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Mathematical modeling and epidemiological analysis of highly pathogenic avian influenza (H5N8) outbreaks in South Korea

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Mathematical modeling and epidemiological analysis of highly pathogenic avian influenza (H5N8) outbreaks in South Korea

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Abstract

Mathematical modeling and epidemiological analysis of highly pathogenic avian influenza (H5N8) outbreaks in South Korea

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Infectious diseases have become important in public health because of their increased socioeconomic impact during epidemics. Attempts are on to understand and predict disease transmission based on mathematical modeling. These models are increasingly being recognized as useful tools for establishing public health policies.

Highly pathogenic avian influenza (HPAI) is one of the major zoonoses transmitted from birds to humans and has been an intermittent disease in South Korea since 2003. Among the multiple epidemics, the HPAI subtype H5N8 is the enormous outbreaks resulting in significant damage to the country's poultry industry. It is, thus, important to study the disease transmission characteristics of the emerging H5N8 subtype in South Korea. However, to date, epidemiological studies have mainly focused on the H5N1 subtype. Therefore, this thesis is aimed to conduct to investigate epidemiologic characteristics of HPAI caused by newly appeared subtypes, including their risk factors, transmissibility, and spatiotemporal dynamics between poultry farms.

First, to understand HPAI H5N8 infection, a retrospective case-control study was conducted to identify and evaluate potential risk factors for HPAI H5N8

infection on broiler duck farms. Duck farms with known H5N8 infections were selected as cases; control farms were matched to cases based on location within a 3-km radius. Potential risk factors were analyzed using univariable and multivariable logistic regression. Fecal removal services (OR = 27.78, 95% confident interval (CI) = 3.89–198.80), farm owner's career (OR = 7.91, CI = 1.69–37.14), large flock size (OR = 6.99, CI = 1.34–37.04), and other poultry farms within 500 m (OR = 6.30, CI = 1.08–36.93) were significantly associated with HPAI (H5N8) outbreaks in the final model. These results indicated that the HPAI H5N8 outbreaks in South Korea were associated with farm owner age, the number of flocks, poultry farm density, and biosecurity. Establishing policies to manage these risk factors may reduce the vulnerability of South Korean poultry farms to HPAI (H5N8) outbreaks.

The second study assessed the transmissibility of the HPAI subtypes H5N1, H5N8, and H5N6 in poultry farms by estimating their basic reproduction numbers (R_0) through mathematical modeling. R_0 calculations used exponential growth and maximum likelihood models based on the susceptible-infected-removed compartment model. The mean R_0 for subtypes H5N1, H5N8, and H5N6 were 1.68-1.95, 1.03-1.83, and 1.37-1.60, respectively. Results of Kruskal-Wallis pairwise comparison tests showed that the mean generation time for H5N8 (7.27 days) was significantly longer than that for subtype H5N1 (4.93 days). These findings suggest that the R_0 differ by HPAI subtype and might be associated with the temperature during the early stage of the infection, species specificity by viral subtype, and prevention policies. Knowledge of these and other factors affecting transmissibility can be used to design practical disease control strategies for future emergent HPAI subtypes.

Finally, H5N8 infections in South Korean poultry farms were analyzed to identify their spatiotemporal distributions, understand the mechanisms of transmission between farms, and evaluate the effectiveness of quarantine policies in addressing outbreaks. The global and local spatiotemporal interactions in the first and second H5N8 epidemics were analyzed using a space-time K function at the national level and a space-time permutation model from 2014 to 2016, respectively. In both epidemics, the space-time K-function analyses revealed significant interactions within three days and up to 40 km distance; excessive risk attributable values (D_0) were maintained despite the distance. Eleven local spatiotemporal

clusters were identified, and results indicated that the regional spread of H5N8 was polarized between small and large spatiotemporal clusters. This global and local spatiotemporal interaction indicates that the HPAI epidemics in South Korea were mostly characterized by short duration of transmission within a small area and then dispersal by long-range jumps.

In conclusion, this study used three epidemiological models to provide a scientific basis for improved effective quarantine policies for HPAI control. Risk factors for the introduction and spread of H5N8 HPAI virus in South Korean poultry farms were identified through analytic epidemiology. Furthermore, differences in disease transmissibility for three HPAI subtypes were demonstrated through mathematical modeling. Results suggest that features of the current quarantine system, such as preemptive depopulation, and the tracking of poultry vehicle movements, need to be continued. The results of this thesis can be used as scientific evidence for evaluating and supplementing HPAI quarantine policies and disease countermeasures. It is also expected that the methodologies used in this thesis can be applied to other infectious zoonosis occurring in South Korea and worldwide.

Keyword: Highly pathogenic avian influenza, poultry farms, risk factors, transmission parameter, mathematical modeling, spatiotemporal analysis

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List of Abbreviations

AI	Avian Influlenza	
APQA	Animal & plant Quarantine Agency	
CI	Confidence Interval	
EG	Exponential growth	
HA	Hemagglutin	
HPAI	Highly Pathogenic Avian Influenza	
IAVs	Influenza A viruses	
IP	Infected premise	
KAHIS	Korea Animal Health Integrated System	
KoROAD	Korean Road Traffic Authority	
LPAI	Low Pathogenic Avian Influenza	
MAFRA	Ministry of Agriculture, Food, and Rural Affairs	
ML	maximum likelihood	
NA	Neuraminidase	
OIE	World Organisation for Animal Health	
OR	Odds Ratio	
PP	Positive premise	
R ₀	Basic reproduction number	
Rt-PCR	Reverse-transcriptase polymerase chain reaction	
SIR	Susceptible-Infected-Removed	
WHO	World Health Organization	

General Introduction

In epidemiology, a risk factor is defined as a factor that increases the probability of developing a disease (Liamputtong, 2019). Case-control studies are among several epidemiologic study designs that identify risk factors by comparing groups (Hess, 2004). Researchers estimate the association of a potential risk factor with the disease by comparing its frequency in a diseased group (cases) with its frequency in a nondiseased group (controls) (Lewallen & Courtright, 1998). It is then possible to estimate the impact of the factor on the disease (World Health Organization, 2018). In infectious disease epidemiology, it is important to identify and quantify associated risk factors through risk-based surveillance and control strategies (Chaudhry et al., 2015). Understanding the factors associated with infections in the host will help in improving actions and policies to reduce the spread of infectious diseases within the population.

The spread of infectious disease is a dynamic process and the amount of the population susceptible to infection changes over time (Grassly & Fraser, 2008). Mathematical approaches have been used to estimate changes in the number of infections by tracking the dynamics of pathogens over time (Grenfell et al., 2004). In recent years, detailed electronic surveillance of infectious disease has become widespread through advances in computing science and rapid diagnostic tests (Delamater et al., 2019). The basic reproduction number (R_0) is a key parameter developed through mathematical modelling to reflect the transmission potential of a disease. R_0 is an average of the number of successful transmission events resulting from a single infection (Dietz, 1993). Estimation of R_0 is a powerful tool for understanding disease dynamics and evaluating the impact of interventions on

infectious disease.

An understanding of the mechanisms driving infectious disease propagation in space and time enable the development of public health policies. Recent technological advances have led to a growing trend of using geographical information system (GIS) approaches for infectious disease epidemiology (Chowell & Rothenberg, 2018). A spatiotemporal analysis is an epidemiologic method that focuses on the relationships between time, space (location), and host or environmental characteristics to detect patterns of disease occurrence (Smith et al., 2015). The occurrence of space-time interactions between outbreak cases located close in time and space varies and can, thus, be considered an infectious disease indicator (Diggle et al., 1995). Measuring and analyzing these indicators provides an understanding of pathogen transmission mechanisms, which enables the development of prevention strategies against disease spread.

Highly pathogenic avian influenza (HPAI) is an avian disease with zoonotic potential. HPAI outbreaks are highly contagious and often fatal to poultry, causing enormous economic damage to the poultry industry (Short et al., 2015). The first case of direct human infection of HPAI from poultry was reported in Hong Kong in 1997 (De Jong et al., 1997). Since then, persistent human infections have been reported and HPAI has become an important public health concern in humans. The novel H5N8 subtype HPAI virus was first reported in poultry farms in January 2014 in South Korea (Lee, 2014), and caused four epidemic waves through April 2016 to become the largest nationwide HPAI outbreak (Animal & Plant Quarantine Agency, 2016). Characteristics of the H5N8 subtype in poultry farms are different from those of the H5N1 viruses previously identified in South Korea. Compared to the high mortality rates of H5N1 virus infections in poultry farms, H5N8 virus infections in

domestic ducks caused lower mortality, furthermore, affected ducks did not display reconizable clinical signs of infection (Kim et al., 2014).

The present study was designed to analyze HPAI (H5N8) in South Korea through three methods to provide a scientific basis for improving effective quarantine policies for HPAI control in the future: an analytic epidemiological model, a mathematical model, and a spatiotemporal model. This thesis is organized into three chapters. In chapter 1, the potential risk factors for H5N8 outbreaks in broiler duck farms are identified and evaluated using a retrospective case-control study design; the results from this study are applicable to policies designed to reduce the spread of the HPAI subtype H5N8 between poultry farms in South Korea. In chapter 2, the R_0 of subtypes H5N1, H5N8, and H5N6 during HPAI outbreaks are estimated and used in mathematical modeling to understand outbreak characteristics and to provide insight into potential control measures. In chapter 3, the spatiotemporal distributions of HPAI (H5N8) in the poultry farms are modeled in order to understand underlying mechanisms of H5N8 HPAI virus transmission between farms.

Literature Review

1. Analytic epidemiology in infectious disease

1.1. Analytic epidemiology

In epidemiologic research, descriptive and analytic studies are the two main types of research designs for describing the distribution of disease incidence and prevalence, studying exposure–disease associations, and identifying disease prevention strategies (Boslaugh, 2007). Analytic epidemiology attempts to evaluate the reasons and mechanisms of the variations in health outcomes observed in different groups, communities, and populations (Dicker et al., 2006). These studies are designed to determine whether differences in outcomes can be attributed to variations between groups in demographic characteristics, socioeconomic status, genetic factors, environmental exposures, and behavioral and other risk factors that are potential direct or underlying causes of the disease (Aschengrau & Seage, 2013).

The term "exposure" is used to represent the potential causes of a health outcome. Depending on the nature of the exposure factor and the study design, the exposure may be a risk factor, a protective factor, or a treatment/intervention. Analytic studies explore the association between exposure status and a health event; comparisons of two or more groups are used to test a study hypothesis to assess whether a relationship exists (Friis & Sellers, 2020). In observational studies such as cross-sectional, case-control, and cohort studies, researchers obtain data on exposure and outcome variables without providing treatments or interventions to the subjects. In contrast, experimental and intervention studies allow researchers to control the exposure factor and test the effect of a treatment or intervention on the outcome (Figure 1) (Liamputtong, 2019).



Figure 1. Epidemiological study designs and the strength of evidence for association between an exposure and health outcome.

Adapted from Liamputtong, 2019

1.2. Case-control study and risk factors

A case-control study uses a retrospective design, that is, both the exposure and the outcome have occurred prior to the study being conducted. The study methods involve the identification of a group with the outcome of interest (cases) and a group free of the same outcome (controls). (Lewallen & Courtright, 1998) Next, the researchers evaluate the frequency of the exposure in the case group with its frequency in the control group (Figure 2) (Hess, 2004). If the amount of exposure in the case group is statistically significantly higher than that in the control group, then the illness (outcome) is considered to be associated with that exposure.

In epidemiology, a risk factor or determinant is a variable associated with an increased risk of disease or infection. Risk factors are associated with the outcome but are not necessarily causal (World Health Organization, 2018). Risk factors relevant to community health policy are those related to a health risk and that are general, abstract, pertain to inequalities, and are difficult for an individual to control; some are also preventable. Case-control studies are one of the epidemiological methods used to assess risk factors.



Figure 2. Case-control study design.

Adapted from Hess, 2004

1.3. Risk factors associated with HPAI outbreaks

The timely identification of risk factors in an outbreak is important to develop strategies to control the disease (Lewallen & Courtright, 1998). Previous epidemiologic studies of AI from a variety of settings and countries have identified several risk factors. However, most of the studies on risk factors were based on the HPAI H5N1 subtype. To date, most of the studies on H5N8 outbreaks have analyzed the genetic epidemiology of the virus, with only a limited number of studies looking at the epidemiology of the disease (Globig et al., 2016; Harder et al., 2015; Shin et al., 2015)

Previous studies of HPAI (H5N1) have identified several factors associated with infection, including contact with the bodies of dead birds (Biswas et al., 2011), methods of carcass disposal (Garber et al., 2016; Liu et al., 2015), distance to case farms (Chaudhry et al., 2015; Wells et al., 2017), equipment sharing (Metras et al., 2013), high poultry densities (Chaudhry et al., 2015; Loth et al., 2010), inadequate biosecurity (McQuiston et al., 2005; Nishiguchi et al., 2007), and a minimal distance to road (Fang et al., 2008; Ward et al., 2008). These identified risk factors include spatial factors. Combining location information with epidemiological analysis is an important tool to fully characterize the occurrence and transmission of HPAI (Iglesias et al., 2010; Martin et al., 2011).

Risk factor	Reference	Odds ratio	<i>P</i> -value
Inappropriate vaccination	Henning et al. (2009b)	85.2	0.01
Visitor access	Henning et al. (2009b)	8.2	0.04
	Osmani et al. (2014)	3.0	0.01
	Fasina et al. (2010)	8.32	< 0.01
Poultry breed mixing	Henning et al. (2009b)	11.5	0.02
Wild animal entering	Henning et al. (2009b)	10.9	0.01
	Biswas et al. (2009a)	4.47	0.032
	McQuiston et al. (2005)	1.9	0.04
Carcass disposal management	Biswas et al. (2009b)	13.29	0.027
Proximity to water	Biswas et al. (2009a)	5.27	0.024
	Paul et al. (2011)	3.48	< 0.001
Selling live poultry	Paul et al. (2011)	3.34	< 0.001
	Fasina et al. (2010)	11.91	< 0.01
	Nishiguchi et al. (2007)	36.6	NA
Distance to road	Paul et al. (2011)	2.44	0.013
Number of workers	Osmani et al. (2012)	12.2	0.001
Equipment sharing	Nishiguchi et al. (2007)	29.4	
Inadequate biosecurity	Nishiguchi et al. (2007)	7	
Distance to case farms	Nishiguchi et al. (2007)	8.6	
Poultry age	McQuiston et al. (2005)	4.9	< 0.001
Managers living outside of the	Fasina et al. (2010)	8.98	0.01
farm	McQuiston et al. (2005)	2	0.03
Contact with dead bird bodies	McQuiston et al. (2005)	7.3	< 0.001

Table 1. Literature references by risk factor of HPAI

2. Pathogen epidemiology in infectious disease

2.1. Pathogen epidemiology

Epidemiology is a population science that studies the patterns of disease incidence, attempting to infer its causes and consequences. Classical epidemiology, for instance, might seek to identify risk factors for a given condition, which might be environmental or genetic. This approach identifies factors that can inform interventions minimizing the risk of disease. However, methods are different for transmissible diseases. The spread of infectious disease is a dynamic process in which an increasing numbers of cases increases the risk to the rest of the population. In contrast, as people recover, they may become immune and be removed from the pool of susceptible. Hence, the numbers of hosts available to be infected changes over time (Figure 3) (Grassly & Fraser, 2008). Infectious disease epidemiology expands the methodological repertoire with the use of mathematical models, comprising sets of differential equations, and statistical models that use a probabilistic framework. These models can then be used to explore the impact of vaccination campaigns or other interventions (Kliman, 2016).



Figure 3. Biological infectiousness overtime after infection for three different human pathogens.

a. Influenza A; based on viral shedding in experimental human infections. b. HIV-1; based on retrospective analysis of HIV-1 discordant couples and viral-load data. c. Malaria; infectiousness of infected humans to mosquitoes based on the detection of infectious gametocytes in the blood after therapeutic treatment of syphilis by inoculation with Plasmodium vivax. Adapted from Grassly & Fraser, 2008

2.2. Mathematical models of infectious disease

Mathematical representation and analyses of infectious diseases have been central to infectious disease epidemiology since its inception as a discipline (Beisner, 2005). In recent years, detailed electronic surveillance of infectious diseases has become widespread, owing to the advent of improved computing, electronic data management, and the ability to share and deposit data. These ongoing developments have increased the application of mathematical models to both the generation and testing of primary scientific hypotheses and to the design of practical strategies for disease control. Such analyses and models have successfully explained challenging observations, such as influenza, HIV, and malaria, and have been key in developing public health strategies in many countries such as influenza, HIV, and malaria (Anderson et al., 1992; Glasser et al., 2004).

Given the growing importance of mathematical epidemiology, the integration of models with rigorous statistical methods has been fundamental to developing methods to estimate key parameters of these models and to test hypotheses using real-life data. In the absence of reliable data, mathematics can help formulate hypotheses, inform data-collection strategies, and determine sample sizes, permitting evaluation of competing hypotheses (Figure 4) (May, 2004). Ideally, data should be analyzed using models that adequately describe the observed dynamics and patterns of interest as well as the mechanisms that generate these observations. Models should be as simple as possible, but not so simple that the consideration of additional realistic complexity alters the conclusions.



Figure 4. Difference between stochastic simulation and deterministic approximation in foot and mouth disease in UK in 2001.

The red curve and shaded region show the mean and range, respectively, for simulations of a detailed stochastic model in which spread of infection is modeled using the actual spatial distribution of farms in England and Wales. The blue curve is from a highly simplified "toy model," using gross averages of relevant parameters. The comparison between the simple model, in which the dynamics can be clearly understood, and the complex computer simulations illuminates both for similarities and differences.

Adapted from May, 2004

2.3. Reproduction number study in infectious disease

The basic reproduction number (R_0) is one of the crucial parameters in infectious disease epidemiology. It is defined as the number of successful transmission events and new infections that result, on average, from one infection. It is possible to relate these numbers to the course of an outbreak. When R_0 . >1, the expected number of new cases will increase, whereas if R_0 . <1, the numbers will fall. It is also essential to define and distinguish incidence and prevalence. Incidence is the number of new cases per unit time, whereas prevalence is the overall frequency of the disease in the population. The incidence can be falling, but the prevalence can continue to rise, albeit at a slower rate.

Figure 5 displays an ideal epidemic curve, which shows prevalence and incidence, and the changes in *R* throughout the outbreak (Kliman, 2016). While in the illustrated case the epidemic diminishes after the pathogen has run out of hosts to infect the disease can become endemic if sufficient susceptible hosts are continually introduced (e.g., by birth or waning immunity). Although the expected final size of the outbreak falls rapidly as R_0 decreases, outbreaks can still occur in the case where $R_0 < 1$. This can happen as R_0 is an average value, and the initial cases can result in an above-average number of new infections. The effects of this phenomenon can be probed using a stochastic approach in which events are modeled as randomly sampled realizations from a probability distribution.



Figure 5. An ideal epidemic curve showing variations in the prevalence, incidence, and reproductive number (R) over the course of an outbreak.

The illustrated case is the result of a Susceptible-Infectious-Recovered model, in which recovered hosts become entirely resistant to infection, and as a result, the prevalence returns to zero. The point at which the decline in the availability of susceptible hosts means each case causes on average just one onward infection is that where R¹/₄1, and is indicated. Note that this is coincident with the peak incidence, which precedes peak prevalence as described in the text.

Adapted from Kliman, 2016

3. Spatial epidemiology in infectious disease

3.1. Application of geographical information system to epidemiology

Over the last few decades, significant progress has been achieved in infectious disease prevention and control; despite this, infectious diseases continue to pose a significant public health burden (Chowell & Rothenberg, 2018). However, the understanding of mechanisms driving their propagation in space and time has advanced exponentially in recent years. Modern quantitative computational tools and highly resolved geospatial demographic, epidemiological, and genomic data are enabling actionable insights for public health in near-real-time (Chowell & Rothenberg, 2018).

Geographic information systems (GIS) have increased the availability and range of tools that can be used to analyze disease outbreaks (Pfeiffer et al., 2008). A GIS is a database designed to handle geographically referenced information and complemented with software tools for the input, management, analysis, and display of data (Longley et al., 2005). It is used widely in epidemiology, with the most straightforward application in the investigation of an outbreak being the creation of maps displaying the locations of cases (Figure 6) (Smith et al., 2015).





Adapted from C. M. Smith et al., 2015

3.2. Spatial research approach

Quantitative methods for investigating infectious disease using spatiotemporal data rely on two broad classes of research methods—spatial statistical modeling (Anselin et al., 2010; Lawson, 2013) and spatial transmission dynamic modeling methods (Sattenspiel & Lloyd, 2009). The application of these methodologies to infectious disease research has increased rapidly over the last two decades. Additionally, there have been major advances in computational power and an increasing amount and diversity of epidemiological and genetic data with spatial and temporal information (Figure. 7) (Chowell & Rothenberg, 2018). For instance, spatial statistical methods are frequently used to uncover relationships between spatiotemporal patterns of a disease and host or environmental characteristics (Lawson, 2013), The resulting detailed maps provide a visualization of the distribution of morbidity or mortality (Zulu et al., 2014), and help identify hotspots or clusters (Kulldorff & Nagarwalla, 1995).



Figure 7. Growth in spatial modeling, 1990–2017 (Web of Science). Adapted from Chowell & Rothenberg, 2018

3.3. Spatiotemporal analysis using clustering

Spatiotemporal modeling of animal diseases has been applied to various outbreak scenarios to identify geographical clusters with higher than expected levels of disease risk. Numerous methods have been developed to detect clusters, including point methods and aggregated data (Pfeiffer et al., 2008). "Global" tests evaluate the entire area for evidence of clustering but without pinpointing specific clusters, whereas "local" (or "cluster detection") tests identify the positions of specific clusters. Cuzick and Edwards' k-nearest neighbor test, for example, is a global method for assessing clustering in case-control point data (Cuzick & Edwards, 1990). The method counts the number of nearest neighbors of cases that are also cases, and compares the result to the number that would be expected under the null hypothesis that cases and controls are randomly distributed. Kulldorff's spatial scan statistic is an additional method used to identify local clustering, usually in point data (Kulldorff, 2007). Observed numbers of cases within spatiotemporal windows of various sizes are compared with numbers that would be expected under a random distribution. Circular or elliptical regions of elevated risk of disease are then located. The scan statistics and k-nearest neighbor test have also been adapted to identify spatiotemporal clustering, testing the null hypothesis that cases that are geographically close to each other occur at random times (Figure 8) (Kulldorff et al., 2005).



Figure 8. Locations and dates of detected diarrhea outbreak signals, using historical data from November 15 to November 14 2002.

The three hospital-based signals are depicted with thicker lines/circles. The stronger residential-based signal was signal C. Note that all the zip-code areas in the residential signal E are also part of signal C.

Adapted from M. Kulldorff, Heffernan, Hartman, Assuncao, & Mostashari, 2005

4. Avian Influenza

4.1. Avian influenza virus

The influenza virus belongs to the *Orthomyxoviridae* family of viruses and are classified into four genera—A, B, C, and D. Among them, B and C infect humans, while D is not known to cause human infections. Influenza A viruses (IAVs) infects various types of vertebrates, such as humans, pigs, horses, dogs, and marine mammals, as well as wild birds and poultry. IAVs are primarily classified into subtypes according to the antigenicity of their surface proteins, hemagglutinin (HA) and neuraminidase (NA) (Figure 9) (Horimoto & Kawaoka, 2005). There are 16 HA and 9 NA subtypes generating 144 subtype combinations. Subtypes are named based on the characteristics of these surface proteins, for example, H5N1, H5N6, H5N8, H7N9 etc. IAVs spread through interspecies transmission and has a variety of hosts for the various subtypes (Figure 10) (Short et al., 2015).

The World Organization for Animal Health (OIE) categorizes AI as having high or low pathogenicity according to molecular characteristics of the virus and its ability to cause disease and mortality in chickens in laboratory settings (Figure 11) (Horimoto & Kawaoka, 2005). HPAI virus is highly contagious in chickens and has a mortality rate of about 90%; infected chickens display clinical symptoms such as shortness of breath, diarrhea, a sharp decrease in egg production, and cyanosis of comb and wattle. It is classified in South Korea as a type 1 livestock infectious disease under the Livestock Infectious Disease Prevention Act (MAFRA, 2015) and designated as a managed disease by the OIE (World Organisation for Animal Health, 2020).



Figure 9. Schematic diagram of influenza A virus.

Adapted from Horimoto & Kwaoka, 2005.



Figure 10. Reservoirs and interspecies transmissions of influenza virus.

Adapted from Short et al., 2015.



Figure 11. Localized low pathogenic avian influenza (LPAI) infection versus systemic highly pathogenic avian influenza (HPAI) infection.

Adapted from Horimoto & Kwaoka, 2005.

4.2. Transmission

AI viruses are transmitted from wild to domestic birds and spill to humans potentially (Figure 12) (Pascua & Choi, 2014). While wild aquatic birds have played the main role in long-distance transmission of AI viruses, ducks are key in the transmission between wild migratory birds and domestic poultry (Lycett et al., 2016). The subtypes that cause HPAI are H5 and H7, and are spread from wild birds to poultry and poultry to humans and pigs via interspecies transmission.

AI is mainly infectious through direct contact with the host animal (Figure 13) (Center for Disease Control and Prevention, 2019). However, mechanical propagation between farms also occurs due to vehicles, people, feed, clothing, shoes, appliances, and equipment contaminated by dust, water, or feces of infected poultry.

Additionally, diseases can occur through animal vectors such as wild mice and wild birds. Further, contaminated water, feed, and aerosolized solids can spread from the affected farms to adjacent farms (Animal & Plant Quarantine Agency, 2011).





domestic birds and potential spill to humans.

.Adapted from Pascua & Choi, 2014.



Figure 13. Avian Influenza Transmission infographic

Adapted from Centers for Disease Control and Prevention, 2019
4.3. HPAI subtype H5N8 outbreaks

The novel H5N8 HPAI virus was first reported in January 2014 in South Korean poultry farms (Y. Lee, 2014). The analysis of virus transmission in migratory birds, and HPAI H5N8 antigens from these birds, revealed that wild water birds introduced this subtype to South Korea during the winter of 2013–2014 (Jeong et al., 2014; Y. Lee, 2014). By the summer of 2014, the H5N8 virus had spread to Siberia and Beringia courtesy of the migratory birds via the East Asia–Australia flyway (Figure 14) (D. H. Lee et al., 2015; Verhagen, Herfst, & Fouchier, 2015). Thereafter, the subtype spread globally through overlapping migratory pathways (Lycett et al., 2016). H5N8 outbreaks have occurred in 37 countries since 2014 and are spread by wild water birds (McLeod & Hinrichs, 2016). In South Korea, the detection rate of HPAI antigens in the feces of wild migratory birds is reported to be high during the winter season (November and December), when they fly to the south, and during the spring season (March and April), when heading to the north for breeding (Song et al., 2017).

The outbreak of HPAI (H5N8) was recorded to be the longest HPAI outbreak in South Korea and occurred over four waves (Animal & plant Quarantine Agency, 2016). The sequencing analysis of the H5N8 to understand their origin and transmission revealed that it entered South Korea from the west via Jeonbuk province, spreading rapidly among western provinces with high densities of overwintering waterfowl and domestic ducks (Figure 15) (Hill et al., 2015). The H5N8 subtype detected in poultry farms has different characteristics than the H5N1 subtype previously occurred in South Korea. First, HPAI (H5N1) outbreaks were

concentrated in commercial chicken farms, whereas HPAI (H5N8) outbreaks occurred more frequently in broiler duck farms. Second, compared with the HPAI (H5N1), HPAI (H5N8) in ducks had no evident clinical signs and lower mortality despite a high level of viral excretion (Kim et al., 2014). These characteristics of the HPAI (H5N8) in domestic ducks present a challenge to virus monitoring. The high density of ducks in farms and the vulnerability of the detection system in live bird markets and poultry farms further increase the difficulty of preventing HPAI outbreaks (Song et al., 2017).



Figure 14. Global movement of the HPAI H5N8 virus.

Geographic map showing the movement of HPAI H5N8 virus in Asia, Europe, and North America in relation to regional waterfowl migration routes. The map, by Dmthoth, is sourced from Wikipedia Commons

(http://commons.wikimedia.org/wiki/File:Blank_Map_Pacific_World.svg).

Adapted from Lee et al., 2015



Figure 15. The estimated trajectory of the H5N8 spread in South Korea.

Arrows connecting locations represent directions of movement with Bayes factor support >10. Arrow colors represent Bayes factor support for rate indictors, with darker blue indicating better support. Arrow thicknesses are proportional to the inferred values of Markov jumps between locations; a wider arrow represents more migration between a pair of locations. Yellow and orange backgrounds show the estimated density (numbers per kilometer) of domestic ducks (colors in key). Adapted from Hill et al., 2015

Chapter 1.

Risk Factors Associated with highly pathogenic avian influenza subtype H5N8 Outbreaks on Broiler Duck Farms in

South Korea

Abstract

Highly Pathogenic Avian Influenza (HPAI) subtype H5N8 outbreaks occurred in poultry farms in South Korea in 2014 resulting in significant damage to the poultry industry. Between 2014 and 2016, the pandemic disease caused significant economic loss and social disruption. To evaluate the risk factors of HPAI infection in broiler duck farms, we conducted a retrospective case-control study on broiler duck farms. Forty-three farms with confirmed laboratories on premises were selected as the case group and 43 HPAI-negative farms were designated as the control group. Control farms were matched based on farm location and were within a 3-km radius from the case premises. Spatial and environmental factors were characterized by site visit and plotted through a geographic information system (GIS). Univariable and multivariable logistic regression models were developed to assess possible risk factors associated with HPAI broiler duck farm infection. Four final variables were identified as risk factors in a final multivariable logistic model: "Farms with \geq seven flocks" (odds ratio (OR) = 6.99, 95% confidence interval (CI) 1.34-37.04), "Farm owner with ≥ 15 yrs. of raising poultry career" (OR = 7.91, 95% CI 1.69–37.14), "Presence of any poultry farms located within 500 m of the farm" (OR = 6.30, 95%CI 1.08–36.93), and "Not using a fecal removal service" (OR = 27.78, 95% CI 3.89– 198.80). This highlights that the HPAI H5N8 outbreaks in South Korea were associated with farm owner education, number of flocks and facilities, and farm biosecurity. Awareness of these factors may help reduce the spread of HPAI H5N8 across broiler duck farms in Korea during epidemics. Greater understanding of the risk factors for H5N8 may improve farm vulnerability to HPAI and other subtypes and help establish policies to prevent re-occurrence. These findings are relevant to

global prevention recommendations and intervention protocols.

1.1. Introduction

Highly Pathogenic Avian Influenza (HPAI) is a major zoonosis between humans and birds, especially domestic poultry (Alexander, 2007). It has caused considerable economic loss to the global poultry industry and is an ongoing major public health threat worldwide (Short et al., 2015). The first case of direct H5 HPAI transmission from chickens to humans was reported during a H5N1 HPAI outbreak on chicken farms and live bird markets in 1997 in Hong Kong (De Jong et al., 1997). Following this first report of human infection, HPAI subtype H5N1 viruses have since infected 859 people with a mortality rate close to 60% (OIE, 2017).

Outbreaks of the new HPAI subtype, H5N8, were first reported in January 2014 on South Korean poultry farms (Lee, 2014). The introduction of the HPAI subtype H5N8 in Korea was associated with wild water birds in winter, determined by transmission factors analyzed through the migration route of migratory birds and HPAI antigen identification in these birds (Jeong et al., 2014; Lee, 2014). As the result of a phylogenetic network analysis of avian influenza viruses worldwide, the H5N8 subtype virus emerged during late 2013 in China, spread in early 2014 to South Korea and Japan, and reached Siberia and Beringia by summer 2014 via migratory birds (Lee et al., 2015; Verhagen et al., 2015). Migratory birds from Korea mainly use the East Asia-Australia route and HPAI H5N8 virus spreads globally through the mutual overlapping of migratory pathways (Lycett et al., 2016). The HPAI H5N8 has occurred extensively in 37 countries globally since 2014 due to spread by wild water birds (McLeod & Hinrichs, 2016). HPAI outbreaks occurred in Korea between from 2003 to 2015 with four outbreaks of HPAI H5N1 in 2003–2004, 2006–2007, 2008, and 2010–2011. The outbreak of HPAI H5N8 was the longest in Korea and occurred over four waves (Animal & Plant Quarantine Agency, 2016). The H5N8 subtype in poultry farms has different characteristics to the H5N1 HPAI that previously occurred in Korea. First, H5N1 HPAI cases were concentrated on commercial chicken farms, whereas HPAI H5N8 occurred more frequently on broiler duck farms. Second, in comparison with the associated high mortality of H5N1 virus on domestic farms, HPAI H5N8 was associated with lower mortality without evident clinical signs on duck farms though there were a large amount of viral excretions (Kim et al., 2014). These characteristics of the H5N8 virus in the domestic duck could present a challenge to virus monitoring. The high density of duck farms and the vulnerability of the detection system in live bird markets or poultry farms are proposed as causes for the continuous HPAI outbreaks (Song et al., 2017).

In order to construct strategies to control diseases, the identification of timely risk factors in outbreaks are important (Lewallen & Courtright, 1998). Previous avian influenza epidemiologic studies have identified several risk factors from a variety of settings and countries (Abbas et al., 2012; Arriola et al., 2015; Beaudoin et al., 2014; Biswas, Christensen, Ahmed, Barua, et al., 2009; Biswas, Christensen, Ahmed, Das, et al., 2009; Biswas et al., 2011; Boender et al., 2007; Bui et al., 2017; Cao et al., 2010; Chaudhry et al., 2015; Desvaux et al., 2011; Dinh et al., 2006; Fang et al., 2008; Fasina et al., 2011; Gale et al., 2014; Garber et al., 2016; Gilbert et al., 2006; Henning et al., 2009; Huang et al., 2016; Iglesias et al., 2010; Kung et al., 2007; Liu et al., 2015; Lohiniva et al., 2013; Loth et al., 2010; Mannelli et al., 2006; Martin et al., 2011; McQuiston et al., 2005; Metras et al., 2013; Mounts et al., 1999; Musa et

al., 2013; Nishiguchi et al., 2007; Osmani et al., 2014; Paul et al., 2011; Tenzin et al., 2017; Thomas et al., 2005; Thompson et al., 2008; Tiensin et al., 2009; Tombari et al., 2013; Tsukamoto et al., 2007; Vong et al., 2009; Wang et al., 2017; Wang et al., 2014; Ward et al., 2008; Zhang et al., 2014; Zhou et al., 2009). However, most risk factor studies are based on the HPAI subtype H5N1. To date most of the HPAI H5N8 studies of the outbreaks have analyzed the genetic epidemiology of the virus with limited epidemiologic studies (Globig et al., 2016; Harder et al., 2015; Hill et al., 2015; Jeong et al., 2014; Lycett et al., 2017; Kim et al., 2015; Song et al., 2014; Lee et al., 2015; Lee, 2014; Lycett et al., 2016; Shin et al., 2015; Song et al., 2017). Phylogenetic analysis is a powerful tool in molecular epidemiology but is enhanced with risk factor studies. To the best of the authors' knowledge, limited epidemiological analysis of H5N8 outbreaks has not been conducted.

In previously published studies, many risk factors were associated with avian influenza infection, such as contact with dead bird bodies (Biswas et al., 2011), carcass disposal management (Garber et al., 2016; Liu et al., 2015; McQuiston et al., 2005), distance to case farms (Chaudhry et al., 2015; Mannelli et al., 2006; Nishiguchi et al., 2007; Wells et al., 2017), equipment sharing (Metras et al., 2013; Nishiguchi et al., 2007), poultry densities (Boender et al., 2007; Chaudhry et al., 2015; Loth et al., 2010; Paul et al., 2011; Tiensin et al., 2009), inadequate biosecurity (Biswas, Christensen, Ahmed, Barua, et al., 2009; Garber et al., 2016; McQuiston et al., 2005; Metras et al., 2013; Nishiguchi et al., 2007; Paul et al., 2017; Paul et al., 2017; Paul et al., 2017; Fang et al., 2008; Paul et al., 2011; Ward et al., 2008), proximity to water (i.e. ponds and lakes), workers and visitor access to barns, and contact with wild birds. Among the identified risk factors, some are related to spatial information. Combining

location information with epidemiological analysis studies is an important tool to fully characterize the occurrence and transmission of HPAI (Fang et al., 2008; Iglesias et al., 2010; Martin et al., 2011). To understand factors for outbreaks on boiler duck farms and to help control the spread of HPAI subtype H5N8, we conducted a retrospective case-control study to identify and evaluate potential risk factors of HPAI H5N8 occurrence during the outbreak that occurred in 2014-2016.

1.2. Material and Method

1.2.1. Case definition and control farm selection

Case farms were selected as infected premises that were confirmed positive by reverse transcription polymerase chain reaction (RT-PCR) by the Animal and Plant Quarantine Agency in Korea. HPAI virus was screened by using the hemagglutination (HA) assay and RT-PCR by using influenza A–specific nucleoprotein (NP) primers (Hoffmann et al., 2001). Infected premises comprised poultry farms with observed clinical signs reported by farmers. Positive premises were diagnosed as positive after culling.

H5N8 outbreaks were the longest HPAI outbreaks in Korea and occurred in four waves: 2014.01.16–2014.07.29, 2014.09.24–2015.06.10, 2015.09.14–2015.11.15, and 2016.03.23–2016.04.5. In the H5N8 outbreak report for 2014 to 2016, a total of 393 case premises were reported as infected (Animal & Plant Quarantine Agency, 2016).

For the HPAI H5N8 outbreaks in the study, the cases occurred mainly on poultry farms in the boundary area of three provinces; Gyeonggi-do, Chungcheongbuk-do, and Chungcheongnam-do, which were surveyed, though the disease eventually

spread throughout Korea. These provinces were considered to have an important role in the early stages of the outbreaks. Sample farms were selected from broiler duck farms because they had a large proportion of the H5N8 cases. Therefore, we decided that comparing the case and control broiler duck farms in these areas would be the most efficient method. In the first wave of HPAI H5N8 outbreaks (2014.01.16– 2014.07.29), 46 broiler duck farms were confirmed as HPAI H5N8-positive in three provinces. Of the 46 HPAI-positive broiler duck farms, three farm owners rejected the questionnaire. The other 43 case farms were enrolled in this study.

Control farms were selected from among farms within a distance of 3 km from the case farm. Control farms were confirmed HPAI-negative by PCR during the outbreak periods from 2014-2016 and were matched in terms of location. Among the non-infected farms, the closest farm was selected as the control farm. The control farms needed to fulfill the selection criteria, which included raising broiler ducks during the HPAI H5N8 outbreaks and not being shut down after the HPAI H5N8 outbreaks. Forty-three case premises and 43 control farms were selected and sampled.

1.2.2. Data collection and survey

It is usually found to determine the risk factors based on the characteristics of pathogens or on research into a similar disease that has been studied. Since there is no previous H5N8 case-control study, potential risk factors related to other subtypes of HPAI were included in the questionnaire for analysis. We investigated 55 possible risk factors associated with HPAI H5N8 infection in broiler duck farms.

Data were collected using a questionnaire comprising 55 binary, multiple choice, and short answer type questions (Appendix A). In the farm characteristic category, we documented farm location, species, and number of flocks on farms, farm area size, density of herds, and the raising of other birds or animals on the farm. Distance between the nearest poultry farms were surveyed by farm owners, and were confirmed using ArcGIS v. 10.0 (ESRI System, Redlands, CA, USA). Demographic factors of owners were also collected and included education level, farm owner age, number of years raising poultry, and any secondary occupations. Farmers were asked to provide details on contact with wild birds and animals in winter to assess the relationship between HPAI and wild animals. Wild bird and animal contacts were estimate through contact probabilities and frequency. The probability and frequency of wild birds and animals were estimated by farm owner response. It was asked whether wild birds were observed on a nearby farm during the winter when HPAI occurred.

Data on the following items were collected from targeted farms as risk factors related to biosecurity: disposal of dead poultry, removal of feces and sewage, use of a biosecurity advisor, fencing, footbaths at entry to the farm, presence of hand sanitizer, change room, log book, individual boots, farm visitor protocols, and disinfection of vehicles entering farms.

Two trained veterinarians conducted interviews of farm owners or senior employee(s) between July and December 2015. In order to confirm the reliability of the survey, on-site farms visits were performed after the interview.

1.2.3. Geographical information

Among the risk factors associated with HPAI, environmental data were calculated via GIS. The basic administrative area was expressed using the shape file

map provided by ArcGIS. The GIS database was constructed through geocoding of the farm address information from the Korea Animal Health Integrated System. Location of poultry farms were analyzed using the GIS database by receiving information from the Animal and Plant Quarantine Agency using the transformation method of Korea Geodetic Datum 2002. To determine if certain geographic features were related to HPAI H5N8 infection, we compared proximity of roads, water environments, and distance to documented migratory bird locations. Data on road networks, such as highways, national roads, and other types of roads were obtained from the traffic management system at the Korea Road Traffic Authority (KoROAD) (2016). Korea Water Resource Management Information Systems was used to obtain information of national canal and local streams (http://www.wamis.go.kr). The location of migratory bird areas and the 2014 winter water bird census reported by the Ministry of Environment were used to estimate the influence of wild birds on HPAI. Breeder poultry farms and duck slaughterhouses were referenced in the statistics from the Ministry of Agriculture, Food, and Rural Affairs (2015). Geographical shape file data and land coverage of the Korea administrative division were downloaded from the Korea National Spatial Data infrastructure portal Open API (http://openapi.nsdi.go.kr).

To assess the impact of environmental risk factors, we estimated minimal distance between the farms and risk factors using the 'near' tools in the GIS proximity toolset, which are used to compute the distance from each point in a coverage to nearest point in another coverage. Land cover spatial information was also surveyed after visualizing and mapping land types such farmland, forest, water, and city.

1.2.4. Statistical analysis

All statistical analyses were performed using SPSS v. 23.0 (IBM, USA) and R software version 3.4.3 (R Project for Statistical Computing, Vienna, Austria). Before being entered into the model, continuous variables were transformed into categorical variables using the quartile of distribution to avoid problems of linearity. The flock numbers and flocks size were changed to dichotomous variables based on whether they were above or below the averages for broiler duck farms in Korea. Farm location and environmental data were analyzed by ArcGIS v. 10.0. The continuous information data, such as distance to environmental factors, were converted into categorical data through quantiles based on control farm data. The population characteristics and continuous variables between case and control farms were examined using Student's t-test. McNemar's chi-squared test was used to compare categorical variables between case and control farms. The level of significance was set at 5% for all comparisons.

Odd ratios (ORs), their 95% confidence intervals (CIs), and p-values were estimated using maximum likelihood methods. Risk factors analysis was carried out in two steps, using univariable and multivariable logistic regression models. As an initial screening, univariable analysis was conducted to test the association between outcome (case-control) and each explanatory variable suspected as a risk factor. The likelihood-ratio test was used to assess the fit of the model.

Multivariable logistic regression was applied to the selection of explanatory variables according to Dohoo et al. (Dohoo et al., 2003). For multivariable analyses, we used the conditional logistic model to assess the effects of risk factors. We used multinomial logistic regression (NOMREG) for 1:1 matches between case and

control farms. Each farm was paired by location. Significance levels of the Wald statistic $p \le 0.2$ in the univariable logistic regression were used to select variables for a multivariable logistic regression. A forward stepwise variable-selection was used to add the variable with the lowest p-value to construct a final model with a significance level of $p \le 0.05$. The new model was compared with the previous one using a likelihood-ratio test after the addition of each variable. The fit of the model was evaluated using the Hosmer-Lemeshow test (Hosmer Jr et al., 2013). For all tests, variables with p-values < 0.05 were considered to be statistically significant.

1.3. Results

1.3.1. Population characteristics

The epidemic curve of HPAI H5N8 outbreaks on case farms in Korea is shown in Figure 16. The first suspected outbreak occurred in January 2014. After this, the number of infected farms rapidly increased and peaked on February 19, 2014.

At the species and breed level, 229 broiler ducks (58.3%), 61 parent stock ducks (15.5%), 47 layer chickens (12.0%), 20 Korean native chicken premises (5.1%), 15 parent stock chickens (3.8%), 11 mixed poultry (2.80%), 3 mallards (0.76%), 2 broiler chickens (0.51%), 2 geese (0.51%), 1 ostrich (0.25%), 1 wild goose (0.25%), and 1 quail (0.25%) were confirmed as positive by PCR for HPAI H5N8.

The geographical distribution of the 43 case and 43 control farms is shown in Figure 17. Most case farms were concentrated in the boundary between the provinces. The Chungcheongbuk-do area and the Eumseong County, is an area where broiler duck farms are concentrated, and an area where most of the farms tested positive for HPAI H5N8.

Case and control farm population characteristics are presented in Table 2. There was a significant difference in the number of flocks between the case and control duck farms (p = 0.009), but no significant difference in the number of ducks, flock size, or farm area size (p > 0.05).

1.3.2. Univariable analysis of risk factors

Fourteen variables were identified as risk factors with the univariable analysis (Table 3). Examining binary variable data, HPAI H5N8 was significantly associated with 11 variables; "Farms with \geq seven flocks" (OR = 4.91, 95% CI 1.79–13.43), "Sizes of flocks \geq 2000" (OR = 2.72, 95% CI 1.05-7.05), "Poultry farms located within 500 m of farm" (OR = 3.82, 95% CI 1.30–11.20), "Farm owners with \geq 15 yrs. of raising poultry career" (OR = 2.99, 95% CI 1.16–7.73) "Hiring new workers" (OR = 58.33, 95% CI 7.33–463.96), "Not using a feces removal service" (OR = 7.14, 95% CI 2.38–20.12), "Not using a biosecurity advisor" (OR = 3.15, 95% CI 1.31–7.60), "No fence around a farm" (OR = 3.71, 95% CI 1.26–10.93), "No footbaths at entry areas to the farm" (OR = 47.31, 95% CI 9.93–255.44), "No hand sanitizer" (OR = 2.58, 95% CI 1.08–6.16), "No disinfection sprayer" (OR = 6.16, 95% CI 2.39–15.86), and "No change area on the farm" (OR = 4.18, 95% CI 1.58–11.05).

Analyzing the categorical data, two variables were significantly related to HPAI. Regarding the method of dead bird disposal, the farms which treated dead birds as "dog food" (OR = 14.40, 95% CI 1.38–150.81) or use of "rendering" (OR = 10.40,95% CI 1.62–66.90) were more vulnerable than the farms that incinerated dead bodies on the farm. In 2014 farms at a distance of less than 13 km from the winter water bird sites were at a higher risk than the farms located more than 24.6 km (OR = 5.87, 95% CI 1.59-21.65). The following factors had the greatest significance (p < 0.001): "Hiring new workers," "Not using a feces removal service," "No footbaths at entry areas to the farm," and "No change area on the farm".

The correlation was analyzed by R using the function cor by using the Spearman rank correlation. Results showed that the only positive correlations found were between "farm workers' boots" and "farm workers' clothes". These two factors were merged into "The worker entry biosecurity,"

1.3.3. Multivariable analysis of risk factors

Twenty-seven variables with $p \le 0.2$ were considered for candidates in the multivariable logistic regression model to estimate effects (Table 4). The final model identified four variables as independent risk factors for HPAI H5N8 infection on broiler duck farms in Korea (Table 5). They were "Farms with \ge seven flocks" (p = 0.021), "Farm owner with ≥ 15 yrs. of raising poultry career" (p = 0.009), "Any poultry farms located within 500 m of the farm" (p = 0.041), and "Not using a feces removal service" (p < 0.001).

1.4. Discussion

The aim of this study was to investigate potential risk factors of HPAI H5N8 infection in poultry farms in Korea. Previous studies have identified risk factors of HPAI infection on duck farms, especially commercial broiler duck farms (Gilbert et al., 2006; Henning et al., 2009). However, these studies focused on small-sized duck farms in Southeast Asian countries. To the best of our knowledge, this is the first case-control study reporting the risk factors for HPAI infection in Korea. It is also

the first epidemiologic report on the risk factors of H5N8 subtypes on commercial broiler duck farms. The results help describe risk factors associated with the HPAI H5N8 infection and transmission in Korea between 2014 and 2016.

The risk factor, "farm with \geq seven poultry flocks," was identified from the multivariable analysis model. Thompson et al. previously suggested that the number of flocks was a risk factor for HPAI occurrence (Thompson et al., 2008). This can be explained by an increased frequency of contacts that have potential for infection (e.g., traders, veterinarian, pharmacy, or feed suppliers). In addition, it might be difficult for farm owners with larger flocks to disinfect their farms. In broiler duck farms, disinfection of flocks is operated after duck shipment. In the case of farms with multiple flocks, the farm owner and employee are more likely to come into contact with other flocks after disinfectant operation. In the univariable analysis, a flock size > 2,000 was also identified as a risk factor. In a previous study, HPAI virus transmission was dependent on an increased number of birds (Tsukamoto et al., 2007). Therefore, a large farm may have a greater chance of infections.

The odds of HPAI infection was eight-fold greater for a farm owner with ≥ 15 yrs. raising poultry career than less those with < 15 yrs. (p = 0.009). One reason for this is that the more experienced a farmer is in the poultry industry, the more likely the facility would be older. There was a significant relationship between the age of the farm and the farmer's poultry industry experience. Second, the longer the farm owner is employed, the older the farmer is likely to be. In the sampled farms, farmer age and years of poultry industry career were found to be positively correlated. The older the farmer, the less likely the farmer may be to accept new information and update their practices (Smith & Buckwalter, 2006). Therefore, it is important to focus on biosecurity and HPAI education with older farm owners in future prevention

activities.

Previous studies have shown that proximity to the nearest case farm was related to avian influenza virus infection in Japan and the Netherlands (Boender et al., 2007; Nishiguchi et al., 2007), which is consistent with our findings. These studies showed that as the distance between farms decreases, the risk of infection increases. The density of poultry farms in the high-risk group was significantly higher than average (Boender et al., 2007). In addition, the greater the number of nearby poultry farms, the higher the probability of HPAI infection. In fact, HPAI H5N8 occurs mainly in areas with a high density of farms, where the proximity of nearby farms is an important factor in the spread of disease (Hill et al., 2015). It may be necessary to reduce the density of poultry farms to control and prevent disease outbreaks.

Our results documented that case farms were unlikely to conduct proper disposal of feces than control farms. Feces are often used as a fertilizer on the owner's farm or neighboring farms in most of the case farms. Inappropriate management of feces has been shown to be an important biosecurity threat in other studies (McQuiston et al., 2005; Musa et al., 2013; Sheta et al., 2014). Feces can be contaminated and serve as a source of HPAI virus allowing spread to wild birds and domestic animals (Stallknecht et al., 1990). Therefore, appropriate management of feces conducted by an approved removal service is important to prevent the virus from entering farms. Although there is no evidence that wild birds have been infected with HPAI through the feces of domestic poultry, there might be a risk that wild animals can come into contact with infected feces and spread the HPAI. This study has some limitations regarding target farm data collection. Because of the ongoing outbreak, it was sometimes difficult to interview farm owners. In addition, control farm owners were also reluctant to be surveyed because of biosecurity concerns. There was some

concern for reporter validation. This concern was minimized by interviewers' observation and it was suspected that no significant bias occurred and that there was high reliability in respondent answers. We also recognized that recall bias may have occurred for some questions. This may especially be true for wild bird related questions. The survey was conducted in July but the questions asked where related to the previous recollection of winter water bird contact. Potential recall differences may increase the possibility of recall bias and explain the lack of statistical significance in wild animal variables.

1.5. Conclusion

We analyzed possible risk factors of the introduction and transmission of HPAI H5N8 on broiler duck farms during the outbreaks in Korea between 2014 and 2016. The risk increased for farms with \geq 7 flocks, owners with \geq 15 years' experience in the poultry industry, poultry farms located within 500 m, and not using a feces removal service. Consideration of these risk factors related to biosecurity of HPAI outbreaks could possibly reduce the risk of HPAI H5N8 infection on broiler duck farms in Korea. This study highlights the importance of farm owner education, reducing flock density, enhancing disease management of large size farms, and proper treatment of feces. Good management practice and strict biosecurity can prevent the introduction of the virus to farms and the transmission within flocks. By managing the risk factors identified through this study, it will be possible to overcome the vulnerability of farms to HPAI and establish policies to prevent the basis for future HPAI H5N8 in Korea. It is believed that this study can form the basis for future HPAI H5N8 analytical research.

Table 2. Case alla colluol lalli	ı populatioi	I CIIAIACICII	surcs						
Variable		C	ase			Con	trol		p-value*
	Min	Mean	Median	Max	Min	Mean	Median	Max	
No. ducks in farms	1000.0	12114.0	10000.0	60000.0	1900.0	11883.2	9500.0	34500.0	0.909
No. flocks	3.0	7.7	7.0	17.0	1.0	5.8	6.0	15.0	0.009
No. ducks per flocks	420.0	2275.9	2000.0	11666.7	828.6	2494.2	2000.0	9000.0	0.565
Farm area size (m^2)	300.0	1824.0	1600.0	3600.0	1000.0	2714.0	2000.0	8000.0	0.389
Farm area size per flocks (m^2)	30.0	266.6	240.0	600.0	142.9	324.2	250.0	666.7	0.323
Birds per m^2	0.9	10.4	9.3	48.3	2.9	7.3	7.0	16.5	0.360
*P-value based on student t-test.									

Table 2. Case and control farm population characteristics

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Variables	Category	No. of case farms (%)(n=43)	No. of control farms (%)(n=43)	OR	95% CI	P-value
Farms with ≥ 7 flocks	Yes	36 (83.7)	22 (51.2)	4.91	1.79-13.43	0.002
	No	7 (16.3)	21 (48.8)			
Sizes of flocks ≥ 2000	Yes	34 (79.1)	25 (58.1)	2.72	1.05-7.05	0.039
	No	9 (20.9)	18 (41.9)			
Poultry farms located within 500 m of farm	Yes	34 (79.1)	22 (51.2)	3.82	1.30-11.20	0.015
	No	9 (20.9)	21 (48.8)			
Farm owner with ≥ 15 yrs. of raising poultry	Yes	19 (44.2)	9 (20.9)	2.99	1.16-7.73	0.024
career	No	24 (55.8)	34 (79.1)			
Hiring new workers	Yes	42 (97.7)	18 (41.9)	58.33	7.33-463.96	<0.001
	No	1 (2.3)	25 (58.1)			
Disposal methods of dead birds	Dog food	10 (23.3)	1 (2.3)	14.40	1.38-150.81	0.026
	Rendering	15 (34.9)	2 (4.7)	10.40	1.62-66.90	0.014
	Burial	12 (27.9)	32 (74.4)	0.55	0.15-2.04	0.371
	Incineration	6 (14.0)	8 (18.6)			0.001^{*}
Using a feces removal service	Yes	22 (51.2)	38 (88.4)			
	No	21 (48.8)	5 (11.6)	7.14	2.38-20.12	<0.001
Using a biosecurity advisor	Yes	16 (37.2)	28 (65.1)			
	No	27 (62.8)	15 (34.9)	3.15	1.31-7.60	0.011
Fence around a farm	Yes	25 (58.1)	36 (83.7)			

Table 3. Univariable logistic regression of risk factors associated with HPAI H5N8 at broiler duck farms in Korea

	No	18(41.9)	7 (16.3)	3.71	1.26-10.93	0.017
Footbath at entry areas to the farm	Yes	13 (30.2)	41 (95.3)			
	No	30 (69.8)	2 (4.7)	47.31	9.93-225.44	<0.001
Presence of hand sanitizer	Yes	17 (39.5)	27 (62.8)			
	No	26 (60.5)	16 (37.2)	2.58	1.08-6.16	0.033
Presence of disinfection sprayer	Yes	15 (34.9)	33 (76.7)			
	No	28 (65.1)	10 (23.3)	6.16	2.39-15.86	<0.001
Changing area on the farm	Yes	8 (18.6)	21 (48.8)			
	No	35 (81.4)	22 (51.2)	4.18	1.58-11.05	0.004
Direct distance to 2014 winter water bird	< 13	24 (55.8)	9 (20.9)	5.87	1.59-21.65	0.008
census of Korea (km)	13-17	7 (16.3)	13 (30.2)	1.19	0.29-4.81	0.813
	17-24.6	7 (16.3)	10 (23.3)	1.54	0.37-6.45	0.555
	≥ 24.6	5 (11.6)	11 (25.6)			0.014^{*}
OR means Odds ratios, CI means Confidential	l Intervals					

*This means total p-value of the categorical variables models.

Table 4. Candidate univariables associated	with HPAI H5N	$48 \text{ with } p \leq 0.2.$				
11. and a state of the state of	Cotocourt	No. of case farms	No. of control	đ	0502 CT	D tuluo
V ariables	Calegory	(%) (n=43)	farms (%) (n=43)	OK OK		r-value
Farms having ≥ 7 flocks	Yes	36 (83.7)	22 (51.2)	4.91	1.79-13.43	0.002
	No	7 (16.3)	21 (48.8)			
Sizes of flocks ≥ 2000	Yes	34 (79.1)	25 (58.1)	2.72	1.05-7.05	0.039
	No	9 (20.9)	18 (41.9)			
When selling poultry, transport all birds	Yes	34 (72.1)	38 (88.4)	0.33	0.07-1.46	0.143
in flocks	No	9 (27.9)	5 (11.6)			
Presence of any poultry farms located	Yes	34 (79.1)	22 (51.2)	3.82	1.30-11.20	0.015
within 500m of the farm	No	9 (20.9)	21 (48.4)			
Farm owner having \geq 15yrs. raising	Yes	19 (44.2)	9 (20.9)	2.99	1.16-7.73	0.024
poultry career	No	24 (55.8)	34 (79.1)			
Farms belong to meat company	No	7 (16.3)	1 (2.3)	8.17	0.96-69.56	0.055
	Yes	36 (83.7)	42 (97.7)			
Farmers determine presence of rodents in	Yes	40 (93.0)	34 (79.1)	2.91	0.57-14.76	0.197
farms	No	3 (7.0)	9 (20.9)			
Having secondary occupation excepts	Yes	13 (30.2)	6 (14.0)	2.61	0.86-7.95	0.092
poultry farms	No	30 (69.8)	37 (86.0)			

Hiring new workers	Yes	42 (79.7)	18 (41.9)	58.33	7.33-469.96	>0.00
	No	1 (2.3)	25 (58.1)			
Hiring foreign farm workers	Yes	16 (37.2)	8 (18.6)	2.59	0.97-6.95	0.058
	No	27 (62.8)	35 (81.4)			
Disposal methods of dead birds	Dog food	10 (23.3)	1 (2.3)	14.40	1.38-150.81	0.026
	rendering	15 (34.9)	2 (4.7)	10.40	1.62-66.90	0.014
	Burial	12 (27.9)	32 (74.4)	0.55	0.15-2.04	0.371
	Incineration	6 (14.0)	8 (18.6)			0.001^{*}
Using a feces removal service	Yes	21 (48.4)	5 (11.6)			
	No	22 (51.2)	38 (88.4)	0.14	0.05-0.42	>0.001
Workers changing cloths before entering	Yes	21 (48.8)	28 (65.1)			
barns	No	22 (51.2)	15 (34.9)	1.96	0.82-4.65	0.129
Workers washing hands before entering	Yes	22 (51.2)	30 (69.8)			
barns	No	21 (48.4)	13 (30.2)	2.20	0.91-5.33	0.080
Using a biosecurity advisor	Yes	16 (37.2)	28 (65.1)			
	No	27 (62.8)	15 (34.9)	3.15	1.31-7.60	0.011
Fence around a farm	Yes	25 (58.1)	36 (83.7)			
	No	18 (41.9)	7 (16.3)	3.71	1.26-10.93	0.017

Individual farm boots	Yes	42 (97.7)	38 (88.4)			
	No	1 (2.3)	5 (11.6)	0.18	0.02-1.62	0.126
Individual barn cloths	Yes	8 (18.6)	10 (23.3)			
	No	35 (81.4)	33 (76.7)	1.33	0.47-3.77	0.060
Footbaths at entry to the farm	Yes	13 (30.2)	41 (95.3)			
	No	30 (69.8)	2 (4.7)	47.31	9.93-225.44	>0.001
Presence of hand sanitizer	Yes	17 (39.5)	27 (62.8)			
	No	26 (60.5)	16 (37.2)	2.58	1.08-6.16	0.033
Presence of disinfection of vehicles	Yes	25(58.1)	33(76.7)			
	No	18(41.9)	10(23.3)	2.38	0.94-6.03	0.069
Presence of disinfection sprayer	Yes	15 (34.9)	33 (76.7)			
	No	28 (65.1)	10 (23.3)	6.16	2.39-15.86	>0.001
Change area on the farm	Yes	8 (18.6)	21 (48.8)			
	No	35 (81.4)	22 (51.2)	4.18	1.58-11.05	0.004
Distance(km) from the farm to closest	< 1.8	16 (37.2)	12 (27.9)	4.44	1.00-19.75	0.050
highway(km)	1.8-3.6	15 (34.9)	10 (23.3)	5.00	1.10-22.82	0.038
	3.6-6.0	9 (20.9)	11 (25.6)	2.73	0.57-13.01	0.208
	≥ 6.0	3 (7.0)	10 (23.3)			0.166*

Distance(km) from the farm to closest	< 0.4	3 (7.0)	12 (27.9)	0.18	0.04-0.81	0.025
national road(km)	0.4-1.2	15 (34.9)	9 (20.9)	1.22	0.39-3.80	0.729
	1.2-2.75	10 (23.3)	11 (25.6)	0.67	0.21-2.12	0.492
	≥ 2.75	15 (34.9)	11 (25.6)			0.083*
Direct distance to Wild birds surveillance	<2	7 (16.3)	11 (25.6)	0.85	0.24-3.06	0.802
area in 2014(km)	2.0-4.8	8 (18.6)	11 (25.6)	0.97	0.28-3.40	0.962
	4.8-11	19 (44.2)	9 (20.9)	2.82	0.87-9.10	0.084
	<u>></u> 11	9 (20.9)	12 (27.9)			0.160^{*}
Direct distance to 2014 winter water bird	< 13	24 (55.8)	9 (20.9)	5.87	1.59-21.65	0.008
census of Korea (km)	13-17	7 (16.3)	13 (30.2)	1.19	0.29-4.81	0.813
	17-24.6	7 (16.3)	10 (23.3)	1.54	0.37-6.45	0.555
	≥ 24.6	5 (11.6)	11 (25.6)			0.014^{*}
*This means total p-value of the categorical	l variable models					

Table 5. Multivariable logistic regression of risk factors associated with I	HPAI H5N8 on	broiler duck farms in	Korea
Variables	OR	95% CI	P-value
Farms having ≥7 flocks	6.99	1.34-37.04	0.021
Farm owner having ≥ 15 yrs. of raising poultry career	7.91	1.69-37.14	0.009
Poultry farms located within 500 m of the farm	6.30	1.08-36.93	0.041
Not Using a feces removal service	27.78	3.89-198.80	0.001
OR means Odds ratios, CI means Confidential Intervals			



Figure 16. Epidemic curve of HPAI H5N8 outbreaks in Korea from January 2014 to April 2016. Infected farms were confirmed as positive by PCR.



Figure 17. Geographical location of case and control broiler duck farms in Korea.

Map was focused on three provinces, Gyeonggi-do, Chungcheongbuk-do, and Chungcheongnam-do. Location of poultry farms were analyzed using the transformation method of Korea Geodetic Datum 2002 Chapter 2.

Estimation of the basic reproduction numbers of the subtypes H5N1, H5N8, and H5N6 during the highly pathogenic avian influenza epidemic spread between farms

Abstract

It is important to understand pathogen transmissibility in a population to establish an effective disease prevention policy. The basic reproduction number (R_0) is an epidemiologic parameter for understanding the characterization of disease and its dynamics in a population. We aimed to estimate the R_0 of the highly pathogenic avian influenza (HPAI) subtypes H5N1, H5N8, and H5N6, which were associated with nine outbreaks in Korea between 2003 and 2018, to understand the epidemic transmission of each subtype. According to HPAI outbreak reports of the Animal and Plant Quarantine Agency, we estimated the generation time by calculating the time of infection between confirmed HPAI-positive farms. We constructed exponential growth and maximum likelihood (ML) models to estimate the basic reproduction number, which assumes the number of secondary cases infected by the index case. The Kruskal-Wallis test was used to analyze the epidemic statistics between subtypes. The estimated generation time of H5N1, H5N8, and H5N6 were between 4.58 and 5.24 days, 6.01 and 8.23 days, and 5.02 and 5.91 days, respectively. A pairwise comparison showed that the generation time of H5N8 was significantly longer than that of the subtype H5N1 (P=0.04). Based on the ML model, R_0 was estimated as 1.68-1.95 for subtype H5N1, 1.03-1.83 for subtype H5N8, and 1.37-1.60 for subtype H5N6. We concluded that R_0 estimates may be associated with the poultry product system, climate, species specificity based on the HPAI virus subtype, and prevention policy. This study provides an insight on the transmission and dynamics patterns of various subtypes of HPAI occurring worldwide. Furthermore, the results are useful as scientific evidence for establishing a disease control policy.

2.1. Introduction

Highly pathogenic avian influenza (HPAI) is a highly contagious viral disease that infects domestic poultry and wild birds (Alexander, 2007). The HPAI virus can cause an epidemic that may spread rapidly, has a high mortality rate among domestic birds, and devastates the poultry industry (Short et al., 2015). Outbreaks of distinct subtypes of HPAI, including H5N1, H5N8, and H5N6, are continually reported worldwide (DeJesus et al., 2016; Gu et al., 2013; Si et al., 2017), and this global HPAI virus dissemination is caused by migratory wild birds (Verhagen et al., 2015). The HPAI crisis appears to be a great threat to not only animal health but also public health worldwide. Furthermore, the World Health Organization reported 860 human infection cases of avian influenza A subtype H5N1 (World Health Organization, 2019) after the first human case of HPAI subtype H5N1, which was reported in Hong Kong in 1997 (De Jong et al., 1997).

In South Korea, outbreaks of three different subtypes of HPAI occurred between 2003 and 2018. The first outbreak of H5N1 occurred from December 2003 to February 2004 and had a high mortality rate at poultry farms, especially among chickens (Lee et al., 2005). Since then, outbreaks of H5N1 have occurred in 2006, 2008, and 2010 (Kim et al., 2012; Kim et al., 2010; Lee et al., 2008). The novel HPAI subtype, H5N8, was first reported in January 2014 at South Korean poultry farms (Lee, 2014). Genetic analyses of viruses isolated from wild birds and poultry farms showed that migratory birds could be responsible for the first wave of H5N8 outbreaks between January and May 2014 (Jeong et al., 2014). After the first wave, two waves of subtype H5N8 occurred during September 2014 to June 2015 and during September 2015 to November 2015 (Animal & plant Quarantine Agency,

2016). It was reported that these sporadic outbreaks were caused by viruses reintroduced into Korea by migratory waterfowl (Kwon et al., 2016). In November 2016, a novel genotype of H5N6 that was first detected in wild birds in Korea and HPAI infectious cases was reported at poultry farms (Lee et al., 2017). Another novel H5N8 virus co-circulated with H5N6 virus during the outbreaks in 2016, from February to June 2017 (Kim et al., 2017). In November 2017, the novel H5N6 virus was detected at a broiler duck farm and in wild mallards, with infection spreading to poultry farms (Lee et al., 2018).

The main strategies used to prevent and control HPAI outbreaks are based on the prohibition of movement, preemptive culling, and vaccinations in infected areas (Yee et al., 2009). Therefore, it is important to understand pathogen transmissibility in a population to establish an effective disease prevention policy. The basic reproduction number (R_0) is one of the important epidemiologic parameters necessary to understand the characterization of disease and the dynamics in a population (de Jong, 1995). R_0 is generally defined as the average number of secondary cases caused by one infectious individual during the entire infectious period in an uninfected population (Thomas et al., 2001). If each infected individual infects more than one other individual, on an average, at any time point, then the epidemic will be sustainable (Dietz, 1993). Various methods are used to estimate the reproduction number (Forsberg White & Pagano, 2008; Wallinga & Lipsitch, 2006; Wallinga & Teunis, 2004), and these have been implemented in the R program (Obadia et al., 2012) and Excel (Cori et al., 2013) as ready-made procedures.

Reproduction number estimation has been used to understand HPAI epidemic characteristics and to provide insight regarding control measures for epidemics. These farm-to-farm reproduction number estimations were targeted to the HPAI subtype H5N1 and were conducted in Nigeria (Bett et al., 2014), Romania (Ward et al., 2009), Thailand (Marquetoux et al., 2012), Bangladesh (Ssematimba et al., 2018), India (Pandit et al., 2013), Italy, Canada, and the Netherlands (Garske et al., 2007). In Korea, there was a mathematical modeling study of the reproduction number for HPAI from 2016 to 2017, but this was limited to the local reproduction number and did not include all epidemics from South Korea (Lee et al., 2019). We aimed to estimate the generation time and R_0 of HPAI subtypes H5N1, H5N8, and H5N6, which were associated with nine outbreaks from 2003 to 2018 in Korea, and demonstrate the characterization of each subtype by analyzing HPAI characteristics, including the epidemic days, number of farms, species distribution, generation time, and R_0 . It is expected that the results of the present study will become a foundation for demonstrating the disease dynamics of each HPAI subtype and its characteristics, as well as for establishing effective HPAI control, not only for traditional HPAI subtype H5N1 but also the emerging subtypes H5N8 and H5N6.

2.2. Material and Method

2.2.1. Data collection

The epidemic data of HPAI outbreaks in Korea were collected by the Animal and Plant Quarantine Agency (APQA) in Gimcheon, Korea. The livestock owner (including the manager) or veterinarian who found an animal with clinical signs and suspected HPAI was required to report the case to the APQA according to the Prevention of Contagious Animal Disease Act. Cloacal, fecal, and blood samples were collected from sick or dead poultry in reported poultry farms, and HPAI virus was confirmed using reverse-transcriptase polymerase chain reaction at the Avian Influenza Research and Diagnosis Department of the APQA. If the suspected farm was confirmed as HPAI-positive and deemed an infected premise (IP), then depopulation of farms with infected poultry and depopulation of all poultry farms in the protection zone were conducted. If a depopulated farm was found to be positive, then it was defined as a positive premise (PP)(Oh, 2018). Both IP and PP were considered cases in this study. The epidemic curve of these HPAI cases was depicted using the "incidence" package in R to illustrate the weekly reported number of poultry farms in the International Organization for Standardization (ISO) week date system (Kamvar et al., 2019) (Figure 18).

2.2.2. Serial interval and generation time

A serial interval is the time between successive cases in a chain of transmission, estimated from the interval between clinical onsets in patients (Forsberg White & Pagano, 2008). We estimated the serial interval of HPAI as the time between the reported date of the first farm with infected cases and secondary farm with infected cases. This estimation was based on the investigation of the epidemic pathway of HPAI transmission, which shows the epidemiologic relationship between the infector and infectee. According to the APQA investigations, HPAI transmission could be possible through wild migratory birds, wild animals, farm owners, managers, staff, vehicles related to the poultry industry, and airborne transmission from nearby infected farms. The epidemic transmission pathway investigation was conducted by an APQA epidemiologic investigator visiting and interviewing the places suspected to be associated with the infected farms, including animal facilities such as hatcheries, feed factories, and live bird markets. The APQA investigated vehicles, people, livestock, and their products that entered an infected farm from 21 days prior to infection and estimated the disease transmissions.

In addition to investigating via interview, the APQA used geographic information to identify HPAI viral transmission by vehicles. In Korea, vehicles related to the poultry industry transporting poultry, poultry products, medicines, feed, and feces must be registered with the Korea Animal Health Integrated System (KAHIS; http://www.kahis.go.kr). The movements of livestock-related vehicles are reported to the KAHIS, making it possible to track the movement of vehicles, people, livestock, and animal products.

Through these interviews and vehicle information, the disease transmission pathway via transportation and human movement was identified. If a clear epidemiologic link to the infected farm could not be found through interviews and movement tracking, then we hypothesized that the farm might have been infected with HPAI by wild migratory birds or wild animals. We then excluded infection thought to be caused by wild birds or wild animals during the estimation of the serial interval because it is not possible to observe the serial interval of virus transmission from wild birds and animals.

The generation time is the modeling term describing the time duration from the onset of transmittable infection in a primary case to the onset of infection in a secondary case infected from the primary case. We defined the generation time as the difference between suspected infection days of the primary farm and secondary farm, which was measured through epidemiologic investigation. The suspected infection day was estimated according to the day reported by the farm owner after clinical symptoms were found in the poultry and the period between the infection and latent period of each HPAI subtype in the poultry species. We estimated the
suspected infection date from the day the clinical symptoms were reported by subtracting the periods between infection and clinical symptoms. For H5N1, the periods between infection and clinical symptoms were assumed to be 2 days for chickens (Lee et al., 2005), 4 days for ducks (Jeong et al., 2009), and 3.8 days for other poultry species (Lee et al., 2005). For H5N8, the periods were 3.2 days for chickens (E.-K. Lee et al., 2016), 8.0 days for ducks (Animal & Plant Quarantine Agency, 2016), and 2.0 days for other species (D.-H. Lee et al., 2016). For H5N6, the periods were 2.6 days for chickens (Park et al., 2019), 4.6 days for ducks (Animal & Plant Quarantine Agency, 2017), and 3.0 days for other species (Animal & Plant Quarantine Agency, 2017).

Based on the generation time between case farms, we calculated the discretized generation time distribution using a function (est.GT) in the R0 package. Discretization is performed on the grid [0, 0.5), [0.5, 1.5), [1.5, 2.5), etc... where the unit is time interval of days (Obadia et al., 2012). Time-to-event data were assumed to follow a parametric distribution with a probability density function (PDF). The distribution of generation time is expressed in the form of parametric distribution such as "gamma," "lognormal," or "Weibull," using maximum likelihood. The mean and standard deviation of generation time is provided in the desired time units. The calculated distribution of the generation time in each subtype and outbreaks is depicted in figure 19.

2.2.3. Model assumption and data analysis

The study model is based on the susceptible-infected-removed (SIR) compartmental model (Iwami et al., 2007), which divides poultry farms into

compartment. A susceptible farm (S) becomes infectious (I) through contact with the possible disease and is then removed (R) by depopulation. The dynamics of an epidemic can be described as the equation given below when N is the sum of S, I, and R.

$$\frac{dS}{dt} = -\frac{\beta IS}{N}$$
$$\frac{dI}{dt} = \frac{\beta IS}{N} - \gamma I$$
$$\frac{dR}{dt} = \gamma I$$

In this model, β is a parameter, which controls how much the disease can be transmitted through the exposure of HPAI virus, and γ is a parameter, which expresses how many poultry farms can be removed in a specific period. In this model, the average number of secondary infections caused by an infected host, R_0 , equals β/γ (Ridenhour et al., 2018).

We constructed exponential growth (EG) and maximum likelihood (ML) models to estimate early reproduction numbers using the R0 package (Obadia et al., 2012) in R (version 3.3.0). The EG model assumes that the initial reproduction ratio can be associated with the EG rate during the early epidemic phase (Wallinga & Lipsitch, 2006). The formula is $R_0 = 1/M$ (-*r*), where *r* denotes the initial EG rate and M stands for the moment generating function of generation time distribution. In the initial EG model, a period from day 1 to day 14 of epidemics was chosen when the outbreak's growth was exponential. A function (est.R0.EG) in the R0 package was used (Obadia et al., 2012). We used a sensitivity test in EG to select the period during which growth is exponential as optimized time windows. We used the "sensitivity analysis" function to compute the deviance R-squared statistic over a range of periods.

The ML estimation model assumes that the number of secondary cases caused by an index case is Poisson-distributed with the expected value R_0 (Forsberg White & Pagano, 2008). This model assume that the number of new cases at time t as N= $\{N_t\}, t=0,...T, \text{ and a generation time distribution } w$. The log-likelihood of R_0 was defined as $LL(R_0) = \sum_{t=1}^{T} \log(\frac{e^{-\mu_t}\mu_t^{N_t}}{N_t!})$, where $\mu_t = R_0 \sum_{j=1}^{t} N_{t-i} w_i$. The likelihood must be calculated on a period of exponential, and the deviance R-squared measure may be used to select the best period that maximized the likelihood. The range was set as 0.01 to 50, in which the maximum must be searched. A function (est.R0.ML) in the R0 package was used (Obadia et al., 2012).

The Kruskal-Wallis test was used to determine the statistical differences in epidemics between subtypes (Breslow, 1970). The epidemic days, number of farms, cases per day, poultry species distribution of farms, generation time, and R_0 estimated by EG and ML of the three subtypes H5N1, H5N8, and H5N6 were analyzed. The significance level was α =0.05. These statistical analyses were performed using SPSS 22.0 (IBM, Armonk, NY, USA).

2.3. Results

2.3.1. HPAI epidemic in Korea

We investigated 12 HPAI outbreaks of three subtypes, H5N1, H5N8, and H5N6, that occurred from 2003 to 2018 in Korea. Table 6 presents a summary of the epidemic data, including the period of outbreaks and the number of infected farms that were investigated. The weekly epidemic curves of HPAI outbreaks are shown in Figure 18 based on the ISO 8601 week date system. The H5N1 HPAI outbreaks (except for the 2008 outbreaks) began between November and February, when the lowest temperature drops below 0°C (Figure 18a). Regarding H5N8 in 2014, the second and third waves recurred in September 2015 and 2016, respectively (Figure 18b). However, the second wave of H5N6 in 2016 occurred in June (Figure 18c). The longest outbreak was the second wave of H5N8 in 2014, which occurred over 260 days. The shortest outbreak was the fourth wave of H5N8 in 2014, which occurred over 14 days. The outbreaks with the most cases (340 poultry farms) and cases per day (3.579 cases per day) were the H5N6 outbreaks in 2016. Regarding H5N8 in 2014, more than 72% of the occurrences were in ducks; however, there was no apparent species specificity for subtypes H5N1 and H5N6.

2.3.2. Generation time and the basic reproduction number

We selected nine outbreaks with sufficient number of premises to calculate R_0 and analyzed the generation time and initial R_0 using the EG and ML methods (Table 2). Generation time distributions are illustrated by each HPAI subtype as the PDF in Figure 19. Generation time of H5N1 were estimated between 4.58 and 5.24 days (Figure 19a), generation time of H5N8 were estimated to have 6 days or more (6.01-8.23 days) (Figure 19b), and generation time of H5N6 were estimated between 5.02 and 5.91 days (Figure 19c). R_0 was estimated as 1.65-2.20 for subtype H5N1, 0.03-1.56 for subtype H5N8, and 1.03-1.24 for subtype H5N6 using EG methods. Using ML methods, R_0 was estimated as 1.68-1.95 for subtype H5N1, 1.03-1.83 for subtype H5N8, and 1.37-1.60 for subtype H5N6.

Most of the R_0 in the EG and ML methods were similar, except for the second

and third waves of H5N8 in 2014. The R value obtained by the EG method was less than 1 for the second and third waves of H5N8 in 2014. To select the optimal time windows, sensitivity results of the time windows and R_0 were used (Table 7). Optimized time windows selected by sensitivity tests accounted for 69.14% of the outbreak periods, on an average, and the optimal R_0 values in optimized time windows were less than 1 for the subtypes H5N1 and H5N6 outbreaks.

2.3.3. Epidemic statistics between subtype

The average values of the number of epidemic days, infected poultry farms, species distribution, and infected farms per day for the three subtypes of nine selected outbreaks were determined (Table 8). The average numbers of epidemic days were 86.0 for H5N1, 108.0 for H5N6, and 143.8 for H5N8. The average numbers of farms were 69.0 for H5N1 (69.0), 107.8 for H5N8, and 181.0 for H5N6. Regarding the species distribution, subtype H5N8 was more highly distributed among duck farms (74.2%) than other subtypes (37.7% for H5N1 and 42.5% for H5N6).

The Kruskal-Wallis H test showed a statistically significant difference in mean generation time among the different subtypes (χ^2 (2) = 6.444; p=0.040), with mean rank scores of 2.33 for subtype H5N1, 7.50 for H5N8, and 4.00 for H5N6. The pairwise comparison showed that the H5N8 generation time (7.27 days) was significantly longer than the H5N1 generation time (4.93 days) (P=0.03) (Table 8). There were no significant differences among subtypes in epidemic days, number of farms, cases per day, species distributions, or reproduction number.

2.4. Discussion

HPAI outbreaks occur continually worldwide and have become a major threat to animal and human public health. In South Korea, eight outbreaks with multiple waves of infections occurred between 2003 and 2018; these involved three different HPAI subtypes, H5N1, H5N8, and H5N6, and massively damaged the poultry industry. Therefore, it is important to understand the HPAI transmissibility at poultry farms to control outbreaks by establishing an effective prevention policy. An effective tool for understanding disease characteristics is the R_0 , which is generally defined as the average number of secondary cases caused by one infected individual (de Jong, 1995). Therefore, we investigated the transmission dynamics of the HPAI subtypes H5N1, H5N8, and H5N6 by estimating the generation time and R_0 . To the best of our knowledge, no previous study has attempted to estimate R_0 of various HPAI subtypes and perform comparative analyses among them. This could be the first study to investigate the disease transmission dynamics of HPAI subtypes H5N1, H5N8, and H5N6, which are emerging worldwide.

The R_0 of HPAI H5N1 in Korea estimated in this study was between 1.68 and 1.95 according to the ML method (Table 7). The R_0 of subtype H5N1 has been estimated in countries such as Italy (1.2-2.7), Canada (1.4-2.7), the Netherlands (1.0-3.0) (Garske et al., 2007), Romania (1.95-2.68) (Ward et al., 2009), Bangladesh (0.85-0.96) (Ssematimba et al., 2018), and Thailand (1.27-1.60) (Retkute et al., 2018). Despite the same subtype of HPAI as that in other countries, the estimated R_0 in Korea was different from that in other countries. We assumed that several factors, such as geographic distribution of poultry farms, mixed farming systems, poultry product supply system, and climate, were associated with this difference. We believe

that unique characteristics of the poultry industry in Korea and climatic differences are the major causes for these observed differences.

Our first hypothesis was that the estimated R_0 may be related to characteristics of the Korean poultry industry, such as the coexistence of large-scale commercial farms and small family farms. Among the Organization for Economic Cooperation and Development (OECD) countries, Korea has the lowest availability of arable land per capita (0.03 hectare in 2016) (Bank, 2020). This land scarcity is an important factor leading to high stocking densities (Statistics Korea, 2015). A previous study suggested that farms with large flocks and the presence of a neighboring farm within 500 m were risk factors of HPAI at Korean broiler duck farms (Kim et al., 2018). This high stocking and local density of large-scale poultry farms could increase the likelihood of massive infections when HPAI outbreaks occur in Korea.

Small family poultry farms also represent a biosecurity risk during HPAI outbreaks. Most of these small farms sell live poultry to local markets without going through slaughterhouses; this could be a pathway for the spread of HPAI viruses. Additionally, there was an obvious lack of information regarding the official statistics of poultry farms too small to be defined as agricultural holders in Korea (OECD, 2017). This includes establishments with less than 0.1 hectares of land or with sales of agricultural products per year or value of agricultural animals less than KRW 1.2 million (USD 1,090).

Our second hypothesis was that climate factors during the epidemic period may affect R_0 in these countries. Climate factors could affect HPAI transmission and persistence by altering bird migration, virus shedding between hosts, and virus survival outside the host (Gilbert et al., 2008). Climate change is considered to influence the wild bird species composition and their migration cycle, and these

changes will affect the transmission intensity of disease (Tian et al., 2015). Furthermore, temperature and humidity could be related to viral persistence in the host and environment. An influenza virus transmission experiment using a guinea pig model suggested that relative low humidity and cold temperature were favorable for spreading influenza (Lowen et al., 2007). Liu et al. showed that the environmental temperature decreased soon before HPAI H5N1 outbreaks in domestic poultry in Eurasia between 2005 and 2006 (Liu et al., 2007). Additionally, AI viral infectivity remained at lower temperatures (<17°C) during an *in vivo* test (Brown et al., 2009). Therefore, it is assumed that our estimated R_0 in Korea is lower than the R_0 in Thailand and Bangladesh, where the average annual temperatures are higher. Based on these results, it is believed that the climate factors were closely related to the R_0 estimated in several countries in terms of virus transmission and survivability. This association between temperature and HPAI viruses was also shown in the R_0 results of the H5N1 subtype. Regarding the HPAI that occurred in 2008 ($R_0 = 1.68$), the R_0 value was less than that of H5N1 that occurred in 2003 and 2010 ($R_0 = 1.95$ and 1.98). For H5N1 in 2003 and 2010, the initial outbreaks occurred in December, and the average temperatures in Korea were -0.2°C and -6.0°C during those periods, whereas the 2008 outbreak started in April and the average temperature was 12.5°C.

In 2016, two novel HPAI subtypes, H5N6 and H5N8, occurred simultaneously. HPAI H5N6 occurred from November 2016 to February 2017, whereas subtype H5N8 occurred from February to April 2016; the first wave and second wave occurred in June. Although these two subtypes occurred simultaneously, both were novel viruses. The genetic clade analysis suggested that Korean H5N6 viruses are novel reassortments of multiple virus subtypes, and it is difficult for H5N6 virus reassortment to occur during outbreaks that could increase the possibility of viral subtype mutation (Si et al., 2017). Additionally, an infection experiment involving wild mandarin ducks demonstrated a difference in viral shedding and viral tropism in H5N8 and H5N6 viruses within the same clade of 2.3.4.4 H5 HPAI viruses (Son et al., 2018). Based on these findings, both subtypes were independent of each other, and the virus infectivity could also be different; therefore, different R_0 was expected.

However, our estimated initial R_0 value in 2016 suggested a similarity between the reproduction number represented in subtypes H5N8 (1.70) and H5N6 (1.60) (Table 7). These results might represent differences between virus transmissions in laboratory experiments and between farms. Apart from the difference in transmissibility of each virus subtype, the level of transmission between farms in the field may be similar between the two subtypes. However, this presumes that the values of R_0 of the two subtypes were similarly calculated because the biosecurity policy implemented during the outbreaks was identical. The basic reproductive number is affected by the rate of contacts in the host population, the probability of infection being transmitted during contact, and the duration of infectiousness (Delamater et al., 2019). Therefore, it can be estimated that the R_0 of two different subtypes were similar due to the reduction of the poultry population through preemptive culling and the reduction of contact between farms because of the standstill (USDA Foreign agricultural service, 2016).

The quarantine against HPAI in Korea has changed over 14 years after the first HPAI epidemic in 2003. The HPAI prevention policy changed dramatically, especially before and after H5N8 epidemics in 2014. Before the outbreaks, Korea Animal Health Integrated System (KAHIS) was established in 2013 to monitor livestock vehicle movement. In this system, all poultry-related vehicles must be registered with KAHIS and equipped with a global positioning system mandatorily (Kim & Pak, 2019). Also during the epidemics, the preemptive depopulation of the protective zone was changed from a radius of 500m to 3km, and inspections were conducted more than once before releasing poultry and poultry products(Oh, 2018), The influence of these quarantine policy can also be seen in the changes in the R_0 values of each wave of subtype H5N8 that occurred between 2014 and 2016. For H5N8 in 2014, the initial R_0 of each wave showed a tendency to decrease as the outbreak progressed gradually (Table 7). This would indicate that the effectiveness of control measures for HPAI were increasing while the waves were passing.

In the Kruskal-Wallis model, H5N1 and H5N8 subtypes showed statistically significant differences in generation time (P=0.03) (Table 8). However, there were no significant differences in the epidemic characteristics of the subtypes. There was also no statistical significance in the R_0 obtained through the EG and ML models. This generation time difference in the two subtypes might be associated with subtype pathogenicity in the poultry species. The spread of H5N1 viruses in the field was quickly controlled as a result of the rapid diagnosis of the infections due to the high pathogenicity of these viruses in poultry. In contrast, subtypes H5N6 and H5N8 clustered as clade 2.3.4. H5NX viruses are usually mild in ducks, leading to delayed diagnosis of infections and persistent spread in the wild (Kwon et al., 2018). Therefore, the H5N8 subtype could possibly spread the HPAI virus over a relatively longer period than the H5N1 subtype which could be driven by sub-clinical spread in ducks.

2.5. Conclusion

In conclusion, this study showed the characterization of each subtype by analyzing the HPAI characteristics, including the epidemic days, number of farms, species distribution, generation time, and R_0 of HPAI subtypes H5N1, H5N8, and H5N6, which were associated with nine outbreaks in Korea between 2003 and 2018. R_0 , which is estimated by the generation time, index case, and secondary cases, is essential for identifying the characteristics of HPAI. In particular, our findings suggest that the estimated R_{θ} might be influenced by the HPAI subtype and might be associated with the temperature during the early stage, species specificity by virus subtype, and prevention policy. We believe that the results of the present study are helpful for demonstrating the disease dynamics of each HPAI subtype and its characteristics and, thus greatly assist in better disease control strategies. It could be possible to establish systematic quarantine policies to reduce the socio-economic losses caused by HPAI, Especially differences observed between countries with different poultry raising systems and climatic conditions. This study provided insight regarding HPAI transmission of the traditional subtype H5N1 and newly emerging subtypes H5N8 and H5N6. Further research on the basic reproduction numbers of the HPAI subtypes occurring worldwide is required to understand the global dynamics of HPAI transmission.

	1		Total		No of		No of other
Clade Date	Date	Days of	niimher of	Cases per	chicken	No. of duck	mo to cort
	Dark	epidemic	Farms	day	farms (%)	farms (%)	farms (%)
2.5 10/12/2003-05/02/2004	10/12/2003- 05/02/2004	58	18	0.310	7 (38.9)	11 (61.1)	0 (0.0)
2.2 25/11/2006-06/03/2007	25/11/2006-06/03/2007	103	L	0.068	4 (57.1)	2 (28.6)	1 (14.3)
2.3.2 01/04/2008- 24/05/2008	01/04/2008- 24/05/2008	54	98	1.815	80 (81.6)	17 (17.3)	1 (1.0)
2.3.2 29/12/2010- 23/05/2011	29/12/2010- 23/05/2011	146	91	0.623	38 (41.8)	50 (54.9)	3 (3.3)
2.3.4.4 16/01/2014-29/07/2014	16/01/2014- 29/07/2014	194	212	1.093	39 (18.4)	166 (78.3)	7 (3.3)
2.3.4.4 24/09/2014-10/06/2015	24/09/2014- 10/06/2015	260	162	0.623	39 (24.1)	117 (72.2)	6 (3.7)
2.3.4.4 14/09/2015-15/11/2015	14/09/2015- 15/11/2015	63	17	0.270	0(0.0)	14 (82.4)	3 (17.6)
2.3.4.4 23/03/2016-05/04/2016	23/03/2016- 05/04/2016	14	7	0.143	0(0.0)	2 (100.0)	0 (0.0)
2.3.4.4 06/02/2017-14/04/2017	06/02/2017- 14/04/2017	58	40	0.690	16(40.0)	23 (57.5)	1 (2.5)
2.3.4.4 02/06/2017- 19/06/2017	02/06/2017- 19/06/2017	18	36	2.000	30 (83.3)	0 (0.0)	6 (16.7)
2.3.4.4 16/11/2016-18/02/2017	16/11/2016- 18/02/2017	95	340	3.579	192 (56.5)	140 (41.2)	8 (2.4)
2.3.4.4 19/11/2017-18/03/2018	19/11/2017- 18/03/2018	121	22	0.182	8 (36.4)	14 (63.6)	0 (0.0)

Table 6. HPAI epidemic in Korea from 2003 to 2018

Subtype	Year of	Distribution	Mean (SD) generation time	Initial R_0 by EG Method	Initial R_0 by ML Method	Optimal time windows	R_{θ} by EG Method
	epidemic		c (Days)	(95% CI)	(95% CI)	(percent in total period)	(optimal) (95% CI)
H5N1	2003	Lognormal	5.24 (3.74)	2.02 (1.02-3.76)	1.95 (0.81-3.86)	9-46 (65.52%)	0.18 (0.01-0.51)
	2008	Gamma	4.98 (4.20)	1.65 (1.02-2.49)	1.68 (0.92-2.76)	9-54 (85.19%)	0.74 (0.65-0.82)
	2010	Gamma	4.58 (4.00)	2.20 (1.51-3.16)	1.93 (1.10-3.10)	9-138 (89.04%)	0.77 (0.72-0.83)
H5N8	2014 1st	Lognormal	7.45 (12.06)	1.56 (0.95-2.23)	1.83 (1.11-2.81)	14-125 (57.73%)	0.72 (0.65-0.79)
	2014 2nd	l Weibull	8.23 (8.35)	0.35 (0.00-1.38)	1.56 (0.70-2.97)	10-248 (91.92%)	1.01 (0.99-1.03)
	2014 3rd	Weibull	7.39 (6.31)	0.03 (0.00-0.98)	1.03 (0.22- 2.88)	10-50 (65.08%)	2.17 (1.26-3.67)
	2016 1st	Weibull	6.01 (4.64)	1.23 (0.50-2.31)	1.70 (0.75-3.22)	2-45 (75.86%)	1.37 (1.13-1.16)
חצאנ	2016	Gamma	5.02 (4.31)	1.24 (0.87-1.73)	1.60 (1.09-2.25)	14-94 (85.26%)	0.71 (0.67-0.74)
ONICH	2017	Lognormal	5.91 (6.62)	1.03 (0.01-2.45)	1.37 (0.34-3.56)	14-107 (77.69%)	0.90 (0.78-1.01)
§ Abbre	eviations: 5	SD, standard c	leviation, EG, ex	sponential growth,	ML, maximum li	kelihood estimation, CI, G	confidence interval

Table 7. Generation time and reproduction number of HPAI by EG and ML method

Table 8.	Epidemic	characteristics, m	nean generati	on time, and	R0 in two	models by l	HPAI subtype H	5N1, H5N8, an	d H5N6
Subtype	Average epidemic days	Average number of farms	Cases per day	Chicken (%)	Duck (%)	Etc. (%)	Mean generation time(days)	$R_{ heta}$ by EG Method	R_{θ} by ML Method
H5N1	86.0	69.0	0.802	41.7 (60.4)	26.0 (37.7)	2.0 (2.9)	4.93	1.96	1.85
H5N8	143.8	107.8	0.750	23.5 (21.8)	80.0 (74.2)	4.3 (3.9)	7.27*	0.79	1.53
H5N6	108.0	181.0	1.676	100.0 (55.2)	77.0 (42.5)	4.0 (2.2)	5.02	1.14	1.49
§ Abbrev	iations: EG	, exponential grow	<i>v</i> th, ML, maxi	mum likeliho	od estimation	, CI, confide	nce interval		
*Mean ge	sneration tin	me of subtype H51	N8 is significa	ntly longer th	an subtype H	5N1 (P-valu	e=0.03)		



Figure 18. Epidemic curve of HPAI outbreaks in Korea from 2003 to 2018. a) Weekly epidemic curves of HPAI subtype H5N1 from 2003 to 2011. b) Weekly epidemic curves of HPAI subtype H5N8 from 2014 to 2017. c) Weekly epidemic curves of HPAI subtype H5N6 from 2016 to 2018. The x-axis represents the week numbers, which were based on the ISO 8601 week date system.



Figure 19. Generation time distribution of HPAI outbreaks from 2003 to 2018 in Korea by HPAI subtype.

A) Generation time distribution of HPAI subtype H5N1 in 2003, 2008, and 2010 B) Generation time distribution of HPAI subtype H5N8 during the 2014 first wave, second wave, and third wave and during the 2016 first wave C) Generation time distribution of HPAI subtype H5N6 in 2016 and 2017. The x-axis represents the days for generation time. The y-axis represents the probability density function (PDF).

Chapter 3.

Spatiotemporal distribution of highly

pathogenic avian influenza subtype H5N8

in Korea

Abstract

In zoonotic disease, highly pathogenic avian influenza (HPAI) is a major threat to human and poultry health worldwide. In January 2014, HPAI virus subtype H5N8 first infected poultry farms in South Korea, and a total of 393 outbreaks were reported with enormous economic damage in the poultry industry. We analyzed the spatiotemporal distribution of HPAI H5N8 outbreaks in poultry farms using the global and local spatiotemporal interactions in the first outbreak wave from January 2014 to June 2015 and the second wave from September 2014 to June 2015. The space-time K-function analyses revealed significant interactions within three days and over 40 km in two study periods in global spatiotemporal interaction. The excess risk attributable value (D_{θ}) was maintained despite the distance in the case of HPAI H5N8 in South Korea. Eleven spatiotemporal clusters were identified, and the results show the HPAI introduction from the southwestern region and the spread to the middle region in South Korea. Six clusters were distributed in 0.46-9.86 km space and 3-19 days in time, while five clusters were distributed in 19.74-72.59 km space and 27-36 days in time. This global and local spatiotemporal interaction indicates that the HPAI epidemic in South Korea was mostly characterized by short period transmission within a small area and dispersed by long-range jumps. This finding supports strict control strategies such as preemptive depopulation, the standstill, and poultry movement tracking. More studies are needed to understand HPAI disease transmission patterns of HPAI in South Korea.

3.1. Introduction

Highly pathogenic avian influenza (HPAI) became a major zoonotic disease that threatens public health (Alexander, 2007). The HPAI virus (HPAIV) is highly contagious to domestic poultry and continuously occurs worldwide, causing enormous damage to the poultry industry(Short et al., 2015). The HPAI subtype H5N8 infection in poultry farms was first reported in January 2014 in South Korea (Lee, 2014). The results of a genetic epidemiologic investigation showed that the transmission occurs through the migration pathway of wild birds in winter season; indicating that the introduction of H5N8 HPAIV is associated with wild water birds(Jeong et al., 2014). Migratory birds that stay in South Korea move through the East Asia-Australia flyway, and the HPAI H5N8 virus has disseminated to other continents, including Europe and United States, through the overlying flyways of migratory birds (Verhagen et al., 2015).

While the wild migratory birds are the source of viral infection in domestic poultry farms (Pandit et al., 2013), the HPAIV transmission and spread between farms occurs mechanically through transport vehicles, people, feeds, clothes, shoes, and equipment contaminated by dust, water, and feces of HPAIV-infected poultry (Dent et al., 2011). In a recent study, HPAIV airborne transmission can be possible between poultry farms and may have played a role in spreading HPAI outbreaks in the United States (Zhao et al., 2019). Considering these various HPAIV transmission pathways, it is important to understanding how HPAI disease is transmitted through time and space to understand and prevent the spread of disease.

The occurrence of space-time interactions between outbreak cases located close in time and space varies and can be considered an infectious disease indicator (Diggle et al., 1995). Measuring and analyzing these indicators provide an understanding of the disease's underlying mechanisms, which enable the development of prevention strategies against disease spread (Baker, 2004). Spacetime interaction analysis using the space-time K function has been used in the following animal disease studies: the foot-and-mouth disease in the United Kingdom (Picado et al., 2007; Wilesmith et al., 2003) and Tanzania (Picado et al., 2011), bovine tuberculosis in New Zealand (Porphyre et al., 2007), Rift Valley fever in South Africa (Metras et al., 2012), and Africa swine fever in Russia Federation (Vergne et al., 2017). The spatiotemporal interaction of HPAI has been studied in France for the H5N8 subtype (Guinat et al., 2018) and in Vietnam for the H5N1 subtype (Loth et al., 2019).

This study aimed to identify the time and space distribution of HPAI H5N8 outbreaks in South Korea from 2014 to 2016. The time-space interaction was analyzed using the space-time K function analysis and the scan statistics of HPAI transmission dynamics. It is believed that this systematic understanding of the spatiotemporal distribution will enable the evaluation of quarantine policies addressing the HPAI outbreaks, thereby providing scientific evidence for future policy development and suggesting the direction for further research.

3.2. Material and Method

3.2.1. Data collection and management

The epidemic data of HPAI subtype H5N8 were collected by the Animal and Plant Quarantine Agency (APQA) in Gimcheon, Korea from January 15, 2014 to April 5, 2016 (Animal & Plant Quarantine Agency, 2016). Following the identification of a bird with clinical signs suspected of HPAI infection by the livestock owners, farm workers, and veterinarians, the case must be reported to the APQA according to the Act on Prevention of Contagious Animal Diseases (MAFRA, 2015) in passive surveillance. Veterinarians from governmental agencies visited the reported poultry farms to collect samples from the sick or dead birds, and then samples were tested to confirm possible HPAI infection. If the suspected farm was confirmed as HPAI-positive, it was deemed an infected premise (IP). Then infected poultry farms and neighboring farms located in a protective zone set to a radius of 3 km were depopulated. A depopulated farm found to be positive for HPAIV was referred to as a positive premise (PP) (Oh, 2018) in active surveillance.

In Korea, all the transporting vehicles related to the poultry industry for transporting either poultry, poultry products, medicines, feed, or feces must be registered with the Korea Animal Health Integrated System (KAHIS; http://www.kahis.go.kr). Based on these vehicles' geographical information, APQA conducted HPAI diagnostic test on the poultry farms visited by the vehicles entering HPAI-infected farms. This active epidemiological investigation of livestock-related vehicle movement makes it possible to detect additional HPAI-infected farms. In this study, all IPs and PPs found through this surveillance were considered as cases. All the geographical data of the poultry farms that were collected at the tong-ri administration, and village level, were projected to WGS84/UTM zone 52N (European Petroleum Survey Group; EPSG: 32652) and processed using QGIS (3.4) (QGIS Development Team, 2020). The dates of each case were based on the date of the first clinical signs observed.

3.2.2. Spatio-temporal analysis

HPAI subtype H5N8 outbreak from January 15, 2014 to April 5, 2016, was classified into four waves in Korea (Animal & Plant Quarantine Agency, 2016). In

this study, the first wave from January 15, 2014 to July 29, 2014, and the second wave that occurred from September 24, 2014 to June 10, 2015, were analyzed. The third (17 cases) and fourth waves (2 cases) of H5N8 were excluded due to the inadequate number of cases for the analysis.

Global and local spatiotemporal interaction analyses were conducted to describe the HPAI subtype H5N8's spatiotemporal characteristics between poultry farms in Korea. The global analysis used the space-time K function to calculate the spatiotemporal interactions of HPAI H5N8 outbreaks (Diggle et al., 1995). The space-time K function, K(s,t), was defined as the number of expected cases (E) if cases are randomly- distributed within a distance s and a time t, then divided by the intensity λ , defined as the mean number of cases per unit of space and time (Equation 1).

$$K(s,t) = \lambda^{(-1)} E$$
 (Equation 1)

If cases occur independently in time and space without space-time interaction, K(s,t) was the product of two K functions in space and time, similar to that shown in Equation 2.

$$K(s,t) = K(s) K(t)$$
 (Equation 2)

We can define D(s,t) as the difference between the observed and randomly expected space-time interactions (Equation 3);

$$D(s,t) = K(s,t) - K(s) * K(t).$$
 (Equation 3)

In this equation 3, D(s,t)>0 means that space-time interactions are presented at a distance s and time t and with higher D(s,t) values showing stronger evidence. D_0 was the value interpreted as the proportional increase, or excess risk attributable to

the space-time interaction to facilitate inference (Equation 4)

$$D_0(s,t) = D(s,t) / (K(s) * K(t))$$
 (Equation 4)

 $D_{\theta}(s,t) > 1$ indicated that the number of observed events was greater than twice the number of expected events (Diggle et al., 1995; Picado et al., 2007).

The null hypothesis of no space-time interaction in the observed cases was tested, the dates of the cases were randomly permuted on a fixed set of the location of the cases, using Monte-Carlo simulation, to generate a distribution of D(s,t) to compare with the D(s,t) of the observed cases.

Suppose D(s,t) value in the observed case exceeds 95% of values derived from the simulation, in that case, we reject the null hypothesis because the probability of observed space-time interaction occurring by chance is less than 5% probability. Therefore, it can be concluded that there was a significant space-time interaction between the observed cases.

In this study, global spatiotemporal clustering of HPAI H5N8 outbreaks was investigated in the first and second study periods using the space-time K function (Metras et al., 2012). The space-time K function analysis was conducted using the maximum space-time window of 40 km and 40 days. Significant space-time clustering was simulated by generating 999 Monte-Carlo random permutations. $D_0(s,t)$ value, the excess risk attributable to the space-time interaction within a distance s and time t, was calculated and visualized in R software version 3.6 (R Core Team, 2020) using the 'splancs' package (Rowlingson & Diggle, 1993).

We used the space-time permutation model of the scan statistics to identify the local spatiotemporal cluster of HPAI H5N8 outbreaks (Kulldorff, 2007) by applying

the spatio-temporal windows shown in global spatiotemporal clustering. This approach was based on creating a series of hypothetical spatiotemporal cylindercentered coordinates of each case (Kulldorff et al., 2005). These cylinder bases and heights represent the space and time dimensions of each potential cluster, respectively. The approach to calculate cylinder is to iterate over a finite number and then gradually to increase the circle radius and height from zero to the maximum space and time value defined by the user. To test the null hypothesis, which assumed a no space-time interaction between cases, randomly distributed permutation of the spatial and temporal attributes of each case were performed using the Monte-Carlo simulation. Through this simulation, the expected disease occurrence is obtained when time and space are assumed to be independent of each other within a given space and time frame. If the observed actual cases are higher than the expected cases calculated through the above process, it can be inferred that the case in region within the cluster was more frequent in space and time than the rest of the geographic areas (Kulldorff et al., 2005; Picado et al., 2011; Porphyre et al., 2007).

The presence of local spatiotemporal clusters in HPAI H5N8 during the two study periods (first and second waves of the outbreaks) between the case poultry farms was investigated using the space-time permutation model of the scan statistic test, implemented using the SatScan (Kulldorff et al., 2005). Statistically significance reported at the level of 5% assessed by the 999 Monte-Carlo replications without overlapping. The maximum spatiotemporal window was set to 25% of the outbreak cases (first wave, 53 cases; second wave, 41 cases) and 25% of the study period (first wave, 49 days; second wave, 75 days).

3.3. Results

3.3.1. Descriptive analysis

In total, 393 HPAI subtype H5N8 outbreaks were reported in poultry farms from January 15, 2014 to April 5, 2016. During the first and second waves, the majority of outbreaks were among the ducks (75.7%, 283/374), followed by the chickens (20.9%, 78/374), and then others (3.5%, 13/374) such as quail or ostriches. In the first (78.3%) and second waves (72.2%), the outbreaks mainly infected ducks (Table 9).

HPAI H5N8 was distributed throughout, nationwide, but was mainly concentrated in the west coastal and southern regions where the duck breeding density was high (Figure 20). The order of the intensities of the distribution by province were Jeollanam-do (JN) (28.6%, 107/374), Chungcheongbuk-do (CB) (24.9%, 93/374), and Jeollabuk-do (JB) (19.0%, 71/374). The order of the case incidence rates was CB (27.4%), JB (22.2%), and JN (22.2%) in the first H5N8 outbreak period; and JN (37.0%), CB (21.6%), and Gyeonggi-do (GG) (19.1%), in the second outbreak period.

The temporal distributions of the first and second outbreaks of HPAI H5N8 in Korea are shown in Figure 21. After the first case farm outbreak was reported on January 16, 2014, the outbreaks increased continuously, peaked in February 2014, and only intermittently spread after March 20, 2014 (Figure 21A). The number of poultry farms infected during the exponential period was 178 (of 212 cases, 83.9%) in the first study period. In the second study period, the HPAIV was reintroduced to Korea on September 24, 2014, with a total of 61 farm outbreaks (37.6%, 61/162), which were infected exponentially for 34 days from January 28, 2015 to March 3, 2015 (Figure 21B).

3.3.2. Spatiotemporal analysis

Out of the 393 outbreaks of HPAI H5N8, a spatiotemporal analysis was performed on 212 and 162 farm outbreaks during the first and second study periods, respectively. The global spatiotemporal cluster of HPAI H5N8 in poultry farms was statistically significant (p<0.05) for each study period (Figure 22). During the first study period (January 15, 2014 - July 29, 2014), the excess risk attributable to spacetime interaction with $D_0>1$ was 40 km and three days; the time was closer to 0, and the D_0 value was higher (Figure 22A). The D_0 value was the highest (21.4) at the spatiotemporal parameters of 2 km and 0 days, and when the temporal parameter was set as 0 days, the D_0 value was maintained at 15, despite the increasing distance.

The excess risk attributable to the spatiotemporal interaction in the second study period (September 24, 2014- June 10, 2015) was 40 km and three days; the time was closer to 0, and the D_0 value was higher (Figure 22B). The excess risk attributable during the second period had a similar pattern to that of the first study period. The D_0 value was the highest (23.4) at 2 km and 0 days, and the value of 6 was maintained, despite the increasing distance.

We identified the 11 statistically significant spatiotemporal clusters from the result of the space-time permutation scan statistic test. The geographical location of each cluster numbered according to the time of occurrence is indicated in Figure 23 and 24. The radius (km), temporal extension (days), number of outbreaks in the cluster, and the observed to the expected ratio of each cluster are shown in Table 10. The clusters were mainly formed around the west coastal area in South Korea. In the first study period, two clusters (Figure 23A) were formed in JN and JB, while three clusters were formed around the border areas of GG, CB, and CN (Figure 23B). The

maximum spatial expansion of the clusters ranged from 2.21 km to 21.84 km, and the maximum time ranged from 3 to 30 days (Table 10). Since the cluster radius was the least at 2.21 km in cluster 3, but the number of farm outbreaks included in the cluster had the highest number of cases at 28. In the second study period, three clusters were found in JN and JB (Figure 24A), two clusters in the northern GG regions, and one cluster in the border areas of CB, CN, and GG (Figure 24B). The maximum space of the cluster was between 0.46 km and 72.59 km, while the duration was between 5 and 36 days (Table 10). All the clusters that showed statistically significant difference during the first study period overlapped with the epidemic exponential growth period (January 16 –March 20, 2014), while no statistically significant clusters were found that overlapped with the second exponential period (January 28 - March 3, 2015). Among HPAI poultry farms, during the study period, the proportion of farms in the cluster that were derived from the scan statistic test was 48.11% (102/212) in the first, and 51.23% (83/162) in the second.

3.4. Discussion

It is important to identify and analyze clustering to detect the area with a higher level of disease risk during outbreak investigations (Pfeiffer et al., 2008). There have been many attempts to apply spatiotemporal modelling to zoonosis, which were estimating the space-time interaction between cases that are spatially and temporally proximate, make it possible to interpret the underlying transmission process (Ahmed et al., 2010; Guinat et al., 2018; Picado et al., 2011). Despite the importance of understanding the spatiotemporal disease dynamics, epidemiological research into HPAI epidemics in Korea was mainly focused on molecular investigations to track the origin of HPAIV strains and pathogens (Kwon et al., 2020; Si et al., 2016). It is important to analyze the global and local spatiotemporal interaction for the HPAI H5N8 outbreak to understand the disease transmission process for effective HPAI controls in poultry farms. This study investigated the spatiotemporal patterns of the first and second waves that occurred after the H5N8 HPAIV was introduced to Korea in January 2014. As far as we know, this is the first HPAI subtype H5N8 study in Korea that analyzed the global and local space-time interaction. This result will be a cornerstone in explaining the spatiotemporal factors related to HPAI H5N8 infection and transmission.

In space-time K function analysis, we identified space-time interactions over a distance of more than 40 km and under two days at the first study period (Figure 22 A). In addition, at 2 km and 0 days, the risk was highest and then decreased, maintaining a constant risk regardless of the increasing distance. This pattern of the space-time interaction was the same in the second study period, but the peak of the risk was highest at 0 days and 0 km, and the D_0 value decreased from 15 to 6 after two days (Figure 22 B). This results showed a different pattern from those of previous research (Guinat et al., 2018; Loth et al., 2019) that have analyzed the spatiotemporal analysis of other HPAIs, and seems to be a characteristic of HPAI disease transmission in Korea. The results of the space-time K function analysis for HPAI in other countries showed significant spatiotemporal clustering less than 13 days and 8 km in France (Guinat et al., 2018), and less than 50 days and 60 km in Vietnam (Loth et al., 2019). Moreover, the excess risk reported in both studies showed a pyramidal shape in which the D_0 decreased as time and distance increased. Conversely, the excess risk was maintained even when the distance was increased in the spatiotemporal interaction in Korea (Figure 22). These results imply that the

spread of HPAI disease in Korea occurs consistently regardless of the distance, especially within two days.

According to the results of our local spatiotemporal cluster analysis, HPAI from our two study periods tends to appear as the clusters in the western coastal area in Korea (Figure 23, 24). Five spatiotemporal clusters showed for 0.46-21.84 km and 5-28 days in JN and JB provinces (Figure 23B & 24B). Among them, the cluster 1 in each outbreak wave appeared in the early stage of outbreaks wave and the southwestern coastal area, major habitats of the wild migratory birds in the winter season. After introduction or re-introduction of HPAIV into Korea, six clusters of 2.21-72.59 km and 3-36 days were formed in the three provinces of GG, CB, and CN (Figure 23A, 24A). The assumption is that the HPAI introduced from the southwestern region has spread to the central area, considering the cluster formation time. These results of spatiotemporal clustering of HPAI H5N8 are consistent with the result of the investigation on the origin and transmission of H5N8 by sequencing analysis, indicating that H5N8 virus entered to western coastal provinces and spread rapidly to other provinces with high densities of winter migratory birds and ducks holding (Hill et al., 2015). In light of these results, the HPAI intensive monitoring is necessary for these regions in winter seasons.

In our results, six clusters were distributed 0.46-9.86 km in space and 3-19 days in time, while five clusters were distributed 19.74-72.59 km in space and 27-36 days in time (Table 10). Furthermore, cluster 3 in the first study period and cluster 3 and 5 in the second study period were less than 3 km in size. It might be that this phenomenon appeared as the size of clusters was suppressed by preemptive depopulation. On the contrary, 5 of the 11 clusters were covering the spatiotemporal extension from 27 to 36 days and from 19.74 to 72.59 km, which is larger than the period (10-25-days) and the distance (16.5 km-52.7 km) of the previous study on H5N8 spatiotemporal cluster study in France (Guinat et al., 2018). This shows that the regional spread of H5N8 in Korea was polarized between small and large spatiotemporal clusters. In other words, the HPAI virus disseminated in the extremely short distance and time, or rather spread over the long-distance and times.

Based on the results of the global and local spatiotemporal interaction, the following were assumed to have affected the spatial and temporal characteristics of the HPAI H5N8 in the poultry farms. First, we assumed that HPAI reporting and depopulation are carried out quickly in Korea, which leads to the prevention of adjacent disease spread (by the preemptive depopulation), from infected farms to the neighboring poultry farms. This can be inferred from the results showing that the time window of excess risk from the time-space interaction analysis was two days, which is shorter than the time reported in other studies (Guinat et al., 2018; Loth et al., 2019). In addition, the third cluster in the first period, the third and fifth clusters in the second period, had small spatial windows greater than 3 km. If the HPAI report from the poultry farms and the disease quarantine were delayed, HPAI would have had sufficient opportunity to propagate adjacent poultry holdings, which would have shown a similar spatiotemporal interaction of other studies (Guinat et al., 2018; Loth et al., 2019). According to APQA, when the suspected poultry with clinical signs is reported and confirmed positive, a 3 km radius depopulation is carried out around infected holdings, and this process, from report to depopulation, is conducted within a short period (Animal & Plant Quarantine Agency, 2016). It can be inferred that the virus short-range contiguous transmission was blocked effectively by removing the host that could cause increase infection spread.

Second, there is a high possibility that the cases that were due to the long-distance

propagation of HPAI through vehicles were relatively due to oversampling, because of the suppression of the adjacent propagation of HPAI. The KAHIS was established in 2013 to integrate the management of animal disease and livestock quarantine information using information and communication technology (ICT) to prevent livestock disease outbreak (MAFRA, 2015). It is possible to collect information on registered vehicle movements related to the poultry industry, such as feces treatment, veterinarian visits, transporting of feed, medicine, poultry, and poultry products. It is mandatory to equip global positioning system (GPS) on registered vehicles under the Korean Act on the Prevention of Contagious Animal Diseases, and their movement information is periodically collected through KAHIS (Kim & Pak, 2019). This systemic tracking makes it possible to track the HPAI long-distance dissemination. If an HPAI case found through the long-distance propagation tracking using this mechanical relationship is included in the analysis, it is judged that a pattern of the D_0 value that is not affected by the distance in the spatiotemporal interaction can appear. Therefore, this result of global spatiotemporal interaction is presumed to rapidly suppression of HPAI outbreaks through active surveillance.

Finally, the excess risk D_0 in the second study period was relatively reduced compared to the D_0 in the first study period (Figure 22). The factors that are estimated to have influenced the decrease in D_0 during the second study period are as follows. First, the livestock owners may be already aware of the HPAI introduction in poultry holdings during HPAI recurrence. Through this recognition, it can be assumed that alertness to HPAI has increased and faster disease reporting has been made. The effect of knowledge and awareness to HPAI reporting was reported from a study of HPAI during the 2006-2008 outbreaks in Nigeria (Ameji et al., 2012). Second, the changed quarantine policy of the Korean government in the second period might be more effective in controlling the outbreak than in the first period. Due to continuous HPAI outbreaks in Korea, the Disease Outbreak Law and infectious disease standard operating procedure was revised in 2015 (MAFRA, 2015) and the systematic investigation of diseases was developed through the manual (Animal & Plant Quarantine Agency, 2015). This change in HPAI biosecurity policy may have resulted in a reduction in excess risk attributable.

The finding of this study may have been affected by several limitations. First, there may have been cases where the presence of HPAI H5N8 was not reported if the sensitivity of the reports from the farms to the government were not optimal. In particular, in case of HPAI H5N8 in Korea, the infected ducks did not show clinical signs, which may have made the detection of H5N8 challenging (Kwon et al., 2020; Song et al., 2017). However, it is already mandatory to sample the poultry at the farm a day before transportation to other farms or slaughterhouses, to inspect them for HPAI using RT-PCR, since 2008 (MAFRA, 2015). Therefore, considering the massive HPAI inspection, the risk of unreported cases may be considered relatively low.

Second, our analysis was performed on the assumption that the date when the clinical signs were first observed was the date HPAIV was first introduced into the poultry farm. This may have had effects on the study results because the incubation period of HPAI H5N8 may differ depending on the poultry species or the condition of the flocks. However, in our study periods, 75.7% of the cases included the ducks; therefore, it can be assumed that the latent period of H5N8 will be similar in most of the poultry farms. The interval from the date of the virus introduction to the flocks, to the date when the first clinical signs were observed is likely to be constant. Therefore, our assumption would not have a significant impact on the temporal

elements in our spatiotemporal analyses to the extent of results bias.

3.5. Conclusion

This study provides insights into the 2014-2016 Korea HPAI epidemic dynamics. This global and local spatiotemporal interaction indicates that the HPAI epidemic in Korea was mostly characterized by short period transmission within a small area and dispersed by long-range jumps. This disease transmission pattern is different from other HPAI spatio-temporal interaction studies. It is believed that these results are closely related to the rapid preemptive depopulation, standstill, and disease tracking policy, using GPS. This finding supports the need for strict control strategies such as the preemptive depopulation, the standstill of poultry transporting, and epidemiological movement tracking in Korea during the H5N8 disease period. Further research is needed to evaluate the optimal culling radius, the spread rate of disease between farms, and the disease transmission pathways by poultry-related vehicles, to help understand HPAI disease transmission patterns.

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Wave	Duck (%)	Chicken (%)	Others (%)	Total number of	Starts	т г Ц	Number of days
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First	166 (78.3)	39 (18.4)	7 (3.3)	212	2014-01-15	2014-07-29	196
Second	117 (72.2)	39 (24.1)	6 (3.7)	162	2014-09-24	2015-06-10	260
Third	14 (82.4)	0 (0.0)	3 (17.6)	17	2015-09-14	2015-11-15	63
Fourth	2 (100.0)	0 (0.0)	0 (0.0)	2	2016-03-23	2016-04-05	14
Total	299 (76.1)	78 (19.8)	16 (4.1)	393			533
TTDAT 1.:-1.1		5					

Table 9. Distribution of HPAI H5N8 outbreaks per species, type and period in Korea

HPAI, highly pathogenic avian influenza

ing HPAI H5N8 outbreaks from 2014 to 2016	Number of Number of Expected Observe to	days outbreaks outbreak expected ratio	14-01-24 10 20 2.3 8.8 0.001	14-02-07 7 15 1.8 8.5 0.001	14-02-19 3 28 4.1 6.8 0.001	14-04-06 28 17 3.9 4.4 0.002	14-04-08 30 11 2.2 5.1 0.030	14-10-12 19 14 1.9 7.2 0.001	14-11-19 34 11 2.3 4.8 0.025	14-12-26 5 5 0.2 32.4 0.001	15-03-20 27 36 10.2 3.5 0.001	15-04-12 18 11 0.8 13.5 0.001	
s from 2014 to	Number of	outbreaks	20	15	28	17	11	14	11	5	36	11	9
H5N8 outbreak	Number of	days	10	L	3	28	30	19	34	5	27	18	36
ns during HPAI F	С* Л Г	הזות	2014-01-24	2014-02-07	2014-02-19	2014-04-06	2014-04-08	2014-10-12	2014-11-19	2014-12-26	2015-03-20	2015-04-12	2015-05-21
Korea poultry farr	Ctout	JIAIL	2014-01-15	2014-02-01	2014-02-17	2014-03-10	2014-03-10	2014-09-24	2014-10-17	2014-12-22	2015-02-22	2015-03-26	2015-04-16
nporal cluster of K	Radius(km)		9.86	6.91	2.21	21.84	19.74	5.95	72.59	1.30	19.76	0.46	30 74
. Spatiotem	Cluster	Clusici	1	7	б	4	5	1	7	б	4	5	ų
Table 10.	W/orro	wave	First					Second					

HPAI, highly pathogenic avian influenza



Figure 20. Location of Highly pathogenic avian influenza case farms in the first and second waves.

Red round dot is the outbreaks in poultry farms at the first wave from January 14, 2014 to June 23, 2014. Green rectangle dot is the outbreaks in poultry farms at the second wave from September 23, 2014 to June 24, 2015.

Province abbreviations are as follows; CB: Chungbuk, CN: Chungnam, GB: Gyeongbuk, GG: Gyeonggi, GN: Gyeongnam, GW: Gangwon, JB: Jeonbuk, JN: Jeonnam


Figure 21. Epidemic curve of HPAI H5N8 from 2014-2015.

A. Epidemic curve of the first wave of HPAI H5N8 from January 2014 to August 2014 in Korea. B. Epidemic curve of the second wave of HPAI H5N8 from September 2014 to July 2015 in Korea.



Figure 22. Excess risk attributable to the space-time interaction (D_0) as a function of space and time.

A. Excess risk attributable to the space-time interaction of the first wave of HPAI H5N8 from January 14, 2014 to June 23, 2014.

B. Excess risk attributable to space-time interaction of the second wave of HPAI H5N8 from September 23, 2014 to June 24, 2015.

The red-shaded area show the space-time interaction for which the observed number of cases was higher than twice the expected number, which assumes no space-time interaction ($D_0 > 1$).

HPAI, highly pathogenic avian influenza



Figure 23. Spatiotemporal cluster of poultry farms during the first wave of HPAI H5N8 outbreaks in Korea.

Left map shows whole area of South Korea

A) Spatiotemporal cluster of the first wave of HPAI H5N8 in the western middle region.

B) Spatiotemporal cluster of the first wave of HPAI H5N8 in the southwestern region

Province abbreviations are as follows; CB: Chungbuk, CN: Chungnam, GB: Gyeongbuk, GG: Gyeonggi, GN: Gyeongnam, GW: Gangwon, JB: Jeonbuk, JN: Jeonnam, HPAI, highly pathogenic avian influenza



Figure 24. Spatiotemporal cluster of poultry farms during the second wave of HPAI H5N8 outbreaks in Korea.

Left map shows whole area of South Korea

A) Spatiotemporal cluster of the second wave of HPAI H5N8 in the western middle region.

B) Spatiotemporal cluster of the second wave of HPAI H5N8 in the southwestern region

Province abbreviations are as follows; CB: Chungbuk, CN: Chungnam, GB: Gyeongbuk, GG: Gyeonggi, GN: Gyeongnam, GW: Gangwon, JB: Jeonbuk, JN: Jeonnam, HPAI, highly pathogenic avian influenza

General conclusion

The present study investigated the epidemiological characteristics of HPAI through risk factors, transmissibility, and spatiotemporal dynamics between poultry farms. Moreover, it provides a scientific basis for improving effective quarantine policies for HPAI control in the future.

The possible risk factors associated with the introduction and transmission of HPAI H5N8 subtype in broiler duck farms were investigated to understanding factors for HPAI H5N8 infection. The result of retrospective case-control study indicated that the HPAI H5N8 outbreaks in South Korea were associated with farm owner career, the number of flocks, poultry farm density, and biosecurity measures. Greater understanding of the risk factors for H5N8 may reduce farm vulnerability to this and other AI subtypes and help establish policies to prevent reoccurrence of infection. Further, awareness of these factors may help reduce the broader spread of H5N8 across broiler duck farms during outbreaks.

Pathogen transmissibility in poultry farms in South Korea during each epidemic was demonstrated in the results of the R_0 of three subtypes, H5N1, H5N8, and H5N6, which were associated with nine outbreaks between 2003 and 2018. The study also characterized each subtype, including the duration of the epidemic, number of affected farms, species distribution, and generation times. In particular, the study findings suggested that the estimated R_0 might be influenced by the HPAI subtype and might be associated with the temperature during the early stage, species specificity by virus subtype, and prevention policy. This study provided information on HPAI transmission of the traditional subtype H5N1 and newly emerging subtypes H5N8 and H5N6.

The study also provided insight into the dynamics of the 2014-2016 South Korean HPAI epidemic. Global and local spatiotemporal interactions indicated that the epidemic was mostly characterized by short period transmission within a small area and dispersal by long-range jumps. This disease transmission pattern is different from results of other HPAI studies assessing spatiotemporal interactions. It is believed that these results are

closely related to the use of GPS for rapid preemptive depopulation of affected farms, the standstill of poultry transporting, and disease tracking policies. These findings support the need for strict, nationwide enforcement of these control strategies during the H5N8 disease period.

Overall, study findings emphasize that HPAI infections of duck farms in South Korea are related to biosecurity, farm and farm owner characteristics, host specificity of HPAI subtype, and strict control strategies. This thesis highlights the value of a multifaceted approach to epidemiological modeling of infectious diseases. Furthermore, the study provided insights into potential data-driven approaches to HPAI control and provides an example for future studies in infectious diseases.

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Appendix

Appendix A. Highly Pathogenic Avian Influenza Investigation-Questionnaire for Evaluation and Improvement of National Biosecurity



I. Premise Information

1. What kind of poultry do you raise on your farms?	2. What kind of animal do you raise on the farm except poultry? (Check all)
 Broiler chicken Layer chicken Breeding chicken Broiler duck Layer duck Breeding duck Korean chicken Other () 	 Dog Cat Pig Cow Chicken (as pet) Duck (as pet) Goat Sheep Horse Other ()
3. What is the size of the farms? (km^2)	 4. Do you raise other species of poultry in the same barn? Yes No
5. How many barns are there in your farm? And how many barns are you raising poultry?	6. Do you practice "all in all out" management on farm level?Yes
(barns / barns)	□ No
7. How many poultry in your farm? (birds)	8. How far is the nearest poultry farm from your farm?(m)
 9. What is your education level? Elementary school graduate Middle school graduate High school graduate University graduate 	 10. Do you manage your own farm by yourself? Yes, I do. No, I have a manager.

 11. What is farm owner's age? Under 30 yrs. 30-39 yrs. 40-49 yrs. 50-59 yrs. Over 60 yrs. 	 12. How many years have you been working in the poultry industry? (yrs.) 13. Does this farm belong to meat company? Pes No
II. Wild Animal	
14. Have you seen migratory birds around the farm this winter?	15. How often have migratory birds been observed this winter?
□ Yes □ No	 Once a day Once every three days Once every week Once every month Once in a winter
16. Is there a migratory bird habitat nearby? If so, how far is it?	17. Have you ever observed migratory birds entering or leaving the poultry farm?
□ 1 km □ 3 km □ 5 km □ 10 km □ No	 Have observed. Never observed before, but there is evidence like feces or feather Never observed before and don't have any evidence, but I think it is possible. Entering is impossible
18. Have you ever observed wild animal around the farm?	19. Have you ever observed domestic bird around the farm?
□ Yes □ No	□ Yes □ No
20. Have the carcass of wild birds been found near farms during the outbreak of HPAI?	21. Have you ever observed mice around the farm?Yes
□ Yes □ No	□ No

22. Do you only work If you have a side work do you do?	on the poultry farm? job, what kind of	23. How farm? there	v many v ? How n ?	workers are nany foreigr	there on the workers are
 Only work on the p Have a side job (Sic Do farm work on th (Main job: 	oultry farm le job:) e side)	(#/	#)
24. Does anyone in your family help farm work? If yes, what is the relationship?		25. Do you have any family or relatives who work in poultry industry? If yes, what is the relationship?			
□ Yes () □ No)		Yes No	()
 Rendering Burial Incineration Other () 					
 Burial Incineration Other ()	□ 28. Do g servie	No you use ce? Yes No	the poultry	feces removal
 Rendering Burial Incineration Other (V. Farm facilit) y and biosecu	28. Do servio	No you use ce? Yes No	the poultry	feces removal
Burial Incineration Other (V. Farm facilit The following question) y and biosecu ns are about farm fa	28. Do servio	No you use ce? Yes No] lease ma	the poultry ark the box ⊐ Closed ty	feces removal es □ Other
 Rendering Burial Incineration Other (V. Farm facilit The following question 29. Barn type: 30. Feeding type:) y and biosecu ns are about farm fa Open type Bucket Whe	28. Do servio servio rity cilities. P Half open eler □	No you use ce? Yes No] lease ma type to Other	the poultry ark the box ⊐ Closed typ	feces removal es be □ Other
Rendering Burial Incineration Other (V. Farm facilit Free following question 9. Barn type: 9. Feeding type: 1. Water supply type:) y and biosecu ns are about farm fa Open type Bucket Bucket Ne	28. Do servio 28. Do servio cilities. Pl Half open eler Nipple	No you use ce? Yes No lease ma type t Other	the poultry ark the box ⊐ Closed typ Dther	feces removal es be □ Other
 Rendering Burial Incineration Other (V. Farm facilit V. Farm facilit Feeding type: 80. Feeding type: 81. Water supply type: 82. Egg collection:) y and biosecu ns are about farm fa Open type Bucket Bucket Hand collecting	28. Do servio servio rity cilities. Pl Half open eler Jipple	No you use ce? Yes No lease ma type to Other Other	the poultry ark the box □ Closed typ Other ther	feces removal es be □ Other
 Rendering Burial Incineration Other (V. Farm facilit V. Farm facilit The following question 9. Barn type: 9. Barn type: 9. Feeding type: 1. Water supply type: 2. Egg collection: 3. Is there any biosecular) y and biosecu ns are about farm fa Open type Bucket Whe Bucket N Hand collecting rity training session f	28. Do servio servio rity cilities. Pl Half open eler = Vipple Inline	No you use ce? Yes No lease ma type Other Other C C S? Y	the poultry ark the box Closed typ Other ther Yes □ N	feces removal es be □ Other o

48. What kind of feed do you use?	49. What kind of vehicle do you use when
□ Self-production feed □ Commercial feed	carrying the poultry feed? ☐ The farmer yourself ☐ Rental car ☐ Feed company delivery car
50. What form of feed do you give?	51. Do you use feed supplements?
□ Mash □ Pellet □ Crumble	□ Yes □ No
52. Do you disinfect the feed box?	53. Where is the source of the water supply?
□ Yes □ No	□ Municipal □ Well □ Other ()
54. Is water treated prior to delivery to poultry? If yes, what is used?	
□ Yes () □ No	
□ Yes () □ No VII. Disease history and o	thers
□ Yes () □ No VII. Disease history and of 55. What kind of disease have your farm infected with last year?	56. Had the farm ever been infected with
 ☐ Yes () ☐ No VII. Disease history and or 55. What kind of disease have your farm infected with last year? ☐ Avian Cholera ☐ Avian Typhus ☐ Neumerthe disease 	<pre>56. Had the farm ever been infected with Highly Pathogenic Avian Influenza? □ Yes (when:) □ No</pre>
 ☐ Yes () ☐ No VII. Disease history and o 55. What kind of disease have your farm infected with last year? ☐ Avian Cholera ☐ Avian Typhus ☐ Newcastle disease ☐ Avian encephalomyelitis 	ther € 56. Had the farm ever been infected with Highly Pathogenic Avian Influenza? □ Yes (when:) □ No 57. Had the farm ever been infected with
 ☐ Yes () ☐ No VII. Disease history and o 55. What kind of disease have your farm infected with last year? ☐ Avian Cholera ☐ Avian Typhus ☐ Newcastle disease ☐ Avian encephalomyelitis ☐ Infectious Bronchitis ☐ Infectious bursal disease ☐ Aspergillosis ☐ Duck virus hepatitis 	 ther € 56. Had the farm ever been infected with Highly Pathogenic Avian Influenza? □ Yes (when:) □ No 57. Had the farm ever been infected with Low Pathogenic Avian Influenza? □ Yes (when:) □ No
 ☐ Yes () ☐ No VII. Disease history and o 55. What kind of disease have your farm infected with last year? ☐ Avian Cholera ☐ Avian Typhus ☐ Newcastle disease ☐ Avian encephalomyelitis ☐ Infectious Bronchitis ☐ Infectious bursal disease ☐ Aspergillosis ☐ Duck virus hepatitis 58. Do you buy live poultry from other farms personally? ☐ Yes 	 ther § 56. Had the farm ever been infected with Highly Pathogenic Avian Influenza? Yes (when:) No 57. Had the farm ever been infected with Low Pathogenic Avian Influenza? Yes (when:) No 59. Do you buy live poultry from traditional markets personally? Yes

국문초록

국내 발생 고병원성조류인플루엔자 (H5N8)의 수학적 모델링 및 역학적 분석

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감염병은 질병 발생 시 사회 경제적 영향이 증가하기에 공중보건학에서 점차 그 중요도가 증가하고 있다. 수학적 모델링을 기반으로 감염병 전파 양상을 이해하 고 예측하고자 하는 시도가 계속되고 있다. 이러한 모델들은 특히 공중 보건 정책 을 세우는데 있어서 유용한 도구로써 점점 더 인식되고 있다.

고병원성 조류 인플루엔자(highly pathogenic avian influenza; HPAI)는 인간과 조류 사이의 주요 인수공통 감염증으로 2003년 이후 한국에서 지속적으로 발생하 고 있다. 특히 H5N8 아형(subtype)에 의한 HPAI는 국내 발생 사례 중 최장기 유 행하여 가금 산업에 막대한 경제적 피해를 초래 하였다. 그러므로 새로운 H5N8 아 형 바이러스의 유입, 전파 및 확산 측면에서의 연구가 필요하나, 현재까지 H5N1 아 형을 대상으로 한 연구만 이루어진 상황이다. 따라서 본 논문은 국내 발생 H5N8 HPAI의 발생 위험 요인, 질병 매개 변수, 그리고 질병 유행 및 확산의 시공간 특성 분석을 통하여 HPAI의 역학적 특성을 조사하였다.

첫 번째로 국내 육용 오리농가에서의 H5N8의 유입 및 발생을 이해하고자 분석 역학 기법인 후향적 환자 대조군 연구(retrospective case-control study)를 사용하여 잠 재적인 위험요인(risk factor)을 선정하고 이를 평가하였다. HPAI H5N8에 감염되었던 양성 농장을 환자군(case)으로 선정하고, 선정 농장을 기준 반경 3km내 음성 농가를 대조군(control)으로 대응 추출(matching) 하였다. 단변수 및 다변수 로지스틱 회귀분 석(univariable and multivariable logistic regression)을 사용하여 육용 오리 농가에서의 잠 재적인 위험요인을 분석하였다. 그 결과 분변 처리 서비스를 이용하지 않는 경우 (OR = 27.78), 농장주의 경력이 15년 이상일 경우(OR = 7.91), 7동 이상의 축사를 가진 경우(OR = 6.99), 마지막으로 농장 주변 500m 이내 다른 가금 농장이 존재하는 경우 (OR = 6.30)가 그렇지 않은 경우에 비해 교차비가 높은 것으로 확인되었다. 이러한 결과는 우선 육용 오리 농장에서 발생한 HPAI H5N8이 농장 방역, 농장 규모 및 시 설, 농장주의 경력과 연관성이 있음을 시사하고 있다. 확인된 위험요인 들을 관리하 기 위한 정책을 수립한다면 가금농가에서의 HPAI 발생 위험성을 줄일 수 있을 것 이다.

두번째로 수학적 모델링을 사용하여 국내에서 발생했던 HPAI H5N1, H5N8 및 H5N6의 기초 재생산 지수(basic reproduction number; R_0)를 추정하여 각 아형에 따른 가금농가간의 전파력을 조사하였다. R_0 의 추정은 SIR compartment model을 기반으로 하여, Exponential growth와 Maximum likelihood 모델을 사용하였다. 세가지 subtype H5N1, H5N8, 및 H5N6의 R_0 는 각각 1.68-1.95. 1.03-1.83, 1.37-1.60 이었다. Kruskal-Wallis pairwise comparison 결과 H5N8의 평균 감염세대기간(generation time) 이 7.27일 로 H5N1의 4.93일보다 통계적으로 유의하게 길다는 결과가 확인되었다. 이러한 결 과는 R_0 가 감염 초기단계의 온도, 가금생산시스템, subtype별 종 특이성 및 방역 정 책과 관련되어 있음을 시사하고 있다. 이러한 질병 전파력에 영향을 미치는 요소를 고려할 시 향후 발생할 수 있는 HPAI 아형에 대한 실질적인 질병 통제 전략을 설 계할 수 있을 것이다.

세번째로 국내 가금류 농장 발생 H5N8의 시공간적 분포를 분석하여, 농장 간의 전파 메커니즘을 이해하고자 하였으며, 방역 정책이 HPAI의 시공간적 분포에 미치 는 영향을 평가하였다. 2014년 1월부터 2016년까지 발생한 H5N8 첫번째 및 두번째 발생 기간에 대한 Global and local 에서의 시공간 상호작용(spatiotemporal interaction)은 각각 space-time K function과 space-time permutation model을 사용하여 분석되었다. 두 발생 기간 모두에서 space-time K function은 3일이내에 40km 이상에서 시공간 상호작 용을 나타내었다. Excessive risk attributable value은 거리와 상관없이 그 값을 유지하였 습니다. 총 11개의 local 시공간 군집(spatiotemporal cluster)가 확인되었으며, 그 결과 H5N8의 지역적 확산은 양극화 되어 작은 시공간적 군집과 큰 시공간 군집이 동시 에 나타났다. 이러한 시공간 상호작용 결과는 한국에서의 HPAI 전염병의 전파가 주 로 작은 지역 내에서 단기간 전파된 이후 장거리 점프에 의해 분산되는 것으로 추 정된다.

결론적으로 본 연구는 향후 HPAI 통제를 위한 효과적인 방역 정책 수립을 위 한 과학적 근거를 제공하기 위하여 세가지 역학적 모델을 사용하였습니다. 국내 발 생 H5N8 HPAI에 대하여 역학적 분석을 통해서 가금농장으로 유입될 수 있는 잠재

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적인 위험요인들을 확인하였다. 또한 수학적 모델링을 사용하여 세 개의 HPAI subtype에 따라 질병 전파력의 차이가 있음을 증명하였다. 국내 발생 H5N8에 대한 시공간적 분석 결과를 볼 때 선제적 살처분, 이동통제 및 가금 차량 이동 추적과 같은 현행 방역 시스템 유지 필요성을 시사한다. 본 연구 결과는 HPAI의 방역 정책 및 HPAI 질병 대응책을 평가하고 보완하는데 있어서 중요한 과학적 근거로써 활용 될 수 있고, 해당 연구의 방법론은 향후 국내외에서 발생하는 타 가축유래 인수공 통전염병에 대하여 접목이 가능할 것으로 기대된다.

- **키워드 :** 고병원성 조류인플루엔자, 가금 농가, 위험요인, 전파매개변수, 수학적 모델링, 시공간 분석.
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