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Avian influenza control strategies in the United States of America

D.E. Swayne[#] *and B.L. Akey*[##]

Abstract

Prevention, control and eradication are three different goals or outcomes for dealing with avian influenza (AI) outbreaks in commercial poultry of the USA. These goals are achieved through various strategies developed using components of biosecurity (prevention or reduction in exposure), surveillance and diagnostics, elimination of infected poultry, decreasing host susceptibility to the virus (vaccination or host genetics) and education. However, the success of any developed strategy has depended on industry–government trust, co-operation and interaction. The preferred outcome for HPAI has been stamping out, for which the federal government has regulatory authority to declare an emergency and do immediate eradication of HPAI, and pay indemnities. For H5and H7LPAI, strategies vary from an immediate control plan followed by an intermediate to long-term strategy of eradication. The state governments have regulatory authority over H5 and H7 LPAI, but work co-operatively with USDA in joint programmes. Stamping out has been occasionally used as has controlled marketing, but inconsistently, indemnities have been funded by the state governments and the poultry industries, and less frequently by USDA. Vaccines have been occasionally used but require USDA license of the vaccine and approval from both state and federal government before use in the field. Non-H5 and -H7 LPAI generally follow a preventive programme, such as H1N1 swine-influenza vaccination for turkey breeders. In other situations, control and eradication strategies are followed but regulatory authority is lacking for USDA. Most programmes for LPAI are voluntary and industry-driven.
Keywords: avian influenza; biosecurity; control; diagnosis; euthanasia; vaccination

Introduction

Poultry and poultry products are a major source of high-quality protein as human food and the per-capita consumption has been increasing around the world during the past two decades. In 2002, the broiler-meat production in the world was 52 million metric tons (MT) with United States (12 million MT), China (9.5 million MT) and European Union (6.4 million MT) being the top three producers (FASonline 2003a). Exports of broiler meat accounted for a little over 10% of total production (5.7 million metric tons), of which the United States (2.2 million MT – 39%), Brazil (1.6 million

[#] Southeast Poultry Research Laboratory, Agricultural Research Service, United States Department of Agriculture, 934 College Station Rd., Athens, Georgia 30605, USA. E-mail: dswayne@seprl.usda.gov
[##] Division of Animal Industry, New York Department of Agriculture and Markets, 1 Winners Circle, Albany, New York 12235-0001 USA

MT – 28%) and European Union (0.9 million MT – 16%) were the leading exporters with 83% of the market. Similarly, world turkey-meat production was 4.9 million MT with the United States (2.5 million MT), European Union (1.7 million MT) and Brazil (0.2 million MT) being the primary producers (FASonline 2003b). World exports of turkey meat are 636,000 MT with European Union (285,000 MT), United States (199,000 MT) and Brazil (85,000 MT) being the principle exporters.

Maintaining poultry free from high-pathogenicity (HP) avian influenza (AI), a list-A disease as defined by the Office International des Epizooties (OIE), is essential to continue trade in poultry and poultry products between nations (Alexander 1997). In addition, some countries specify freedom from avian influenza viruses of low pathogenicity (LP), principally H5 and H7, before importing poultry and poultry products. Over the last decade, the impact of trade on national animal-health policies has increased. As a result, national policies have focused not only on disease control as a national need, but also on the expectation for continuing or expanding exports. Therefore, national control strategies are impacted by the right of importing nations to protect their own poultry populations from introduction of catastrophic diseases, such as HPAI, through implementation of sanitary and health standards to assure freedom in the importing product from such disease-causing agents. However, at times, some countries have used non-tariff trade barriers as strategies to protect domestic poultry production when legitimate sanitary and health issues do not exist. In implementing non-tariff trade barriers, only scientifically sound risk assessments should be used to identify real threats for disease introduction and distinguish these from perceived threats or political protective intents. For example, in 2002, the US had embargoes imposed by a trading partner against pasteurized egg products from the Midwest after the US reported H7N2 LPAI in the state of Virginia. This incident was not a legitimate trade barrier because: 1) H7N2 LPAI virus was not covered under the World Trade Organization by the OIE international health code, 2) the infection was compartmentalized to a small geographic region in the Eastern United States, and 3) the pasteurization process used on the product would inactivate any influenza virus that might have been present.

Strategy components for dealing with avian influenza

In dealing with avian influenza in the United States, different strategies have been developed and used. Each strategy has been designed with one of three different goals or outcomes in mind: preventing the introduction of avian influenza into poultry, controlling losses by minimizing the negative economic impact of avian influenza when present, or total elimination of avian influenza (eradication), especially the highly pathogenic form. These goals are achieved through various strategies developed using universal components that include: 1) biosecurity (management procedures to prevent introduction or escape of AI virus), 2) diagnostics and surveillance (detection of AI virus infections), 3) elimination of AI-virus-infected poultry, 4) decreasing host susceptibility to the virus (vaccination or host genetics), and 5) education. In developing and implementing specific strategies, multiple factors are considered and include virus strain (pathotype and haemagglutinin subtype), which poultry commodity or commodities are affected, the density of poultry in a geographic area, the demands of export markets, federal verses state regulatory authority, and availability of financial compensation. The success of any strategy is dependent on industry–government trust, co-operation and interaction. In the USA,

the federal government has regulatory authority over eradication of HPAI viruses, while the state governments have jurisdiction over LPAI viruses.

Biosecurity

Biosecurity is the utilization of best management practices to reduce the risk of introducing avian influenza virus in a poultry house, farm or operation, either for the initial case or secondary cases in an *ongoing* outbreak, or preventing movement of avian influenza virus off a premise containing infected birds to a new site. In most situations, these practices focus on preventing movement of the avian influenza virus on contaminated equipment, clothing and shoes off of farms with infected birds; preventing movement of infected poultry or their by-products (e.g. manure); or preventing exposure of poultry to wild waterfowl. Farm quarantine is included in biosecurity practices. In many instances, practising biosecurity means controlling the movement of people including restrictions to minimize the number of visitors to farms. This is best achieved by restricting *inbound* and *outbound* movements through circumferential fencing of the farm and locking of the gates, or even better, a manned guard shack to ensure adherence to biosecurity policies (Figure 1). Other high-risk activities must be managed by proper cleaning and disinfection (C&D) of equipment

Figure 1. Guard house which adds an additional layer of biosecurity by screening out visitors and assuring proper biosecurity procedures are followed for entry and exit

shared between farms, decontamination of clothing and shoes of workers (preferably having work clothing and shoes left on the farm with laundering locally), having employees dedicated to one farm, and having strict rules prohibiting employees from owning backyard or recreational poultry or from visiting other poultry farms or establishments. For shared employees such as vaccination crews, catch crews, feed truck drivers, service personnel etc., they must diligently practise C&D of equipment (including vehicles), clothing and shoes, and minimize their exposure to the birds. Ideally, poultry farms should be of low density in a geographic area to reduce the ease of farm-to-farm transmission (Capua and Marangon 2003). Regional biosecurity plans

to co-ordinate movement of LPAI-recovered birds to slaughter; disposal of AI-contaminated manure and AI-infected carcasses, C&D of farms and repopulation have been developed and used in California and Minnesota with success (Halvorson 1998; Cardona 2003). In the Virginia H7N2 LPAI outbreak (2002), the movement of daily mortality off the farm to a rendering facility was associated with spread of avian influenza from farm to farm (Akey 2003). This necessitates finding alternative methods for disposal of daily mortality such as on-farm composting or implementation of revised biosecurity procedures to prevent rendering trucks from entering the farm. The latter is best achieved by placement of the daily mortality in rigid containers at the end of farm entry roads and practising proper C&D.

The most important aspect of biosecurity is the development of a 'biosecurity culture' on the premise and within the company. Employees will not practise biosecurity unless they understand the procedures and importance of biosecurity, and how they affect the company and ultimately their jobs. An education component is essential, as will be discussed later. In developing a biosecurity culture, the ownership and management must take it seriously and practise biosecurity in order for the employees to abide by biosecurity policies. Similarly, communication of the location of influenza cases between companies is important in developing regional biosecurity plans.

Preventing direct or indirect exposure to AI-virus-infected birds is very important; including preventing exposure to potentially infected wild birds. Between 1978 and 2000, poultry farmers in Minnesota experienced 108 introductions of LPAI viruses of various haemagglutinin and neuraminidase subtypes from migratory ducks into turkeys (Halvorson 2002). Twenty of these introductions were from H5 or H7 LPAI viruses, but none of these mutated to HP as occurred in Chile in 2002 (H7N3), Italy in 1999 (H7N1), Mexico in 1994 (H5N2) and Pennsylvania in 1983 (H5N2), possibly because the industry eliminated the viruses in less than 6 months. These Minnesota cases resulted from close direct contact between seasonal migratory juvenile ducks (September to November) with range-reared turkeys, or usage of AI-virus-contaminated lake or pond water for indoor-reared turkeys. Although the range-reared or semi-confinement method has represented historically less than 5% of turkey production, this minor production method has been the introduction point for LPAI viruses into Minnesota commercial turkeys with disastrous results. The worst outbreak year was 1995 with 178 farms having LPAI-virus-infected turkeys, predominantly the H9N2 subtype. With the H5N1 HPAI poultry outbreak and human infections in Hong Kong, the Minnesota production companies agreed to stop range rearing of turkeys to eliminate introduction of waterfowl LPAI viruses and a potential public-relations problem should an outbreak of LP or HPAI occur in Minnesota. As a result, from 1997-2000 only 28 flocks had infections with LPAI viruses, mostly from swine H1N1 influenza virus. However, with the development and usage of organic standards for poultry, outdoor rearing will increase in popularity and enhance the risk for introduction of avian influenza into farming systems.

Diagnostics and surveillance

The speed with which a new disease is eliminated is dependent upon how rapid the index case is detected and how fast eradication strategies are implemented. The presence of high mortality is suggestive of an exotic disease such as highly pathogenic avian influenza or velogenic Newcastle disease, but a definitive diagnosis requires the identification of the causative agent (Swayne, Senne and Beard 1998; Swayne and Halvorson 2003). Other causes of high mortality include some toxins, water

deprivation and heat exhaustion. In addition, the presence of respiratory problems or drops in egg production could be consistent with LPAI, but other more common viral, bacterial, fungal and non-infectious causes need to be excluded. Historically, diagnosis of avian influenza has required isolation in embryonating chicken eggs and identification by antigen testing, but this is a slow laboratory process requiring 1-3 weeks depending on the number of negative back passages performed and the availability of embryonating eggs. In the past 5 years, the United States Department of Agriculture (USDA) has begun using two alternatives to virus isolation and identification for screening and diagnosing AI: 1) direct detection of type-A influenza virus proteins or antigens, and 2) amplification and detection of AI virus genes.

During the Virginia H7N2 LPAI outbreak of 2002, management decisions had to be made rapidly in the field to quarantine flocks based on a reasonable suspicion of AI virus infection, and identify AI-virus-negative flocks to allow low-risk movement to slaughter (Akey 2003). A membrane-bound, sandwich enzyme-linked immunosorbent assay (antigen-ELISA) to detect Type-A influenza virus antigen (Directigen®, Becton Dickinson, Cockeysville, Maryland) was used as a screening test to detect the presence of AI virus in tracheal swabs from poultry. This assay was useful in several situations. First, if poultry flocks were showing clinical signs of respiratory disease or experienced an abrupt drop in egg production, the antigen-detection system was effective as a diagnostic screening tool to identify AI virus infections in poultry. In previous studies, this antigen-ELISA had 100% specificity and 79% sensitivity for detecting AI virus from such poultry sampled during the 1997 H7N2 LPAI outbreak in Pennsylvania (Davison, Ziegler and Eckroade 1998). Second, the test was useful in the Virginia surveillance programme within the quarantine zone to identify infected birds using the daily mortality removed from the farms, and to allow movement of clinically normal flocks to slaughter when the test results were negative (Akey 2003). Similarly, this test is being used in the current H7N2 LPAI outbreak in Connecticut layers by screening chickens from the daily mortality for the presence of AI virus infections. Previous experimental studies have shown the value of the antigen-ELISA to detect AI virus in tracheal and cloacal swabs of chickens showing clinical respiratory disease and in identifying AI virus in allantoic fluid of inoculated embryonating chicken eggs (Slemons and Brugh 1997). However, during the Virginia H7N2 LPAI outbreak, a few farms had false-positive results on the antigen-ELISA when the birds sampled were moderately to severely autolysed. In the 2003 Connecticut H7N2 LPAI outbreak, a similar problem of false-positive results occurred in autolysed birds, possibly resulting from an alkaline-phosphatase reaction produced by saphrophytic bacteria (Mary J. Lis, personal communication). All tracheal samples screened with antigen-ELISA in Virginia and Connecticut H7N2 LPAI outbreak were tested for AI virus by RRT-PCR and virus isolation to confirm the results of the antigen-ELISA.

Although the antigen-ELISA was an effective field-screening test, a rapid more sensitive and specific diagnostic test was need for avian influenza. A USDA co-operative effort between Southeast Poultry Research Laboratory (Athens, Georgia) and National Veterinary Services Laboratories (Ames, Iowa) developed and validated a laboratory-based, one-step, real-time reverse-transcriptase polymerase chain reaction (RRT-PCR) assays for detection of avian influenza virus in field specimens (Spackman et al. 2002). These RRT-PCR tests have been used for diagnosis in three different H7N2 LPAI outbreaks: live poultry markets of New England during 2002, commercial poultry in Virginia during 2002, and commercial layers of Connecticut during 2003.

The RRT-PCR assays utilized a single nucleic-acid extraction system (Rneasy kit, Qiagen, Valencia, California) from transport media containing pooled tracheal or cloacal swabs, hydrolysis primer and probe sets, and a portable thermocycler. The first assay used an influenza virus matrix-gene-specific PCR primer set and a hydrolysis probe designed for a conserved region present in all type-A influenza viruses; whether avian, swine, equine or human. For all samples which were matrix-gene positive, H5- and H7-specific primer sets to conserved regions of the North-American H5 and H7 haemagglutinin gene sequences, respectively, were used in secondary tests. For all assays, the probes used a 6-carboxyfluorescein reporter dye and 6-carboxytetramethylrhodamine quencher dye.

The RRT-PCR assays took less than 3 hours for completion, which includes sample preparation time, and the answer was produced in real time (Figure 2). On a flock or market basis, the RRT-PCR tests performed well compared to virus isolation with sensitivity of 94% for matrix-gene assay and 97% for H7 haemagglutinin-gene assay during the live-poultry market eradication programme (Spackman et al. 2002). The detection limit for the matrix-gene test was 10^{-1} 50% egg infectious doses (EID_{50}) while the H5 and H7 assays detected 10^{1} EID_{50}. These RRT-PCR tests performed equally well in Virginia during the summer of 2002 using tracheal swabs from meat turkeys, turkey breeders, broiler breeders and broilers. In Connecticut during February 2003, the RRT-PCR test was used to detect the H7N2 LPAI virus in Connecticut egg layers. This USDA-developed and -validated RRT-PCR assay is laboratory-based and has been disseminated to several state veterinary diagnostic laboratories within the National Animal Health Laboratory Network. However, hand-held instrumentation is under development that will make the test usable in the field or at pen side (Perdue, Swayne and Suarez 2003).

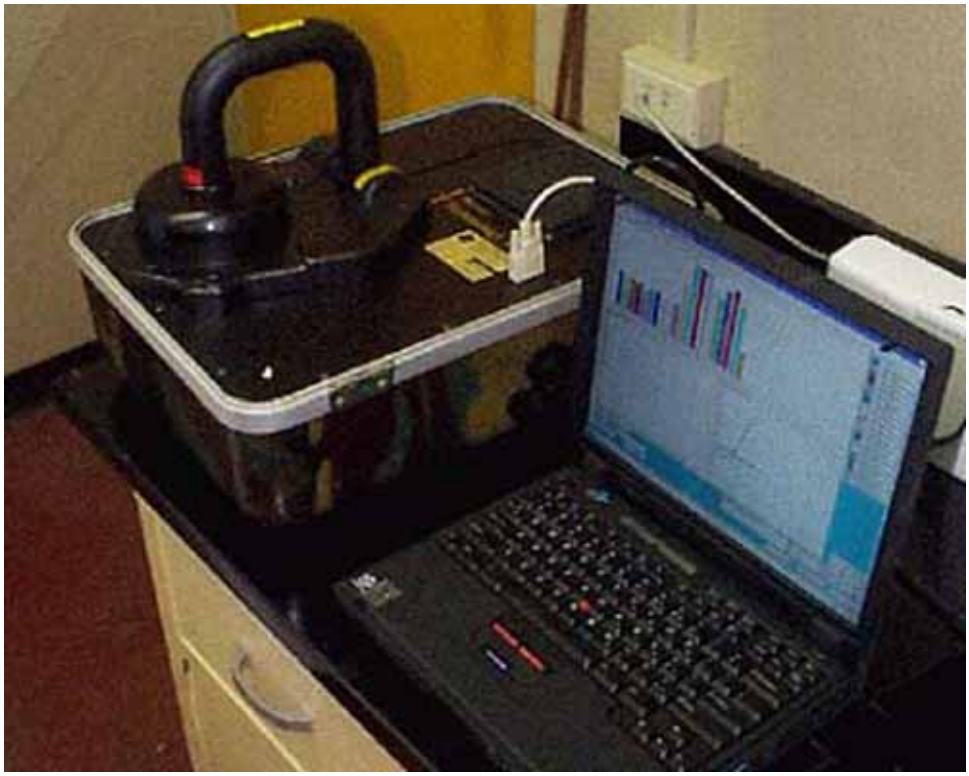

Figure 2. Portable thermocycler with laptop computer operating system displaying the results of RT-PCR reactions in real-time (Courtesy of David Suarez)

National surveillance for avian influenza in commercial poultry is accomplished through three systems: 1) National Poultry Improvement Plan, a Federal–State–Industry partnership, to certify chicken and turkey breeders flocks as AI free; 2) testing of broiler and meat-turkey flocks for product export to Mexico; and 3) state programmes to detect AI in high-risk areas (Myers et al. 2003). Specifically, these programmes use the agar-gel immunodiffusion (AGID) and two commercial ELISA (IDEXX, Westbrook, Maine; and Synbiotics, San Diego, California) tests that detect antibodies against the nucleoprotein and matrix protein of all Type-A influenza viruses. All positive results from the ELISA tests are confirmed by AGID test. All AGID-positive samples are forwarded to NVSL for haemagglutinin and neuraminidase subtyping. Serological testing has been used to certify an area or farm as free of AI, or during an AI outbreak to determine the extent of the infected zone for quarantine purposes.

Elimination of infected poultry

AI-virus-infected poultry flocks have been eliminated through two systems: 1) controlled marketing of convalescent or recovered flocks, and 2) *on-farm* depopulation and disposal of infected flocks. The method of elimination depends on the production company's procedures; local, state and federal agricultural and environmental regulatory policies; availability of indemnities; and accessibility to different disposal methods.

Historically, some LPAI virus-infected meat-turkey flocks have been allowed to recover from infection and were marketed through routine processing (Halvorson 2002). However, for processing, the convalescent flocks have been handled differently from non-AI-virus-infected flocks; i.e. with processing occurring at the end of the day followed by disinfection of the plant and delivery trucks before the beginning of the next day of transport and processing of AI-virus-negative flocks.

Euthanasia and disposal is the preferred method of eliminating flocks acutely infected with HPAI virus or recovered from such an infection. In addition, in some situations, euthanasia and disposal of LPAI-virus-infected poultry has been used such as in Virginia during the 2002 H7N2 LPAI outbreak. Depopulation requires two processes: 1) rapid, humane euthanasia of large numbers of poultry, and 2) disposal of the carcasses in an environmentally sound way.

Euthanasia

Usage of carbon-dioxide gas is the preferred method for euthanasia, but administration can be a logistic problem. With caged layers, individual birds must be removed from cages and manually placed in large airtight containers and CO_2 added. With small numbers of birds, portable, self-contained euthanasia chambers can be constructed and moved to the site for use (Figure 3) (Webster, Fletcher and Savage 1996). With large numbers of layers, large steel trash containers covered with airtight tarps and filled with CO_2 have been used effectively for euthanasia. For broilers, meat turkeys and breeders, portable panels were used to construct enclosures, the birds were herded into the enclosures and covered with plastic. Upon introduction of CO_2 (0.08 to 0.11 lbs of CO_2/ft^3) (Figure 3), the birds were euthanized in less than 15 minutes (Akey 2003).

Figure 3. Self-contained stainless steel CO_2 chamber for humane euthanasia of poultry

Carcass disposal

Disposal of euthanized birds has utilized various methodologies depending on local circumstances, including local, state and federal environmental laws. During the 2002 H7N2 LPAI outbreak in Virginia, 4.7 million birds on 197 farms were affected and these farms were depopulated (Akey 2003). Elimination of the infected birds utilized various methods including *on-farm* burial, incineration, composting, landfill disposal and controlled marketing (Table 1). **First**, *on-farm* burial was used in the Virginia

Table 1. Elimination methods used in H7N2 LPAI outbreak in Virginia during 2002 (Bruce Akey).

Disposal method	Number of birds	Total (%)
Composting	43,000	0.9
Incineration	641,000	13.4
Landfill	3,103,000	65.5
Controlled marketing	943,000	19.9
On-farm burial	15,000	0.3
Total	4,732,000	100.0

H7N2 LPAI outbreak only on the first affected turkey farm using an emergency permit and a lined-pit system. Future use of *off-farm* burial will be greatly limited because of public concern and environmental regulations designed to prevent contamination of the ground water. **Second**, forced air-curtain, wood-burning

incinerators were used in the Virginia H7N2 LPAI outbreak, but on a limited basis because of complaints about smoke, and because of the high cost for fuel and maintenance of equipment. This incineration process cost $500 per ton plus cost for transportation of the carcasses and disposal of the ash. In other situations for poultry disposal, gas-fired incinerators with afterburners have been used because they are designed to meet air-quality emission standards (Figure 4), but high fossil-fuel costs still made incineration one of the most costly options for disposal of poultry carcasses.

Figure 4. Stationary natural-fired incinerator with afterburners used for disposal of poultry carcasses at a research facility

In The Netherlands 2003 H7N7 HPAI outbreak, incineration was the preferred method of disposal with shipping of euthanized birds in sealed trucks to large stationary incineration plants that meet air-quality emission standards. **Third**, a small number of birds in the Virginia H7N2 LPAI outbreak were composted in the houses by a windrow method or in the commercial Ag-Bag® system (Ag-Bag International, Warrenton, Oregon). The latter method cost $13 per ton for the bagging materials, but required the fixed overhead cost of purchasing a specific loader for the system ($50,000). The windrow method of composting has a similar low cost, but requires the addition of an auxiliary carbon source (such as hay or wood-chip litter), proper construction of the compost windrow, control of vermin to prevent removal of infected carcasses, and periodic turning of the compost pile (Murphy 1992). One advantage of the Ag-Bag® system is the lack of any requirement for turning the compost pile because aerobic digestion is maintained by forced-air and not passive ventilation. In experimental studies, H5N2 HPAI virus was totally inactivated at the

end of the first 7-10 days of the composting process using a windrow system (Mixson 1992). On some poultry farms, continuous composting of daily mortality is used as a primary disposal method (Merka et al. 1994). On-farm composting of AI-virus-infected carcasses and manure during AI outbreaks has a good potential for more use in future AI or Newcastle-disease eradication efforts. **Fourth**, most birds in the Virginia H7N2 LPAI outbreak were disposed of by discarding in an approved landfill, but the primary location was a 3-hour drive outside the quarantine zone causing the transportation costs to be enormous. The landfill tipping fees varied from $45 to $140 per ton ($730,000 total cost) plus transportation charges and cleaning and disinfecting the transport trucks. **Fifth**, some birds in the Virginia H7N2 LPAI outbreak were marketed. These birds originated from flocks detected at slaughter but which had shown no symptoms before, or from flocks detected early during the outbreak which had recovered from AI infection. They were marketed because at that time landfill space was unavailable for disposal.

The Minnesota AI Control plan is based on recovery of grower cost by marketing the recovered, asymptomatic birds (Halvorson 1998; 2002). This has worked effectively since 1978, and when compared with the cost to control the H7N2 LPAI outbreak in Virginia by stamping out, the 25 years of LPAI in Minnesota (1097 farms) cost growers $22 million versus $130 million to the industry in losses for 2002 Virginia LPAI outbreak (197 farms) plus eradication costs of $82 million. For LPAI, marketing of recovered birds should be given more serious consideration in the future as a method of elimination. In experimental studies using the H7N2 LPAI virus, meat from intranasally inoculated broilers did not contain any AI virus based on virus isolation attempts, and feeding the meat obtained from inoculated chickens did not transmit the LPAI virus to other chickens (David Swayne, unpublished data). By contrast, broilers inoculated intratracheally with H5N2 HPAI virus from 1983 resulted in AI-virus isolation from meat, and feeding of the meat to other broilers resulted in seroconversion to the AI virus. For LPAI, marketing of recovered birds should be given more serious consideration in the future as a method of elimination since the raw meat produce has a negligible potential for transmitting AI viruses. An alternative would be to use meat from infected flocks for further processing and pre-cooked products. This would be effective for both LP and HPAI viruses since they are thermally labile (Swayne and Halvorson 2003).

Rendering of carcasses was an option used in the Italian H7N3 HPAI outbreak (Capua and Mutinelli 2001). However, in the USA, the rendering industry has been reluctant to accept birds from an AI outbreak because of stigma associated with the product source. Although the rendering process will kill the virus, extra precautions must be taken to prevent reintroduction by cross-contamination of transport vehicles.

Decreasing host susceptibility

Vaccination

Vaccination with homologous haemagglutinin AI vaccines has been shown to decrease susceptibility of poultry to infection by avian influenza viruses. Studies with a fowlpox recombinant virus containing an H5 AI-virus gene insert prevented transmission of AI virus between *in-contact* vaccinated chickens (Swayne, Beck and Mickle 1997). In other studies, vaccination with inactivated whole AI-virus or recombinant live AI-virus vaccines prevented clinical disease and mortality, and decreased replication and shedding of the field virus from respiratory and digestive tracts (Swayne 2003). However, vaccines do not completely prevent infection,

especially in the field, thus biosecurity practices are essential to prevent spread between vaccinated flocks that may become infected. Reviews have been written covering AI vaccines and should be referred to for more detail (Capua and Marangon 2003; Swayne 2003).

AI vaccines have been developed, licensed and used in the USA during the last 25 years. AI inactivated vaccines have been produced and licensed under both the autogenous and conditional (limited) licensing authorities (Myers and Morgan 1997; Myers et al. 2003). Full licensure has been granted to a fowl poxvirus recombinant containing an H5 AI-gene insert and an inactivated H5 whole-AI-virus vaccine. However, USDA-licensed vaccines can only be used under permit and in an official government control programme. Specifically, usage of H5 and H7 vaccines requires approval by USDA and the state government, but usage of USDA-licensed AI vaccines of the other 13 haemagglutinin subtypes (non-H5 and non-H7) only requires approval of the state government (Myers et al. 2003). AI vaccines have not been routinely used in the USA for AI prevention, control or eradication. Most vaccines have been used in turkeys, but usually only during individual outbreaks and primarily in turkey breeders (Halvorson 2002). However, in the case of turkey breeders, H1N1 influenza vaccine has been used in some states to prevent egg-production drops from infection by H1N1 swine influenza viruses. During 2001, 2,697,000 doses of H1N1 vaccine were used in 5 states (Illinois, Michigan, North Carolina, Minnesota and Ohio) and an additional 100,000 doses of H1N2 autogenous H1N2 vaccine were used in Missouri turkey breeders (Swayne 2001). In the same year, 677,000 doses of H6N2 inactivated AI vaccine were used in California on one layer farm. Since 1978, a variety of different haemagglutinin-subtype AI inactivated vaccines have been used in Minnesota turkey breeders and meat turkeys (Halvorson 1997).

Host resistance

In vitro studies using transfection technologies have shown that Mx genes from some chicken breeds conferred resistance to influenza infection in mouse 3T3 cells, but not from a White leghorn line (Ko et al. 2002). In an in-vivo study, mild differences in virulence of an LPAI virus were noted between specific-pathogen-free White leghorn, commercial White Leghorn and broiler chickens (Swayne et al. 1994). The impact of host genetic selection on resistance to AI virus infections has not been fully determined.

Education

Education is an essential component of any AI prevention, control or eradication strategy. This involves providing information to the industry concerning the biology of avian influenza viruses, how the virus is introducted and spread on farms, and application of methods and practices in biosecurity to prevent introduction of AI virus onto a farm (exclusion biosecurity practices) and, on infected premises, biosecurity practices to prevent the AI virus from leaving (inclusion biosecurity practices). The education process involves all employees in the company who are provided information on what avian influenza is, how it is transmitted, identification and elimination of behaviours that put the farm at risk for AI introduction (e.g. owning backyard poultry, working or visiting on multiple poultry farms), biosecurity procedures to protect the poultry (e.g. farm-dedicated clothing and shoes left on the farm at the end of the workday, employee showering facilities before entry on the farm, C&D of equipment used between farms) and consequences to the company and their jobs if an AI outbreak involves their workplace. Risk communication is essential

between companies when AI-infected premise is identified. In the Minnesota turkey industry this has been accomplished by a telephone and mail alert system to the poultry industry for suspicious and confirmed cases of AI (Poss 1997). The industry then develops and implements a responsible response for eradication of AI (Poss 1997; Halvorson 2002).

Specific strategies used in USA for avian-influenza prevention, control and eradication

HPAI

There have been three outbreaks of HPAI in the USA during the 20th century: 1924-25 fowl plague in live-poultry markets of Northeast cities, and supplying farms in Northeast and upper Midwest; 1929 fowl plague in a few New Jersey farms; and 1983-84 H5N2 HPAI, principally in Pennsylvania, but also limited involvement in Maryland and Virginia (Swayne and Halvorson 2003). The basic goal has been rapid eradication accomplished through quarantine of infected flocks, depopulation of flocks and disposal of carcasses, C&D of equipment and farms, diagnostics and surveillance testing (1983-84) and indemnities paid for destruction of poultry (1983-84). Federal laws and regulations give USDA the authority to declare an animal-health emergence, to quarantine and destroy flocks, and to pay indemnities (Myers et al. 2003). These eradication programmes can be conducted in co-operation with state governments and are outlined in the USDA Emergency Response Plan (APHIS 1998), also called the 'Red Book'. This document provides for the potential future use of vaccines as part of eradication strategies for HPAI.

H5 and H7 LPAI

The goal or outcome for H5 and H7 LPAI has been control or eradication. Strategies to accomplish the goal of eradication or control have varied with the individual situation. Such strategies utilized components of biosecurity, diagnostics and surveillance, elimination of infected birds and in some situations, vaccination. Prior to 1995, use of USDA-licensed AI vaccines only required a decision by the poultry industry and state governments, but in 1995, USDA implemented the requirement of federal approval for field use of USDA-licensed H5 and H7 vaccines (Myers et al. 2003). In most situations, indemnities have not been paid for elimination of H5 and H7 LPAI-infected poultry. Currently, federal regulations do not provide for indemnities to cover H5 and H7 LPAI. In the next few paragraphs, control or eradication strategies for specific outbreaks of H5 and H7 LPAI will be covered, with primary focus on the use or non-use of vaccines, payment or non-payment of indemnities, and elimination methods used. Biosecurity enhancements, quarantine and surveillance are common components used in all the strategies to control or eradicate AI.

Minnesota, 1978-2002

Since 1978, the Minnesota turkey industry has experienced 108 outbreaks with AI viruses in turkeys involving 1097 farms, twenty of these outbreaks were from waterfowl-origin H5 or H7 LPAI viruses (Halvorson 2002). In each instance, the industry implemented an AI-eradication strategy utilizing components of education (includes risk communication within the poultry industry), monitoring, responsible response (includes controlled marketing and enhanced biosecurity practices), and vaccination with the outcome of eradication of AI in less than six months (Halvorson

2002). Prior to 1995, H5 and H7 vaccines were allowed without USDA approval, but since 1995 none have been used. The Minnesota control programme was a voluntary co-operative industry–state programme and did not include any indemnity from the federal or state governments, but relied on marketing of recovered turkeys as financial compensation for participation. Moving or marketing of turkeys during the acute phase of infection, i.e. period of high AI-virus excretion, was reported as a high-risk activity associated with spreading of the virus to other farms; therefore, infected flocks are required to sit on the farm under quarantine for
1-2 weeks before sending to processing.

Utah, 1995

On 26 April 1995, an H7N3 LPAI virus was isolated from commercial meat turkeys in a single production company in the Sanpete Valley, Utah (Halvorson et al. 1997). Control measures implemented included informing growers of the outbreak, enhanced biosecurity, controlled marketing of recovered flocks, and C&D of housing. An H7N3 autogenous inactivated AI vaccine was produced and, beginning on 20 June 1995, uninfected 3-8-week-old turkeys were given a single dose of the vaccine. Within 6 weeks, 150 flocks were vaccinated. Sentinel birds placed at the time of immunization did not seroconvert over the next 6 months. The company concluded the vaccine was effective in reducing susceptibility of turkeys to AI virus and, along with biosecurity measures, ending the outbreak. No state or federal indemnities were paid as compensation.

Live-poultry markets, 1994-2002

The H7N2 AI outbreak in live-poultry markets of the Northeastern USA (1994-2002) and in commercial poultry inVirginia (2002) are covered by Dennis Senne in this volume and will only be discussed below in brief. Between 1994 and 2001, several attempts at eliminating H7N2 LPAI by identifying markets with infected birds, closure of infected markets, eliminating infected birds, and C&D were ineffective (Mullaney 2003). In April 2002, a federal–multiple state co-operative programme was launched with goal of a simultaneous closure of 123 retail markets in six Northeastern states. The owners sold down their bird inventories the day before closure and killed all remaining birds on the day of closure. Each market owner was compensated $3000 for the three days of closure if the establishment handled only poultry, and a $1000 supplement was paid if they handled both poultry and red-meat livestock. The establishments were cleaned and disinfected by the owners and inspected by the task force before being allowed to repopulate with certified AI-free birds.

Pennsylvania, 1996-97 and 2001-2002

In 1996-97, H7N2 LPAI spilled over from the live-poultry markets into 18 commercial layer farms, two commercial layer pullet farms and one commercial meat-turkey farm in Pennsylvania (Davison et al. 2003). The control strategy was placement of quarantine, immediate depopulation with *on-farm* burial or delayed depopulation with landfill burial, C&D of premises, and surveillance. Depopulation was voluntary without any federal compensation on 21 farms. However, on two large layer farms, chickens were allowed to recover and continue to produce market eggs. Within 6 months, AI virus was again recovered from chickens within both flocks. Ultimately, the flocks were depopulated and buried in a landfill. Request by the industry to vaccinate against H7 AI was not approved by USDA because of broiler

industry's concerns over a potential trade embargo on broiler-meat exports if vaccine was used. Partial indemnity was paid by the state government and from an industry indemnity pool. In 2001-02, H7N2 LPAI virus spilled over from live-poultry markets to infect broiler breeders on two farms and broilers on five farms in Pennsylvania (Dunn et al. 2003). Several of the broiler farms did partial load-outs and sold birds to wholesalers for live-poultry market distribution. The broiler breeders were euthanized in the houses with CO_2 gas and transported to landfill for disposal. For the broiler flocks, one was marketed, one was euthanized on site with landfill disposal, and three flocks were euthanized and composted within the houses. No vaccine was used and federal indemnity was not paid. Other components of the control programme were quarantine and surveillance. The area was declared AI free within 6 months.

Virginia, 2002

H7N2 LPAI virus moved from live-poultry markets to infect 197 commercial farms of turkey and broiler breeders, meat turkeys and broilers during the spring 2002 in the Shenandoah Valley of Virginia. An eradication programme was undertaken with a USDA–Virginia-State co-operative programme funded by USDA (Akey 2003). Stamping out was the strategy used for eliminating infected birds and, for the depopulation, the Federal government paid indemnity. This unusual action by the Federal government to fund eradication of H7N2 LPAI resulted from the progressive change in the LPAI virus; i.e. between 1994-2002 substitutions of non-basic with basic amino acids near the haemagglutinin proteolytic cleavage site. One additional change by a single nucleotide could alter the genetic code and substitute a fifth basic amino acid at the hemagglutinin proteolytic cleavage site. This genetic change would mean an accompanying change in virulence from LP to HP. The turkey industry requested use of inactivated H7N2 AI vaccine in repopulated turkey breeders, which was approved by USDA, but concerns by the broiler industry of an embargo of meat sales by trading partners resulted in non-approval of vaccination by the state government. Initially, diagnosis was made by virus isolation, but during the outbreak sufficient samples were examined to validate the sensitivity and specificity of the RRT-PCR test for detecting AI virus. During the second half of the outbreak, the RRT-PCR assay became the primary test for diagnosing AI virus and virus isolation became a secondary test. The total federal cost of eradication was $81 million and losses to poultry farmers were in excess of $130 million.

Connecticut, 2003

In February 2003, H7N2 LPAI was diagnosed in chickens within a large layer company in Connecticut (Swayne et al. in press). Over the next 3 months, three farm sites in the company involving 2.9 million layers became infected. USDA requested the owner depopulate the infected flocks, but indemnities were not available. The state government and company developed an alternative control strategy to prevent the infection from spreading and eliminate infected birds over their production cycles with the 1-year goal of eradication. The basic strategy is to isolate the farms through biosecurity practices, increase the immunity in infected layers to a uniform level by a single inactivated H7N2 AI-virus vaccination, over the normal production cycle replace infected layers with twice-vaccinated pullets (H7N3 AI vaccine), and establish a monitoring programme using unvaccinated sentinels and normal daily mortalities for virus detection. A 'DIVA' serological monitoring strategy using neuraminidase is under consideration. As of 12 October 2003, the last H7N2 LPAI virus detected by RRT-PCR was on 26 June 2003. The control strategy will continue through one

laying cycle with periodic evaluation of progress. Isolation or detection of AI virus by RRT-PCR will result in re-evaluation of the control strategy and possibly total depopulation.

Rhode Island, 2003

On 26 April 2003, a 32,000-bird mixed layer operation in Rhode Island became positive for AI virus on RRT-PCR test. Seven days earlier, a live-poultry market dealer visited the farm to purchase birds. The farm is under quarantine with no addition of new birds. The farm can continue to sell sanitized eggs, but at the end of lay the farm will be depopulated, C&D and repopulated with AI-free layers.

Concerning control and eradication of H5 and H7 LPAI, specific issues continue to create problems in the development of consistent and effective eradication strategies: 1) lack of federal authority over H5 and H7 LPAI; 2) inconsistent availability of funds for the eradication efforts, especially for indemnities; 3) continued concerns over potential trade embargoes should vaccines be used in a control or eradication programme; 4) the penalty for using vaccines under OIE health code which require twice the length from last positive case to be declared AI-free (6 versus 12 months); and 5) concerns that if H5 and H7 LPAI are made reportable to OIE, this will necessitate immediate eradication strategies using expensive and disruptive stamping-out policies.

Non-H5 and non-H7 LPAI

Prevention, control and eradication have been strategies used for dealing with non-H5 and non-H7 LPAI. In Minnesota since 1978, the same control and eradication strategy has been used with non-H5 and non-H7 LPAI as with H5 and H7 LPAI, except usage of vaccination continues to be a component in non-H5 and non-H7 LPAI eradication strategies. By contrast, with H1N1 swine influenza, prevention has been the preferred strategy in turkey breeders where vaccination is the primary component used in the prevention strategy. In the recent H6N2 LPAI outbreak in California, the Minnesota control and eradication plan was modified and used. However, success was elusive because biosecurity practices on some individual farms were inconsistent. H6N2 inactivated vaccine has been used in layers but not broilers. The outbreak of exotic Newcastle disease in 2002-03 has temporarily delayed the H6N2 LPAI control programme.

Conclusions

The development and implementation of new control programmes for ‘reportable AI’ will require courageous steps by all countries that are members of OIE. The addition of H5 and H7 LPAI along with HPAI to the list of ‘reportable AI’ should reduce the number of HPAI outbreaks in the future by providing governments with incentives to control LPAI before it mutates to HP. However, if trading partners use the addition of H5 and H7 LPAI as a non-tariff trade barrier, this will have the opposite of the intended effect by encouraging nations not to report but to hide LPAI, and this could possible lead to increased HPAI outbreaks in the future. Specific steps need to be taken to make H5 and H7 LPAI reportable:

1. OIE needs to embrace the idea that control methods besides stamping out can be effective and less costly for eradicating H5 and H7 LPAI. LP and

HPAI have different pathogenesis of infection, different virus-shed rates and different rates of transmission, thus their risks are different.

2. Acceptance of the compartmentalization concept is critical for developing new control and eradication methods for HPAI and for including H5 and H7 LPAI as 'reportable AI'.
3. Federal–state co-operative control and eradication programmes need to be developed with financial incentives for rapid detection and elimination of index cases of H5 and H7 LPAI.
4. USDA needs legal authority to control H5 and H7 LPAI including financial resources to pay indemnities.

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