United States Department of Agriculture

Reduction of Infectious Highly Pathogenic Avian Influenza Virus In Animal Agricultural Settings

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Introduction

As a result of the experience with cleaning and chemical disinfection of poultry facilities in the spring 2015 highly pathogenic avian influenza (HPAI) outbreak, an examination of less labor- and resource-intense methods of virus elimination is needed. Factors contributing to viruses' persistence or degradation in natural and agricultural environments must be included in such an examination.

There is a large body of literature regarding influenza contamination of, transmission by, and elimination from – materials and surfaces. Publications may focus on specific situations or interventions, but the information may be applicable to other situations. Scenarios include hospital surfaces, equipment, and materials (e.g., pajamas) (Jeong et al., 2010), food and food preparation surfaces (Anon, 2005), and bodily fluids and surfaces (Bean et al., 1982), due to the concern with pandemic and seasonal human influenza. Transmission of influenza in wildlife by the fecal-oral route through surface water has led to another body of literature (Stallknecht et al, 1990; Brown et al., 2009). The threat and cost of avian influenza outbreaks in poultry has produced many publications (Tiwari et al., 2006; Wood et al., 2010) applicable to that problem (including studies using chicken feces, soil, wood, etc.), which will be the primary focus of this review. A review of this brevity can include only a sampling of the available literature.

Disinfection, inactivation, and natural degradation

Avian influenza virions are comprised of eight single-stranded negative-sense RNA genome segments (coated by nucleoprotein) surrounded a matrix protein layer and finally by a host-cell derived lipid envelope with virus-encoded imbedded proteins. Damaging the viral host-recognition molecules, disrupting the lipid envelope, or damaging the nucleic acid or other structures of the genome segments so they cannot be replicated will render the virus particle non-infectious. These are the known or presumed (Wigginton et al, 2012) mechanisms of action of many of the chemical and physical disinfectants employed for decontamination or inactivation for vaccine production (Budowsky et al., 1971). Similar effects can be observed in the natural environment (degradation) when certain physical (heat, irradiation) or chemical (e.g., pH, salinity) influences apply. Note that some applied 'disinfection/inactivation treatments' are the same as natural influences, e.g., heat, UV irradiation, high or low pH, and desiccation.

Some mainly chemical disinfectant studies include heat, UV, or acid/base (Zou et al., 2013) and because of the standardization of disinfectant studies, they can be used as models for natural degradation. Further, even studies focused solely on chemical disinfectants may provide info on the effects of carrier, test system, etc. in their positive controls. Assays usually can be

categorized into ‘suspension’ vs. ‘surface.’ In ‘suspension’ trials the treatment is applied to the virus in suspension – e.g., mixing with a disinfectant, or putting a test tube of virus suspension in a water bath. In ‘surface’ trials the virus is dried on a ‘coupon’ of the matrix, and the treatment applied. Virus-killing procedures are often less effective on dried-on virus samples.

Factors in virus persistence and natural degradation studies

Irwin et al. (2011) performed a systematic review of “literature describing persistence of influenza virus in environmental samples, i.e., air, water, soil, feces, and fomites.” They evaluated 19 suitable reports for inclusion of 17 types of information, including such things as experimental matrix (19/19), method of inoculation (19/19), sampling interval (7/19), virus assay and limits (4/19), and estimates of decay rate with variance (0/19). They concluded in part that “although there is a significant amount of published literature regarding influenza virus, there are very few studies that can be used to support decision-making and policy formation. Although this study was comprehensive, the resultant data extracted for this synthesis leave a great deal of uncertainty for field application or management decisions and are outdated for certain matrices.” It is outside the scope and purpose of this review to analyze information gaps in the cited publications. Rather publications with noted and unnoted gaps are included in an attempt to provide sample data across a variety of experimental situations.

Persistence or degradation in the environment is usually a factor of time, temperature, pH, salinity, light (UV), desiccation or relative humidity, virus/strain/isolate, and matrix. In experimental conditions, multiple variables may be held constant (e.g., strain/isolate, pH, salinity, UV, and RH), while others are then varied (e.g., time and temperature). Although this helps isolate the effect of treatments, the interactions of treatments (Stallknecht et al., 1990) may be missed and the results may therefore apply less well to field conditions. Some studies focus on treatments in the expected/physiologic range – for example the virus concentration observed in infected chicken feces (Kurmi et al., 2013), or the pH and salinity found in wild waterfowl habitat (Stallknecht et al., 1990) – and often measure virus persistence. Others expand the treatment range to observe rapid inactivation by extremes in pH or temperature (Zou et al, 2013).

Selection of matrix is important for the relevance of data obtained. For example, treating virus in cell culture media may serve well as a reference point, but neither that trial nor one in sea water may translate well to treatment in a manure pit. Matrices may: a) protect the virus from certain treatments (e.g, UV); b) deliver certain stressors (e.g., acid). In most cases trials are done with ‘pure’ matrices – e.g., clean and sterile stainless steel for EPA surface disinfection trials. Although in some cases an ‘adulterant’ (serum, feces, soil) is added to a matrix to gain information (e.g., measure the impact of organic load on disinfectant action), in other trials the ‘adulterant’ is tested by itself, as a separate ‘pure’ matrix. Although testing non-sterile matrices such as feces or soil is desirable to more closely approximate real-world conditions, it introduces reproducibility issues as well as complications in virus detection that often reduce the sensitivity of the assays.

Influenza viruses are generally considered equivalent for disinfection studies/prediction. That is, using a LPAI or a mammalian influenza as a surrogate for HPAI is often considered acceptable. However, difference in lability or stability for certain treatments has consistently been observed

between strains and isolates in individual avian influenza trials (Brown et al., 2009; Wanaratana et al., 2010). This is consistent with observations with certain other closely related viruses (Wigginton et al, 2012).

pH

It is believed the action of acid on avian influenza is on the HA protein and is related to its low pH-dependent (in the endosome) fusogenic activity. This activity varies between strains/isolates which in turn is related to residues at specific amino acid positions (Mair et al., 2014).
Zou et al. (2013) did not observe virus H7N9 virus loss (7.7 logs starting concentration) held at pH 4-12 for 24 hours (longest observation time). However, no virus was detected after 30 min at pH 1, 1 hour at pH 2, and 24 hours at pH 3. Stallknecht and Brown (2009) reported a "rapid loss of infectivity below pH 6.5" for 12 wild bird-origin influenzas.

UV

Zou et al. exposed a suspension of H7N9 with 7.7 logs EID to the germicidal lamp in a biosafety cabinet (75 cm). No virus was detected after 30 min. Songserm et al., 2005 detected no virus in allantoic fluid or feces spiked with 6.3 logs EID of H5N1 after 30 min at 32-35 C. Chumpolanchorn et al. (2006), in an H5N1 chicken manure suspension test, exposed the samples to ambient sunlight and observed virus survival at 4 hours, but not at 5.5 hrs at 25 C.

Salinity

Brown et al. (2009) observed virus stability at "fresh to brackish salinities" (0-20,000 ppm) but shorter durations of persistence at high salinity (>25,000 ppm) for 12 wild bird origin influenzas. Irwin et al. (2011) noted "Salinity and pH were significant predictors of persistence in water conditions." Stallknecht et al. (1990) evaluated the persistence of an H6N2 at three pHs and 2 salinities. They observed strong interaction between pH and salinity.

Time x temperature virus reduction studies, by matrix

A sampling of publications reporting time and temperature virus reduction data for matrices relevant to animal agriculture is described below, with inclusion of RH and virus subtype where known/appropriate. Many food (meat, egg) and compost studies have been published and are of obvious outbreak response and control interest, but will not be included here. Standard laboratory matrices (allantoic fluid, cell culture media, and peptone water) are included for reference information.

Allantoic fluid

In a suspension trial conducted by Zou et al. (2013), with 7.7 logs of H7N9 virus, no virus was detected after 30 min at 56 C, 10 min at 65 C, and 1 min at 70, 75, or 100 C. Jeong studied H1N1 virus (8.02-8.08 logs) in a suspension trial. Virus was undetectable after 5 min at 70 C, 2.5 min at 80 C, and 1 min at 90 C. Wanaratana et al. (2010) studied three H5N1 reference viruses (7.6 – 9.34 logs/ml) in a suspension trial. The most temperature-labile virus was reduced but detectable after 60 min at 55 C, and undetectable after 60 min at 60 C and 10 min at 65, 70, and 75. The most temperature-stable virus was still detected after 60 min at 65 C and 30 min at 75 C. It should be noted that results with this virus preparation were not linear across time-temperature treatments.

Cell culture media
In a suspension room temperature (no UV) study Tiwari et al. (2006) recovered H13N7 virus (7 logs starting concentration) at 15 days. No virus was detected at 18 days.

Water
Shahid et al. (2009) suspended H5N1 virus in peptone water at a concentration of 4 HA units. Virus was not detected after 30 min at 56 C. Stallknecht et al. (1990) evaluated the persistence of an H6N2 at two temperatures (17 and 28 C), three pHs, and 2 salinities. They observed strong interaction between pH and salinity. Persistance was longest (100 day) at 17 C (pH 8.2, 0 ppt) and shortest (9 days) at 28 C (pH 8.2, 20 ppt). Brown et al., (2009) studied optimum temperature, pH, and salinity conditions for survival using 12 wild-bird origin AI viruses including 12 H subtypes and 5 N types. Rt values (days for a 1 log reduction to occur) were calculated. Temperatures studied ranged from 4 C to 37 C (with pH and salinity held constant at 7.2 and 0 ppm). At 4 C the Rt value ranged from 18.0 to 176.2. Variation between strains was reduced with increased temperature, and at 37 the overall Rt was ~5 days.

Soil
Nazir et al. (2011) placed filter germ carriers with H4N6, H5N1, H6N8, and H1N1 in lake sediment at four temperatures. T_{90} (time for 90% loss, or 1 log reduction) value ranges were 46-394 days at 0 C, 19-54 days at 10 C, 13-18 days at 20 C, and 4-11 days at 30 C. In surface inoculation trials conducted by Wood et al. (2010) with 6-8 logs of H5N1 virus, on soil no virus was detected at 2 days at room temp and low RH (30-50%). Conversely, at low temps (4-6 C) and low RH, virus was still detectable at 13 days (the longest time tested. When UV irradiation was added to the latter condition, virus was detected at 4 days (the longest time tested). In this study the results were similar between soil and chicken feces.

Feces
In suspension inoculation trials conducted by Kurmi et al. (2013) with 6-6.5 logs of H5N1 virus, in wet and dry chicken feces no virus was detected at 24 hours at 42 C, 30 hrs at 37 C, 5 days at 24 C, and 7 (wet) or 8 (dry) weeks at 4 C. Nazir et al. (2011) placed filter germ carriers with H4N6, H5N1, H6N8, and H1N1 in duck feces at four temperatures. T_{90} value ranges were 47-75 days at 0 C, 14-21 days at 10 C, 4-7 days at 20 C, and 1-2 days at 30 C. In surface inoculation trials conducted by Wood et al. (2010) with 6-8 logs of H5N1 virus, on chicken feces no virus was detected at 1 day at room temp and low RH (30-50%). Conversely, at low temps (4-6 C) and high RH (79-97%), virus was still detectable at 13 days (the longest time tested). When UV irradiation was added to the low temp, low RH condition, no virus was detected at 4 days. In this study the results were similar between chiken feces and soil.

Feathers
Yamamoto et al. (2010) plucked feathers from H5N1 infected ducks and stored them at two temperatures. Virus was detected at 15 days (not detected at 20 days) at 20 C, and 160 days (not detected at 200 days) at 4 C.

Wood
In surface trials conducted by Wood et al. (2010), 2-3 logs less virus was recovered from basswood (hardwood), and no recovered virus was recovered from pine (a softwood) after inoculation with 6-7 logs of H5N1 AI virus. Although it is disappointing that assays of the impact of treatment were therefore not possible, it implies that wood partially inactivates or sequesters virus by itself, perhaps serving as a virus elimination aid.

Concrete
Concrete is reported in multiple virus disinfectant trials to be difficult to recover virus from with no treatment (Biechler, 2006). In surface trials conducted by Wood et al. (2010), no recovered virus was detected after inoculation with 6-7 logs of H5N1 AI virus. Although it is disappointing that assays of the impact of treatment are therefore not possible, it implies that concrete inactivates or sequesters virus by itself, perhaps serving as a virus elimination aid.

Plastic/Rubber
In a surface room temperature (no UV, room RH) study Tiwari et al. (2006) recovered H13N7 virus from latex (at nearly the same titer as 0 hr) at 6 days, the longest sampling time. They recovered virus from gumboot and plastic after 3 days, but no virus was recovered after 6 days.

Glass
In surface inoculation trials conducted by Wood et al. (2010) with 6-8 logs of H5N1 virus, on glass no virus was detected at 1 day at room temp and low RH (30-50%). Conversely, at low temps (4-6 C) and low RH, virus was still detectable at 13 days (the longest time tested). When UV irradiation was added to the latter condition, no virus was detected at 2 days. In this study the results were similar between glass and metal.

Metal
In surface inoculation trials conducted by Wood et al. (2010) with 6-8 logs of H5N1 virus, on galvanized metal no virus was detected at 1 day at room temp and low RH (30-50%). Conversely, at low temps (4-6 C) and low RH, virus was still detectable at 13 days (the longest time tested). When UV irradiation was added to the latter condition, no virus was detected at 2 days. In this study the results were similar between metal and glass.

Practical application of time-temperature data

Although many of the trials described have intended to model specific field situations and/or contribute information for field application, translation of laboratory studies to natural environments can be problematic. The variety of conditions, synergy or competition of treatments and conditions, and lack of control at large scale are concerns.

The impact of non-linear inactivation curves (Brown et al., 2009) on field application should also be considered (i.e., 'overkill' may be necessary to eliminate residual live virus). The availability of non-degraded virus is a complex one. It is difficult to recover virus from concrete, wood, and cloth by physical means in laboratory studies (Tiwari et al., 2006; Wood et al., 2010). Whether the 'lost' virus is available for infection is more difficult to determine. The matrix and likely routes of infection (e.g., stirred up dust and inhalation, contaminated feed or water and oral

inoculation, contaminated caging and scarification) should be considered. Further, the infectious dose by any of these routes is usually not 1 EID, and may vary by strain – so residual levels of virus may not result in infection.

Time-temperature degradation is of special interest because of its natural occurrence, and because it appears to have fewer issues with 'shadowing' than UV, spray or even some gaseous disinfectants. Irwin et al. (2011) noted "Temperature was a significant predictor of persistence over all matrices." As a result of the data for 1 hour at 56 C in key matrices, multiple sources have recommended materials be held at 56 C (~133F) for 3 hours to inactivate avian influenza. Higher temperatures could be employed for shorter times (e.g., 75-90 C for 1-5 minutes). The more frequent question would be the time required for complete elimination at the lower temperatures (e.g., 37C, 25C) that would be easier to attain (naturally in more months in temperate zones, and/or require less added energy). As noted previously, the influence of humidity on virus survival at moderate temperatures is significant (Wood et al., 2010) and is likely to be uncontrolled. Under such conditions, days or weeks may be necessary for elimination from some matrices. Prolonged (months) virus persistence at low temperatures (4C) and high humidity has been documented, and virus persistence in frozen environments has been described as 'indefinite.'

The variety of field situations faced would require design and possibly measurement specific to the situation, should time-temperature be employed for virus elimination. For example, variation in building insulation in cold ambient conditions, cul-de-sacs, variation in RH resulting from the closing and heating procedure, large manure piles, mixtures/combinations of materials in production equipment could require special placement of heating equipment, extensions of time, and decisions on depth of HPAI contamination of materials as well as on what materials to remove or sacrifice to the procedure. This review did not deal with potential virus elimination from the carcasses themselves, including composting. That is the topic of other literature but could be important as part of the overall response to a zoonotic virus using time-temperature treatment.

Placement of recording thermometers for initial operations and in complex or problematic situations could provide confidence in the procedures. Validation of virus elimination could be accomplished using filters (Nazir et al., 2011), coupons or media spiked with a virus surrogate (a virus similar in sensitivity to degradation but not pathogenic and non-reactive on HPAI diagnostics). Additional, well controlled and documented research at the laboratory (model) and pilot field trial level would be helpful to better understand the mechanics of time-temperature achievement and influences on virus survival.

Conclusions

Trials using high levels of influenza viruses and a variety of materials commonly found in animal agricultural settings have demonstrated that influenza virus survives for up to a few weeks at room temperature (approximately 21 C) depending on the matrix, and for much longer (weeks to months) at lower temperatures. However, data from many surface and solution experiments indicate that heating to 37 C reduces survival to days, and heating to 56-60 C reduces survival to minutes to a few hours.

Not all desirable combinations of temperature, time, and matrix have been assayed, and the variability introduced by matrix, strain, humidity, pH, and availability to poultry suggest caution should be exercised in applying the experimental results to real-world practice. However, appropriately applied protocols employing elevated temperature and time to reduce and eliminate infectious influenza virus in poultry facilities are supported by the available data

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