

## Investigation of H7N2 Avian Influenza Outbreaks in Two Broiler Breeder Flocks in Pennsylvania, 2001–02

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**SUMMARY.** An avian influenza (AI) outbreak occurred in meat-type chickens in central Pennsylvania from December 2001 to January 2002. Two broiler breeder flocks were initially infected almost simultaneously in early December. Avian influenza virus (AIV), H7N2 subtype, was isolated from the two premises in our laboratory. The H7N2 isolates were characterized as a low pathogenic strain at the National Veterinary Services Laboratories based on molecular sequencing of the virus hemagglutinin cleavage site and virus challenge studies in specific-pathogen-free leghorn chickens. However, clinical observations and pathologic findings indicated that this H7N2 virus appeared to be significantly pathogenic in meat-type chickens under field conditions. Follow-up investigation indicated that this H7N2 virus spread rapidly within each flock. Within 7 days of the recognized start of the outbreak, over 90% seroconversion was observed in the birds by the hemagglutination inhibition test. A diagnosis of AI was made within 24 hr of bird submission during this outbreak using a combination of virus detection by a same-day dot-enzyme-linked immunosorbent assay and virus isolation in embryonating chicken eggs. Follow-up investigation revealed that heavy virus shedding (90%–100% of birds shedding AIV) occurred between 4 and 7 days after disease onset, and a few birds (15%) continued to shed virus at 13 days post-disease onset, as detected by virus isolation on tracheal and cloacal swabs. AIV was not detected in or on eggs laid by the breeders during the testing phase of the outbreak. The two flocks were depopulated at 14 days after disease onset, and AIV was not detected on the two premises 23 days after depopulation.

**RESUMEN.** Investigación de brotes de influenza aviar con virus de tipo H7N2 en dos parvadas de pollo de engorde en Pennsylvania, 2001–2002.

Se observó un brote de influenza aviar en pollos de engorde en el área central del estado de Pennsylvania durante el periodo de Diciembre 2001 a Enero de 2002. Dos parvadas de pollos de engorde fueron afectadas en forma casi simultánea a principios del mes de Diciembre. Se aislaron a partir de estas parvadas virus de influenza aviar del tipo H7N2. Estos aislados fueron caracterizados por el laboratorio nacional de servicios veterinarios (NSLV por sus siglas en inglés) como virus del tipo H7N2 de baja patogenicidad, basado en la secuencia de nucleótidos del gen que codifica por el sitio de desdoblamiento de la proteína hemagglutinina y en los estudios de desafío hechos en pollos libres de patógenos específicos. Sin embargo, los síntomas clínicos y hallazgos histopatológicos indicaban que estos virus del tipo H7N2 eran significativamente patógenos para pollos de engorde bajo condiciones de campo. Investigaciones subsiguientes indicaron que estos virus se diseminaron rápidamente en las parvadas infectadas. Se observó seroconversión, mediante la prueba de inhibición de la hemagglutinación, en el 90% de las aves en las parvadas dentro de los primeros 7 días de la aparición de la enfermedad. El diagnóstico de influenza aviar se realizó mediante una combinación de detección viral por prueba puntual de inmunoabsorción ligada a enzimas y aislamiento viral en embrión de pollo, dentro de las primeras 24 horas después de ser sometida para pruebas diagnósticas aves enfermas de las parvadas afectadas. Otras investigaciones revelaron una alta incidencia de diseminación viral (de un 90 a un 100% de las aves) en un periodo de 4 a 78 días luego del inicio de la enfermedad, y algunas aves (15%) todavía diseminaban el virus 13 días luego de la aparición de la enfermedad, la cual se detectó mediante aislamiento viral e hisopos cloacales. El virus de influenza aviar no se

detectó en los huevos procedentes de las parvadas de reproductoras durante el brote de la enfermedad. Las dos parvadas fueron despobladas a los 14 días de la aparición de la enfermedad y no se pudo detectar el virus en las granjas 23 días después de la despoblación de las mismas.

Key words: avian influenza virus, H7N2 subtype, virus isolation, dot-ELISA

Abbreviations: AGID = agar gel immunodiffusion; AI = avian influenza; AIV = avian influenza virus; CAF = chorioallantoic fluid; dot-ELISA = dot-enzyme-linked immunosorbent assay; ECE = embryonating chicken eggs; HA = hemagglutination; HI = hemagglutination inhibition; MAb = monoclonal antibodies; NVSL = National Veterinary Services Laboratories; VTM = viral transport medium

Four avian influenza (AI) outbreaks have occurred in multiple flocks of domestic poultry in Pennsylvania during the last two decades. The most extreme (1983–84) outbreak, caused by the high pathogenic H5N2 strain of avian influenza virus (AIV), led to the depopulation of 16 million birds at a cost of greater than \$60 million (13). In the 1985–86 outbreak (8), a low pathogenic H5N2 subtype was identified, and depopulation of 350,000 birds was conducted. Between 1996 and 1998, an outbreak of low pathogenic H7N2 subtype of AIV affected 47 flocks comprising 44 egg layer flocks, 1 turkey flock, 1 quail flock, and 1 mixed species flock on 27 premises, representing 2.73 million birds (7). Between December 2, 2001, and January 3, 2002, an outbreak of low pathogenic H7N2 subtype of AIV occurred initially in two broiler breeder flocks (each flock consisted of approximately 8000 birds) and subsequently infected several commercial broiler flocks in central Pennsylvania (6). Approximately 140,000 broilers and broiler breeders were depopulated because of infection or as a precautionary measure. This case report describes investigation of H7N2 AIV outbreaks in two broiler breeder flocks and evaluates the methodologies that led to rapid diagnosis and control of the AIV outbreak.

## MATERIALS AND METHODS

**Flock visit and sample collection.** Two broiler breeder flocks, A and B (approximately 8000 birds each flock, located 1 mile apart in central Pennsylvania), were initially visited on December 4 and 5, 2001, 4 days after disease onset. Depression, respiratory disease symptoms, declining egg production, and increasing mortality were present in the two flocks. Blood samples were collected for serology. Specimens of tracheas and lungs were collected from dead and euthanatized sick birds for virus isolation. Avian influenza virus, H7N2 subtype, was isolated within 24 hr following the first visit. A second visit followed 3 days later (7 days after disease onset). Blood samples, tracheal swabs, and cloacal swabs were collected from 30 live birds in each flock. Watery

manure samples, dust, and other environmental swabs were also collected. Seven fresh dead birds were necropsied on site to examine gross lesions and to collect more tissue samples for detection and isolation of AIV. Prior to depopulation 13 days after disease onset, a third visit was made to collect specimens, including tracheal and cloacal swabs from live birds, tissue specimens from dead birds, and environmental swabs. Eggs were also collected for virus isolation and yolk antibody detection at 7 and 13 days (the second and third visits) after disease onset. Additionally, trachea, lung, kidney, liver, brain, proventriculus, gizzard, pancreas, oviduct, cecal tonsils, and duodenum were collected at days 4, 7, and 13 of clinical onset (the first three visits) and were fixed in 10% neutral buffered formalin for histopathology. A fourth visit was made 23 days after depopulation. Various environmental swabs, including manure samples, fan and heater dust, wall swabs, nest swabs, and floor drag swabs, were collected during the fourth visit. A fifth visit was made to collect floor and wall swabs after the premises were cleaned and disinfected 4 wk after depopulation.

Sterile cotton swabs were used for collecting tracheal swabs, cloacal swabs, and other environmental swabs. Three to five swabs of a single source were pooled for one sample and placed in one 15-ml sterile tube containing 5 ml viral transport medium (VTM). Drag swabs of cheesecloth moistened with phosphate-buffered saline (PBS) were used for floor samplings.

**Sample preparation.** All swab samples and tissue specimens were processed for virus isolation following standard procedures (13,15). Briefly, tracheal, cloacal, and other environmental swabs in VTM broth in tubes were vortexed, and then swabs were squeezed and removed. These samples were centrifuged at 1200 rpm for 10 min at 4 C, and the supernatants were filtered through 0.45- $\mu$ m syringe filters. Tissue specimens were diluted with VTM at a dilution of 1:5 (w/v) and were then placed in a stomacher bag and homogenized in a Stomacher blender (model 80, Seward Ltd., Thetford, Norfolk, UK) for 3 to 5 min. The tissue homogenate was then transferred to a centrifuge tube, followed by centrifugation and filtration. The filtered samples were used for virus detection by a monoclonal antibody (MAb)-based dot-enzyme-linked immunosorbent assay (ELISA) (9) and

a commercial Directigen™ Flu A test and for virus isolation in embryonating chicken eggs (ECE). Three sources of egg samples were prepared for virus isolation from egg shell swabs, albumen, and yolk. Egg shell swabs were obtained from egg shell surfaces and treated as other swabs described above; albumen and yolk were diluted at 1:1 (v/v) with sterile PBS, five eggs per pool of each sample source. Tissue specimens fixed in 10% neutral buffered formalin were sectioned and processed for routine hematoxylin and eosin stains for histopathologic examination.

**Virus detection by dot-ELISA and Directigen Flu A test.** A MAb-based dot-ELISA (9) has been developed in the Animal Diagnostic Laboratory of Pennsylvania State University that detects antigens of all AIV subtypes using group-specific MAb to AIV nucleic proteins and that specifically detects H7 subtype using subtype-specific MAb to HA proteins of H7N2 virus (17). Briefly, the dot-ELISA procedure was performed as follows: 1) 5 µl of each test sample was applied to a strip (10 × 60 mm in size, 5–6 samples per strip) of nitrocellulose membrane (Bio-Rad Laboratories, Hercules, CA); 2) the test strips were treated with two solutions, A (150 mM citric acid prepared in dH<sub>2</sub>O) and B (1.5% mucolytic agent, 6% detergent, and 0.2% sodium azide), respectively, each for 3–5 min and then washed twice with a wash solution (western blot kit from Kirkegaard & Perry Laboratories; Gaithersburg, MD; each wash 30–60 sec); 3) a block solution (western blot kit from Kirkegaard & Perry Laboratories) was then applied to the strips, which were incubated for 10 to 30 min; 4) primary MAb to H7 subtype or AIV group was added to the strips, the strips were incubated for 15–30 min, and then washed three times for 1 to 2 min each wash; 5) a secondary antibody of goat anti-mouse conjugate was added to the strips, with an incubation for 15–30 min; the strips were then washed as described in step 4; and 6) alkaline phosphatase substrate was added, followed by 5–10-min incubation in the dark for color development; the reaction was stopped by the addition of dH<sub>2</sub>O or tapwater when a clear purple dot developed on the positive control strip. All reactions were conducted at ambient temperature (18–24 C) within clean Pitch dishes. The test strips were placed on a chromatography paper to air dry before and after each reaction. The dot-ELISA was employed in the detection of AIV directly from clinical specimens and chorioallantoic fluid (CAF) samples harvested from inoculated ECE. As a comparison, a commercial Directigen Flu A test (Becton Dickinson Microbiology Systems, Cockeysville, MD), which was studied (5,11) and widely used for AIV detection during recent outbreaks (2,19), was used on testing selected specimens.

**A modified procedure of virus isolation in ECE.** Each clinical or field specimen was inoculated into 9–11-day-old specific-pathogen-free (SPF) ECE via chorioallantoic cavity route, 0.2 ml per egg, four to five eggs per specimen. The inoculated ECE were incubated

at 37.5 C for 72–96 hr and CAF was harvested and screened for the presence of hemagglutinin agents of AIV or other viruses by the hemagglutination (HA) test, following standard procedures (13,15). In addition, at 20–24 hr and again at 40–48 hr postincubation, 0.2–0.5 ml of CAF was withdrawn (using a 1-cc syringe with 25½-gauge needle, through the inoculation hole, using sterile technique) from the ECE that had been inoculated with a specimen that was positive for AIV by dot-ELISA or Directigen test or from a case that was clinically suspicious for AIV infection. The ECE were resealed and placed back into egg incubator to continue incubation after CAF samples were withdrawn. These early incubation CAF samples were tested for AIV by the dot-ELISA and HA tests. CAF showing hemagglutinating activity was assayed by a dot-ELISA using AIV group- and H7 subtype-specific MAbs, and it was also tested for AIV H7 subtype and Newcastle disease virus (NDV) by the hemagglutination-inhibition (HI) test (16) using reference antisera to these viruses. All AIV-positive isolates were sent to the National Veterinary Services Laboratories (NVSL) in Ames, IA, for confirmation and pathotyping.

**Serologic tests of sera and egg yolk samples.** The agar gel immunodiffusion (AGID) test (16) was used to detect antibodies to AIV from chicken blood sera and egg yolks. Positive antiserum and antigen to AIVAGID test were supplied by NVSL. The HI test was also used to detect serum antibody titers to H7N2 virus.

**Control strategies.** Quarantines and depopulation were used as essential strategies to control this H7N2 AIV outbreak in the two broiler breeder flocks. Increased surveillance for virus isolation and serologic tests were applied in surrounding areas.

## RESULTS

**Flock observations and pathologic lesions.** Disease onset in the two broiler breeder flocks occurred almost simultaneously, and the personnel managing the two flocks had frequent contact. Clinical signs of depression, rales, dyspnea, cyanosis, watery eyes, facial edema, dehydration, and diarrhea were observed in the infected broiler breeder flocks at days 4 and 7 of clinical onset. At the time of visit, morbidity was started at approximately 60%–70%. On necropsy, tracheas showed reddened and thickened mucosa, and some contained thick yellow exudates. Lungs were dark red and congested. Air sacs of some birds were thickened with yellowish serous or caseous exudates. Some birds had hemorrhagic foci on the mucosa of the proventriculus adjacent to the esophageal junction. Regression of ovarian follicles and free yolk material in the peritoneum were observed in some hens. *Escherichia coli* was isolated from caspous lesions with air sacs, lungs, and

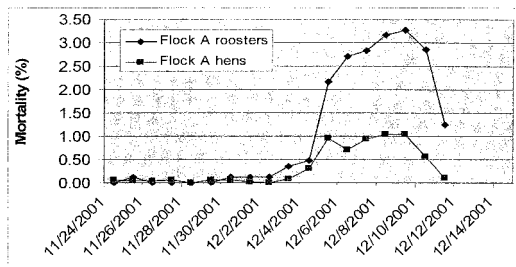


Fig. 1. Daily mortalities (%) of hens and roosters in broiler breeder flock A before (12/01/01), during, and after the clinical phase of avian influenza H7N2 infection.

peritoneum of several males and few females. Microscopic examination of lung, trachea, kidney, liver, brain, proventriculus, gizzard, pancreas, cecal tonsils, and duodenum showed that lesions in these organs consisted of diffuse, predominantly lymphocytic inflammation, with areas of hemorrhage and necrosis also present, especially in the trachea, lung, proventricular/gizzard junction, kidney, and cecal tonsils. No significant lesions were observed in the oviduct, pancreas, or brain. These microscopic findings are consistent with lesions reported by others with mildly pathogenic avian influenza infections (13). Daily mortalities in both flocks increased to more than 10 times normal values, from less than 0.1% to 1% in hens, and less than 0.2% to greater than 2%–3% in roosters at peak (Figs. 1, 2). The death loss in the males was considerably higher than that of the females in both flocks. Daily egg production dropped over 40% from 50%–55% to less than 10%–12% after the onset of the disease in both flocks (Fig. 3).

**Virus detection by dot-ELISA and Directigen Flu A test.** The dot-ELISA effectively detected the H7 subtype of AIV directly from

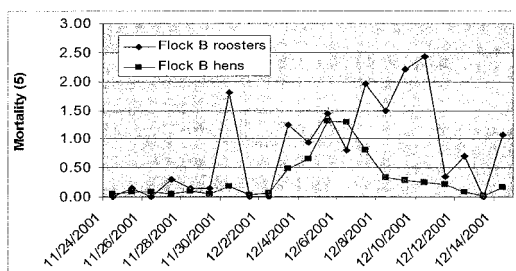


Fig. 2. Daily mortalities (%) of hens and roosters in broiler breeder flock B before (12/01/01), during, and after the clinical phase of avian influenza H7N2 infection.

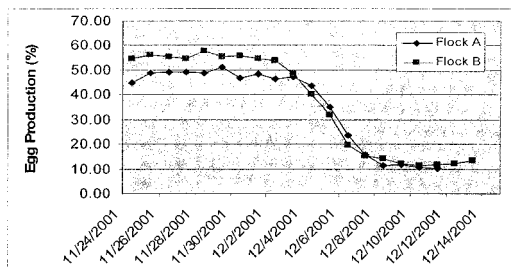


Fig. 3. Daily egg production (%) in two broiler breeder flocks, A and B, before (12/01/01), during, and after the clinical phase of avian influenza H7N2 infection.

various clinical specimens collected between 4 and 7 days post-disease onset. In comparison with virus isolation, the dot-ELISA identified 88% (15/17) positives on tracheal swabs, 33% (5/15) on cloacal swabs, 100% (3/3) on manure samples, 33% (1/3) on dust swabs, and 90% (9/10) on respiratory tissue specimens from dead birds. The Directigen Flu A test provided virtually equivalent results to the dot-ELISA in testing these samples (Table 1).

**Virus isolation in ECE.** The tracheal and lung tissue samples and tracheal swabs collected 4 days after clinical onset of the disease, which were positive for AIV H7 subtype by the dot-ELISA using MAb to H7N2 virus, were confirmed positive for AIV by virus isolation in ECE following the modified procedure of early withdrawal of CAF within 24 hr. The CAF harvested from the inoculated ECE within the first 24 hr postincubation was positive for H7 subtype of AIV by the dot-ELISA using H7 subtype-specific MAb and was also positive by HA and HI tests using reference antiserum produced from SPF chicken inoculated with H7N2 virus. In this case, the virus HA titer reached 1:64 within 24 hr and 1:1024 within 48 hr in CAF. The AIV isolates from both flocks were identified as H7N2 subtypes and were characterized as low pathogenic strains in SPF chicken pathogenicity test at NVSL. Sequencing analysis at NVSL indicated that the amino acid profile at the hemagglutinin cleavage site was compatible with that of low pathogenic AIV.

A large number of clinical specimens from various sources were collected in the second visit 7 days after clinical onset in the two flocks. Virus isolation results from both flock A and flock B revealed a high percentage of AIV-positive samples from various sources. In flock A, 90% (9/10) of tracheal swab pools, 90% (9/10) of cloacal swab pools, 100% (11/11) of tissue specimens of cloacal swabs, 60% (3/5) of

Table 1. Comparison of dot-ELISA and Directigen test in the detection of AIV from specimens collected at 4 and 7 days after clinical onset in two broiler breeder flocks.

Type of specimens	Number of specimens	Virus isolation positives	Directigen test positives (%)	Dot-ELISA positives (%)
Tracheal swab pool	17	17	15 (88) <sup>A</sup>	15 (88) <sup>A</sup>
Cloacal swab pool	16	15	5 (33)	5 (33)
Manure sample	5	3	3 (100)	3 (100)
Dust swab	6	3	0 (0)	1 (33)
Lung and trachea	11	10	9 (90)	9 (90)

<sup>A</sup>Percentage of AIV-positive specimens detected by Directigen test or dot-ELISA based on positive virus isolation results.

manure samples, and 50% (3/6) of environmental dust swabs were positive for AIV. In flock B, 100% of tracheal swab pools (5/5) and 100% of cloacal swab pools (4/4) obtained from 30 live birds were positive for AIV. Eggshell swabs, albumen, and yolk samples from 60 eggs collected in flock A and 60 eggs in flock B were all negative for AIV by virus isolation (Table 2).

A third visit was made to flock A 13 days after clinical onset and prior to depopulation. A total of 46 samples from various sources were collected, which included 7 tracheal swab pools and 7 cloacal swab pools from 21 live birds; 8 environmental dust swabs; 8 manure samples; 4 oral swabs from dead birds (not fresh); and 12 tissue pools from 10 sacrificed birds. Only 3 (1 cloacal swab pool and 2 manure samples) of the 46 samples were positive for AIV. The other 43 were all negative.

A fourth visit was made 23 days after flock depopulation and before any clean-out had taken place. Various environmental samples including manure samples, floor drag swabs, floor litter, fan dust and wall dust swabs, and nest box swabs were collected in flock houses A and B, with a total of 16 and 14, samples, respectively. All samples from both houses were negative for AIV. After cleaning and disinfection of both flock houses 4 wk after depopulation, 10 environmental swabs were collected from each house in the fifth visit, and all were negative for AIV.

**Serologic results.** During the first visit to flock A (4 days after clinical onset), 17 serum samples were collected, and all were negative for AIV by AGID and HI tests. In the second visit (7 days after disease onset), 28 of 29 serum samples collected in flock A were positive for AIV H7 subtype by HI test. At this time, HI titers ranged from 1:32 to 1:512, with a mean titer of 1:128. By AGID test on the 29 serum samples, 23 were positive and 6 were negative. In flock B, serum samples were not collected at the initial visit (4 days after disease onset). At the second visit (7

days after disease onset), 27 of 30 serum samples were positive for AIV H7 subtype by HI, with titers ranging from 1:8 to 1:512, with a mean titer of 1:128. By AGID, 24 samples were positive and 6 were negative. In addition, 60 eggs were collected from each flock 7 days after disease onset during the second visit. Egg yolk antibodies to AIV were not detected by the AGID test in any of these eggs. At 13 days after disease onset, during the third visit to flock A, 60 eggs were collected, and 5 of the 60 egg yolk samples were positive for AIV antibodies by the AGID test.

**Quarantines and depopulation.** The Pennsylvania Department of Agriculture placed immediate quarantines on both sites at the time of diagnosis of AIV in the two broiler breeder flocks. Portable spray washers were placed at the driveway entrances to both flock houses, and access was restricted. No hatching eggs were moved off site after the clinical onset on December 4, 2001. All poultry companies with flocks in these areas were notified, and alternate travel routes were used for flock servicing and for feed and processing plant deliveries to avoid the immediate areas at risk for AIV transmission. Decisions were made to depopulate the two broiler breeder flocks and any subsequent flocks positive for AIV during the outbreak. The broiler breeders were euthanized by CO<sub>2</sub> gas in the houses at 14 days post-clinical onset, by which time clinical signs had abated. Carcasses and hatching eggs remained in the houses for 3 to 4 days and then were removed in covered containers on trucks to disposal in a landfill. Removal of litters and clean-out of the houses were not allowed until houses were virus isolation negative (the fourth visit) after 23 days depopulation. Repopulation was not begun until a final test proved the houses were virus isolation negative (the fifth visit) after cleaning and disinfection had taken place on the two premises.

## DISCUSSION

A 24-hr diagnosis of AIV was achieved during the 2001–02 outbreak in Pennsylvania, which provided

Table 2. Virus isolation results in follow-up investigation of two broiler breeder flocks.

Flock visit (date) and days after disease onset	Type of specimen	Flock A positives/total	Flock B positives/total
First visit: 12/4/01 4 Days after disease onset	Tracheal swab pool	1/1	1/1
	Cloacal swab pool	1/1	1/1
	Trachea and lung	1/1	1/1
Second visit: 12/7/01 7 Days after disease onset	Tracheal swab pool	9/10	5/5
	Cloacal swab pool	9/10	4/4
	Tissue swab	11/11	Not collected
	Manure sample	3/5	Not collected
	Dust swab	3/6	Not collected
	Eggs	0/60	0/60
Third visit: 12/13/01 13 Days after disease onset	Tracheal swab pool	0/7	Not collected
	Cloacal swab pool	1/7	Not collected
	Dead bird oral swab	0/4	Not collected
	Tissue specimen	0/12	Not collected
	Dust swab	0/8	Not collected
	Manure sample	2/6	Not collected
	Eggs	0/60	Not collected
	Fourth visit: 23 days after depopulation and before cleaning and disinfection	Floor swab	0/3
Dust swab		0/3	0/3
Nest swab		0/2	0/2
Manure sample		0/6	0/6
Fifth visit: after cleaned and disinfected	Floor swab	0/6	0/6
	Wall swab	0/4	0/4

the essential information required to initiate immediate actions to control this outbreak. This rapid diagnosis was accomplished using a MAb-based dot-ELISA (9) for rapid screening for AIV directly from clinical specimens in combination with a modified procedure of virus isolation in ECE. Results of this study show that the MAb-based dot-ELISA is virtually equivalent to the Directigen Flu A test with regard to the detection of AIV antigens from clinical and field specimens. Additionally, the dot-ELISA detects all AIV subtypes when group-specific MAb to AIV nucleic proteins are utilized and specifically detects H7 subtype when using subtype-specific MAb to HA proteins of H7N2 virus (17). The dot-ELISA is a low-cost (<\$0.50/sample) test and is feasible for use in mass testing. One individual can screen up to 100 clinical samples in one run within 2 hr. During the 2001–02 AI outbreak in Pennsylvania, the dot-ELISA detected AIV directly from clinical specimens before virus isolation from the two broiler breeder flocks and four of five infected broiler flocks. Isolation of AIV in ECE has been the standard procedure to date (13,15), although the process is labor intensive and time consuming. However, with use of dot-ELISA, results during the 2001–02 AI outbreak, we confirmed the presence of AIV by virus isolation in

ECE within 24 hr by means of a modified procedure, in which a small amount of CAF sample (0.2–0.5 ml/egg) was withdrawn and tested every 20–24 hr, with incubation continued until 72–96 hr. The harvested CAF was used to screen for virus HA activity and to identify the presence of AIV by dot-ELISA. Findings in this study showed that the H7N2 virus present in a clinical specimen grew rapidly in ECE and yielded sufficient HA titers for AIV identification within 24 hr postinoculation if the clinical or field specimen was positive or suspicious for AIV by the dot-ELISA. Therefore, the combination of AIV screening test by dot-ELISA and virus isolation in ECE made a more rapid and effective diagnosis possible during this AI outbreak.

A serologic immune response was not detectable by AGID and HI test within 4 days of onset on clinical signs. However, relatively high serum HI titers (mean 1:128) were detected by day 7 following clinical onset. It appears that AIV-infected birds require more than 4 days or a minimum of 7 days to develop detectable antibodies by HI and/or AGID tests. Zhou *et al.* (18) studied antibody responses of emus to AIV infection and found that all AIV-infected birds developed antinucleoprotein antibodies as early as 7 days postinfection, as detected

by a competitive ELISA. In this AI outbreak in broiler breeders, seroconversion was not detected in egg yolks at day 7. Only 5 of 60 eggs collected 13 days after clinical onset were positive for AIV antibodies by AGID test. This result indicates that egg yolk antibody appeared at least 1 wk after serum antibody was detectable by the HI or AGID tests. Recent studies by Beck *et al.* (3) that evaluated antibody response in serum and yolk collected from experimentally AIV-infected SPF layers showed that serum antibody was detected from all AIV-infected birds by day 7 postinoculation, but yolk antibody was not detectable until 14 days postinoculation. AGID, HI, and ELISA are commonly used tests for the detection of antibodies to AIV infection. Some studies indicated that ELISA was the most sensitive test (1,18); AGID and HI tests were equally effective in detecting AIV antibodies (14).

Virus isolation results showed that more than 90% of live birds, 100% of dead birds, and greater than 50% of environmental swabs were positive for AIV between day 4 and 7 during the AI outbreak in the two broiler breeder flocks. However, the number of AIV-positive specimens decreased to less than 10% (3/34) when samples were collected 13 days after disease onset in flock A. These findings indicate that the first week postinoculation is the best time to collect samples for virus isolation during an outbreak. Two weeks after clinical disease onset or disappearance of clinical signs, virus recovery is more difficult because of the development of flock immunity.

Though classified as a low pathogenic strain of AIV, this H7N2 virus caused respiratory disease, over 40% loss in egg production, and a more than 10 times increase in mortality in the week following clinical disease onset. Such findings indicate how this low pathogenic strain of H7N2 AIV is able to cause clinical disease in broiler breeder chickens under field conditions. Roosters suffered higher mortalities than hens, which indicates the severity of AIV infection in broiler breeders may be gender or body weight related.

Hemorrhages and ulcerations at the proventricular/esophageal junction were seen in several infected broiler breeders in the first week of clinical onset during the outbreak, but such lesions were not seen in commercial layer chickens infected by H7N2 AIV in the 1997–98 outbreak in Pennsylvania. Conversely, the H7N2-infected broiler breeders did not show the oviduct lesion (acute edema) that was very characteristic of the disease in reproductively active layer chickens (leghorn) in the 1997–98 outbreak (19).

Negative virus isolation results from eggs laid by AIV-positive birds indicate that vertical transmission from eggs is unlikely. Fertile eggs collected 2 and 3 days prior to the outbreak were transported to the hatchery, incubated, and hatched. The progeny were raised on Pennsylvania farms and they did not develop disease. Antibodies to AIV were not detected from market-age progeny of the infected broiler breeders. During the 1997–98 H7N2 AIV outbreak in Pennsylvania, in 9930 eggs sampled over a period of several months from three layer premises that had H7N2 AIV-infected chickens, viruses could not be isolated from swabs taken from the egg shell surfaces or albumen from the eggs (4). These results indicate that the subtype H7N2 of AIV is not transmittable via the egg.

Immediate and coordinated reporting and action by government authorities, poultry industry personnel, field veterinarians, and laboratory diagnosticians proved successful as strategies to quickly control the 2001–02 AI outbreak in Pennsylvania. Immediate quarantines and depopulation of AIV-positive flocks were critical steps to control this H7N2 AIV outbreak. At the time of depopulation, closing house for several days between bird euthanatization and opening the premise to loading and transport of carcasses was very important to allow inactivation of any live virus in the house and birds. Results of our previous studies (10) indicated that H7N2 AIV was effectively inactivated by field chicken manure in less than 2 days at 28–30 C and in less than a week at an ambient temperature (15–20 C). Increased surveillance in surrounding areas was another essential component of control strategies. During the outbreak, two surveillance areas within a 10-mile radius based around each area of case(s) were established and affected approximately 3.5 million poultry raised in these areas. The increased surveillance measures, including submission of dead birds for virus isolation and serologic tests (AGID), were extended for a period of 57 days after the detection of the last AIV-positive broiler flock. Although the H7N2 virus was detected in five broiler flocks within 4 wk following the two broiler breeder flocks, the 2001–02 AI outbreak in Pennsylvania was quickly controlled by actions such as immediate quarantines and increased surveillance, rapid 24-hr laboratory diagnosis of AIV, and depopulation of AIV-positive or suspicious flocks.

Sources of infection for the two broiler breeder flocks were undetermined. At least four companies with flocks and many “backyard” flock owners in this region supply poultry into the northeastern live bird

marketing system. Panigrahy *et al.* (12) tested the presence of AIV inside and outside the live bird markets of the northeastern United States between 1993 and 2000. Their test results indicated the H7N2 virus was presented simultaneously in both live bird markets and non-live bird market premises during the years from 1994 to 1999. The H7N2 virus that infected the two broiler breeder flocks presumptively represented the same source virus circulating in the live bird markets. Airborne transmission, travel of farm equipment, and social contact among family relatives were likely to maintain the spread among the five closely located infected flock premises involved during the outbreak.

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