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Highlighting the complexities of a groundwater pilot study during an avian influenza outbreak: Methods, lessons learned, and select contaminant results

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ABSTRACT

The highly pathogenic avian influenza (H5N2) outbreak in the Midwestern United States (US) in 2015 was historic due to the number of birds and poultry operations impacted and the corresponding economic loss to the poultry industry and was the largest animal health emergency in US history. The U.S. Geological Survey (USGS), with the assistance of several state and federal agencies, aided the response to the outbreak by developing a study to determine the extent of virus transport in the environment. The study goals were to: develop the appropriate sampling methods and protocols for measuring avian influenza virus (AIV) in groundwater, provide the first baseline data on AIV and outbreak- and poultry-related contaminant occurrence and movement into groundwater, and document climatological factors that may have affected both survival and transport of AIV to groundwater during the months of the 2015 outbreak. While site selection was expedient, there were often delays in sample response times due to both relationship building between agencies, groups, and producers and logistical time constraints. This study's design and sampling process highlights the unpredictable nature of disease outbreaks and the corresponding difficulty in environmental sampling of such events. The lessons learned, including field protocols and approaches, can be used to improve future research on AIV in the environment.

1. Introduction

During the spring of 2015, a highly pathogenic strain of avian influenza virus (HPAIV) H5N2 infected poultry in the US and Canada that led to significant economic loss (estimated $3.3 billion; Greene, 2015) to this multi-billion dollar industry. This historic outbreak was the largest and most costly foreign animal disease in US history. The particularly virulent strain of avian influenza A virus (AIV) led to near 100% mortality among poultry, with most birds dying within 3–5 days of infection. The outbreak began in the US in December 2014 and by the end of June 2015 led to the loss of 50.4 million birds (primarily chickens and turkey) in 15 states (21 states including wild birds) across the US, with roughly 67% of the impacted birds (32 million) in Iowa alone (IDALS, 2015). Most of the birds were intentionally culled in an attempt to contain this deadly virus and prevent the further spread of the outbreak. The impact of the outbreak on egg production, both the sharp decline and gradual recovery, are seen in layer numbers from both Iowa and the US in 2015 during the months of April to June (Fig. 1A, B).

Since 1997, the US has experienced sporadic incidents of low pathogenic avian influenza (H5 or H7) (OIE, 2016). However, only one outbreak of HPAIV H5 virus in commercial poultry was reported between 1997 and the 2015 outbreak; H5N2 was reported in February of 2004 in a flock of 7000 chickens in south-central Texas and was the first HPAIV outbreak in the US in 20 years (Pelzel et al., 2006). While AIV outbreaks are not new to the US, the 2015 epornitic was the largest to date.

Waterborne viruses are known water quality contaminants that can threaten both human and animal health. Groundwater has been previously documented as an environmental reservoir for enteric viruses such as enteroviruses (i.e., poliovirus, echoviruses, and coxsackieviruses), adenovirus, norovirus, rotavirus, and hepatitis A virus (Hynds et al., 2014; Borchardt et al., 2003). In addition, previous studies have concluded that land-applied livestock manure can be a source

Abbreviations: AIV, Avian Influenza Virus; HPAIV, Highly Pathogenic Avian Influenza Virus

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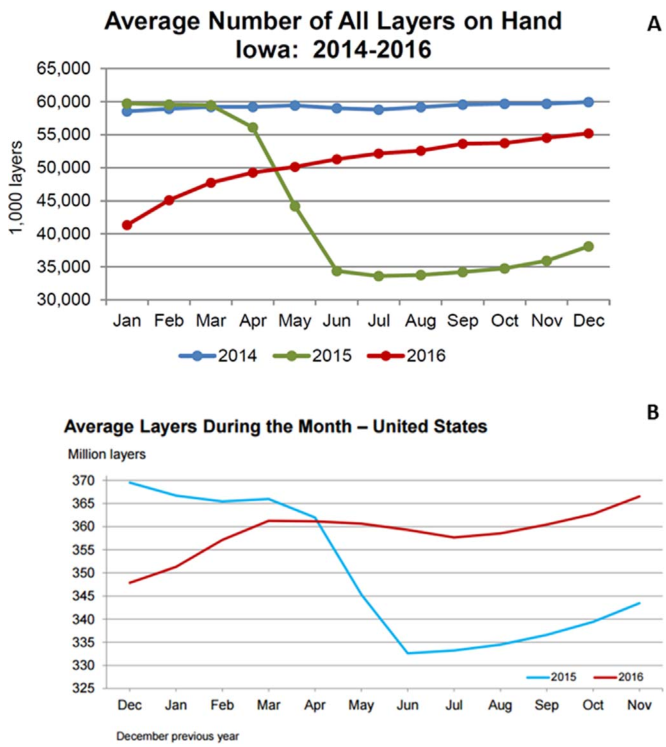

Fig. 1. Average number of layers by month in Iowa (A) and US (B).

of such pathogens in groundwater (Gerba and Smith, 2005; Close et al., 2008). Key factors for virus transport to groundwater include manure from virus-infected hosts (source component), sufficient precipitation to drive subsurface transport, and cool groundwater temperatures to slow virus inactivation (Azadpour-Keeley et al., 2003; Borchardt et al., 2007; Bradbury et al., 2013; Wallender et al., 2014). In groundwater, previous research of AIV has been limited, although substantial research has been conducted on enteric pathogens in groundwater. A review of 55 enteric pathogen groundwater studies (Hynds et al., 2014), identified four primary categories of investigations: 1) repeat sampling based fieldwork, 2) "snapshot" sampling studies, 3) laboratory method-based studies, and 4) outbreak investigations (the most frequent type of study design). Previous research on pathogen contamination in groundwater has documented the importance of study design and the identification of contributing factors such as local hydrogeology and climatic factors (*e.g.*, heavy precipitation) to pathogen transport (Hynds et al., 2014; Wallender et al., 2014). While limited research has been conducted on AIV in surface water, research in groundwater as environmental reservoirs during AIV outbreaks is even less common. Previous research documented influenza A (H5N1) in environmental samples (*e.g.*, poultry feces, feathers, soil, mud, water plants, and pond water) during a 2006 outbreak in Cambodia (Vong et al., 2008). This study, however, provided minimal information on the environmental study design other than stating a portion of the households sampled ($n = 43$; located within a 1-km radius of the outbreak site) had corresponding environmental samples ($n = 176$) selected by proximity to the household. For enteric pathogen studies, the importance of standard approaches in reporting study design and results has been highlighted previously (Hynds et al., 2014).

Study design during this 2014–2015 historic epornitic was affected by the spatio-temporal pattern of the outbreaks on the commercial operations. Si et al. (2013) noted distinct spatial patterns between poultry and wild bird HPAIV H5N1 outbreaks and suggested that environmental factors such as increased population density, increased proximity to lakes and wetlands, and increased air temperature and reduced precipitation increased the probability of poultry outbreaks. While the introduction of HPAIV in the US has been linked to timing of waterfowl migration and shedding of the virus by migratory waterfowl, the spread of the disease from west to east during the 2014–2015 outbreak did not correlate with typical waterfowl migration (Bui et al., 2016). Instead, the spatial patterns of outbreaks in Iowa and Minnesota (the most impacted states) suggested local transmission rather that migratory waterfowl was driving the spread of the HPAIV outbreaks (Bui et al., 2016). While there is insufficient evidence to determine the exact mechanism(s) for the spread of the 2014–2015 historic outbreak, it has been hypothesized that lapses in biosecurity practices (*e.g.*, movement of people, animals, vehicles, and equipment) and environmental factors (*e.g.*, aerosolization of the virus by high wind speeds) may have contributed to the operation-to-operation transmission of HPAIV H5N2 (USDA APHIS, 2015a).

The role of water as environmental reservoirs (*e.g.*, groundwater, surface water, wastewater lagoons, etc.) in latent storage and transmission of the HPAIV virus is not well understood. Water is suggested to be an environmental reservoir as controlled experiments have demonstrated the persistence of AIV of up to several months in water (Stallknecht et al., 2010). In addition, previous research suggests that HPAIV H5N1, a subtype of avian influenza A, would have an increased environmental persistence at colder temperatures and less sunlight (Woods et al., 2010). While little data exists regarding HPAIV presence in water, previous research detected HPAIV H5N1 viral RNA in 35% of pond water samples collected within 1 km of an outbreak site (Vong et al., 2008).

Other contaminants such as pathogens, antimicrobials, antibiotic resistance genes, and hormones are known to be excreted with and found in chicken litter (Brooks et al., 2016; Lu et al., 2014; Munaretto et al., 2016; USEPA, 2013). Although few studies have examined poultry litter derived hormone transport to groundwater, hormones have been detected in both sediment and groundwater under a dairy wastewater lagoon (Arnon et al., 2008). Studies have documented the presence of hormones in chicken litter (Velicu et al., 2007; Bevacqua et al., 2011; Lu et al., 2014) and in runoff from agricultural land amended with litter (Finlay-Moore et al., 2000; Jenkins et al., 2006; Dutta et al., 2010). In many cases, the detections in runoff were at concentrations that may contribute to endocrine disruption of various species in aquatic systems (Nichols et al., 1997; Finlay-Moore et al., 2000; Dutta et al., 2010).

Movement of disinfectant (and disinfection by-products) and other outbreak-related contaminants to groundwater was also an environmental concern. During the 2014–2015 H5N2 outbreak, large quantities of water with EPA-approved disinfectant was used during the disinfection process of vehicles and equipment entering and exiting the HPAIV-infected operations. At the time of the outbreak, approximately 200 disinfectants were registered for use against AIV (USEPA, 2016). According to a cleaning and disinfection equipment and supply list, however, chemicals commonly included: Virkon® S, sodium hypochlorite [$NaOCl$], anhydrous sodium carbonate [Na_2CO_3], sodium hydroxide [$NaOH$], quaternary ammonia disinfectant, and iodine (USDA APHIS, 2013). Decisions on the disinfectant most appropriate for the each specific HPAIV-infected operation was made on-site (USDA APHIS, 2015b). Many of these disinfectants are known to form associated disinfection byproducts, including sodium hypochlorite and iodine.

As the 2014–2015 H5N2 outbreak continued to escalate to epidemic levels in Iowa (totaling 77 cases across 18 counties by May 2015) and the uncertainty of how H5N2 was spatially transmitted between poultry operations, the need for scientific data on H5N2 in and around affected operations became paramount. Birds infected with AIV shed large quantities of the virus in their feces. Layer chickens on average, produce 4536 kg/AU/year of manure (Chastain et al., 2003). Therefore, a 1 million chicken layer operation would produce approximately 49,895 kg per year of manure. Thus, one objective of this proof-of-concept study was to determine the baseline occurrence of HPAIV and other poultry-related contaminants in underlying groundwater and surface water lagoons on outbreak-affected poultry operations (virus