male turkeys in the first flock decreased to 50 per cent of normal, followed by respiratory signs becoming evident within two days. Postmortem examination revealed airsacculitis. Although the results of bacteriological culturing were negative, the practitioner decided to treat the turkeys via their drinking water with amoxicillin (Paracilline; Intervet), followed by tylosin (Tylan; Elanco). Their food intake increased, but their respiratory signs remained until the end of the fattening period. Mortality was not significantly increased. The turkeys were slaughtered at 20 weeks of age, when they still had respiratory signs.

Clinical problems also developed in the second flock, starting on December 15, when they were 24 days old, with minor respiratory problems. The flock showed signs of general depression and respiratory distress. Up to day 25 the average daily mortality among the hens and toms had been approximately 0·1 per cent, but on day 26 the mortality among the hens was 4 per cent, and among the toms 11 per cent. Airsacculitis, pericarditis and sinusitis were observed postmortem. Bacteriological culturing demonstrated the presence of *Escherichia coli* in the airsacs, and treatment with enrofloxacin (Baytril; Bayer) in the drinking water was therefore started.

Because the daily mortality remained high (approximately 4 per cent in both hens and toms), intramuscular injections of enrofloxacin were given on days 28 and 29. On day 29, birds were sent to the Animal Health Service, where airsacculitis was diagnosed and *E coli* and *Pseudomonas aeruginosa* were cultured from the airsacs. Drinking water treatment was started on day 30 with gentamicin (Gentamicinesulfaat; Dopharma) and doxycyclin (Doxycycline-hyclaat 50 per cent; Dopharma) followed by an intramuscular gentamicin injection (Gentaject; Dopharma) on day 32. Treatments were terminated on day 33, when the birds' food intake increased and daily mortality had decreased to 0·7 per cent in the toms,

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and 0·6 per cent in the hens, and the situation seemed to have normalised (Fig 1).

It was assumed that a viral infection might have caused the problems, followed by secondary bacterial infection with *E coli* or *Ornithobacterium rhinotracheale*. Turkey rhinotracheitis or Newcastle disease virus from the first flock, or Newcastle disease vaccine virus used in the second flock were considered as possible sources of the virus.

## FOLLOW-UP DURING THE EPIDEMIC OF HIGHLY PATHOGENIC AVIAN INFLUENZA

### Serological findings

On February 28, 2003 an outbreak of H7N7 was reported on a layer farm in the central part of the Netherlands (Elbers and others 2004, Stegeman and others 2004), which resulted in the infection of 255 flocks and the slaughter of over 30 million poultry (Den Boer and others 2004). During this epidemic, a serological surveillance programme was carried out to determine whether strains of avian influenza virus might have circulated beyond the borders of the infected areas. The programme was based on the guidelines for surveys implemented by the European Union (Elbers and others 2004, Stegeman and others 2004). In each province a number of flocks was sampled on the basis of a flock prevalence of 5 per cent, and 20 samples were taken from each flock.

One of the flocks included in the programme was the turkey farm described above. The serological screening revealed that all 40 serum samples taken from the two houses were positive for antibodies against avian influenza A, subtype H7. Serum samples were also taken from two neighbouring flocks, and all these samples were also positive for antibodies against subtype H7. The farmers on these two farms did not notice or did not report any signs of avian influenza at any time during or after the clinical problems on the turkey farm.

### Virological findings

In December 2002, five turkeys from the farm described previously were killed and stored at –20°C, and in April 2003 these cadavers were sent to the national reference laboratory CIDC-Lelystad for virus isolation. Trachea and lung samples were prepared for further testing, and the suspensions were cultured in specified pathogen-free fertilised eggs according to World Organisation for Animal Health (OIE) standards (OIE 2000). The allantoic fluid was harvested and tested in a reverse transcriptase-PCR (RT-PCR), and the fluid was also tested in an intravenous pathogenicity test using chickens according to the OIE manual (OIE 2000). The virus from the lungs and trachea of the chickens used in the intravenous pathogenicity test was subsequently isolated, and tested in a RT-PCR.

Virus was isolated from the lungs and trachea of all five turkeys. Sequencing of the haemagglutinin gene showed that the H7 contained only a single basic amino acid at the cleavage site (PEIPKGR*GLF) and the virus was therefore considered to be of low pathogenicity, according to the OIE manual (OIE 2000). The allantoic fluid was also tested in an intravenous pathogenicity test, resulting in an index of 2·4 and the virus was therefore characterised as a strain of high pathogenicity according to the OIE manual. In addition, a RT-PCR was carried out on the lungs and trachea of the birds used for the intravenous pathogenicity test, and showed multiple basic amino acids at the cleavage site (PEIPKGSRVRR*GLF), thereby also characterising this strain as of high pathogenicity, by both criteria in the OIE manual. Later, the European Union Community Reference Laboratory at Weybridge, confirmed that the virus isolated from the turkeys was considered to be of low pathogenicity and also sequenced the neuraminidase gene, which proved to be a N3 subtype (J. Banks, personal communication). Furthermore, the intravenous pathogenicity test was repeated, resulting in an index of 2·8.

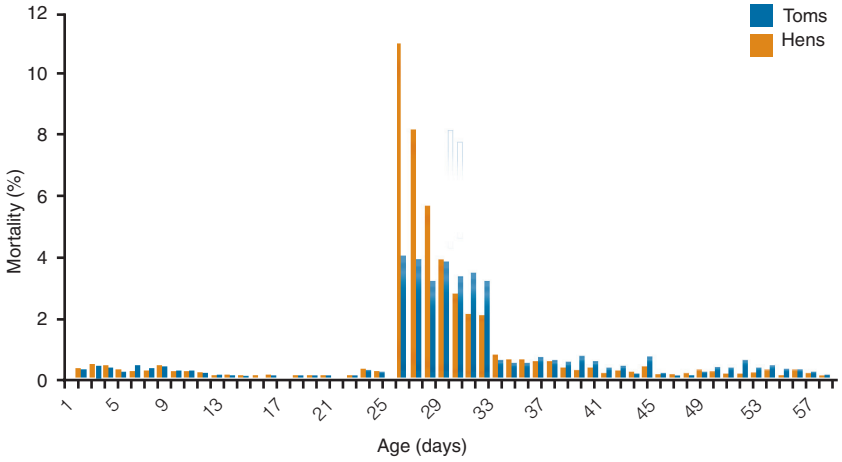


**FIG 1: Daily percentage mortalities among the hens and toms of the turkey flock infected with avian influenza**

## DISCUSSION

An outbreak of avian influenza occurred on a Dutch turkey farm in December 2002, a few months before the outbreak of highly pathogenic avian influenza in the Netherlands in 2003 (Elbers and others 2004). The clinical signs were not recognised as such during this outbreak, and the final diagnosis of avian influenza was made by RT-PCR and an intravenous pathogenicity test months later. The RT-PCR initially indicated that a strain of low pathogenicity was involved whereas the pathogenicity test showed that a highly pathogenic strain was involved. Since the RT-PCR was carried out twice, with similar outcomes, the most likely explanation is that the isolate was a mixture of strains of both types and that the intravenous pathogenicity test selected for the highly pathogenic strain. This finding suggests that a molecular assessment alone is insufficient to confirm an avian influenza virus as a low pathogenicity, although this is implicit in the current OIE and EU definitions.

A possible explanation for the failure of the RT-PCR to detect the highly pathogenic strain is that there was much more of the strain of low pathogenicity present in the isolate. In general, it is assumed that highly virulent strains of a virus have a higher transmission capacity than less virulent strains (Van der Goot and others 2003a, b), suggesting that, once it has mutated into a highly pathogenic strain an isolate would contain mainly that strain. However, the findings in this paper suggest otherwise, because the highly pathogenic strain was isolated after intravenous passage in chickens but not identified by RT-PCR carried out twice. The rapid death of birds infected with highly pathogenic strains might reduce the excretion of virus by a flock and could be an explanation for the smaller quantity of the highly pathogenic strain in the isolate (Westbury and others 1979).

An explanation for the observation that the intravenous pathogenicity test indicated a highly pathogenic strain is that such strains can replicate in several types of cells, whereas strains of low pathogenicity replicate only in cells where trypsin-like enzymes are present, inducing a selective advantage for the highly pathogenic strain (Bosch and others 1981).

Nevertheless, a highly pathogenic strain was most probably present in the turkey flock, but not recognised as such. The primary cause of the clinical problems may have been an infection with a strain of low pathogenicity, originating from

wild fowl, which mutated into a highly pathogenic strain some weeks after it had been introduced into the flock; the immune response to the initial infection might have reduced the severity of the clinical signs induced by the highly pathogenic strain, as has been described for H5N2 by Van der Goot and others (2003b). However, it could not be determined from the course of the infection whether this happened. The isolate did not spread to more than three farms, possibly owing to the low density of poultry farms in the area. Because each introduction of a strain of low pathogenicity may result in an outbreak of disease due to a highly pathogenic strain it is important to detect an initial outbreak as quickly as possible. As a result, it should be considered whether an outbreak due to a strain of low pathogenicity should be treated as if it were highly pathogenic; changes of the definitions have been proposed (Alexander 2003, Pearson 2003). The question is whether outbreaks due to strains of low pathogenicity can be recognised before they mutate into strains of high pathogenicity. They are difficult to recognise because the clinical signs and mortality can vary widely (Bano and others 2003, Mutinelli and others 2003). Monitoring programmes based on reductions in feed intake or decreases in egg production and increases in mortality might help to detect outbreaks quickly, and such programmes have been implemented in the Netherlands, since the outbreak of highly pathogenic avian influenza in 2003. It is not clear, however, whether such programmes would prevent a highly pathogenic outbreak in the Netherlands in the future. A comparison of the costs of different monitoring programmes with the potential costs of outbreaks may help to select the optimal monitoring programme.

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