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Highly Pathogenic Avian Influenza Outbreaks in Canada in 2014-2015

Introduction

Avian influenza (AI) is a contagious viral infection that can affect poultry as well as pet and wild birds. AI viruses are classified into two categories: low pathogenicity (LPAI) and highly pathogenic (HPAI), based on the level of severity of the illness caused in birds (Diseases of Poultry, 13th ed. 2013).

Canada has had both HPAI and LPAI outbreaks on commercial poultry farms in the recent past (Bowes et al, 2004 and CFIA, 2010). In the fall of 2014 and the spring of 2015 Canada had outbreaks of H5N2 HPAI in commercial poultry farms located in the provinces of British Columbia and Ontario. The USA also had outbreaks of HPAI (mainly H5N2) across many states causing the depopulation of an unprecedented number of birds. HPAI's characteristics, epidemiology, detection, control strategies, depopulation and composting methods are reviewed in this paper.

Pathogen and Disease Characteristics

Avian influenza viruses are divided into subtypes (or "pathotypes") based on two proteins found in the viruses: hemagglutinin, or "H" protein, and neuraminidase, or "N" protein. There are 16 different H types and 9 different N types which create a total 144 possible combinations.

The virus is part of the viral family Orthomyxoviridae, genus type A. The genomic material is negative sense RNA arranged into 8 gene segments. The capsid is roughly spherical and size is 80 – 120 nm in diameter. The virus is enveloped and sensitive to the following: common disinfectants, detergents, heat, extreme pressure, non-isotonic solutions and dryness (Diseases of Poultry 13th ed., 2013).

The H5 and H7 subtypes of the virus are of particular concern, given the ability of these two H-types to mutate from low to high pathogenicity after they infect domestic birds. These two H-types have been known to cause serious disease or mortality in domestic poultry, yet low pathogenic H5 and H7 viruses are quite common in wild waterfowl (Takekawa, 2010). AI pathotype designations are derived from the intravenous inoculation of eight susceptible chickens (6 week-olds) for the IVPI (intravenous pathogenicity index). If the virus causes at least 75% mortality (kills at least 6 of the 8 chickens) then it is considered an HPAI virus. LPAI viruses usually do not result in death of any of the chickens following intravenous inoculation. H5 and H7 viruses that cause less than 75% mortality in an intravenous lethality test should be sequenced to determine whether multiple amino acids are present at the cleavage site of the haemagglutinin (HA) protein. Most HPAI viruses have multiple basic amino acids in the HA cleavage site (CFIA website and Diseases of Poultry 13th ed, 2013).

Some or all of the following clinical signs are evident in HPAI infected birds

- a drop in production of eggs
- egg quality issues with soft-shelled or shell-less eggs observed
- diarrhea
- hemorrhages on the hock
- high and sudden mortality rate
- quietness and extreme depression
- swelling of the skin under the eyes
- wattles and combs become swollen and congested

The incubation period of AI ranges from 2 to 14 days. Differential diagnoses for HPAI infection of chickens include Velogenic Newcastle Disease (Avian Paramyxovirus type 1), Infectious Bronchitis, Infectious Laryngotracheitis and Mycoplasmosis among others.

Detection Methods

HPAI should be suspected on the basis of clinical signs and relevant history. Laboratory testing is needed to confirm the presence of AI. The signs of AI are very similar to those seen with Velogenic Newcastle Disease (also reportable in Canada) and other poultry diseases.

Antibodies to hemagglutinin (H), neuraminidase (N), matrix (M) and nucleoprotein are produced of which the antibodies to H are considered the most important for immune protection. ELISA (Enzyme-linked immunosorbent assay) testing, AGID (Agar gel immune-diffusion), HI (hemagglutinin inhibition) and NI (neuraminidase inhibition) are all serological methods of detecting AI infection that have been described. Commercial ELISA kits (Zoetis ProFlok Plus AIV+ and IDEXX AI MultiS-Screen) are licensed and in use for serology based surveillance testing of domestic poultry flocks in Canada (CFIA Veterinary Biologics Website). Depending on the kit or reagents used identification may be general (AI exposure in the flock) or subtype specific (both H and N type specific typing is possible). The CanNAISS (Canadian Notifiable Avian Influenza Surveillance System) program is run by CFIA (Canadian Food Inspection Agency) and provides (among other things) regular surveillance for notifiable AI in longer lived birds (such as broiler breeders, turkeys and leghorn type chickens) (CFIA Avian Influenza Surveillance Website, 2015).

PCR testing to detect the matrix gene (type A influenza), or for specific H or N genes can be conducted very rapidly in suitable diagnostic laboratories. Both traditional RT-PCR and RT-RT-PCR tests have been developed for detecting AI in clinical samples (Spackman et al, 2003). The preferred samples for domestic poultry include tracheal and cloacal swabs and in wild birds cloacal swabs. Swabs should be placed in media (brain heart infusion (BHI)) with antibiotics for virus isolation and/or PCR testing. Swabs and tissues should be stored at 4C (fridge temperature) or ice for shipment and testing should be performed promptly. PCR based tests are well suited for diagnostics during an outbreak of HPAI while ELISA based serology tests are useful for routine monitoring and post-outbreak surveillance.

Virus isolation in SPF chicken embryos is considered the gold standard diagnostic method. Virus isolation takes longer to perform and is much more difficult to automate and run on a larger scale than PCR or ELISA based testing. PCR positive samples may be used for virus isolation in an attempt to minimize unsuccessful isolation attempts. Virus isolation is necessary for further study of a given AI virus (for example to obtain IVPI information or for developing vaccines).

Epidemiology

Wild birds, especially waterfowl, are natural reservoirs of influenza viruses (Takekawa et al, 2010). They are not normally sickened by the disease, but can still pass it on to domestic birds. Wild waterfowl excrete virus in their feces and can also spread virus through direct contact. Two man-made AI reservoirs are live bird markets and commercial swine facilities with live bird markets not common in Canada and commercial swine facilities having high biosecurity standards. The disease can spread to birds through contact with infected poultry and poultry products. It can also spread through contaminated manure, litter, clothing, footwear, vehicles, equipment, feed and water (fomites – inanimate objects) (DJ Alexander, 2000 and 2007).

Different strains of the same type of virus can exist, particularly in different parts of the world. Such strains can have very different characteristics and structure. For example, the H5N1 strain that has been reported in various parts of Europe is low pathogenic and is distinctly different from the Asian strain, which is highly pathogenic. Not all H5N1s are similar. AI viruses, such as the highly pathogenic H7N9 virus present in China, may, on rare occasions, cause disease in humans (Kalthoff et al, 2010). Transmission to humans (of many different H and N type AI viruses) has occurred through very close contact with infected birds or heavily contaminated environments (Kalthoff et al, 2010).

Control Strategies (Biosecurity and Vaccination)

In Canada, highly pathogenic avian influenza and low pathogenicity H5 and H7 avian influenza viruses are considered to be Notifiable Avian Influenza, which is a reportable disease under the federal Health of Animals Act. All cases must be reported to the Canadian Food Inspection Agency (CFIA). Under the Notifiable Avian Influenza Hazard Specific Plan, the CFIA responds to both highly pathogenic and low pathogenic H5 and H7 viruses by reporting disease outbreaks to the OIE (World Organization for Animal Health), establishing quarantines, ordering the humane destruction of poultry, conducting trace-out activities, overseeing the cleaning and disinfection of premises, and verifying that the affected farms remain free of avian influenza according to OIE standards (CFIA website, 2015).

Poultry producers need to use strict biosecurity practices in order to prevent introduction of the virus to their flock. Farmers should develop a comprehensive biosecurity plan that would include: keeping poultry away from wild birds through keeping barns secure (for example: keeping netting on any barn openings), maintaining strict control over access to poultry barns (both human and animal), making sure that equipment is cleaned and disinfected before taking it into poultry houses, keeping wild birds away by not keeping bird feeders or creating duck ponds close to poultry barns because they attract wild birds

and by maintaining high sanitation standards. Currently a very small percentage of commercial poultry production is free range but free range producers should pay even closer attention to their biosecurity programs because they have a higher risk of wild bird exposure and they should be ready to move birds indoors in the face of an outbreak (CFIA website, 2015). Small flock producers should register their flocks with the relevant provincial authority so that they can participate in AI control activities. In the event of a notifiable disease outbreak careful investigation is needed to find any and all poultry populations in affected areas for control as backyard flocks are often not registered (and producers may not know that a neighbor has backyard poultry).

Vaccinating birds for AI may play a role in reducing the spread of the disease but does not eliminate the virus (Capua and Maragnon, 2007). No commercially licensed AI vaccines are available commercially in Canada currently (CFIA website, 2015). Vaccination would have serious trade implications (CFSPH, 2009). AI vaccination has provoked much debate over the years internationally and maintenance of trade with uninfected poultry and products during vaccination is scientifically feasible by using DIVA (differentiation of infected and vaccinated) surveillance strategies (Horrox, 2007).

Depopulation of Affected Flocks

Canada's emergency response strategy to an outbreak of avian influenza would be to eradicate the disease and re-establish Canada's disease-free status as quickly as possible (CFIA website, 2015). This type of strategy is referred to as “stamping out” (CFSPH, 2009).

The CFIA's AI emergency response strategy includes the following measures (CFIA website, 2015)

- the humane destruction of all infected and exposed animals
- surveillance and tracing of potentially infected or exposed animals
- strict quarantine and animal movement controls to prevent disease spread
- strict decontamination of infected premises
- zoning to define infected and disease-free areas

Depopulation of affected flocks in Canada during the 2014-2015 HPAI outbreak was accomplished by using whole barn CO_2 gassing. Whole barn CO2 gassing has been used to depopulate poultry houses in Europe in response to AI outbreaks (Van den Berg et al, 2008, Raj et al, 2006 and Ryan et al, 2006) as well as in Canada (Bowes et al , 2004 and CFIA, 2010) and the USA in response to velogenic NDV (Kingston et al, 2005).

The procedure generally followed for CO_2 gas poultry barn depopulation in Canada was described by Taylor et al in the 2012 Poultry Science paper. The CO_2 gas is flooded into the barn through manifolds deployed around the barn, flooding the barn with high levels of CO_2 gas within a few minutes. Gas monitoring units (both CO_2 and O_2 gas levels) are deployed at bird level in the barn and monitored from outside the barn. CO_2 gas levels are maintained at high levels (above 45%) until no bird noises are heard from outside the barn and then for several minutes (at least) after that. Technicians with enclosed space

training and wearing self-contained breathing apparatus (SCBA) may remove temporary fan covers and enter the house to turn the barn ventilation system on. The CO_2 gas is then vented through the barn ventilation system. All equipment and trucks are washed and disinfected before leaving the farm.

This technique may be used in Canada to depopulate leghorn-type chicken operations when no slaughter operations are available or to depopulate other types of poultry in non-reportable disease or compromised welfare situations (such as severe rickets were loading of poultry might cause broken bones) as well as in emergency depopulation. Provincial marketing boards have protocols for planning a safe and effective CO_2 gas based depopulation operation. These protocols and documents are used by the poultry industry in many different provinces for depopulation. Protocols used by CFIA, private industry and provincial poultry marketing boards may be slightly different and are updated as new technologies or techniques are developed for any part of the process.

Animal welfare concerns are an important consideration when depopulating poultry barns with CO_2 and have been reviewed (Raj et al, 2006) and investigated extensively (Taylor et al, 2012). Killing birds in large numbers for depopulation has many practical differences from individual bird euthanasia and in-barn whole house CO_2 gassing has been characterized as a high welfare (or welfare friendly) method of emergency depopulation (Raj et al, 2006 and Taylor et al, 2012). Immediate insensibility (unconsciousness) or as rapid insensibility as possible during depopulation/euthanasia is desirable as welfare ceases to be a concern in the affected birds when they are rendered insensate and thus unable to feel pain or to suffer (Alphin et al, 2010). Insensibility of poultry in depopulation procedures may be measured by loss of posture or directly by observing brain electrical activity through the use of EEG (electroencephalogram) data analysis. No statistically significant difference has been observed between time to loss of posture as measured by accelerometers attached to birds or time to unconsciousness measured using EEG data analysis (Benson et al, 2012B and 2012C). Gas based depopulation does not induce instant insensibility in exposed birds, taking an average time of 4 minutes to induce unconsciousness in the Gerritzen et al model (Gerritzen et al, 2007), an average time of 90 seconds in the turkey model in used in Rankin's thesis (Rankin, 2010) and an average time of approximately 80 seconds for unconsciousness and 5 minutes to brain death in field trials (Taylor et al, 2012). The behavior of poultry exposed to up to 45% CO_2 was described by Gerritzen et al in their laboratory based experiments to simulate whole house CO_2 gassing of birds and included head shaking, gasping, sitting-jumping before loss of posture and consciousness and ultimately death. Head shaking and open mouth breathing or gasping was observed by Taylor et al in their field trials. The interpretation of this behavior is unclear and may not indicate a significant aversion to the gas by the affected poultry (Taylor et al, 2012). Time to insensibility in a whole barn gassing situation will be linked to the actual CO_2 concentration in the barn which may be limited by the available gas flow from the mobile gas source (Gerritzen et al, 2007). Loss of consciousness and brain death were seen at CO_2 gas levels of >20% and O_2 gas levels <20% in field testing (Taylor et al, 2012). One of the important conclusions of Gerritzen et al's paper is that different types of commercial poultry such as broilers, leg-horn type chickens, turkeys

and ducks do not need to be managed differently for whole barn gassing to be effective, although there are behavioral differences between the different types of poultry (Gerritzen at al, 2007).

Water fire-fighting foam based depopulation techniques have been developed in the USA for depopulating poultry houses and were used widely in the USA 2014-2015 HPAI outbreaks (Benson et al, 2012, Rankin 2010, Rankin et al 2013, and Alphin et al, 2010). Fire-fighting foam based depopulation techniques compare very positively when compared to CO_2 based whole barn gassing and may actually cause faster insensibility and brain death (Rankin 2010, Rankin et al, 2013) or at least have similar results (Elphin et al, 2010). Raj et al. raised welfare concerns with this method because water-based foam with ambient air causes airway occlusion, a form of suffocation (Raj et al, 2008). Many different mass depopulation techniques have been investigated such as using different gasses (such as CO or N_2), using partial-house gassing, using enclosed canisters for gas exposure instead of the barn, and adding gasses to fire-fighting foam to name a few examples (Raj et al, 2006). A discussion of the plusses and minuses of each system is beyond the scope of this document.

Composting

Composting of poultry carcasses on-site during notifiable disease outbreaks is coordinated and monitored by the CFIA and performed according to CFIA protocols. Protocols used by CFIA in the province of British Columbia (BCMA, 2009) are typical of the composting protocol used in response to HPAI across Canada, with consultation from the relevant provincial authorities. As new techniques and technologies are developed the CFIA and provincial authorities may update their composting plans and procedures. These protocols have similarities and differences from carcass composting techniques that have been reported from use in outbreaks in Virginia, USA (Flory and Peer, 2010).

The first stage of composting is equivalent to an in-barn Biologic Heat Treatment (BHT) process. This process requires the temperatures of the compost to be greater than or equal to 37°C and be sustained for six consecutive days. A base layer of wood shavings 30cm thick is put down in the barn. Then mixed carcasses and an appropriate carbon source such as litter are piled on top in windrows up to 1.5m high. Then the windrow is covered again with a top 30cm layer of wood shavings. Water is added to the mixture of carcasses and shavings in the windrow to achieve a desired moisture level of 50-60%.

CFIA disposal specialists record temperatures throughout the compost pile on a daily basis. Once the BHT is completed the compost pile is moved outside the barn for further composting and temporary storage. A plastic liner is placed on the ground for the outside stage and chipped wood is used for a base layer 30cm deep. PVC piping is placed in the compost pile to provide for aeration. The BHT treated contents from the barn are put down in windrows and then the windrows are covered with structural wrap (an air permeable water shedding liner). CFIA disposal specialists record temperatures throughout the compost pile on a daily basis again looking for temperatures above 37C for 6 consecutive days. After CFIA sign-off the material is then suitable for use as fertilizer on farmland or it can be transferred to a provincially approved landfill with approval from the relevant provincial authority (BCMA, 2009 and CFIA, 2010).

Conclusions

HPAI outbreaks have had a devastating impact on the North American poultry industry in 2014-2015. A thorough knowledge of the virus and disease characteristics, detection methods, epidemiology, control strategies, depopulation techniques and composting of affected poultry carcasses and litter is essential for continuing control of this disease. Knowledge of these topics continues to grow as researchers in the lab and workers in the field research and battle HPAI.

This article was written by the veterinarians of Poultry Health Services Ltd. Poultry Health Services is a private veterinary practice providing diagnostics for Alberta poultry producers as members of the Poultry Health Centre of Excellence (PHCE). Bird submissions can be submitted to the PHCE via Government offices in Edmonton, Airdrie and Lethbridge. Please call 403-948-8577 if you have a mortality problem or want help making a submission.

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