

Case Reports—

The Highly Pathogenic Avian Influenza A (H7N7) Virus Epidemic in the Netherlands in 2003—Lessons Learned from the First Five Outbreaks

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SUMMARY. Clinical signs and gross lesions observed in poultry submitted for postmortem examination (PME) from the first five infected poultry flocks preceding the detection of the primary outbreak of highly pathogenic avian influenza (HPAI) of subtype H7N7 during the 2003 epidemic in the Netherlands are described. The absence of HPAI from the Netherlands for more than 75 yr created a situation in which poultry farmers and veterinary practitioners did not think of AI in the differential diagnosis as a possible cause of the clinical problems seen. Increased and progressive mortality was not reported to the governmental authorities by farmers or veterinary practitioners. It took 4 days from the first entry of postmortem material to notify the governmental authorities of a strong suspicion of an AI outbreak on the basis of a positive immunofluorescence test result. The gross lesions observed at PME did not comply with the descriptions in literature, especially the lack of hemorrhagic changes in tissues, and the lack of edema and cyanosis in comb and wattles is noted. The following lessons are learned from this epidemic: a) in the future, increased and progressive mortality should be a signal to exclude AI as cause of disease problems on poultry farms; b) intensive contact between the veterinary practitioner in the field and the veterinarian executing PME is necessary to have all relevant data and developments at one's disposal to come to a conclusive diagnosis; c) in an anamnesis, reporting of high or increased mortality should be quantified in the future (number of dead birds in relation to the number of birds brought to the farm to start production, together with the timing within the production cycle), or else this mortality cannot be interpreted properly; d) if clinical findings such as high mortality indicate the possibility of HPAI, the pathologist should submit clinical samples to the reference laboratory, even if PME gives no specific indications for HPAI; e) the best way to facilitate early detection of an HPAI outbreak is to have the poultry farmer and/or veterinary practitioner immediately report to the syndrome-reporting system currently in operation the occurrence of high mortality, a large decrease in feed or water intake, or a considerable drop in egg production; f) in order to detect low pathogenic avian influenza infections that could possibly change to HPAI, a continuous serologic monitoring system has been set up, in which commercial poultry flocks are screened for antibodies against AI virus of subtypes H5 and H7.

RESUMEN. *Reporte de Caso—*Epizootia del virus de la influenza aviar altamente patógeno (H7N7) en Holanda en el año 2003. Lecciones aprendidas de los primeros cinco brotes.

Se describen los signos clínicos y las lesiones macroscópicas en aves remitidas para examen postmortem de las primeras cinco parvadas infectadas que precedieron a la detección del brote primario de influenza aviar de alta patogenicidad del subtipo H7N7 durante la epizootia en Holanda. La ausencia de influenza aviar de alta patogenicidad en Holanda por más de 75 años creó una situación en la cual avicultores y médicos veterinarios no incluían esta enfermedad dentro de los diagnósticos diferenciales para los problemas clínicos observados. Los avicultores y médicos veterinarios no reportaron a las autoridades gubernamentales los casos de mortalidad

umentada y progresiva. Desde la primera remisión de material para necropsia, transcurrieron cuatro días para notificar a las autoridades gubernamentales de la fuerte sospecha de un brote de influenza aviar con base en un resultado positivo mediante la prueba de inmunofluorescencia. Las lesiones observadas en el examen postmortem no coincidieron con las descritas en la literatura, especialmente por la ausencia de cambios hemorrágicos en los tejidos y por la ausencia de edema y cianosis en la cresta y barbillas. Las siguientes lecciones se obtuvieron de esta epizootia: a) En el futuro, la mortalidad aumentada y progresiva será un indicador para incluir influenza aviar como causa de enfermedad en las granjas avícolas; b) Se requiere de comunicación continua entre el médico veterinario del campo y el veterinario que realiza los exámenes postmortem para reunir toda la información importante y los elementos disponibles para obtener un diagnóstico concluyente; c) En la anamnesis, los reportes de mortalidad alta o incrementada deberán ser cuantificados en el futuro (número de aves muertas en relación con el número de aves con que se inició la producción, junto con tiempo dentro del ciclo productivo), de otra manera, la mortalidad no podrá ser interpretada adecuadamente; d) Si los hallazgos clínicos como la presencia de mortalidad alta indican la posibilidad de virus de influenza de alta patogenicidad, el patólogo deberá remitir muestras a un laboratorio de referencia, aún cuando los hallazgos postmortem no proporcionen indicaciones específicas del virus patógeno de influenza; e) La mejor manera de facilitar la detección temprana de los brotes de influenza de alta patogenicidad es hacer que los avicultores y los médicos veterinarios notifiquen inmediatamente al sistema de reporte que se encuentre en operación, de la ocurrencia de alta mortalidad, de un decremento en el consumo de agua y alimento o de una caída considerable en la producción de huevo; f) Para detectar la influenza aviar de baja patogenicidad que posiblemente pueda cambiar a alta patogenicidad, se debe establecer un sistema de muestreo serológico continuo, en donde las parvadas comerciales sean sometidas a una prueba de escrutinio para detectar anticuerpos contra influenza aviar del subtipo H5 y H7.

Key words: highly pathogenic avian influenza, H7N7, poultry, pathology, clinical signs

Abbreviations: AHS = Animal Health Service; AI = avian influenza; BGA = brilliant green agar; DFCAD = Directive Flock Control Avian Diseases; EU = European Union; FP = fowl plague; H = hemagglutinin; HPAI = highly pathogenic avian influenza; HRP = high-risk period; IB = infectious bronchitis; IFT = immunofluorescence test; ILT = infectious larynchotracheitis; LIMS = Laboratory Information Management System; LPAI = low pathogenic avian influenza; MAI 2003 = Monitoring Avian Influenza 2003; N = neuraminidase; ND = Newcastle disease; NIC = National Influenza Center; NRI = National Reference Institute; OIE = Office International des Epizooties; PME = postmortem examination; PPE = Product Boards for Poultry and Eggs; RT-PCR = reverse transcriptase-polymerase chain reaction; RVV = National Inspection Service for Livestock and Meat; WHO = World Health Organization

Outbreaks of fowl plague (FP), an often fatal disease caused by influenza A viruses, were first described in the Netherlands in poultry in 1924 in the municipalities of Achterveld, Scherpenzeel, and Woudenberg (the same area that was struck during the 2003 epidemic). The last time FP was observed in the Netherlands was in 1927, and the FP was observed in the same area as in 1924 (29).

FP was never reported before by the Netherlands to the Office International des Epizooties (OIE) in Paris. However, after an absence of FP from the Netherlands for more than 75 yr, a serious suspicion of an infection with highly pathogenic avian influenza (HPAI) virus was reported on February 28, 2003, on several poultry farms in the “Gelderse

Vallei,” an area in the central-eastern part of the Netherlands with a high density of poultry and poultry farms (Fig. 1). Consequently, the governmental authorities were informed. It was the start of a large HPAI epidemic, in which especially the high-density poultry areas were severely hit.

Despite control measures like movement restrictions, establishment of protection and surveillance zones, stamping out of infected flocks, preemptive culling of flocks within a radius of 1 km of an outbreak, establishment of buffer zones between defined areas by complete depopulation of poultry flocks in the buffer zone, and compartmentalization of the Netherlands into separate regions, a total of 255 flocks became infected, and 1381 commercial



Fig. 1. Location of the "Gelderse Vallei" within the Netherlands during the avian influenza epidemic in 2003. Reprinted with permission of Gezondheidsdienst voor Dieren.

flocks and 16,521 backyard/smallholders were depopulated. The total number of AI-susceptible animals euthanatized amounted to approximately 30 million: 175,035 birds from backyard/smallholders, approximately 25 million birds lost as a result of depopulation of infected flocks and pre-emptive culling, and 4.5 million birds culled for welfare reasons. In addition, the virus was transmitted to people directly involved in handling infected

poultry, with evidence for a few person-to-person transmissions and the death of a poultry veterinarian (12,17).

The objective of this case report is to describe the clinical signs seen in the flocks and the pathologic lesions observed at postmortem preceding the suspicion of an AI infection in the first five infected flocks.

We will discuss our findings in the light of experiences in other countries. Another objective of

this case report is to extract valuable lessons regarding how to detect index cases better and sooner.

MATERIAL AND METHODS

Poultry farms. A description of the poultry farms involved in the first five outbreaks preceding the detection of the index case follows. Case 1 had two large floor-raised houses (free-ranging facilities) and a small groundfloor house. A total of 7175 layers were placed on the farm in house 1 on September 10, 2002; in the weeks preceding the AI outbreak, the birds were kept inside. A total of 21,350 layers were placed on the farm in house 2 on April 10, 2002; the birds used free-range facilities in the weeks preceding the AI outbreak. A total of 550 layers were placed on the farm in house 3 on October 8 and 9, 2002.

Case 2 had two poultry houses with caged layers. A total of 37,375 layers were placed on the farm in house 1 on November 8, 2002. A total of 38,012 layers were placed on the farm in house 2 on September 3, 2002.

Case 3 had three poultry houses with free-ranging facilities. A total of 7600 layers were placed on the farm in house 1 on February 13, 2002. A total of 12,100 layers were placed on the farm in house 2 on February 13, 2002, and a total of 6800 layers were placed on the farm in house 3 on February 13, 2002.

Case 4 had two poultry houses with free-ranging facilities. A total of 17,000 layers were placed on the farm in house 1 on June 26, 2002. A total of 10,000 layers were placed on the farm in house 2 on June 26, 2002.

Case 5 had two poultry houses (without free-ranging facilities). A total of 4504 broiler breeders were placed on the farm in house 1 on February 22, 2002, and a total of 4300 broiler breeders were placed on the farm in house 2 on February 22, 2002.

These five cases were located in the "Gelderse Vallei," an area in the central-eastern part of the Netherlands with a high density of poultry and poultry farms (± 4 poultry farms/km²). The premises of case 1 and case 5 were situated opposite to each other at the same road (± 500 m distance) in the same municipality. The premises of case 3 were located at the same road as the premises of case 1 and case 5. Case 2 was situated in the same municipality as case 3; the premises of case 2 and case 4 were situated close to each other (± 600 m distance).

Postmortem Examination (PME). A facility for PME is available at the Animal Health Service Ltd. (AHS) in the Netherlands to assist farmers and veterinary practitioners in supporting a clinical diagnosis for poultry diseases. AHS is an independent private organization with a staff of veterinary consultancy specialists and a large veterinary diagnostic laboratory, executing control programs for animal diseases for cattle, swine, and poultry.

PME of birds submitted to the AHS comprised macroscopic examination by a veterinary poultry specialist. Gross lesions noted were recorded on an individual animal basis. PME was executed according to standard operating procedures, and the registration of the pathologic findings in the Laboratory Management Information System (LIMS) was also conducted according to a standardized protocol. PME started with an external macroscopic inspection. After removal of the skin, the abdominal cavity was opened, and trachea, heart, and liver were evaluated. The alimentary tract was removed, and all organs and serous membranes in the abdominal cavity were evaluated. To accomplish this, the proventriculus and the intestinal tract were opened.

Immunofluorescence test (IFT). An IM-AGENTM Influenza virus A test (DakoCytomation Ltd., Ely, United Kingdom) was used by the AHS to detect influenza A virus in clinical specimens from case 1 and case 3 on February 28, 2003. This is a qualitative direct IFT for the detection and differentiation of influenza A virus. The test was validated for poultry at the AHS and was able to detect AI virus subtypes H1 through H12. The test utilizes monoclonal antibodies (originating from the Department of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention, Atlanta, GA) to detect epitopes of influenza virus glycoproteins and fusion proteins specific to influenza A viruses. An IFT to detect Newcastle disease (ND) (15) or infectious bronchitis (IB) (32) was also used by the AHS.

Virus isolation and characterization. Virus isolation was performed according to the procedures laid down in Annex III of Council Directive 92/40/EEC on measures for the control of AI. Reverse transcriptase-polymerase chain reaction (RT-PCR) specific for AI viruses of H5 and H7 subtypes was executed on RNA isolated directly from tissue samples from dead birds of cases 1 to 5 at the National Reference Institute (NRI) in Lelystad and of birds from case 3 at the National Influenza Center (NIC), Erasmus Medical Center, in Rotterdam.

CASE REPORT

In the following case reports, the chronology of clinical signs seen and pathologic lesions observed during PME of poultry in the first five HPAI outbreaks preceding detection of the primary outbreak in the Netherlands is described.

Case 1. Saturday, February 22. The poultry farmer observed a decrease in feed and water intake in poultry from house 1.

Sunday, February 23. The decrease in feed and water intake was enhanced and mortality increased (in poultry house 1, a total of 112 dead birds: approximately 1.6%, see course of mortality in Fig.

2). In the evening, the poultry farmer contacted a representative of the poultry supplier by telephone with respect to the increased mortality. On the basis of the observations by the farmer, it was suggested that *Escherichia coli* might be involved. It was agreed that the representative would visit the farm the next morning.

Monday, February 24. The increased mortality in poultry house 1 was further enhanced (465 birds, see Fig. 2), and there was almost no feed and water intake anymore by the birds. The representative of the poultry supplier visited the flock at 10:30 AM. Observation of the clinical signs in this flock did not remind the representative of an infection with *E. coli*; he thought that rather he might be observing an infection with turkey rhinotracheitis (TRT) virus, a respiratory virus. The same morning, seven dead layers were submitted for PME to the AHS by the representative of the poultry supplier. The anamnesis accompanying the birds reported the following clinical signs: mild respiratory problems, severe diarrhea, severe apathy, and severe egg-production problems; decrease in average feed intake from 140 to 50 g per bird per day, water intake likewise decreased, eggs with bad egg-shell quality; and increased mortality.

PME showed a clear peritonitis in five birds, and in two birds, a beginning and slight tracheitis was seen (Table 1). Because the AHS suspected that an *E. coli* infection was the cause of increased mortality, a general bacteriologic examination was begun. Because of the slight tracheitis, without a direct suspicion for ND, an investigation into ND as well as IB was executed using an IFT on tissues sampled during the PME.

Tuesday, February 25. In the supplementary investigation of postmortem material, an *E. coli* infection was confirmed, the IFT on ND and IB was negative (Table 1). These results were, for the veterinary practitioner of the poultry supplier, also reason enough to suspect an *E. coli* infection as the cause of the problems observed. In the evening, this veterinarian contacted the poultry farmer and prescribed an antibiotic treatment with oxytetracyclin. At the end of the day, this veterinarian brought four other boxes for submitting other dead birds for PME to the AHS. This veterinarian did not visit the poultry houses to have a look at the clinical situation, but he deposited the boxes at the edge of the premises.

Wednesday, February 26. A total of four boxes with layer hens (50% dead, 50% alive) were submitted for PME to the AHS. Preceding the submission, the veterinarian of the flock reported

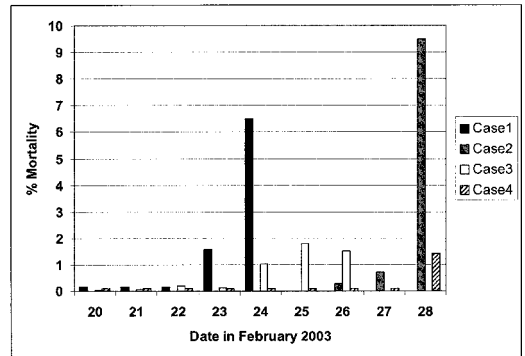


Fig. 2. Development of mortality in cases 1 to 4 in the last week of February 2003.

excessive mortality in the flock by telephone to the AHS. PME showed a large number of birds with slight peritonitis, swollen liver, and swollen spleen (Table 1). Besides the *E. coli* infection, another (primary) bacterial agent was considered to be responsible for the enhanced mortality, and supplementary laboratory investigations were started.

Thursday, February 27. In the supplementary investigations, general bacteriology yielded negative results, coliforms were detected in the peritoneum, and suspected colonies were found from one sample in the direct salmonellae culture (Table 1).

Friday, February 28. To exclude possible other causes, the AHS started testing samples of trachea, spleen, and liver from submitted birds on ND and AI by means of IFT. Furthermore, a veterinary poultry specialist from the AHS was sent to the farm to investigate the clinical situation and to collect additional information with respect to the possible cause of the clinical problems.

The telephone report in the afternoon of the veterinary poultry specialist to the laboratory of the AHS of a dramatic clinical situation at the farm coincided with a positive IFT result on AI from clinical samples. The governmental authorities were notified immediately of a strong suspicion of an AI outbreak. A specialist team from the National Inspection Service for Livestock and Meat (RVV) subsequently visited the case 1 site in the afternoon, and birds collected during clinical inspection were submitted to the NRI in Lelystad.

Case 2. *Wednesday, February 26.* Dead birds were submitted for PME that afternoon to the AHS by the poultry farmer. The anamnesis accompanying the dead birds reported acute mortality in one specific part of the caged laying hen facility February 25: three dead birds; February 26: >100 dead birds;

Table 1. Results of postmortem and supplementary investigation of poultry from the first five outbreaks submitted to the Animal Health Service preceding the detection of the index case of highly pathogenic avian influenza A (H7N7) virus in the Netherlands in 2003.

Date	Case 1	Case 2	Case 3	Case 4	Case 5
Feb. 24	<p>From seven dead layer hens: Five birds with clear peritonitis; Two birds with beginning and slight tracheitis</p> <p>Supplementary investigation: Coccidiosis: negative; Ascaridia: positive</p>				
Feb. 25	<p>Supplementary investigation of Feb. 24: <i>E. coli</i>: positive ND: negative IB: negative</p>				
Feb. 26	<p>From seven dead layer hens: Seven birds with peritonitis; In three birds also pericarditis; In five birds swollen liver In seven birds swollen spleen; Ascaridia infection</p>	<p>From two dead layer hens: Two birds with acute peritonitis; In one bird also a swollen liver</p>			
		<p>From four euthanized layer hens: Three birds with beginning peritonitis; Three birds with slight liver degeneration; Two birds with hemorrhages in the stomach</p>			
		<p>Supplementary investigation: Coccidiosis: negative Ascaridia: negative</p>			

Table 1. Continued.

Date	Case 1	Case 2	Case 3	Case 4	Case 5
Feb. 27	<p>Supplementary investigation of Feb. 26:</p> <p>General bacteriology (24 hours): negative and to be continued;</p> <p>Peritoneum: coliforms detected;</p> <p>supplementary investigation on salmonellae (BGA);</p> <p>Direct salmonellae culture: suspected colonies found from one sample;</p> <p>Indirect salmonellae culture: immediate grafting to BGA plates</p>	<p>Supplementary investigation of Feb. 26:</p> <p>General bacteriology (24 hours): negative and to be continued;</p> <p>Direct salmonellae culture: nothing detected and to be continued;</p> <p>Indirect salmonellae culture: immediate grafting to BGA plates</p> <p>IFT on ND: negative</p> <p>IFT on AI: positive</p>	<p>From four dead layer hens:</p> <p>Four birds with a beginning peritonitis;</p> <p>Three birds with swollen liver;</p> <p>In one bird also hemorrhages in the liver;</p> <p>From three euthanatized layer hens:</p> <p>One bird with chronic ovary inflammation;</p> <p>One bird with disturbed ovary function, beginning peritonitis, slightly swollen liver, and also slightly swollen spleen</p> <p>Supplementary investigation:</p> <p>Coccidiosis: negative</p> <p>Ascariid: positive</p>	<p>From four dead layer hens:</p> <p>In four birds ovary disturbed;</p> <p>In two birds beginning peritonitis;</p> <p>In three birds abundance of lice detected;</p> <p>Supplementary investigation:</p> <p>Coccidiosis: negative</p> <p>Ascariid: negative</p>	
Feb. 28	<p>Supplementary investigation of Feb. 26:</p> <p>General bacteriology: nothing detected;</p> <p>Direct salmonellae culture: nothing detected;</p> <p>Indirect salmonellae culture: nothing detected yet;</p> <p>IFT on ND: negative;</p> <p>IFT on AI: positive</p>	<p>Supplementary investigation of Feb. 26:</p> <p>General bacteriology: a few plates with coliforms;</p> <p>Direct salmonellae culture: nothing detected and to be continued;</p> <p>Indirect salmonellae culture: immediate grafting to BGA plates;</p> <p>IFT on ND: negative</p>	<p>Supplementary investigation of Feb. 27:</p> <p>General bacteriology (24 hr): a few coliforms detected, immediate grafting to BGA plates;</p> <p>Direct salmonellae culture: nothing detected and to be continued;</p> <p>Indirect salmonellae culture: immediate grafting to BGA plates;</p>	<p>Supplementary investigation of Feb. 27:</p> <p>General bacteriology (24 hr): one sample a suspicion of <i>Mannheimia haemolytica</i>; one sample a few coliforms, other organs: no growth.</p> <p>Direct salmonellae culture: nothing detected and to be continued;</p>	<p>From two dead layer hens:</p> <p>Two birds with severe hemorrhagic inflammation of trachea;</p> <p>In one bird beginning peritonitis</p>

Table 1. Continued.

Date	Case 1	Case 2	Case 3	Case 4	Case 5
Feb. 29	Indirect salmonellae culture: nothing detected	Indirect salmonellae culture: nothing detected	<i>Mannheimia haemolytica</i> infection suspected; coliforms typed as <i>E. coli</i> IFT on AI: positive	Indirect salmonellae culture: immediate grafting to BGA plates	
Mar. 3			<i>Pasteurella</i> from ovary: positive <i>Mannheimia haemolytica</i> : positive		IFT on ND: negative IFT on ILT: negative IFT on AI: positive

development of mortality shown in Fig. 2); egg production of 94%–95%; and average feed intake of 118 g per bird per day. Results of PME are shown in Table 1. The preliminary diagnosis of the AHS on the basis of PME was peritonitis caused by a *Salmonella gallinarum* infection. On the basis of this suspicion, several bacteriologic investigations were started, among which were direct and indirect *Salmonella* cultures. The *S. gallinarum* suspicion was based on the fact that identical clinical problems had been seen in two other flocks earlier that year (not in the same area), yielding a final diagnosis of *S. gallinarum* infection. The AHS did not succeed in culturing salmonellae from the submitted birds the next day. But, because the group of birds was medicated with an antibiotic at the time of sampling, and because literature indicated that some *S. gallinarum* strains take a long time to culture, this result did not remove the suspicion of a *S. gallinarum* infection in the opinion of the AHS. However, more supplementary investigations aimed at *S. gallinarum* were started (see Table 1). The suspicions of *S. gallinarum* infections were reported to the Product Boards for Poultry and Eggs (PPE), because the eradication of *S. gallinarum* in parent stock is based on the regulation of the PPE.

Thursday, February 27. The enrichment culture for salmonellae was accelerated by means of grafting from the enrichment culture directly on a brilliant green agar (BGA) plate (Table 1). General bacteriology and the direct salmonellae culture yielded negative results. Browsing the literature also indicated that *S. gallinarum* infection could cause mortality up to and above a rate of 50%.

Friday, February 28. Supplementary laboratory investigations showed the detection of coliforms; the direct salmonellae culture was negative (Table 1). The enrichment culture for salmonellae (indirect salmonellae culture) was accelerated by means of grafting from the enrichment culture directly on a BGA plate. An IFT on ND was negative.

Because of the notification to the governmental authorities of a strong suspicion of an AI outbreak in case 1 and case 3, a specialist team from the RVV also visited the site of case 2 in the afternoon, and birds collected during clinical inspection were submitted to the NRI in Lelystad.

Case 3. *Thursday, February 27.* In the afternoon, birds for PME were submitted to the AHS (see Fig. 2 for development of mortality). The anamnesis accompanying the birds reported high mortality and decrease in feed intake. PME showed four birds out of four with a beginning

of peritonitis, three birds with a swollen liver, and one bird that also exhibited small hemorrhages in the liver as well as two birds with ovary dysfunction (Table 1). Supplementary bacteriologic investigations were begun. A preliminary diagnosis on the basis of PME was peritonitis and a suspicion of *E. coli* infection.

Friday, February 28. Supplementary laboratory investigations showed the detection of a few coliforms; the direct salmonellae culture was negative. A *Mannheimia haemolytica* infection was suspected. To exclude possible other causes, the AHS started testing samples of trachea, spleen, and liver from the birds submitted on the day before for ND and AI by means of IFT. Furthermore, a veterinary poultry specialist from the AHS was sent to the farm to investigate the clinical situation and to collect additional information with respect to the possible cause of the clinical problems.

The telephone report in the afternoon of the veterinary poultry specialist to the AHS of a dramatic clinical situation at the farm coincided with a positive IFT result on AI from clinical samples. The governmental authorities were notified immediately of a strong suspicion of an AI outbreak. A specialist team from the RVV subsequently visited the site of case 3 in the afternoon, and birds collected during clinical inspection were submitted to the NRI in Lelystad.

Case 4. Thursday, February 27. In the afternoon, birds for PME were submitted to the AHS (see Fig. 2 for development of mortality). PME showed birds with disturbed ovaries and the beginning of peritonitis (Table 1). Supplementary bacteriologic investigations were begun. A preliminary diagnosis on the basis of PME was peritonitis and suspicion of *E. coli* infection.

Friday, February 28. Supplementary laboratory investigations yielded a suspicion of a *Manheimia haemolytica* infection, and a few coliforms were detected; a direct salmonellae culture was negative. Because of the notification to the veterinary authorities of a strong suspicion of an AI outbreak in case 1 and case 3, a specialist team from the RVV also visited the site of case 4 in the afternoon, and birds collected during clinical inspection were submitted to the NRI in Lelystad.

Case 5. Monday, February 24. The poultry farmer observed increased mortality in poultry house 1 (15 birds, see Fig. 3 for development of mortality).

Tuesday, February 25. The poultry farmer observed enhanced increased mortality in poultry house

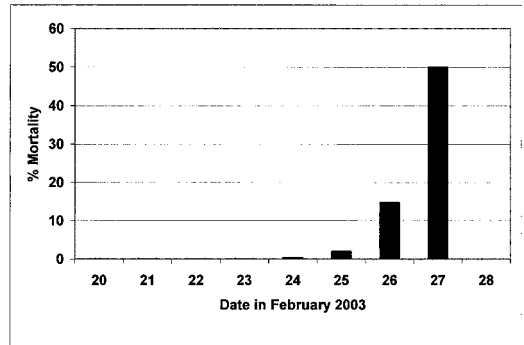


Fig. 3. Development of mortality in case 5 in the last week of February 2003. Mortality was so severe after February 27 that the actual number of dead birds could not be counted anymore.

1 (81 birds) and increased mortality in poultry house 2 (30 birds). The poultry farmer contacted a representative of the feed supplier, and they subsequently asked a veterinarian to visit the flock for a clinical inspection. This veterinarian visited the flock the same day. During his clinical inspection he contacted a poultry veterinarian at the AHS by telephone because of the increased mortality. He described the following clinical signs: hemorrhagic and red inflamed trachea in layer chickens. The AHS endorsed—on the basis of the clinical signs described by telephone—a first preliminary diagnosis of the visiting veterinarian, infectious larynchotracheitis (ILT). The AHS recommended the visiting veterinarian to submit birds for PME to come to a conclusive diagnosis. Unfortunately, this was not done by the visiting veterinarian. Only on Friday, February 28, when the majority of birds present at the farm had died, were birds submitted for PME to the AHS by the poultry farmer himself.

Wednesday, February 26. Mortality increased: in poultry house 1, a total of 588 birds died, and in poultry house 2, there were a total of 132 dead birds. In poultry house 1 a decrease in feed intake was also observed; in poultry house 2, feed intake was still normal.

Thursday, February 27. Mortality was dramatic, with 2000 dead birds in poultry house 1 and approximately 660 dead birds in poultry house 2. In both houses there was no feed intake at all. Flumequine was added to the drinking water by the poultry farmer as a therapy to control the clinical problems.

Friday, February 28. In the afternoon, two dead birds were submitted for PME to the AHS. In the anamnesis, severe respiratory problems, severe di-

arrhea, and severe apathy were reported, with high mortality and suffocation of birds. PME showed severe hemorrhagic inflammation of the trachea, and in one bird also a beginning peritonitis (Table 1). A preliminary diagnosis on the basis of PME was an ILT infection. Because of the notification to the governmental authorities of a strong suspicion of an AI outbreak in case 1 and case 3, a specialist team from the RVV also visited the site of case 5 in the afternoon, and birds collected during clinical inspection were submitted to the NRI in Lelystad.

Disease emergency phase. After the veterinary authorities were notified in the afternoon of Friday, February 28, an emergency plan was activated based on an animal health problem in five poultry flocks located in the "Gelderse Vallei," with a suspicion of AI as the cause of the clinical problems. Specialist teams from the RVV, consisting of a state veterinarian, the local veterinary practitioner, and a veterinary poultry specialist of the AHS, subsequently visited the sites of cases 1 to 5 on the same day, and birds collected from these farms during clinical inspection were submitted to the NRI in Lelystad. The clinical picture from the PME on birds submitted resembled those earlier seen during PME at the AHS. The same evening, a total standstill was announced. RT-PCR specific for AI viruses of H5 and H7 subtypes was executed on RNA isolated directly from tissue samples from dead birds of cases 1 to 5 at the NRI in Lelystad and of case 3 at the NIC on Saturday, March 1, 2003. RT-PCR on H7 was positive. The hemagglutinin (H) and neuraminidase (N) genes were sequenced on Sunday, March 2, providing evidence for viral subtype H7N7 (12). Sequencing indicated the presence of multiple basic amino acids at the protease cleavage site of the H gene, which is a characteristic associated directly with virulence (33). The presence of HPAI in the Netherlands was reported to the OIE on March 2, 2003 (21). Intravenous pathogenicity tests performed at the NRI on the isolate of case 3 in 6-wk-old chickens resulted in a value of 2.94 (i.e., HPAI viruses).

Hypothesis on introduction of AI virus into primary outbreak. Through monitoring of wild water fowl in the Netherlands, it has become clear that several different AI viruses circulate in these groups (10,11). In 1999 and 2000, AI viruses were isolated from ducks within the AIV surveillance studies in migratory birds by the NIC (12,20): A/Mallard/Netherlands/12/00 (H7N3), A/Shoveler/Netherlands/18/99 (H11N7), and A/Mallard/Netherlands/2/00 (H10N7).

It is suggested that H7N7 subtype of the 2003 epidemic in the Netherlands might be the result of reassortment in a wild reservoir (ducks, geese) of AI virus isolates, as mentioned above (20). It is hypothesized that LPAI of subtype H7N7 was transmitted to the free-range layers of case 1 by means of (wild) water fowl that frequented a large pond on a recreational camping site close (<1000 m) to case 1 (Fig. 4). It is further suggested that in the free-range layers of case 1, a shift of LPAI to HPAI occurred after introduction of LPAI of subtype H7N7.

DISCUSSION

Clinical signs. As early as 1992, De Boer *et al.* (5) warned that in the case that an HPAI outbreak occurred in commercial poultry in the Netherlands, all involved parties would be presented with large problems, because clinical signs are difficult to distinguish from a large range of other poultry diseases, and the time needed for ultimate detection of the new infection would provide time for the disease to spread quickly. Clinical signs associated with HPAI virus infections can vary considerably and depend on species, age, sex, concurrent infections, virus strain, and environmental conditions (7). Chickens and turkeys infected with a HPAI virus are mainly found dead (within only a few days, up to 100% mortality) and only a limited number of clinical signs appear, such as general depression, apathy, and absence of vocalizations inside the house; decreased feed and water intake; swollen head and wattles; diarrhea; and in turkeys, often a lack of coordination, head shaking, paralysis of the wings, abnormal gait, loss of their balance, and ending up in a recumbent position with pedaling movements (4,7,28). In exceptional cases, cyanosis of the comb and wattles is observed. In breeders and layers, egg production drops quickly to almost 0% in only a few days. Furthermore, disease progresses more slowly in caged layers, compared to birds located in other housing systems, such as free-range and floor-raised systems.

The (atypical) clinical signs described in literature were comparable with those observed in the first five outbreaks during the HPAI epidemic in 2003 in the Netherlands. However, clinical signs are not specific and can be caused by a range of other poultry diseases. The absence of HPAI from the Netherlands for more than 75 yr created a situation in which poultry farmers and veterinary practitioners did not think of AI in the differential diagnosis as a cause of

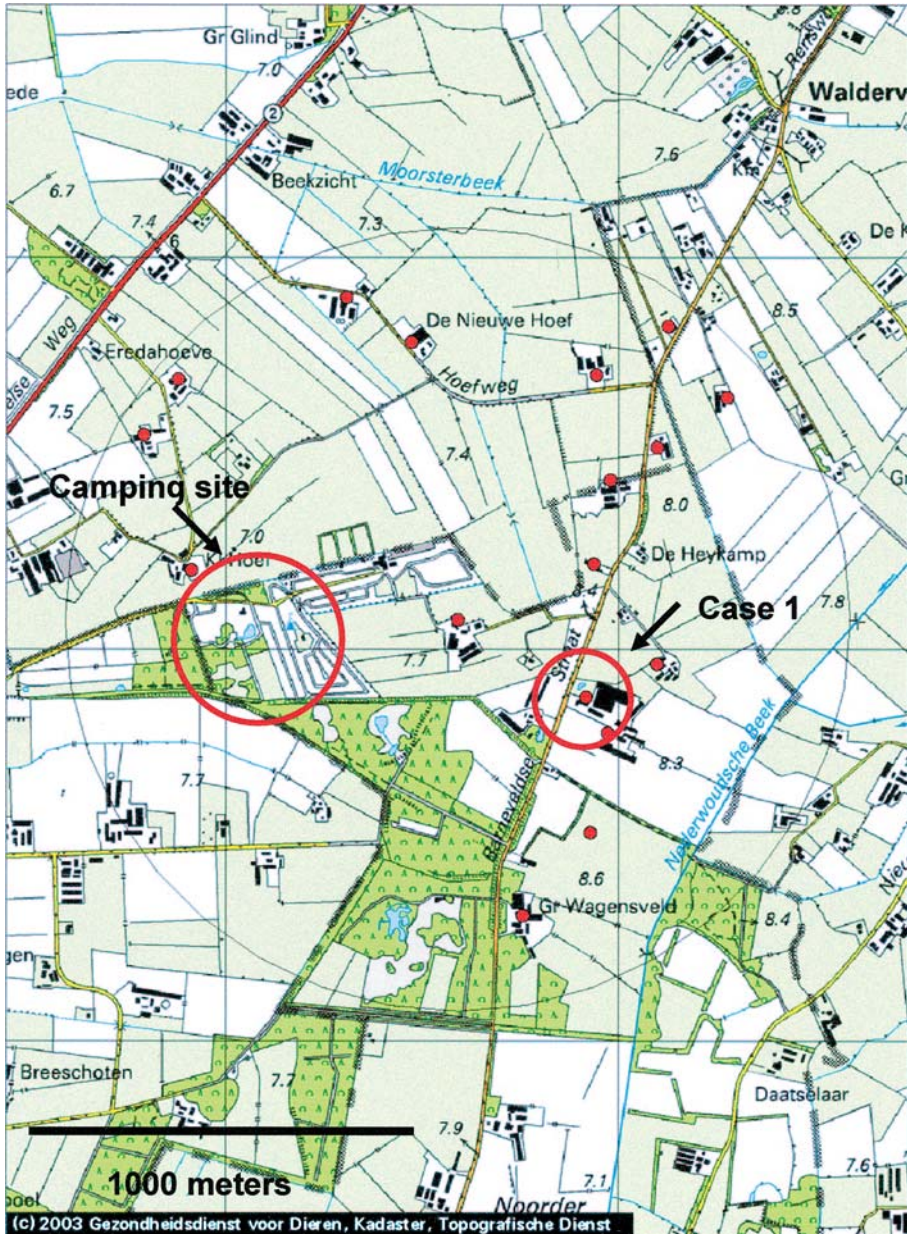


Fig. 4. Geographical location of case 1 in relation to the location of the recreational camping site. The red dots represent premises with commercial poultry flocks. Reprinted with permission of Gezondheidsdienst voor Dieren.

the clinical problems seen. We can hope that a lesson is learned from this. Furthermore, in the future, progressive and excessive mortality should be a signal to exclude AI as cause of disease problems on poultry farms. The use of clinical signs to detect HPAI

outbreaks during the 2003 epidemic will be the subject of further research in our group.

Gross lesions. In the case of HPAI viruses, there may be no prominent lesions because the birds die very quickly, before gross lesions can develop

(16). However, a variety of congestive, hemorrhagic, transudative, and necrobiotic changes have been described: in turkeys and layers, the internal organs (spleen, liver, kidneys, and intestines) are enlarged, hemorrhagic, and blotched by necrotic foci. Furthermore, pancreatitis, tracheitis, pneumonia, and hemorrhagic cecal tonsils can be observed, but also observed are petechial hemorrhages on epicardium, on abdominal fat, and occasionally in the muscles. Initial changes may include edema of the head, with swollen sinuses and cyanotic, congested, and hemorrhagic comb and wattles (1,4,7,16,34).

The gross lesions observed in this case report—and for the complete 2003 epidemic (8)—did not comply with the descriptions in literature. Of special note is the fact that a lack of hemorrhagic changes in tissues and a lack of edema and cyanosis in the comb and wattles was noted. This finding represents one major reason why it took 4 days from the first entry of postmortem material to the postmortem laboratory to report a suspicion of AI on the basis of a positive IFT. An investigation into the performance of gross lesions to detect HPAI outbreaks during the 2003 epidemic revealed that presence of peritonitis or tracheitis or hemorrhages in the proventriculus or edema of neck and/or wattles at PME was the most efficient diagnostic test, combining a sensitivity of 80% with a specificity of 84% (8). If pathology is a fixed part of the AI diagnosis, false-negative pathology results will postpone the diagnosis of HPAI-infected herds in approximately 20% of the cases. Therefore, a lesson from this case report is that if clinical findings such as high mortality indicate the possibility of HPAI (28), the pathologist should submit clinical samples to the NRI for use of specific HPAI laboratory tests, even if PME gives no specific indications for HPAI.

In the poultry industry, the existence of a certain baseline level of mortality—dependent on poultry species and timing within production period—is a general phenomenon. Another lesson learned from this case report is that in an anamnesis, reporting of high or increased mortality (a qualitative measure) should be quantified in the future; otherwise it cannot be interpreted properly. Therefore, mortality should be described in the anamnesis in terms of the number of dead birds in relation to the number of birds brought to the farm to start production, together with the timing within the production cycle.

Furthermore, this case report demonstrates that intensive contact between the veterinary practitioner in the field and the veterinarian executing PME is much needed to have all relevant data and develop-

ments at one's disposal. The mutual communication on new developments—progression in mortality and deterioration of the clinical situation over time—is very important to redirect PME and laboratory investigations. Furthermore, the threshold to submit birds for PME or to ask for a farm visit in such a clinical situation should be very low.

Early warning system—syndrome reporting. Experiences teach that it is rather common that HPAI outbreaks are detected at least a week or more after clinical signs have begun (2,26,27,31). An important requirement for the development of a large epidemic is a high density of poultry farms in a given area. The Italian experiences show that an HPAI epidemic in a high-density poultry area is very difficult to control, especially if the detection of the infection is delayed (2,4,34). This points out a considerable risk, because in times without AI outbreaks in a free country, there will be a tendency by the farmer or veterinary practitioner not to report AI-suspect situations. The length of the high-risk period (HRP) is one of the most important parameters that determine the magnitude of an outbreak, because it defines the period in which the virus can circulate freely and is able to infect poultry flocks. The period begins when the first animal is infected and ends when all eradication measures are in full operation (i.e., the region concerned no longer presents any risk for other regions). The consequence of not reporting AI-suspect situations (because of low specificity of clinical signs) will be a longer HRP. A longer HRP increases the risk of spread of infection to other flocks, especially in high-density poultry areas, and this will seriously hamper the eradication of AI after introduction into a free country.

Because of the threat of a large HPAI epidemic in Italy in 1999–2000 (2), stringent measures were established by the veterinary authorities (Directive Flock Control Avian Diseases 2000 [DFCAD-2000]) to recognize possible AI outbreaks (including LPAI infections) as quickly as possible (13,14). According to DFCAD-2000, every poultry farmer was obliged to counsel his veterinary practitioner when a flock was treated because of a possible infectious disease or mortality of a flock was 0.5% or more per 24 hr. Call-in of the veterinary practitioner was also required when, in reproduction flocks or layers, the mean egg production decreased by 5% or more in a period of 1 wk. The veterinary practitioner clinically inspected the flock and took 20 blood samples per poultry house for detection of antibodies against AI virus. The results of the clinical inspection were

collected into a central database. At the end of 2000, when the HPAI epidemic in Italy was under control, the DFCAD-2000 in the Netherlands was dismantled.

In 2002 and the beginning of 2003, AI outbreaks in domestic poultry were reported from the United States (LPAI subtype H7N2) (19), Chile (LPAI and HPAI subtype H7N3) (25), and Italy (LPAI subtype H7N3) (3). This situation did not lead to the reestablishment at the beginning of 2003 of measures like the DFCAD in 2000. However, during the 2003 epidemic—and up to the present—a syndrome-reporting system combined with a serologic monitoring system (Directive “Monitoring Avian Influenza 2003” [MAI-2003]) (6) was set up by the Dutch Ministry of Agriculture, Nature Management and Food Safety, which is comparable to the DFCAD-2000.

According to MAI-2003, every poultry farmer is obliged to report mortality of more than 3% per week to the veterinary authorities. Furthermore, a poultry farmer has to consult a veterinary practitioner when a clinical problem is observed in AI-susceptible animals or when a reduction in feed or water intake of more than 20% is observed. If the veterinary practitioner concludes that no AI or ND is involved in the disease problems, this is reported and administered in a database at the AHS.

Retrospectively, one could argue that if measures such as DFCAD-2000 in the Netherlands had been in permanent operation from 2000 on—and had been properly executed—there might have been a better chance in 2003 to detect the primary case earlier than actually happened. Furthermore, one can conclude from this case report that poultry farmers and veterinary practitioners did not report high and progressive mortality observed in their flocks to the governmental authorities. We hope that the dramatic consequences of this large epidemic have served as a wake-up call to the poultry industry and the veterinary practitioners, with the result that excessive mortality or a large decrease in feed or water intake or a considerable drop in egg production will be reported immediately to the syndrome-reporting system presently in operation in the Netherlands.

Early warning system—serologic monitoring. In 2000, a one-time nationwide serologic survey was executed by the AHS in breeding flocks, broilers, domestic ducks, and turkeys (9,18) in response to the HPAI outbreak in Italy. In the blood samples collected, no antibodies against AI virus were detected.

In domestic and in wild waterfowl—especially in ducks, geese, and shorebirds—influenza A viruses are present that are not pathogenic to them. AI viruses are spread all over the world and are subject to genetic changes (30). Through monitoring of wild water fowl in the Netherlands it has become clear that a large variety of AI viruses circulate in these groups (10,11).

On the basis of agreements within the European Union (EU) in 2002, member states were obliged to execute a serologic survey for avian influenza in poultry flocks in 2003 to investigate the possible presence of infection with LPAI H5 or H7 viruses (EU Directive 2002/649/EC). However, before the EU survey in the Netherlands was actually started, the Netherlands was struck by an HPAI epidemic at the end of February 2003, after a period of decades of being AI free. Two weeks after the detection of the primary outbreak of the 2003 epidemic, the serologic survey was executed on a much larger scale than proposed in directive 2002/649/EC (22). Approximately 1200 commercial poultry flocks from all over the country were screened for antibodies against AI virus. Three poultry flocks (two layer and one turkey) in the southwestern part of the Netherlands showed antibodies against AI virus of subtype H7 (most likely of low pathogenicity); no clinical disease was seen at the time of sampling (unpublished data).

In response to the 2003 epidemic, a serologic monitoring system (Regulation “Monitoring Avian Influenza 2004”) was set up by PPE in conjunction with the Dutch Ministry of Agriculture, Nature Management and Food Safety. In this monitoring system, broiler flocks, duck flocks, layer breeder and broiler breeder flocks, and layer flocks without free-range facilities are screened once a year; turkey flocks are screened once every fattening period; and layer flocks with free-range facilities are screened every 3 mo. Serum samples are screened for antibodies against (low-pathogenic) AI virus of subtypes H5 and H7. This monitoring system started halfway through February 2004, and a month later the detection of antibodies against AI virus of subtype H7 (likely of low pathogenicity) was reported in free-range commercial layer hens from a flock in the northern part of the Netherlands (23). Furthermore, antibodies against AI virus of subtype H5 (likely of low pathogenicity) were reported in free-range ducks from a trader in the western part of the Netherlands and in ducks from a flock in the southwestern part of the Netherlands that had links to the other duck flock (24).

Within Europe, it is the most intensive system to monitor possible avian influenza introductions into commercial poultry flocks. In order to detect in an early phase an introduction of AI virus that could possibly lead to an epidemic comparable to that observed in 2003 in the Netherlands, the sampling frequency should be much higher in the serologic monitoring system, especially for the poultry flocks with free-range facilities. However, in theory, the operational syndrome-reporting system should be far better able to detect in an early stage an introduction of AI virus into a poultry flock. Time will tell whether this is a correct presumption.

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