CURRENT AND FUTURE CHALLENGES OF PREVENTING OUTBREAKS OF HIGHLY PATHOGENIC AVIAN INFLUENZA

by

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Abstract

Avian influenza (AI) is a zoonotic disease that has garnered much attention in recent years due to its detrimental effects on poultry, producers and potentially human health. This disease can be extremely fatal to domestic poultry, killing as high as 90-100% of the flock. This virus has the potential to cause devastation to and loss of entire flocks. AI is typically spread between wild fowl and domestic poultry with a zoonotic potential to also affect human health as well as other animals. Its spread also has a massive economic impact due to the decreased amounts of available poultry products to consumers around the world. This report will examine the worldwide history and epidemiology of highly pathogenic avian influenza (HPAI). In the last ninety-two years, there have been five recorded outbreaks of HPAI in the United States (US). Globally, notable outbreaks have occurred in Italy (1997-2001), the Dutch region of Europe (2003), Canada (2004), and more recently, in Asia.

Preventative measures will be examined in this report. In particular, biosecurity, quarantine, surveillance, and eradication are some of the most widely recognized and accepted ways to help prevent and control HPAI outbreaks. However, none of these methods are failsafe strategies to completely prevent or control the spread of HPAI. This report will focus on an additional preventative measure - currently available and potential future vaccination programs. There is a global shift toward procuring poultry that are AI-free as well as unvaccinated for AI. This is, in part, due to the limitations of currently available vaccines in completely ridding poultry of this disease. Vaccinations may reduce the amount of virus in infected birds, but this does not prevent birds from becoming infected.

When addressing the control and eradication of HPAI, some future challenges include viral mutations, intermingling of domesticated and wild birds, and vaccine development.

Because of the current limitations of vaccines and future challenges in controlling the spread of infection, there is no one single solution to this problem. It will require a multi-faceted approach.

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Dedication

To my uncle, Dave.

He inspired me to make my childhood dreams about a career in veterinary science a reality.

Chapter 1 - Introduction

Background

Avian influenza (AI) is a zoonotic disease that has garnered much attention in recent years due to its detrimental effects on the economy and human health. Much of this increased attention is due to the fact that the impact of AI outbreaks have sharply increased over the past few years. This impact could be attributed to the number of birds affected as well as the escalated economic costs related to controlling the disease.

AI may be characterized into two different biotypes, strains of a virus which inflict different physiologic symptoms. These biotypes are Low Pathogenic Avian Influenza (LPAI) and High Pathogenic Avian Influenza (HPAI). The differences between these two biotypes is based mainly on differences in their virulence. (Rebel, 2011) HPAI was first defined in domestic poultry in 1959. (Clark, 2006) According to the Office International des Epizooties (OIE),

> "high pathogenicity avian influenza viruses have an intravenous pathogenicity index (IVPI) in six-week-old chickens greater than 1.2 or, as an alternative cause at least 75 percent mortality in four-to-eight-week-old chickens infected intravenously. H5 and H7 viruses which do not have an IVPI or greater than 1.2 or cause less than 75 percent mortality in an intravenous lethality test should be sequenced to determine whether multiple basic amino acids are present at the cleavage site of the haemagglutinin (sp) molecule (HA); if the amino acid motif is similar to that observed for other high pathogenicity avian influenza isolates, the isolate being tested should be considered as high pathogenicity avian influenza virus. Low pathogenicity avian influenza viruses are all influenza A viruses of H5 and H7 subtypes that are not high pathogenicity avian influenza viruses." (Infection, 2013 p.492)

HPAI has been limited to H5 and H7 subtypes, even though not all viruses that include these subtypes inflict disease. The remaining viruses can inflict a minor, mainly respiratory disease. (Alexander, 2000) Most known HPAI viruses have emerged by mutation after a LPAI precursor has been introduced into poultry from the wild bird reservoirs. Throughout this disease's documented history, the factors that underlie the mutation from LPAI to HPAI are unknown. It is recognized that generally all HPAI viruses should have a LPAI progenitor, even if this has only been found and documented in a small number of cases. (Capua, 2013). For the purposes of this report, HPAI will be the main focus.

Classification

Influenza viruses belong to the viral family Orthomyxoviridae. There are three types of influenza – influenza viruses A, B, and C. These viruses are distinguishable based on alterations in their nucleoprotein antigen. Both influenza viruses A and B have the ability to initiate genetic variation. Influenza C virus, on the other hand, is antigenically stable. (Couch, 1996) All forms of avian influenza are exclusively classified as influenza virus A. (Clark, 2006)

Properties

Influenza is an enveloped, negative-sense, single-stranded ribonucleic acid (RNA) virus. (Clark, 2006) These viruses are about 80 to 120 nm in diameter. (Couch, 1996 and CIDRAP, 2013) The virus is generally spherical in structure; however, filamentous forms have been discovered. (Couch, 1996) The genome is comprised of eight segments. (Wu, 2012) The genome can encode for different proteins; eight structural proteins – one nucleocapsid protein (NP), two surface glycoproteins hemagglutinin (HA) and neuraminidase (NA), two matrix proteins (M1 and M2) and three transcriptases (PA, PB1, and PB2), as well as, two nonstructural proteins - NS1 and NS2. (CIDRAP, 2013)

> “The three largest RNA segments (RNA 1, 2, 3) code for subunits of the viral RNA polymerase (PB2, PBl, and PA, respectively) and are responsible for transcription and amplification of the viral genome. A "cap-snatching" function of the viral polymerase, where the 5' methylated ends of cellular mRNAs are cleaved and used as primers for transcription of viral mRNAs, is a unique feature of these viruses.” (Clark, 2006 p.6)

Viral proteins, HA, NA and M2, are embedded within the lipid envelope of the virus. As illustrated in Figure 1.1. HA and NA will be discussed in more depth later on; however, it should be noted that M2 is a transmembrane protein that is crucial for viral replication. During cell entry and viral maturation, M2 assists to equalize pH across both the viral and trans-Golgi membranes of infected cells. (Pielak, 2011)

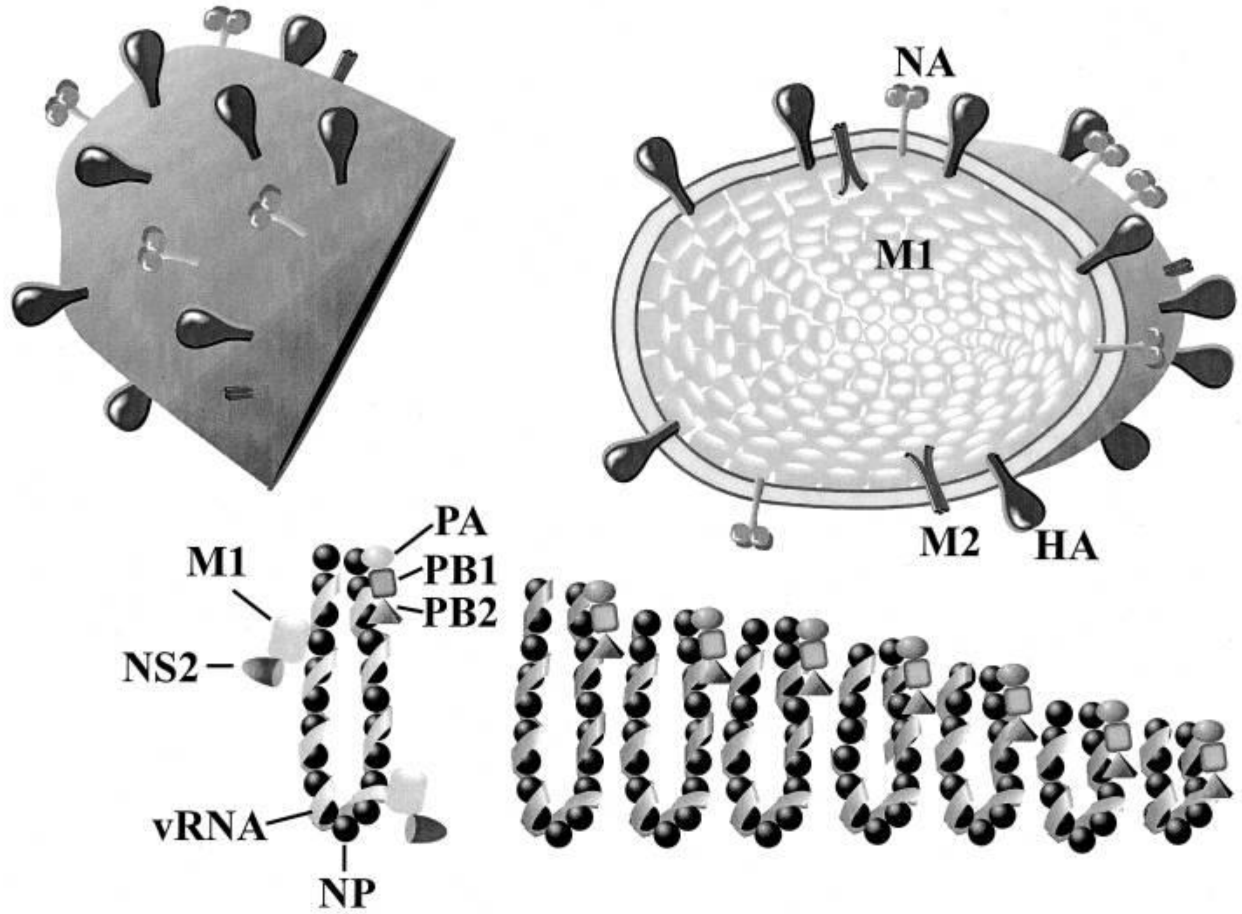

Figure 1-1 Structure of influenza A virus virions.

Two glycoprotein spikes, HA and NA, and the M2 protein are embedded in the lipid bilayer derived from the host plasma membrane. The RNP consists of a viral RNA segment associated with the NP and the three polymerase proteins (PA, PB1, and PB2). The M1 protein is associated with both RNP and the viral envelope, while NS2 is associated with RNP through interaction with M1. NS1 is the only nonstructural protein of influenza A virus. Adapted from Horimoto, T., & Kawaoka, Y. (2001). Pandemic Threat Posed by Avian Influenza A Viruses. *Clinical Microbiology Reviews, 14*(1), 129-149. Retrieved February 24, 2016.

Nomenclature

HA and NA are essential membrane proteins. HA is involved in helping the virion bind to host cells as well as assists in fusion between the two. NA is involved with virus budding and shedding, binding, and host-range determination. Both HA and NA are highly variable and are significant pathogenicity determinants. Based on these two viral proteins, influenza virus A can be further classified into subtypes. HA has 16 different subtypes, H1 through H16, whereas NA only has 9 subtypes, N1 through N9. (Clark, 2006) Table 1.2 identifies the subtypes of HA, NA and the predominant host of each subtype for influenza virus A.

Table 1.2 Natural Hosts of Influenza A Viruses

Hemagglutinin		Neuraminidase	
Subtype	Predominant Hosts	Subtype	Predominant Hosts
H1	Human, pig, birds	N1	Human, pig, birds
H2	Human, pig, birds	N2	Human, pig, birds
H3	Birds, human, pig, horse	N3	Birds
H4	Birds	N4	Birds
H5	Birds, (human)	N5	Birds
H6	Birds	N6	Birds
H7	Birds, horse, (human)	N7	Horse, birds
H8	Birds	N8	Horse, birds
H9	Birds, (human)	N9	Birds
H10	Birds	N10	Bats
H11	Birds	N11	Bats
H12	Birds		
H13	Birds		
H14	Birds		
H15	Birds		
H16	Birds		
H17	Bats		
H18	Bats		

Table 1-1 Natural hosts of influenza A viruses.

The table indicates the subtypes of hemagglutinin (HA) and neuraminidase (NA), and the hosts in which they have been identified. Adapted from Lamb RA, Krug RM. *Orthomyxoviridae*: the viruses and their replication. In: Knipe DM, Howley PM, Griffin DE *et al*., editors. *Fields Virology*, 4th edn. Lippincott Williams & Wilkins, 2001; pp. 1487–1531.

Modes of Transmission and Pathogenesis

Within the wild bird population, AI is generally a mild or insignificant disease. Very few cases of severe disease or mortality have been reported. (Clark, 2006) In fact, many wild fowl are a primary reservoir for AI, serving as a healthy carrier of the disease. Susceptible birds can become exposed to HPAI either directly or indirectly. According to Mubareka et al., "Potential modes of transmission of influenza virus include direct contact with infected individuals, exposure to virus-contaminated objects (fomites), and inhalation of infectious aerosols." (2009 p.858)

Most commonly, contaminated bodies of water aid in the spread of HPAI through the fecal-oral route. Domestic poultry are in jeopardy of contracting HPAI anytime a water source is shared with wild birds. Disease-ridden birds can shed the virus in their feces into the water, other birds can then contract HPAI by consuming the contaminated water. Halvorson et al, also demonstrated that contamination of surface-water ponds may be due to the viral contamination of nearby groundwater supplies and vice versa. This contamination may result due to using contaminated manure to fertilize fields etc. (1984) Alternatively, the inhalation of aerosolized fecal dust can be another form of transmission. Through this route, the bird can become infected through the oral and or respiratory routes. However, this is a less effective mode for infection. (Clark, 2006)

AI viral replication is restricted to the intestine and respiratory epithelium. Once the virus has gained entry, it will begin to rapidly replicate producing large quantities of virus progenies. These progenies are consequently released from cells and shed from the host. (Lekcharoensuk, 2008) To provide an example, if the virus were to initiate replication within the epithelial tissue of the intestine, this process could take between two to fourteen days after ingestion. Once the virus has produced enough progenies, the infected bird will subsequently start shedding the virus through the cloaca. This process, in particular with regard to viral replication within the intestine, is important for the transmission of HPAI virus to spread through the fecal-oral route. (Clark, 2006)

Symptoms

For domestic poultry species, AI symptoms can vary among different breeds of birds, such as chickens, ducks and turkeys. HPAI has an incubation period of three to five days, with symptoms potentially developing once its incubation is complete. (Clark, 2006) These symptoms can vary from cyanosis of the comb and waddles, a decrease in egg production, diarrhea, edema in the head or face, an excessive flow of tears, respiratory signs, ruffled feathers, sinusitis and distress upon the central nervous system, such as, head tilt or a lack of coordination,. (Horimoto, 2001) In addition to these symptoms, upon onset of disease, these birds may also experience fever and lethargy. (Clark, 2006) This disease can lead to death within hours of the onset of clinical symptoms and mortality rates can be as high as one hundred percent. (Clark, 2006 and Horimoto, 2001) Currently, there are no specific treatment plans for HPAI infections in poultry. Those flocks infected with the virus are generally eradicated.

Zoonotic Potential

AI epidemics pose risks to not only domestic poultry but humans and other animals as well. In 1997, Hong Kong had the first documented case of an individual contracting H5N1 from a chicken. Humans contracted the disease by coming into close contact with infected birds or their feces. This was primarily through the inhalation of aerosolized fecal dust or contaminated water. There are very few cases of this strain of AI spreading through human-to-human transmission. By December 1997, the outbreak had resulted in the hospitalization of eighteen individuals and six deaths. Since that initial outbreak, there have been multiple incidents of humans contracting AI from domestic poultry. Other human cases of AI have been recorded since the 1997 outbreak. These cases involved a variety of strains such as: H5N1, H7N2, H7N3, H7H7 and H9N2. (Imperato, 2005) Most recently, a H7N9 strain was reported in China. The impact these outbreaks could have on public health has called for an increase in surveillance of wild and domestic birds, improved communication with officials, educating the public on potential effects of the disease and working toward improving medical treatment.

AI can present a variety of symptoms in humans. These symptoms can range from classical influenza symptoms, including cough, fever, muscle aches and fever. However, symptoms as severe as “eye infection, pneumonia, acute respiratory distress syndrome (ARDS), multiple organ failure, lymphopenia, elevated liver enzyme levels and abnormal clotting profiles” can occur. (Weir, 2005 p.785)

The main cause for concern is the potential for AI to participate in cross-species transmission, leading to reassortment of the virus. (Ferguson, 2004) Reassortment is the recombination of the virus’ genetic material, and can be performed through antigenic variation. In fact the classic hallmark of influenza is its ability to undergo antigenic variation. Antigenic variation can present itself in the form of antigenic drift and antigenic shift. (Treanor, 2004) Antigenic drift develops gradually over a period of time. It can occur in both influenza virus A and B. (Treanor, 2004) Antigenic drift occurs due to faults within the viral replication process, over time these mutations allow subtypes of the virus to adapt and cross species. Fortunately, antigenic drift does not normally increase the pathogenicity or virulence. (United, 2015) Antigenic shift can express variations in host susceptibility, pathogenicity and virulence. It can occur in a multitude of ways, but most commonly as direct species adaptation and genetic reassortment. Direct species adaptation is that process in which a species previously unknown to be affected by a particular subtype now has that strain circulating within its species and may subsequently become infected with the disease. (United, 2015) Host cells that are co-infected with two different viral strains of AI virus can result in the genetic reassortment of the virus, producing an entirely new virus. (United, 2015)

Concurrent infections of human and avian influenza could, in principle, produce novel influenza viruses. “These hybrid viruses would have the potential to express surface antigens from avian viruses to which the human population has no preexisting immunity.” (Kaye, 2005 p.108) Overall, these hybrid viruses could instigate a devastating pandemic and amplify already high rates of mortality. (Ferguson, 2004)

Purpose

Historically, HPAI is a globally devastating disease and currently continues to have a massive impact. The purpose of this report is to understand avian influenza by examining its background, history and epidemiology. Furthermore, it is necessary to explore the available preventative measures and future challenges of controlling HPAI outbreaks. Such a synopsis is essential to set the stage for researchers to continue to combat its effects.

Chapter 2 - Worldwide History and Epidemiology of HPAI

This chapter of the report will focus on notable HPAI outbreaks worldwide. These outbreaks have caused a significant impact on the health of domestic poultry as well as financial losses. In the last ninety-two years, there have been five recorded outbreaks in the US. Outbreaks have also occurred in Italy (1997-2001), the Dutch region of Europe (2003), Canada (2004), and more recently, Asia. To add, other previously reported outbreaks of HPAI are displayed in table 2.1.

Table 2.1 Previous Outbreaks of Highly Pathogenic Avian Influenza Worldwide

Year	Country/Area	Domestic Birds Affected	Strain
1959	Scotland	Chicken	H5N1
1963	England	Turkey	H7N3
1966	Ontario, Canada	Turkey	H5N9
1976	Victoria, Australia	Chicken	H7N7
1979	Germany	Chicken	H7N7
1979	England	Turkey	H7N7
1983–1985	Pennsylvania, United States	Chicken and Turkey	H5N2
1983	Ireland	Turkey	H5N8
1985	Victoria, Australia	Chicken	H7N7
1991	England	Turkey	H5N1
1992	Victoria, Australia	Chicken	H7N3
1994	Queensland, Australia	Chicken	H7N3
1994–1995	Mexico*	Chicken	H5N2
1994	Pakistan*	Chicken	H7N3
1997	New South Wales, Australia	Chicken	H7N4
1997	Hong Kong (China)*	Chicken	H5N1
1997	Italy	Chicken	H5N2
1999–2000	Italy*	Turkey	H7N1
2002	Hong Kong, China	Chicken	H5N1
2002	Chile	Chicken	H7N3
2003	Netherlands*	Chicken	H7N7

*Outbreaks with significant spread to numerous farms, resulting in great economic losses. Most other outbreaks involved little or no spread from the initially infected farms.

Table 2-1 Previous Outbreaks of Highly Pathogenic Avian Influenza Worldwide.

Adapted from Avian influenza A (H5N1) - update 31: Situation (poultry) in Asia: Need for a long-term response, comparison with previous outbreaks. (2004, March 2). Retrieved February 26, 2016, from http://www.who.int/csr/don/2004_03_02/en/

United States

Before the most recent outbreak in 2016, the US has had only four recorded outbreaks of HPAI in the last ninety-two years: in 1924, 1983, 2004, and 2015.

Outbreak in 1924

In June 1924, the first HPAI outbreak was recorded in the US. Throughout this outbreak, two subtypes were in circulation, H7N1 and H7N7. (Swayne, 2013) Prior to this outbreak, HPAI was not acknowledged to be present in the country. It began with an undiagnosed high rate of mortality affecting birds in the East Coast live bird markets. This primarily occurred in the state of New York. By August of 1924, the disease had spread within poultry to both New Jersey and Pennsylvania. In December, New York began rejecting chickens imported into the live bird markets from numerous Midwestern states. Officials wrongly believed that these birds were the source of infection. The rejected chickens were then sent to other regions, continuing to spread the disease through different means, such as contaminated crates and the railway system. There was no indication that wild birds played a role in the transmission of this particular outbreak. In addition, no human cases of infection were reported. By January and February 1925, six more states were affected by the disease: Connecticut, Illinois, Indiana, Michigan, Missouri, and West Virginia. Eventually, through preventative measures, such as biosecurity, depopulation and quarantine, the United States Department of Agriculture (USDA) was able to control the multi-state outbreak and the disease was eradicated that spring. It has been estimated that within New

York City alone, 500,000 to 600,000 chickens died. Furthermore, the financial impact of this outbreak was projected to be one million dollars. (Halvorson, 2009)

Outbreak in 1983

In April 1983, a LPAI H5N2 infection was detected in a Pennsylvania flock. Symptoms observed were typical to an LPAI infection, including the initial onset of respiratory symptoms followed by a reduction in egg production and low mortality rates. As the disease carried on, there was an increase in the severity of symptoms, including a decline in food consumption, dehydration, lethargy, edema, cyanosis and hemorrhages. This raised the concern of whether the initial LPAI infection had converted into an HPAI virus. Diagnostic tests were performed and the virus was isolated as a HPAI H5N2 virus. This is the first documented case within the United States of LPAI mutating into HPAI.

The disease continued to spread to flocks in Pennsylvania (HPAI and LPAI), New Jersey (LPAI), and Virginia (LPAI). Similar to the outbreak in 1924, the infection was accredited to have spread through contaminated equipment, coops and trucks used to transport birds. Contaminated eggs, feed and water were also thought to have contributed to the spread. Again no zoonotic cases of disease were reported. By November 1983, biosecurity, containment and eradication efforts were instituted by the USDA; however, it was not until February 1984 that the decision to eradicate all flocks that exhibited signs or symptoms of H5N2 infection was finalized. More significantly than the outbreak of 1924 with the destruction of 500,000 to 600,000 chickens, this outbreak resulted in the depopulation of 17 million birds throughout 448 flocks. The economic impact of this outbreak was estimated to be fifteen million dollars in non-insured losses for poultry producers and sixty-three million dollars for the government. (Halvorson, 2009)

Outbreak of 2004

In February 2004, the USDA confirmed the presence of a HPAI H5N2 outbreak near Gonzalez, Texas. The disease affected one farm of approximately 6,600 chickens. Observations were made that the infection arose due to poultry species returning to the farm from live bird markets. Clinical symptoms presented were similar to an LPAI infection with respect to respiratory symptoms and low mortality rates. Those infected and at-risk birds were culled as directed by the USDA and the outbreak was controlled by April. There was no indication that wild birds played a role in the transmission of this particular outbreak. Nor were any cases of human infection reported. (Lee, 2005 and Halvorson, 2009)

Outbreak in 2015

In December 2014, in Douglas County, Oregon, the first case of HPAI was confirmed. It occurred in a backyard operation, consisting of approximately 130 mixed species of poultry. (Newton, 2015) Throughout mid-January 2015, the USDA identified fourteen more cases of an Asian-origin HPAI strain. Of the fourteen HPAI cases identified, several strains were detected, including H5N1, H5N2 and H5N8. (Jhung, 2015) Five months after the initial outbreak, a ‘state of emergency’ had been declared in Iowa, Minnesota and Wisconsin. Preventative measures were implemented to control this outbreak including, biosecurity and eradication. (Greene, 2015) The final detected case of HPAI came on June 17th.

It is believed that the epidemic was transmitted by wild birds migrating from Eurasia. These birds traveled along the Pacific flyway and continued to move into the Central and Mississippi flyways. Flyways represent the predispositions of wild birds to confine their movements within expansive geographic bounds. (Clark, 2006) They most commonly utilize these routes when traveling from their breeding grounds to winterized areas. Additionally, APHIS advised that gaps in preventative measures, such as biosecurity could have also contributed to the spread of this disease. (Greene, 2015) This outbreak devastated domestic egg and poultry producers affecting more than 48.8 million birds within 21 states. The economic impact from this outbreak was estimated to be 3.3 billion dollars, classifying it as one of the worst animal diseases in US history. (Greene, 2015 and USDA, 2015)

Outbreak in 2016

On January 15, 2016, APHIS confirmed the year's first case of HPAI in Dubois County, Indiana. The H7N8 strain was discovered in a commercial turkey flock and different than the HPAI strain that resulted in countless numbers of outbreaks in 2015. Officials immediately began the quarantine and depopulation of the affected area. (United, 2016) Furthermore additional surveillance and testing of birds within the affected area was implemented. These methods included epidemiologic, geospatial, genetic, and wildlife investigations. APHIS began the testing and sampling of wild birds within the region, no detection of H7N8 has been found. (USDA, 2016) At the present time, this outbreak has been considered contained.

Italy

From October 1997 to February 2001 two extremely devastating HPAI epidemics affected regions of Italy.

Outbreak of 1997

In October 1997, unexpected high mortality rates of rural flocks were being reported. HPAI was suspected and an H5N2 subtype was isolated. This epidemic spread to eight backyard and semi-intensive flocks throughout the Veneto and Friuli-Venezia Giulia regions of north-eastern Italy. Additionally, this region of Italy lies on a major migratory flyway and wetland for wild birds and is heavily populated with intensive poultry operations, making it a prime location for this particular strain to spread rapidly. However, control and prevention measures were implemented immediately to prevent the further spread of this disease into more well-established poultry facilities. The epidemic, affecting approximately 6,503 birds, was declared over by January of 1998. (Capua, 1999)

Outbreak of 1999

One of the most important HPAI outbreaks throughout Europe, the 1999 epidemic originated from an LPAI strain. In March 1999, an H7N1 strain was detected in parts of northern Italy. Despite attempts to control and eradicate the disease, 199 flocks had become infected. The H7N1 strain continued to spread to other facilities until April of 2000. At this point the disease was thought to be eradicated. (Monne, 2014) However, by the following August, the disease had reemerged and seventy-eight more outbreaks were identified. In November 2000, a vaccination program was instituted to prevent further losses. The vaccination program was mainly targeted for meat turkeys, due to their increased susceptibility of the disease. In addition, poultry with a longer life span, such as layers, were also vaccinated. Through the implementation of this vaccination program, there was an immense reduction in the number of outbreaks as well as a decrease in the extent of the epidemic. By February of 2001, this devastating epidemic was declared over (Monne, 2014), but not before resulting in 415 outbreaks and the loss of approximately fourteen million birds. (Alexander, 2007)

The Dutch Region of Europe – Belgium, Germany and the Netherlands

In February 2003, an H7N7 outbreak occurred in the Netherlands. The initial outbreak was reported in a heavily populated poultry farm near Geldersei Vallei. (Van Berm, 2014 and Alexander, D.J., 2007) The outbreak rapidly spread to a province near the Belgium border. By mid-April, eight farms in Limburg on the Belgium-Netherland border had also become infected.

Only one farm was affected in Germany. Extensive stamping out procedures were implicated to gain control and eradicate this disease. The Dutch epidemic resulted in 241 outbreaks and the eradication of twenty-five million birds. (Alexander, D.J., 2007)

In addition to HPAI H7N7 infections in birds, there were also several zoonotic cases. Officials originally thought that the risk of contracting the disease was low; however, an investigation was launched and found that eighty-nine individuals developed the disease. In addition there was one case fatality, a veterinarian who had visited several of the farms. (Koopmans, 2004)

Canada

In February 2004, British Columbia, Canada reported its first case of HPAI. The LPAI H7N3 virus shifted to an HPAI strain. The epidemic began at a well-established broiler-breeder farm located in Fraser Valley. By mid-February the affected flock had been culled. Despite the enactment of control and prevention methods such as surveillance and depopulation, by March the disease had continued to spread to other local farms. Eventually, the disease encompassed the densely populated valley, which contained approximately 85-90% of the area’s poultry production. The decision was finally made to cull all birds that displayed signs of symptoms of the disease. The epidemic resulted in 53 outbreaks between commercial and backyard flocks and the depopulation of seventeen million birds. (Pasick, 2009)

Asia

This section of the report will not have specific epidemiological statistics nor highlight significant years because outbreaks occur frequently in Asia. Thus, bringing to light the almost endemic situation that currently faces Asian countries in regard to AI. For example, Capua et al. notes about HPAI H5N1:

> “At the moment it is unclear whether or not HPAI H5N1 is truly endemic in the Eurasian wild bird population or merely limited to spill-over events from domestic birds. If the latter is true, then provided the domestic source of infection is eliminated, and the infections are responsible for the death of the wild avian hosts, presumably the prevalence of infection will gradually be reduced to zero. In contrast, if HPAI infection does not bring about the death of the wild bird host and becomes compatible with normal behavioral patterns and migration in at least some species, this will result in the development of an endemic cycle in wild bird, mimicking the well-known LPAI ecology. The consequences of such a situation are unpredictable.” (Capua, 2007 p.5646)

Over the last thirty years, various strains of AI have spread across Asia. Due to lax preventative measures, these strains have spread across numerous Asian countries. Alexander (2007) qualifies the situation as endemic in many of those countries. Specifically, the HPAI H5N1, originally isolated in China in 1996, spread in poultry or wild birds into the rest of Asia, Europe, and Africa. Because of this uncontrolled spread, extreme measures, such as eradication were employed resulting in the culling of hundreds of millions of birds. Furthermore, this spread posed an increased risk to human health via direct species adaptation. Between December 2003 and February 2004, outbreaks of AI have been reported in Korea, Cambodia, China, Indonesia, Japan, Lao People’s Democratic Republic, Thailand, and Vietnam. (Reinhardt, 2004)

Due to these events, the H5N1 infections are now considered endemic within the commercial poultry in Bangladesh, China, Egypt, India, Indonesia, and Vietnam. Even though, other countries have also reported outbreaks of the disease. “This unprecedented situation has occurred certainly via the trade of infected poultry and poultry products, but infection of wild birds has also most probably contributed to carrying the virus over large distances.” (Capua, 2007 p.5646) The poultry industry has experienced devastating consequences due to the various strains of AI reaching endemic qualities. These consequences include economic issues, negative public opinion, the creation of human health issues, such as a pandemic zoonotic virus, and the loss of millions birds through culling. (Capua, 2007)

Chapter 3 - Preventative Measures

Naturally, the best method for reducing the catastrophic effects of a HPAI epidemic is to avoid having any outbreaks to begin with. There is no single solution to controlling the spread of the HPAI virus. It will require using a variety of programs and interventions simultaneously. Some of the most effective approaches to date have included: 1) enhancements in biosecurity, 2) quarantine of suspected HPAI cases, 3) surveillance of wild birds, 4) eradication of affected flocks, 5) efforts to communicate and educate professionals in the poultry industry and 6) vaccination. These measures will be discussed in detail, including the strengths and weaknesses of each approach.

Biosecurity

When assessing potential preventative measures for HPAI outbreaks, biosecurity may appear to be an obvious solution. According to the Food and Agriculture Organization of the United Nations (FAO), "Biosecurity is the implementation of measures that reduce the risk of the introduction and spread of disease agents. Biosecurity requires the adoption of a set of attitudes and behaviors by people to reduce risk in all activities involving domestic, captive exotic and wild birds and their products." (Biosecurity, 2008 p.1) However, this solution can be difficult to implement. Biosecurity requires additional planning and understanding. For example, in the outbreak in Indiana in 2016, the USDA launched a questionnaire to examine biosecurity measures of the infected farms. The initial investigation found that while some measures were implemented others were not. This investigation is ongoing and will look at those farms that were not affected. This analyses should be able to help determine the cause of the outbreak in regards to the lack of biosecurity. (USDA, 2016)

The concept of biosecurity encompasses three basic principles: segregation, cleaning and disinfection. Segregation is the practice of keeping one flock away from another flock. In the instance that both flocks are housed on the same farm, it may be required of personnel to shower, and change scrubs before moving from one flock to the other. Cleaning is a very important aspect of biosecurity. Influenza is easily inactivated by most soaps and disinfectants. This principle must be performed on all equipment. This may include: feeders, waterers, nest boxes, perches and crates. Disinfection can be performed through thoroughly spraying the equipment and room or by fogging.

Simply put, segregation is a matter of keeping one flock of birds completely separate from another. It is crucial to keep wild birds and domestic flocks from intermingling. It is also important to ensure that barriers to limit exposure are maintained. For example, when working with two flocks of birds housed separately, it is essential to follow protocol on the following principles of cleaning and disinfecting so that the segregation remains in place. The basic principle of cleaning involves ensuring that all materials potentially infected by passing through a virus site must be cleaned. Typically, if all visible dirt is removed, the majority of the virus will also be removed. The last basic principle is disinfection. This principle typically occurs after cleaning because if one attempts to disinfect an item without first removing the visible debris, the process will be ineffective at removing the virus. Most disinfection agents do require appropriate proportions when added to another liquid and a certain amount of contact time before it can completely kill the virus. For example, sodium hypochlorite needs at least ten minutes of contact time to ensure the material is fully sanitized. Also, for appropriate sanitation, the concentration of sodium hypochlorite in liquid form depends on targeted microorganism and the object being sanitized. Therefore, a different proportion of sodium hypochlorite to water may be required to be effective against AI than for other diseases. (Biosecurity, 2008)

As a preventative measure, absolute biosecurity is nearly impossible to achieve. However, attaining a level of biosecurity that reduces the risk of infection is possible. This measure is critical for preventing disease, as well as hindering its continuing spread. (Biosecurity, 2008)

Quarantine

Quarantine may prove to be an additional preventative measure with significant value. This is due to the fact, that by establishing a quarantine it controls the movement and spread of the disease. According to the Centers for Disease Control and Prevention (CDC), quarantining "separates and restricts the movement of people who were exposed to a contagious disease to see if they become sick." (2015) Animal quarantine works exactly in the same manner. When applying this concept to an HPAI outbreak, it may possibly prevent the further spread of disease from those infected to susceptible birds. The quarantine period depends on a variety of factors which can include; the amount of virus a bird is exposed to, the mode of transmission, the species affected and the presentation of clinical signs. However, for international regulatory purposes the OIE recognizes twenty one days as the incubation period for AI. This time frame may differ from what is actually implemented in the field. These differences are due to a different interpretation of incubation period. More specifically, in the field incubation period is defined as the period from exposure to the initiation of clinical disease. (Swayne, 2013)

There are a variety of serological diagnostic tests available to test birds for the presence of an HPAI infection. Agar gel immunodiffusion (AGID) tests, enzyme-linked immunosorbent assays (ELISA), hemagglutination inhibition (HAI), and neuraminidase inhibition (NAI) assays have all been utilized to detect HPAI. (OIE) These tests are utilized to identify, type and characterize an AI infection. Additionally, rapid detection tests have been made available to detect the presence antigens, specifically H5, H7 and N1. However these have low to moderate sensitivity. And are more highly utilized when there are multiple birds showing symptoms. (Swayne, 2008) Diagnostic tests such as virus isolation, genetic sequencing, and real time reverse transcription polymerase chain reaction (rRT-PCR) have also been highly utilized. If those quarantined birds are found to be infectious, it is to be expected that the rest of the flock would be culled. (Biosecurity, 2008)

As with biosecurity there must be strict boundaries between quarantined and non-quarantined zones. This would include, but are not limited to, using clothing, footwear, personal protective equipment (PPE) and other supplies at separate locations. These materials would also need to undergo cleaning to ensure quarantine remains unbroken. In some cases, laborers may be assigned to designated areas so that no cross-contamination occurs. Without this separation, HPAI can easily spread to the remaining birds in the flock. (Biosecurity, 2008)

The effectiveness of this preventative measure was tested and demonstrated in England in 2005. This outbreak resulted due to an Asian-Origin HPAI H5N1 strain, and resulted in the quarantine of caged birds. These birds had displayed a high rate of mortality due to the disease which led to trade restrictions within the European Union (EU) and beyond. (Capua, 2013)

Surveillance

Additional means of preventing HPAI may be through the implementation of surveillance. Surveillance is an essential preventative measure that can effectively contribute to the planning, implementation and potential control of a future outbreak. This process can be performed through the constant sampling, analysis and interpretation of data. (Introduction, n.d.)

Samples are generally collected from cloacal swabs of live wild birds, wild fowl killed by hunters during hunting seasons, or other unexplained wild bird deaths. (Robert, 2009) Surveillance should be targeted at those susceptible poultry within a particular region with the goal of identifying the disease and/or infection. (Infection, 2013) Surveillance, while an ongoing process, can be categorized into two types; passive and active surveillance. Passive surveillance relies on collecting data from previously reported cases. (Introduction, n.d.) Alternatively, active surveillance conducts current sampling from those points, which may be affected by an outbreak. (Phan, 2013) According to the OIE, active surveillance should be performed every six months at the minimum. (2013)

Surveillance has become globally recognized as a means to detect HPAI early within the poultry population, and prepare for potential future threats. More notably, using surveillance on a global scale, while studying AI, may give new insight as to the variations in where the disease spreads to and how much time the disease takes to circulate. (Munster, 2006) These results, whether discovered due to research or from government surveillance, are shared through online databases and scientific journals. Often times, these studies may lack standardization and some results may not be reported due to its negative findings. This can reduce the value of these reports, slowing the prevention and control of a future outbreak. Luckily, there are a couple of online databases, such as GenBank and the Influenza Research Database, that provide detailed and accurate reporting. Nonetheless, despite the benefits of surveillance, there is unfortunately no standardized, comprehensive reporting system. (Machalaba, 2015)

Munster and colleagues bring up a good point when stating the benefits of utilizing wild bird studies as an early warning system. (2006) Early warning systems are most often utilized to quickly identify the introduction or rapid surge in the occurrence of disease. These systems are mainly based on surveillance and accurate reporting of collected data. (Martin, 2006) For example, if the person facilitating the study places some "sentinel" birds (unvaccinated) within each flock, it is surmised that once one of these sentinel birds contracts the disease, the facilitator can act accordingly and report it to the authorities. Therefore, the authorities are warned much earlier than in other situations. This early action can help prevent further spread or mutation. (2006) (Capua, 2007) Currently organizations such as the OIE, FAO and the EU have been actively monitoring AI. For example, the FAO uses a web-based program to record, analyze and monitor the AI situation. This program monitors both wild and domestic birds. Other programs that are effective with early warning systems are the Global Early Warning and Response System. This works to monitor transboundary animal diseases. (Martin, 2006)

An understanding of animal movements is important to prevent and control future HPAI outbreaks. By gaining a better understanding of these movements, surveillance techniques can become more targeted in their approach. This is of note for those countries that do not have the resources to conduct detailed surveillance plans. These countries include Cambodia, China, Hong Kong, Thailand and Vietnam. Surveillance in these countries has proved effective by establishing that a HPAI H5N1 strain is circulating in live bird markets. (van Kerkhove, 2009)

Eradication

Eradication appears to be the only practical resolution to quickly controlling an HPAI outbreak. This generally leads to mass depopulation of the infected flock and those flocks within that region. According to the American Veterinary Medical Association (AVMA), "mass depopulation refers to methods by which large numbers of animals must be destroyed quickly and efficiently with as much consideration given to the welfare of the animals as practicable." (2016) The most common means to depopulating a flock are cervical dislocation, avicide poisoning, gas and water-based foam. Cervical dislocation leads to an immediate state of unconsciousness due to the dislocation of the head and the spinal cord. This procedure requires each bird to be handed individually. Additionally each person must be properly trained to ensure that they are performing the procedure as humanely as possible. Avicides are a poison that are highly toxic to avian species. This method of depopulation would require the avicide to be delivered within the drinking water. This method is much slower, due to the bird needing to consume enough poison to be lethal. Depopulation of a flock with gas is typically performed with carbon dioxide. This can be achieved by creating a fully sealed chamber and filling it with the gas. Water-based foam is a medium that fully covers the birds. This medium lodges within a bird's trachea and inhibits it from breathing. Fortunately, this technique spreads and builds up well allowing for a quick and humane method of depopulation. To add, other means of depopulation such as electrocution and maceration have been performed but are not ideal. (Webster, 2007) Ideally, through means such as surveillance, the detection and potential eradication of a HPAI infected flock should be performed as quickly as possible. Through eradication, the reduction of HPAI virus in the environment can be achieved thus preventing the spread to other flocks.

Unfortunately, eradication does have its faults in attempting to control an HPAI outbreak. According to Song et al., "the eradication of HPAI virus-infected poultry is a temporary measure to prevent virus exposure to humans, but ducks and geese in nature are asymptomatically infected with these avian influenza viruses making it impossible to completely avoid contact with the virus." (2009 p.3145) Generally, the culling of birds that are considered healthy and un-infected, but also at risk of HPAI infection, may be considered a fault of eradication. This is primarily due to the fact that it can cause economic constraints on the poultry industry and can be a cause for public concern. For example in Egypt in 2006, educational programs were deployed to various groups such as poultry industry officials, the media, and veterinarians to deal with alarm reactions in the public. Later, door-to-door education was implemented by volunteers. (Abdelwhab, 2011)

Communication and Education

In today's global economy, it is not sufficient for countries to only focus on controlling HPAI outbreaks within their country. In order to control the devastating effects of HPAI, a continuing strategy involving multiple invested parties will be necessary. (Alders, 2007) As Alders and colleagues point out, "a common understanding of the problem and effective education and communication components are important elements of the control strategy." (2007 p.143) Effective communication must be clear, consistent, credible, practical and correct. If communication is unclear, it could slow the response to an HPAI outbreak. (Alders, 2007) With multiple countries working together, they can preemptively prepare for, control and eradicate future HPAI outbreaks. (USDA, 2015, April) To date, there is no universal procedure to report results of other preventative measures such as surveillance. This limits the clear communication between agencies, state officials and/or poultry producers needed for controlling outbreaks.

Chapter 4 - Vaccination Programs

A strong vaccination program is one of the most important prevention strategies when looking to reduce the likelihood of a HPAI outbreak from occurring; however it is highly under-utilized. Vaccines provide numerous benefits including: 1) increasing resistance in poultry to developing HPAI infection, 2) reducing the environmental load and transmission of HPAI, 3) reducing viral replication, 4) preventing illness and death of infected birds, and 5) reducing human exposure and infections. (Swayne, n.d.) Due to its importance as a preventative strategy, HPAI vaccines and the current state of vaccine development will be discussed in detail, including the worldwide implications of different vaccine strategies.

Available Vaccines

Currently, birds are inoculated with vaccines by needle injections - intramuscularly or subcutaneously. This requires vaccinations be done by hand, which takes many hours. Studies are being conducted to research the efficacy of administering vaccines through either air or water supplies, or via egg injection. These studies are promising in that entire flocks of birds can be vaccinated in a short amount of time. For example, researchers are working to develop an AI vaccine that would be capable of differentiating infected from vaccinated animals (DIVA). This vaccine could be administered through water and would protect birds against Newcastle Disease virus (NDV) as well as H5N1, H5N2 and H5N8. Furthermore, the vaccine has the potential to be administered *in ovo*, resulting in automatically vaccinated chicks. This study was initiated in 2015 at Kansas State University. (Prairie, 2016)

Ideally, after initial vaccination, a vaccine should be able to prevent future infections as well as induce lifelong immunity to the disease. (Rahn, 2015) Unfortunately, no such vaccine is currently available. The inactivated whole virus vaccine, is widely available and accepted to control HPAI infections and outbreaks. Even though this type of vaccine is widely used, it is by no means a universal vaccine as vaccines tend to be strain specific. (Rahn, 2015) It must be noted that reassortment can be a cause for a potential vaccine to be ineffective, which leads to the need to constantly develop new vaccines.

Inactivated Vaccines

Inactivated vaccines are the most commonly used vaccines, and they are commercially available. Although they do reduce the spread and transmission of the virus, there are limitations in their use. Unfortunately, inactivated vaccines are inefficient against maternal antibodies. In birds, chicks absorb maternal antibodies through the yolk. While these antibodies serve as a source of passively acquired immunity, they can also inhibit vaccines from inducing an immune response. (Forrest, 2013) Additionally, inactivated vaccines have to be subtype specific. For example, a vaccine for H5N1 would not be effective for an outbreak of H7N1. This leads to the constant development of vaccines that will be effective against each strain. Another significant concern of using this type of vaccine is interference due to vaccine-initiated antibodies when employing surveillance to predict how the disease spreads. (Lee, 2004)

Inactivated vaccines are usually administered at about three weeks of age and can require between two to four injections. These injections are administered approximately three weeks apart, and if given properly, these vaccines can confer adequate immunity. However, immunity takes approximately seven to ten days to develop, and would not become sterilizing. (Capua, 2013 and USDA, 2015) Inactivated vaccines for H5, H7 and H9 virus subtypes are commercially available. These vaccines have been approved for use in numerous countries, such as China, Egypt, Indonesia and Vietnam to name a few. (Capua, 2013 and Roth, 2012)

Experimental Vaccines

Other vaccines that are being studied and are gaining momentum as novel approaches to controlling HPAI in poultry, include live attenuated vaccines, DNA-based vaccines, RNA replicon particle-based vaccines, subunit vaccines, virus-like particles and vectored vaccines. These experimental vaccines are capable of producing antibodies, which then provide protection against losses associated with decreased egg production, disease, and death. Live attenuated vaccines initiate antibodies on system-wide and mucosal levels. Mucosal immunity works to protect the host from pathogen invasion within the mucus membranes. Additionally, the immune system can elicit a systemic response throughout the host body generally in response to a pathogen. They can also elicit a significant cellular immune response. Therefore, live attenuated vaccines can provide widespread and consistent influenza protection. (Wu, 2010) Additionally, vectored vaccines are a type of modified live attenuated virus obtained by utilizing reverse genetics technology. Researchers in this field are becoming more inclined to use vectored vaccines as they have a strong ability to combat interference from maternal antibodies. (Kapczynski, 2015) More so, they can effectively stimulate the immune system through cell mediated immunity.

In order to be successful, the vectored vaccine needs to be effective against a wide range of host species and there should be no preexisting antibodies against the vector within the host. (Rahn, 2015) The host range of the vectored vaccine coincides with the ability of the virus vector to successfully infect the animal. For example, if the virus vector has a wide host range, there is a greater chance of protecting a large number of birds from HPAI than a virus vector that has a narrower host range. Additionally, the animal should not have preexisting antibodies to the vector as this can hinder the effectiveness of the vaccine.

Differentiation of Infected from Vaccinated Animals (DIVA) Strategy

The DIVA strategy allows for immunological differentiation of infected from vaccinated animals. This is most often achieved by a vaccine based upon a different strain than the outbreak strain. A DIVA strategy uses the aforementioned inactivated whole virus vaccine, which contains the same HA subtype as the HPAI causing an outbreak but uses a different NA subtype. For example, vaccinating for H5N2 when the outbreak is truly being caused by H5N1. Overall, this strategy works to induce an immune response to the vaccinated strain. By means of an ELISA test, researchers can differentiate between the vaccine strain and the outbreak strain. Commercially, this means the poultry industry could actually be able to identify which poultry are infected by the outbreak, wild-type strain versus those that have been vaccinated. The poultry industry can then act accordingly to effectively control the current outbreak through eradication, quarantine, and/or biosecurity. The effectiveness of this strategy was tested in Italy during the LPAI H7N1 outbreak that occurred between 2000 to 2002. The vaccine administered was a H7N3 strain that helped to supplement additional control measures to eradicate the disease. (Lee, 2004)

There are some limitations to utilizing the DIVA strategy. It is imperative that the combination of influenza subtypes for the vaccine are different from the outbreak. Also, the vaccine should closely match the challenge strain; otherwise, having too many differences in the HA protein will reduce the effectiveness of the vaccine (i.e., reduced cross protection). The DIVA strategy works best when there is only one NA subtype difference from the NA subtype of the vaccine currently in the field. It is problematic, in endemic countries, where several NA subtypes of the AI virus circulate at the same time and wild bird reservoirs continue to introduce new NA subtypes. The increasing use of vaccines can create more subtypes of the virus within the bird population, thus decreasing the efficacy of the readily available vaccines through antigenic drift. This is why it is important, as highlighted in the Italy example, that a vaccine bank be created in order to quickly respond when outbreaks occur. (Lee, 2004) Unfortunately, the use of this strategy is not ideal, specifically in unindustrialized countries whose main concern is to contain the outbreak. (Peyre, 2008)

Current State of Vaccine Development in the United States

Vaccination is an effective preventative measure that could help control and eradicate HPAI infections. Within the poultry industry, “universal” vaccines do not exist for the prevention of this disease. It is clear that no single vaccine is entirely safe and/or efficacious on its own, nor is this ideal vaccine available. As of September 2015, the United States had six vaccines commercially available. Of the six vaccines, four of them are fully licensed and the remaining two have conditional licenses. Those that do have full licenses are two inactivated vaccines, a live herpesvirus-vectored vaccine and a fowlpox-vectored vaccine. The remaining two conditionally licensed vaccines are both inactivated. All of these vaccines have label claims to deliver protection within chickens, but not in turkeys. (USDA, 2015)

There are many experimental vaccines in development. The studies being performed show promise in regard to novel approaches to vaccine technology. Potential vaccines that may be developed include an inactivated vaccine developed from the most recent HPAI outbreak, an LPAI seed virus that utilizes reverse genetics, an RNA particle vaccine as well as DNA and other live-virus vectored vaccines. Promise has been shown in the development of the many of these vaccines. The inactivated vaccine has proven to be effective in preventing HPAI infections as well as reducing shedding. The LPAI seed vaccine uses reverse genetics inserting the HA molecule from the 2015 outbreak, and has provided adequate protection in both chickens and turkeys. The RNA particle vaccine has previously demonstrated protection of AI. Other potential vaccines are still under investigation. (USDA, 2015) With these studies and the current knowledge on AI, researchers are well on their way to, as Rahn et al. opines, developing “a safe and efficacious vaccine with a very broad reactivity against several subtypes, with DIVA-features and which allows mass application e.g. by oral immunization, (which) would be the perfect tool in the future.” (2015 p. 2420) While researchers are on their way to developing this vaccine technology, there is still much work to be done before this can be scientifically proven.

In the future, the ultimate goal is to develop a universal HPAI vaccine. Ideally, this vaccine would not be hindered by maternal antibodies. It would provide sterilizing immunity after one injection as well as mucosal and systemic responses. It would be protective against a broad range of AI subtypes, providing protection against many strains of the disease. Additionally, administration of this vaccine would be performed quickly and efficiently.

Chapter 5 - Future Challenges

There are many challenges facing the control and eradication of the HPAI virus in poultry around the world. Among these challenges are: 1) antigenic variation, 2) climate change, 3) intermingling of domesticated and wild birds, and 4) the current state of vaccine development.

Antigenic Variation

The classic hallmark of influenza is its ability to undergo antigenic variation. As previously mentioned over time antigenic drift and antigenic shift will allow for the adaptation of the virus and allow it to have the potential to cross species. Furthermore, antigenic shift will allows for these variations to influence host susceptibility, pathogenicity and virulence. It must also be noted that host cells that are co-infected with two different viral strains of AI virus can result in the genetic reassortment of the virus, producing an entirely new virus. (United, 2015)

Within the wild bird population, numerous AI strains exist and are continuing to interact and evolve. When a new mixed origin virus emerges, it is unpredictable in its emergence, yet not unanticipated. Antigenic variation can allow the AI virus to escape immunity previously established by vaccination or an earlier infection. Immunity induced in a bird is solely dependent upon the strain they are exposed. This can prove to be problematic when developing AI vaccine technologies. Thus the exploration of antigenic variation is imperative for the selection of strains vaccines are developed. (Smith, 2004)

Climate Change

Climate change can prove to be challenging when assessing control measures of HPAI. Research suggests that there may be a link between climate change and the likelihood of an HPAI outbreak in poultry. This can be attributed to climate change directly altering environmental conditions in which the virus can thrive within and transmit the disease. Lowen and colleagues provided experimental evidence that influenza is most favorable in conditions in which there is low relative humidity, between 20-30%, and colder temperatures, around 41 °F. Furthermore they proved that transmission of influenza was entirely blocked when relative humidity reached 80% or temperatures warmed to 86 °F. (2007) To summarize, AI can persist longer in the environment and maintain its infectivity in these climates. (Clark, 2006) Additionally, it was suggested that seasonal outbreaks of influenza could attribute to these two factors. (Lowen, 2007)

Climate change, particularly colder temperatures, could also indirectly contribute to alterations in the migration pattern of wild birds. The migration patterns, otherwise known as flyways represent the predispositions of wild birds to confine their movements within expansive geographic bounds. Birds most commonly utilize these routes when traveling from their breeding grounds to winterized areas. Globally there are nine recognized flyways, these are displayed in Figure 5.1.By altering their traditional patterns, wild birds could carry and introduce HPAI into new regions. In Russia and Mongolia, circumstantial evidence was provided that indicated the spread of disease was in fact due to the migratory patterns of wild birds. These birds became infected with HPAI and then traveled extended distances prior to dying. (Mu, 2014).

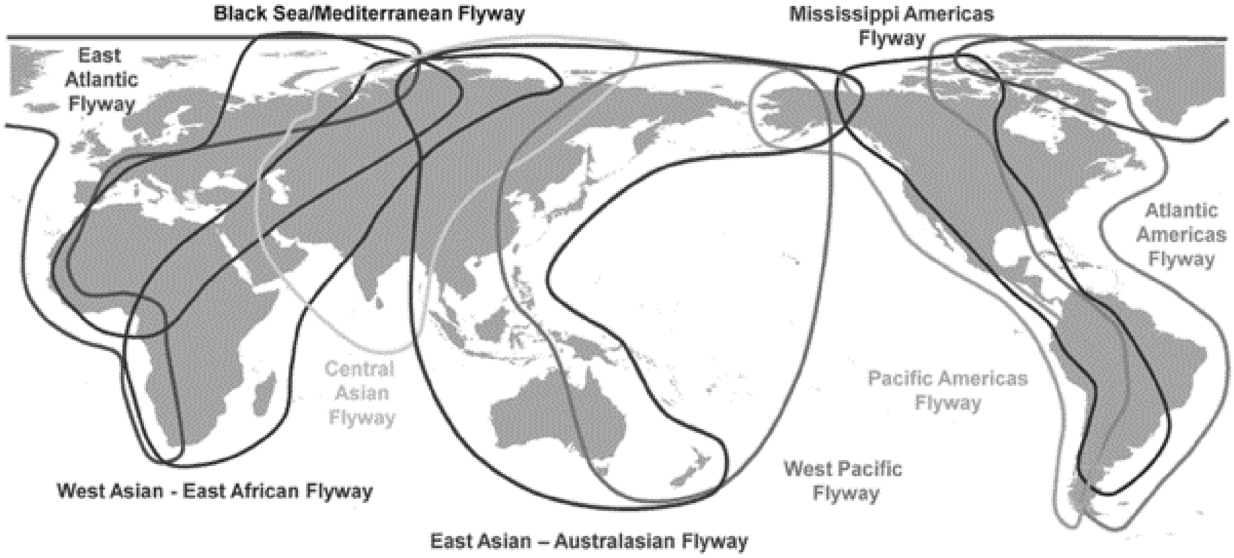

Figure 5-1 Nine major migratory water bird flyways largely based on shorebirds

Adapted from The Flyway - East Asian-Australian Flyway Partnership. (2016). Retrieved March 27, 2016, from http://www.eaaflyway.net/about/the-flyway/ Intermingling of Domesticated and Wild Birds

Intermingling of Domesticated and Wild Birds

Preventing the intermingling of domesticated and wild birds would be an effective, yet difficult in significantly reducing HPAI outbreaks. The fact that wild birds can serve as healthy asymptomatic carriers and reservoirs of HPAI presents a significant challenge in achieving this separation. This does not necessarily mean direct contact of domestic and wild birds, in fact indirect contact can prove to be just as challenging to avoid. As previously mentioned, the fecal-oral route is the most common mode of transmission of AI. Alexander and colleagues confirm this:

> "This may not necessarily involve direct contact as infected waterfowl may take the viruses to an area and these may then be introduced to poultry by humans, other types of birds or other animals, which do not need to be infected but may transfer the virus mechanically in infective feces from the waterfowl." (2007 p.5640)

Through the development of preventative measures, such as biosecurity, outbreaks initiated by the intermingling of domestic and wild birds may be significantly reduced. Possible biosecurity measures to reduce interactions between wild and domestic birds, include keeping domestic flocks either indoors or in fenced in areas. In 2012, a study performed in Chile, analyzed the segregation and biosecurity measures implement to keep backyard poultry systems separate from wild birds within the flyway. Their study concluded that most farms consisted of small flocks that used mixed/partial confinement with little to no biosecurity. If systems had been implemented there would likely be a decrease in direct contact between backyard flocks and wild birds. (Hamilton-West, 2012)

The Challenges and Controversy of Vaccines

Despite the fact that there are many benefits to developing a safe, effective and widely accepted vaccine for HPAI, there are also several challenges in its development.

As previously mentioned within antigenic variation, AI frequently goes through antigenic variation, which makes previously developed vaccines ineffective. As well as allowing those developed vaccines only effective for those strains that they are developed for. To provide an example, a vaccine for the most recently detected (January 2016) HPAI, H7N8, would not work for last year's outbreak strains, H5N8. Furthermore, Maas et al. mentioned that, "It is important to realize that when the antigen dose in an AI-vaccine is suboptimal, vaccination can result in clinical protection after a field infection, but may not prevent virus circulations within and between flocks nor potential exposure of people to this virus. Especially when immunity after vaccination in a field situation varies and a subpopulation of the vaccinated animals may not be optimally protected, virus may circulate in vaccinated flocks unnoticed." (Maas, 2009 p.3596)

HPAI vaccines may reduce sickness, clinical symptoms, and death in domestic poultry, however they may not completely protect the birds from becoming infected with the AI virus. Vaccine may not provide full protection from the disease. Previously conducted studies, have discovered unacceptable and variable vaccine protection. For example, a study was performed in Egypt HPAI H5N1 is considered to be endemic. To be more specific, in a backyard flock the protection induced by a vaccine offered as low as 1% protection and as high as 25-30%. (Capua, 2013)

Ideally, vaccination would be able to produce complete protection. Unfortunately, this can be difficult to achieve, more practicable is the ability to reduce the amount of virus excreted from the bird. Since February 2006, HPAI H5N1 severe outbreaks have been reported in Egypt. A recent study demonstrated the ability of a vaccine to reduce the amount of virus shed from infected birds. In the experiment, viral shedding was reduced for all groups when compared to those groups who were not vaccinated. (Abdelwhab, 2011) Ensuring that vaccines can decrease virus excretion to levels inadequate for transmission within poultry flocks is vital to the future development of successful vaccination programs.

AI vaccines are increasingly used as a tool to control HPAI outbreaks. Yet there is some controversy on whether these vaccines could be responsible for the further spread of the virus. Routine and improper use such as, vaccines not administered as instructed and improper storage, may contribute to the "persistence" of the virus in the field. (Roth, 2012) This could be due in part to clinical disease becoming less obvious following vaccination, and its continued unnoticed spread in the population of partially immune birds. In those countries that have approved the usage of vaccination, HPAI H5N1 has become endemic or continues to persist in the environment for lengthy periods of time. (Capua, 2013)

Stockpiling AI vaccines can prove to be challenging. For example, at this time the USDA does not approve HPAI vaccine use as a precautionary measure. Much of this has to do with economic reasons, both domestically and internationally. It is more cost effective to use the vaccine to curb current outbreaks rather than use the vaccine as a blanket preventative measure in healthy flocks. Currently, only the USDA and official state veterinarians can authorize vaccine use and monitor its administration once an outbreak occurs. These officials will take the following into consideration when authorizing vaccine use: the extent of the outbreak (including the spread rate and current response effectiveness), the poultry operations that are affected (backyard or commercial), the possible economic impact (i.e., both domestic and internal supplies and markets, and the ability to export poultry products overseas), and the availability of the vaccine. (APHIS, 2015) However, this is not with great regard for the restrictions that could be placed on imports and exports. While many countries do approve the usage of vaccines, others such as Angola, China and South Korea do not. These countries will not allow the importation of US poultry products that have been moved through regions where HPAI is thought to exist either naturally or through vaccination. This can cause severe economic distress. For example, in 2015 the ban from these three countries alone cost the US almost $700 million dollars. (Plume, 2015)

All of these challenges and/or controversies can prove to be deterring when attempting to produce a vaccine that will aid in the prevention of future HPAI outbreaks. Nonetheless, HPAI has proved time and time again that is has the potential of developing to epidemic and endemic proportions, it can lead to serious socio-economic consequences and zoonotic potential. HPAI outbreaks continue to display that further efforts are needed to control this disease.

Chapter 6 - Discussion and Conclusion

Recently, avian influenza has garnered much attention due to increased outbreaks over the last several years. To truly understand avian influenza, an understanding of its background, history and epidemiology are provided in this report. Such a synopsis is essential to set the stage for researchers to continue to combat its effects rather than allowing it to continue its economic, health, environmental and physical devastation. This devastation is not limited to poultry, but it can also affect human health due to its zoonotic potential.

Multiple preventative measures are available for use, such as biosecurity, quarantine, surveillance, eradication, vaccinations and communication/education. As previously noted, there is much focus on available vaccines and how they can be improved to create the "ideal" vaccine. There is a ways to go before this is realized; however, more promising and novel work is being performed. It is clear in the research that no one preventative measure is fully effective by itself. For example, surveillance alone would not be as efficacious without communicating the results to poultry officials at agencies such as the USDA, OIE, FAO, etc. Each approach to every outbreak should involve a multi-faceted approach using as many preventative measures as realistic in each outbreak situation.

In my opinion, there is not a single prevention method that will prevent the occurrence of HPAI outbreaks. First and foremost, there must be biosecurity measures in place. It should be noted that biosecurity does not just protect the flock. It also protects those individuals who work with the flock as well as protecting the spread of disease to wild birds. The effectiveness of other prevention methods also depends on sound biosecurity measures. Surveillance can predict the occurrence of HPAI, but it cannot solely prevent an outbreak. Eradication is the best means to temporarily rid a region of HPAI during an outbreak. Vaccination has proven to be an effective tool to preventing infection, yet there are still many challenges in its development.

While new and innovative measures, such as vaccines, are designed and implemented, researchers must take into account the future challenges of antigenic variation, climate change, intermingling domestic and wild birds, and the current challenges/controversy of vaccines. Without taking these challenges into account, the history of HPAI outbreaks will continue to repeat itself.

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Appendix A - Acronyms

AGID - Agar Gel Immunodiffusion Tests

AI – Avian Influenza

ARDS – Acute Respiratory Distress Syndrome

APHIS – Animal and Plant Health Inspection Service

AVMA – American Veterinary Medical Association

CDC – Centers for Disease Control and Prevention

DIVA – Differentiation of Infected from Vaccinated Animals

DNA - Deoxyribonucleic Acid

ELISA - Enzyme-Linked Immunosorbent Assays

EU – European Union

FAO – Food and Agriculture Organization of the United Nations

HA – Hemagglutinin Antigen

HAI - Hemagglutination Inhibition Assays

HP – Highly Pathogenic

HPAI – Highly Pathogenic Avian Influenza

IVPI – Intravenous Pathogenicity Index

LP – Low Pathogenic

LPAI – Low Pathogenic Avian Influenza

NA – Neuraminidase Antigen

NAI – Neuraminidase Inhibition Assays

NDV – New Castle Disease virus

NP – Nucleocapsid Antigen

OIE	Office International des Epizooties
PPE	Personal Protective Equipment
RNA	Ribonucleic Acid
rRT-PCR	Real Time Reverse Transcription Polymerase Chain Reaction
US	United States
USDA	United States Department of Agriculture