PREVALENCE OF INFLUENZA A VIRUS IN CHICKEN AND DETERMINATION OF RISK FACTORS OF ITS PRESENCE AND SPREAD IN UASIN GISHU COUNTY, KENYA

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DECLARATION

Declaration by Student

This thesis is my original work and has never been submitted for any academic award in any institution; and should not be reproduced in part or full, or in any format without prior written permission from the author and/or University of Eldoret.

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Declaration by Supervisors

This thesis has been submitted for examination with our approval as University supervisors.

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DEDICATION

This thesis is dedicated to my beloved husband Jeremiah and my lovely children Fortunate, Precious, Debbie and Steve for being a source of encouragement and motivation. No word can express my gratitude for them and this resulting master's thesis is a symbol of what we have accomplished as a united family.

ABSTRACT

Avian Influenza is a zoonotic disease of birds, caused by influenza A virus. It's highly contagious with high mortality rates in chicken. It is a disease of global concern spread by migratory birds from Asia and Europe to Africa. Although avian influenza a virus infection was detected in Kenya in 2005, the actual prevalence and risk factors associated with avian influenza Virus in Uasin Gishu is unknown. This study aimed at determining the prevalence and assessing risk factors that are associated with influenza A virus infecting chicken in Uasin Gishu county, Kenya. The study was conducted at the Regional eterinary Investigation Laboratories in Eldoret during the months of May and June (2020), February and March (2021) to cover the wet and dry seasons. Oropharyngeal swabs were collected from 305 sampled chicken brought in by farmers to the laboratory for screening of suspected zoonotic diseases from all the sub-counties of Uasin, Gishu County. Real-time reverse transcriptase polymerase chain reaction (rtRT-PCR) was used to diagnose the virus infection. Face to face interviews with farmers who brought their chicken for screening in the laboratory were conducted to gather information on possible confounding factors such as sex, age, breed, management system, seasonal weather variation, restocking source and vaccination status of chicken which were recorded using a structured questionnaire. The results showed that the overall prevalence of Influenza A virus in chicken in the study area was 1.3%. Out of all the possible risk factors that were assessed, there was significant difference in influenza A virus prevalence between hybrid and indigenous ($p = 0.000$), while age ($p=0.6992$), sex ($p=0.879$), management systems ($p=0.5747$), vaccination status ($p=0.81$), restocking source ($p=0.549$) and seasonal weather variation ($p=0.42$) did not affect the prevalence of Influenza A virus in chicken. The study concluded that, although the observed prevalence was low (1.3%), the presence of this highly contagious virus indicates a potential epidemic outbreak, and that the breed of chicken was a significant risk factor on the prevalence of Influenza A virus in chicken in Uasin Gishu County, Kenya. The study recommends public health education by veterinary sectors within the ministry of agriculture from the County to create more awareness to farmers on vital signs and symptoms, control and prevention of Influenza A virus among hybrid and indigenous breeds of chicken and methods of improving chicken breeds. The finding of this study are a useful guide to draw policies on detection, control and prevention of avian influenza in Kenya.

Key Words: Influenza A virus, Prevalence, Risk factors, Chicken, Diagnosis, rtRT-PCR, Uasin Gishu County, Kenya

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LIST OF ABBREVIATIONS

AI - Avian influenza

AIVs -Avian Influenza viruses

CDC -Centers for Disease Control and Prevention

DVS -Department of Veterinary services

FAO -Food and Agriculture Organization

HA -Hemagglutinin

HPAIVs -Highly pathogenic Avian Influenza virus

KEMRI/CDC -Kenya Medical Research Institute Centre for Disease Control

RVIL- Regional Veterinary Investigation Laboratory

LBMS - Live bird markets

LPAIVs -Low Pathologic Avian Influenza Virus

HPAIVs -Low Pathologic Avian Influenza Virus

NA -Neuraminidase

OI E -World Organization for Animal Health

RNA -Ribonucleic acid

RSP- Royal Society for the Prevention of Cruelty to Animals

rtRT PCR -Real time-Reverse Transcription Polymerase Chain Reaction

USA -United States of America

USDA -United State Department of Agriculture

WCF -World Compassion Farming

WHO -World Health Organization

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CHAPTER ONE

INTRODUCTION

1.1 Background Information

Influenza A virus causes the highly infectious Avian Influenza Virus (AIV) infection in poultry, posing severe economic and public-health implications (Hasni *et al.*, 2021). The virus is one of only a few orthomyxoviruses that may spontaneously infect birds and it is composed of eight different genes as mono negative-sense RNA. (Spackman, 2020). The virus circulates widely in several wild waterbirds reservoirs habitats with occasional introduced into domesticated chicken industry (Paul *et al.*, 2019). AIVs have caused significant financial losses in the chicken industry over the last 3 decades, their zoonotic potential has made them a serious veterinary and public health concern (Pusch *et al.*, 2019).Many bird species, including chickens, have been demonstrated to be susceptible to influenza A virus infection and Birds in the water act as a significant reservoirs for these viruses, however, the vast majority of isolates have been found to be of low pathogenicity (low virulence) for chicken (Yoon, *et al.*, 2014).

The virus infects poultry birds in two ways: highly pathogenic avian influenza (HPAI), which causes subtypes H_5 and $H_{7,}$ and low pathogenic avian influenza (LPAI), which is mostly caused by subtype H_9 (Peacock *et al.*, 2019). In the United States of America, the Department of Agriculture (USDA) reported an outbreak of AIV affecting almost 42.2 million birds (Firger *et al.*, 2015). Data from Thailand revealed varying rates of influenza virus prevalence and backyard chickens were found to have a higher infection rate (56%) than broilers (6%), and layers (5%) (Ali *et al.*, 2021). Confined chicken on the other hand, have a high pathogenicity viral infection because of the settings in which hens are crowded together in cages and large warehouses. The virus was particularly prevalent in high stocking, heavy poultry and egg production farms, where subtype H_5N_1 had evolved remarkable virulence among chickens (Fasanmi *et al.*, 2017).

Various AIVs infestations was documented in Pakistan, affecting commercial and backyard chicken populations (Kausar *et al.,* 2018). Most poultry farmers in the United State of America have biosecurity knowledge of avian and pandemic influenza, but the Midwestern epidemic of 2006 showed that the existing controls were insufficient (USDA-APHIS Veterinary Services, 2006). Large virus loads of influenza have been found in commercial poultry production facilities using severely confined and overcrowded chicken management systems. A virus can easily be transmitted from one bird to another, enabling the birds to acquire adaptable variations that cause high infection rates (Beato *et al.,* 2009). However, HPAI H_5N_1 outbreaks appeared to be intermittent, with no outbreaks during the hot summer season, implying the existence of an environmental reservoir that keeps H_5N_1 viruses alive during the summer (Hasan *et al.*, 2019).

Very little is known about the immunity that develops with age in the virus's main reservoir, wild birds, despite the significance of this information in understanding the behaviour of avian influenza viruses (Hill *et al.*, 2016). The age-related frequency of AIVs, however, differs based with the largest isolation rates found in young birds (Stallknecht *et al.,* 1988). Because they enable the reassortment of AIVs adapted to various host species, markets with their daily intake of new birds, serve as significant farm surrogate reservoirs in low-income nations like Kenya (Otte *et al.,* 2017). It is critical to identify these routes in order to design effective prevention and control strategies (Paul *et al.*, 2019).

Additionally, there are numerous accounts of H_5N_1 in poultry in Africa, including Egypt, Nigeria, Sudan, Ghana and Kenya, since the original 2006/2007 outbreak of influenza A virus in chicken in Nigeria (Monne *et al*., 2008).The infection spread to Burkina Faso, Niger, Benin, Ivory Coast Djibouti, Cameroon, and South Africa (Ofula *et al.*,2013). South Africa has had several epidemics of various influenza A subtypes in chickens (Abolnik *et al*.. 2010), Although avian influenza viruses (AIVs) have not been widely detected in Kenyan poultry populations,

risk evaluation studies done in 2007–2008 in reaction to the potential of the spread of highly virulent avian influenza in 2005 (HPAI) H_5N_1 into the poultry population demonstrated that if the virus was introduced, there would be a considerable potential of spreading (Omiti *et al.*, 2008).

When correctly implemented with accurate epidemiologic investigation and control methods, vaccination policy may be a viable option for preventing IAVs. Chickens are protected against highly pathogenic avian influenza viruses by use of only one dose of multi-clade virus-like particle vaccine (Kang *et al.*, 2021). Vaccination in China minimized the impact of outbreaks, which is a positive sign. In 2017, China set a requirement for all poultry to be vaccinated against a H_7N_9 strain that has the ability to transmit to humans. Through vaccination, the virus's prevalent infections were eradicated. (Stokstad *et al.*, 2022). Because of antigenic drift of field viruses and loss of vaccination protection, inactivated vaccine seed strains have been replaced twice since 1994 with inactivated oil-emulsified AI vaccination employing the officially certified vaccine seed strain (Swayne *et al.*, 2016). In places with a large concentration of small-scale poultry keepers that raise birds in "traditional" ways primarily for household consumption, publically supported mass vaccination campaigns against HPAI are expensive and seem unsuccessful (Otte *et al.*, 2017).

In partnership with the Kenyan Department of Veterinary Services (DVS), the Kenya Medical Research Institute Centre for Disease Control and Prevention Kenya (KEMRICDC-K) launched a surveillance to assess the existence of avian influenza. A virus was found in birds traded in live bird markets (LBMS), Influenza A virus was discovered with a prevalence of 0.8% of chicken sampled (Munyua *et al.*, 2013).

In 2005, the fear of Highly Pathogenic Avian Influenza (HPAI) caused by H_5N_1 virus infection caused the Kenyan people to lose an estimated KSh2.3 billion in poultry production (OIE,

2015). Kenya is highly at risk of being infected with influenza A (H_7N_9) virus, which was discovered in China where it had a widespread in poultry and other species, (Ngotho, 2014).

Therefore the spread of influenza A virus in chicken from Asia and Europe to Africa countries including Kenya gives an opportunity for the spread of the virus to many regions in the country including Eldoret in Uasin Gishu, County. No study has been undertaken in this region regarding the risk of disease posed by the activities of the international airport. Kenya has little data on the surveillance of Influenza A virus in chicken with limited knowledge on potential risk factors, thus, the actual prevalence and risk factors of influenza A virus in the regions is not yet established. The ~~present~~ study set out to determine the prevalence of Influenza A virus and assessed the risk factors associated with the virus such as age, sex, breed, vaccination status, restocking source, management systems of chicken and seasonal weather variations on the prevalence of influenza A virus in chicken in Uasin Gishu, County, Kenya.

1.2 Statement of the Problem

Avian Influenza caused by influenza A virus is a threat to poultry production in Kenya as indicated by previous risk assessment (Omiti *et al.,* 2008; ILRI, 2010).The poultry farmers in Kenya are highly susceptible to the introduction and spread of the highly pathogenic avian influenza (HPAI) due to the country's location along key wild bird's migratory routes and the absence of strong mechanisms to deal with the outbreak of the disease (Thurlow, 2010). Clinical and post-mortem manifestation of H5N1 is identical to that of Newcastle disease and farmers may not know the difference. The fact that AIVs is zoonotic makes it more dangerous disease in both poultry and the people handling or eating the poultry or its products.

AIVs has been detected in Uasin Gishu County by the RVIL but a proper prevalence study has not been undertaken and the risk factors. Therefore, a research study on prevalence of influenza A virus and risk factors on chicken screened at the RVIL Eldoret, Uasin, Gishu County is very important in understanding its occurrence. Such knowledge is useful in directing focused

interventions by recommending proper biosecurity management of chicken in Eldoret Uasin Gishu County.

1.3 Justification of the Study

The global chicken industry has showed considerable promise for increasing and economically producing food to address national concerns with food security. The production of chicken has greatly contributed to economic growth in Kenya. The infection of chickens with highly pathogenic and lowly pathogenic avian influenza viruses has posed a danger to the poultry industry, causing significant losses in chicken production. In Kenya including Uasin Gishu County region, large-scale and small-scale chicken rearing are still among the most popular farming activities for people living in rural and urban areas where they produce 80% of the national poultry production (Miriam *et al* ., 2014).

The knowledge of the prevalence of AIV and the risk factors that contribute to its infection and spread determination among chicken reared in Uasin Gishu is desirable. The finding will assist veterinary department in laying down preventive measures that will contribute towards poverty reduction among poultry farmers through proper management. Avian influenza is a zoonotic disease; therefore managing it will reduce transmission from chicken to human. This study aimed at determining the prevalence of influenza A virus in Uasin Gishu County and assessing the risk factors that are associated with its occurrence in chicken. The result of the study will assist in providing recommendations on management of the disease in both poultry and man.

1.4 Objectives

1.4.1 General objective

To investigate the risk factors associated with the prevalence of influenza A virus in chicken screened at the regional veterinary investigation laboratory Eldoret, Uasin Gishu County, Kenya

1.4.2 Specific objectives

i. To determine the prevalence of influenza A virus in chicken screened at the regional veterinary investigation laboratory Eldoret, Uasin Gishu, County Kenya.

ii. To assess the risk factors associated with outbreak and spread of influenza A virus in chicken screened at the regional veterinary investigation laboratory Eldoret, Uasin Gishu County.

1.5 Null hypotheses

i. There is no influenza A virus in chicken screened at the regional veterinary investigation laboratory Eldoret, Uasin Gishu, County Kenya.

ii. There are no risk factors associated with Influenza A virus prevalence in chicken screened at the regional veterinary investigation laboratory Eldoret, Uasin Gishu County, Kenya.

CHAPTER TWO

LITERATURE REVIEW

2.1 The Influenza A virus and the disease it causes

Influenza A virus is a segmented, negative sense, mono-stranded RNA virus that belongs to the Orthomyxoviridae family. It's a strain of H_5 and H_7, of the avian influenza virus (A) and are very lethal in birds, (Poovorawan *et al.,* 2013). According to the antigenic associations between their membrane-bound surface, influenza "A" viruses are categorized by their glycoproteins, haemagglutinin (HA), and neuraminidase, further categorized by the antigenic connections between the surfaces of glycoproteins, haemagglutinin, and neuraminidase subtypes (Fouchier *et al.,* 2005). The inner membrane is covered with two different glycoprotein spikes and projections that connects to the haemagglutinin and neuraminidase activities of the virus (Fodor *et al.,* 2020). It has helical symmetry and is surrounded inside a protein matrix. Eight single-stranded RNA segments make up the segmented genome of the influenza virus, which is enclosed (Laksper *et al.* 2014). According to the stated process of transcription and replication, the virus enters the cell through endocytosis after being identified by receptors for sialic acid coupled to galactose. The eight viral RNA segments separate from their lipid sheath and go into the cell nucleus where they begin to replicate and be transcribed. The host's mRNA serves as the source of the primers that are used to create the vRNA. The Golgi apparatus is where the proteins HA, NA, and M2 mature before moving to the cytoplasm. In the cytoplasm, where they start the viral assembly, the other vRNA segments also travel. Finally, they take use of the cell membrane to create viral envelope and bud within extracellular environment (Madsen *et al.,* 2018).

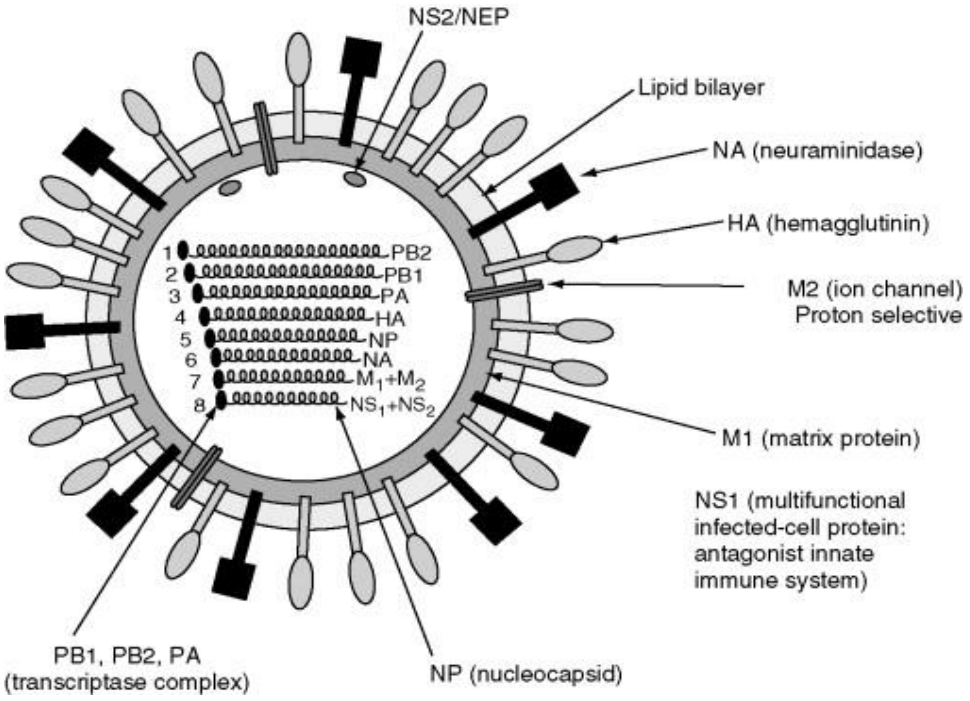


Figure 2.1: Influenza A Virus morphology - Source-(Fodor *et al.*, 2020)

Numerous influenza strains and subtypes can be found in the primary reservoirs, and domesticated ducks raised in constructed wetlands serve as the logical link between those reservoirs and other domestic poultry, wild birds are known to carry influenza viruses, especially migrating ducks and geese (Ahrens *et al.*, 2022). Although the majority of the 16HA and 9NA influenza A subtypes cause asymptomatic infections in avian hosts, the H_5N_1 and H_7N_8 subtypes can develop into HPAI strains through the acquiring of a novel variable region that allows maturation by ubiquitous host proteases and spread of the virus outside the respiratory and intestinal epithelia to multiple organs, including the brain. These infections can be fatal in chicken, turkey, and some breeds of domesticated duck (Hulse-Post *et al.*, 2005).

In "backyard farming," Influenza virus spreads very fast in chicken population, when they are reared alongside one another and in live poultry markets, later due to lack of hygiene and viral, transfer to large bird farms may occur (Fournie *et al.*, 2013). Domestic poultry are typically exposed to avian influenza virus either directly or indirectly through contact with ill birds due to the closeness of the chickens, avian influenza viruses are passed in the fecael and respiratory

secretions causing illness to spread very quickly in the farm by fecael-oral route transmission (Alexander *et al.,* 2007; Swayne *et al,*. 2016). The albumen and yolk of eggs from chickens, turkeys, and quail have been found to contain highly pathogenic viruses, and a tiny portion of the flock may excrete low pathogenic avian influenza viruses for up to two weeks. Additionally, the movement of infected birds, contaminated equipment, and contact with contaminated infectious organic material all contribute to transmission (Ssematimba *et al.,* 2013).

2.2 The behaviour of the disease in susceptible population including man

Influenza A virus infection vary depending on the environment, age, the presence and co-existence of other diseases, and the aggressiveness of the viruses with regard to the affected species (Tombari *et al.,* 2013). Highly pathogenic avian influenza causes birds to develop enlarged heads, a blue comb and wattles, lethargy, lack of appetite, respiratory discomfort, diarrhoea, and a significant drop in egg production (Dudo *et al* ., 2007). However, the low pathogenic avian influenza virus may cause no symptoms or just moderate ones such as ruffled feathers and a decrease in egg laying. The majority of avian influenza A viruses are also low pathogenic and only occasionally infect wild birds, which results in few symptoms of the disease and some viruses can evolve into extremely dangerous avian influenza viruses in poultry (Rebel *et al.,* 2011). The common signs in poultry are depression, loss of appetite, a reduction in egg production, nervous behaviours, swelling and blue colouring of the combs and wattles due to blood circulation issues, coughing, sneezing, and diarrhoea, it's possible to pass away suddenly with no warning, the fatality rate may be as high as 100% based on the species, age, type of virus involved, and environmental conditions such recurrent bacterial infections (Capua *et al.,* 2006).

The severity of the viral infection can range from mild to more rapid; the lethal virus strains can sometimes kill young chickens suddenly without causing any other clinical symptoms and similarly, influenza A virus that quickly causes death in birds tend to produce little symptoms than when the viral dose allows the birds to survive for a long period (Hinchliffe, 2015). In addition to respiratory distress, swelling, purple discoloration of the head, comb, wattles, and neck, coughing, sneezing, or rasping respiration, a sharp drop in feed intake, water intake, and egg production, as well as ruffled feathers, drowsiness, closed eyes, and diarrhoea in chickens (Lawes-Wickwar *et al*., 2021). Avian influenza symptoms can also include respiratory distress (Lierz and Heffels-Redmann, 2019). Clinical symptoms of LPAI includes a drastic reduction in feed consumption, water usage, and egg output, ruffled feathers, drowsiness, and closed eyes, as well as the 3–15% fatality rate in small segments of chicken flocks (Cameroon, 2012).

Figure 2.2: Chicken with H_5 N_1 highly pathogenic Influenza A virus symptoms on the head (Lawes-Wickwar *et al*., 2021).

Figure 2. 3: Chicken with H5 N1 highly pathogenic Influenza A virus symptoms on feet (Lawes-Wickwar et al., 2021).

Due to their alleged capacity to infect people, it is also recognised that many H9N2 LPAIV lineages pose a risk to the public health and environment (Pusch and Suarez, 2018). There isn't any conclusive proof that migratory birds disseminate the H_9N_2 virus internationally; instead, commerce and transit of live chickens may help the virus spread (Lee *et al.,* 2017)

Since its discovery in Libya in 2006, the G1 lineage of H_9N_2 LPAIV has spread throughout Africa. Since then, reports of the G1 lineage viruses in poultry populations have been made in Egypt, Burkina Faso, Tunisia, Morocco, Ghana, and Uganda (Awuni *et al.,* 2019;). AIVs was found in 0.8% of the chickens collected at Kenya's live bird markets, according to earlier AIVs surveillance, however no virus was successfully isolated from any of the birds, and the detected samples were not further subtyped (Munyua *et al*., 2013).

2.3 The Prevalence of Influenza A virus

The avian influenza A virus was originally identified in 1996 (Duan *et al.,* 2008). It spread to more than 60 nations and has been circulating among many bird species for more than two decades (Li *et al.,* 2020). Domestic poultry is a significant factor in the transmission and spread of H_5N_1 AIVs as well as the virus's tendency to occasionally infect people, posing a serious

threat to public health (Van Kerkhove *et al.,* 2011). Because of the virus' longevity in domesticated poultry hosts, it is possible for new avian influenza viruses to evolve (Li *et al.,* 2022). Recently, two H5 AIV subtypes have re-emerged in Asia, including H_5N_8, which caused epidemics in local poultry after spreading to Europe, North America, and Africa (Naguib *et al.,* 2019)

AIVs transmission and the trading of live chickens with other associated poultry foods have been the subject of prior investigations, but there are few empirical data on the dynamics of transmission (Moyen *et al.,* 2021). Despite the fact that many retrospective epidemiological studies have evaluated the typical risk factors for AIV outbreaks, only a small number have incorporated poultry trade patterns, which are regarded as being challenging to gather (Gilbert and Pfeiffer, 2013). By compiling the travel paths of traders among live poultry markets, chicken trading networks were examined at the local level, and it was discovered that these trade patterns are related to AIVs outbreaks (Martin *et al.,* 2011). Other researchers have used Bayesian phylogeographer methods to analyse AIV genome sequences and have discovered that the number of poultry markets and the density of poultry populations are factors that affect the spatial dissemination of AIVs in endemic areas (Yang *et al.,* 2019). IAVs is a viral disease that is highly infectious and affects several species of birds, including chickens (Kuchipudi *et al.,* 2014). Low pathogenic avian influenza (LPAI) viruses induce a mild sickness with ruffled feathers and a decline in egg production that may go undetected but highly pathogenic avian influenza (HPAI) viruses are known for their propensity for rapid propagation, catastrophic illness outbreaks, and high rates of poultry mortality (Globig *et al.,* 2018).

2.3.1 Prevalence of Influenza A virus in Chicken in Developed Countries

After first appearing in late 2003 in Vietnam and Thailand and causing substantial mortality in affected flocks, the virus returned in 2004 and 2005 and was deemed endemic in the region (Pfeiffer *et al.,* 2010). The United States Department of Agriculture (USDA) verified in 2014

that domestic birds in the country have H_5N_1 viruses associated with highly pathogenic avian influenza (OIE, 2015). Since 2003, a type of highly contagious avian influenza (H5N1) has swept throughout Asia and then entered Europe. Over 250 million chickens have perished or been killed worldwide due to this virus, which has primarily attacked poultry and wild birds. Migrating birds from Asia could spread the infection to Alaska and infect birds from the Americas on common nesting sites. The newly sick birds can spread the disease through canal towpaths in North America (Monke *et al.*, 2007).

A total of roughly 42.2 million birds got diagnosed with the avian flu virus following the reports of avian influenza outbreaks by the U.S. Department of Agriculture (USDA) received from May 2014 onward, largely from farms in the Midwest (Hurt *et al,*. 2014). And over 60 virus detection reports were sent to the USDA from the Iowa region, the biggest egg producer in the country, with more than 28.1 million chicken, as a result of other farms from various regions being affected by the outbreak that also started in December 2014 and leading to the multi-state outbreak (Firger *et al,*. 2015). In addition, incidences of the H_5N_1 in the US and Canada, a large number of flocks have now been found to be infected with the avian influenza virus (Pa'gina *et al.*, 2015). Additionally, in 2003, the influenza A virus re-emerged as a significant pathogen with a high epidemic that was unheard of in scope and geographic reach, infecting poultry in a number of Asian nations, including Cambodia, China, Indonesia, Japan, Laos, South Korea, Thailand, and Vietnam (Sampathkumar *et al* ., 2005).

However, HPAI subtype H_5N_1 appeared to be more virulent in poultry, and it was present in populations of domestic and wild birds in Asia and Europe (Lee et al., 2017). Avian influenza, a highly contagious form of poultry virus, was originally discovered in Guangzhou Province in southeast China in 1996. In 1997, a virus became a potentially fatal animal disease after internal genes from the H_9N_2virus were isolated from domestic quail (Nguyen et al., 2005). In addition, Pakistan had numerous outbreaks of the highly virulent variant H_7N_3 from 1995 to

2003 caused 3.2 million birds to perish and 1999, the H9N2 virus was responsible for epidemics of avian influenza in young and laying birds in Pakistan (Sarwar *et al.*, 2013).

The H5N1 virus has indeed been recurring at sporadic intervals throughout all continents, with the most major outbreaks being reported in South East Asia, Chile, Chile in 2002, and Hong Kong in 2003. 2004-2006 (Swayne *et al.*, 2016).

2.3.2 Prevalence of Influenza A virus in Chicken in Africa

Although there is very little information on influenza A virus infections in chickens in Africa, highly pathogenic avian influenza H_5N_1 had first been discovered there in Nigeria in 2006 (Metras *et al.*, 2013). In 2006–2007, the nation got plagued down by serious outbreaks that had a negative impact on the poultry population (Metras *et al.*, 2013); Monne *et al*., 2008). The first H_5N_8 HPAI virus detections were reported in Egypt and Tunisia in November 2016, and Nigeria, Niger, Cameroon, and Uganda in January 2017 (Sims *et al.*, 2017). There is ample evidence to conclude that seasonal migration of wild aquatic birds, particularly that of Palearctic dipping ducks, was the key cause of the transcontinental spread, based on prior incidents utilizing a different comparable H5N8 HPAIV strain in 2014-2015 (Global Consortium for H_5N_8 and Related Influenza Viruses) (Lycett *et al.*, 2020).

Following the first HPAI incursion ever documented in Eastern Africa, series H5N8 HPAI outbreaks were also noted in the Democratic Republic of the Congo's north-eastern region in April 2017 (Roche *et al,*. 2017). Up until this time, the seasonal migration of Palearctic waterfowl and virus spill over from wintering ducks to what has also happened in Europe, populations of Afro-tropical aquatic birds and fowl, may have been used to explain the virus's spread (Napp *et al.*, 2018). On the shore of Lake Albert in the Democratic Republic of the Congo, the disease first appeared in domestic poultry in April 2017. By June, the situation had changed into a typical chicken pandemic, with clusters of affected areas (Wade *et al.*, 2018).

Uncertainty surrounds whether the cases in the Democratic Republic of the Congo in April represented the first incidences or whether there were further cases that hadn't been discovered (Isoda *et al.,* 2020).

With epidemiological indications of spill over within a wetland leak, the HPA H5N8 haplotype was discovered on May 17, 2017, at Harare, Zimbabwe, at a factory that breeds commercially broilers (Tehrani *et al,*. 2021). The town of Villiers, which is also close to a waterway, the Vaal River, was where the first outbreak in South Africa was discovered on June 19, 2017 (Roche *et al.,* 2018). Although no epidemiological connections to earlier outbreaks could be found, H_5N_8 HPAI also resulted in fatalities at an industrial breeder facility in Standerton, 35 miles distant (Roche *et al.,* 2018). Furthermore, the Rivers region of Nigeria has had two further outbreaks of highly virulent avian influenza; these outbreaks, which both involved broilers and layers of unknown age and affected farms, were both of the H_5 N_1 serotype, which has been ravaging West Africa for a while (Sulaiman *et al*., 2021). In Khartoum the results of prevalence showed that 19 serum samples out of 160 sera (11.9 %) from layer chicken were positive while 141 sera (88.9 %) were negative. In broiler chicken, 1 serum samples out of 90 sera (1.1 %) as positive while 89 sera (98.9 %) were negative (Mohammed, 2008)

According to reports, Tanzania experienced a bird flu outbreak that reduced poultry production and killed millions of birds nationally (Sonaiya *et al.,* 2007). In Uganda prevalence was 0.4 % in a chicken population of 1865 where 7 tested positive, for the wet season prevalence was 0.2% in the entire poultry and 1.4 for the wet season (Kirunda *et al .,* 2015)

2.3.3 Prevalence of Influenza A virus in Chicken in Kenya

Indigenous chicken (*Gallus domesticus*), Chicken production systems especially the backyard chicken farms in rural Kenya are dominated by bantams of diverse origins, Mediterranean egg

types, and unremarkable combinations of Asian meat and game types, being responsible for about 50% of egg output and for more than 80% of the chicken populations in most African nations, including Kenya, the production of chicken is important to household and national economies (Kemboi *et al.,* 2013).

The first H_9N_2 LPAIVs identification from Kenya in 2013 was confirmed to be contagious and spread by direct contact in hens, posing a new hazard to poultry and maybe to humans (Kariithi *et al,.* 2020). The characterization of influenza A viruses in domesticated animals in kenya, discovered a sera-prevalence of 0.1% in chickens sampled at live bird markets in Nairobi (Munyua *et al.,* 2015).

A risk evaluation conducted after the threat of HPAIV's entrance to the nation in 2005 revealed a sizable risk of avian influenza A virus transmission into chicken farms in Kenya (Omiti *et al.,* 2008). However, The Kenyan government issued a warning about the dangers of the virus following an outbreak of the deadly H5N1 virus in southern Sudan that caused the deaths of many birds in the provinces of Khartoum and Jazeera (Mohammed, 2008). To combat the avian influenza virus, Kenya as a nation established a ban on the importing of chicken between the two nations (Omiti *et al.,* 2009). Chicken in Kenya have been found to have influenza A virus that was discovered with a prevalence of 0.8% in chicken sampled at live bird market (Munyua *et al.,* 2013).

However the information on the avian influenza virus in Kenyan chicken is very limited. The Kenya Medical Research Institute Centers for Disease Control and Prevention - Kenya (KEMRICDC-K) and the Kenya Department of Veterinary Services (DVS) started surveillance to evaluate the positivity of avian influenza viruses in birds that were sold in live bird markets in Kenya and influenza A virus was detected at a rate of 0.8% in chicken samples (Munyua *et al.,* 2013).

2.3.4 Diagnosis of Influenza A Virus infection in Chicken by use of rtRT-PCR

Real-time reverse transcriptase polymerase chain reaction (rtRT-PCR) assays were developed employing hydrolysis probes to identify influenza A viruses in chicken secretions as a result of the increased public understanding of the danger of an influenza outbreak (Pabbaraju *et al .,* 2011). The technique was developed for single-tube reverse transcription-PCR and employed sets of primers based on highly conserved sections of the matrix gene for the identification of influenza A viruses from distinct species (Qiu *et al.,* 2009). It was totally reactive with a panel of 25 genetically varied bird virus isolates, including all known influenza A virus subtypes, and it was more sensitive than other tests, chicken swab samples proved that rtRT-PCR was quicker and or accurate than other processes (Chaharaei *et al.,* 2009).

2.3.5 Control and Prevention of Influenza A Virus infection in Chicken

Capua *et al.,* (2006) observed the huge rise in avian influenza epidemics over the past few year, with epidemics affecting 200 million birds between 1999 and 2005, up from 23 million in 1959, 1998, and 2005. The outcome has been huge economic losses for both the public and commercial sectors (Capua *et al.,* 2006). Therefore, a variety of tactics have been used globally to prevent and manage avian influenza virus, the most crucial preventive measure is surveillance, which allows for prompt discovery of virus variations and assessment of the present contamination situation to enable the implementation of efficient virus control methods (Wang, *et al.,* 2017).

Infected chickens should be quarantined, depopulated, and disposed of inside the quarantine area using composting, burning, or burial in line with environmental regulations and legislation. Before being removed from the sick farm, equipment needs to be cleaned and disinfected. Only once the illness has totally been resolved and the viral excretion titres have decreased may non-infected birds can be relocated. Additionally, HPAI outbreaks necessitate

programs for killing diseased hens; this is the greatest line of defence against the disease's spread and eradication (Swayne *et al.,* 2011). The OIE has created guidelines for depopulating poultry in contaminated areas (Alexander *et al*., 2003). It is crucial to prevent and manage HPAI in poultry ((Fasanmi *et al.,* 2017). Reduce the flow of people, vehicles, or equipment into and out of places where poultry are housed when there are diseased wild birds in the vicinity. Make sure guests change their clothes before and after contact with the flock, and disinfect frequently (Suarez *et al.,* 2005). Highly pathogenic bird flu strains have been contained by control measures, which should also entail eradicating sick flocks and immunizing healthy birds (Trampuz *et al.,* 2004)

2.4 Intrinsic and Extrinsic Risk Factors Associated with Influenza A Virus Infection in Chicken

Extrinsic (environmental) factors like restocking source, management system, and seasonal weather variation, as well as intrinsic (host factors) factors like breed, age, vaccination status and sex of the chicken have also been studied in relation to the ecological risk factors associated with the effective transmission of AIVs (Koutsos *et al,* .2014). In order to better understand the prevalence of influenza A virus and its control, it is important to understand the elements that influence virus infection in poultry. Host and environmental characteristics of viral infections are valuable in forecasting disease incidence (Robertson *et al.,* 2020). People who are frequently exposed to processing plants, poultry houses, and meat processing facilities where poultry is handled are at a greater risk of getting the avian influenza virus (Nyaga *et al.,* 2007). According to a study by: Gharieb *et al.,* 2019 on the risk factors in Egyptian poultry farms, the presence of the highly pathogenic AI H_5 virus was most frequently influenced by chicken breed, worker movement between flocks, a lack of utensil disinfection, and the introduction of new birds to the farm. The transmission of the influenza virus in poultry has been identified to be influenced by a variety of factors for instance raising various breeds of free-range poultry

and residing close to polluted commercial farm buildings both enhance the risk of illness (Fasina *et al.*, 2011).

2.4.1 Effect of Breed

Breeder, broiler, and layer poultry farms are all present in the Netherlands. The farms are anticipated to have a greater risk of acquiring the avian flu virus because outdoor farming increases the likelihood that LPAIVs would spread to domesticated birds from wild birds (Gonzales *et al,*. 2013). Among Pakistan poultry, severe outbreaks of highly deadly avian influenza killed 3.2 million birds by 1999, primarily in layers (Sarwar *et al.*, 2013). In the Iowa region of the United States, another incident of virulent H_5N_2 avian influenza was discovered in a breed of layer chickens with broiler and layer breeder farms being more severely affected by the avian influenza virus (Zhao *et al.*, 2019). In 1983, a highly virulent avian influenza virus infection with a variety of symptoms affecting Pennsylvanian broilers and layers which included Serosal petechiae and unpleasant lesions in broilers and in layers there was Vesiculation, necrosis, and comb edema, as well necrotizing pancreatitis, with external ocular muscles and limbs in local chicken (Acland *et al.*, 1984).

In Ghana, low egg production layer birds in farms with affected broilers and layers, including day-old chicks and layers older than 21 weeks was associated with a viral antibody prevalence that was high for breeder and layer chicken (Asante *et al,*. 2016). Between 2006 and 2008, Commercial breeds of layer, cockerel, and broiler chickens in Nigeria infected with the HPAI H5N1 virus had mortality rates of 11.11% for layer breeds, 45.51% for cockerel breeds, and 73.92% for broiler breeds (Akanbi *et al.*, 2014).

When the first H_5N_1 -positive local poultry bird was discovered in Uganda, individuals responded to the risk posed by poultry, but they underreacted to the pandemic risk. Prior to the

discovery and isolation of the first virus in local birds, nobody knew that local birds posed a concern for the H_5N_1 pandemic (Sandman *et al*., 2006).

Around 16 million day-old chicks are produced annually in Kenya by big farms breeding broilers and layers. Few Kenyans are involved in the commercial poultry industry than in raising local chicken, nevertheless. This suggests that the consequences of avian flu on livelihoods are likely to arise through effects on locally produced poultry feed and native breeds acting as the risk factors (Thurlow *et al*., 2010).

2.4.2 Effect of Age

In Alaska, the prevalence of AIV infection more often than not, adult birds significantly had higher AIVs seroprevalence than juvenile birds due to their generally low immunological state, which makes them more likely to be vulnerable to infection and have higher shedding rates (Cheema *et al.,* 2011). The significant discrepancies between adults and sub-adults point to either significantly different rates of annual encounter with highly virulent avian influenza (Wilson *et al,*. 2013). The bird flu outbreak in Iran affected broilers between the ages of 3 and 7 weeks with clinical signs of Anorexia and decreased water intake followed by sadness, coughing, sneezing, and dyspnea (Nili *et al.*, 2002).

Due to their underdeveloped immune systems, younger hens are more susceptible to contagious diseases than older ones, age had little effect on the expression of the majority of host factors that interact with viral components to prevent virus replication, suggesting that the intensity of host transcriptional responses may play a significant role in age-dependent vulnerability to bird flu infection (Reemers *et al.,* 2010). However sometimes older birds are more susceptible to AIVs than are younger bird and recovering birds grow poorly in the future (Cheema *et al.,* 2011). Furthermore the viral infection has been reported to be common in flocks of 60-week-

old birds than in flocks of 3 week-old birds, according to a review on the disease (Nooruddin *et al.,* 2006).

2.4.3 Effect of sex

The avian influenza virus predicted differential infections probabilities, with female chicken being less likely to test positive than males which may be due to increased testosterone levels during the breeding season, which has been shown to impair males' immune systems, making them more susceptible to the virus (Farnsworth *et al.,* 2012). A newly re-emerged AIV strain A/chicken/Vietnam/AI-1606/2016 (H5N6), that has been positively identified as being a member of clade 2.3.4.4 H5N6 highly pathogenic virus, was isolated from female chickens in the Vietnamese poultry market (Le *et al.*, 2021).

Additionally, females had a little lower likelihood of having the H_9N_9, H_6N, or H_5N_2 viruses in their system, this was according to some sex-specific seroprevalence which may be caused by inborn differences in immunological function or antibody persistence (Hill *et al.,* 2016)

2.4.4 Impact of Seasonal Weather Variation

The distribution and pressure of infections and diseases affecting the hosts will be affected by changes in average temperatures, rainfall, and climate extremes, some infectious diseases, like Newcastle , avian influenza, infectious bronchitis, and infectious bursal , are more likely to spread in cold temperatures (Olabisi *et al.,* 2021). Seasonal influenza virus outbreaks can occur at different times around the world, with the bulk of cases in temperate nations occurring in the winter and in tropical regions during the rainy season (Hirve, *et al*., 2016). Research using animal models has demonstrated that lower temperatures in temperate countries are more favourable for the respiratory droplet dispersion of seasonal influenza viruses (Lowen, *et al.,* 2007).

On the other hand, in many tropical regions, influenza virus outbreaks happen during the local rainy seasons (Petrova *et al.,* 2018). This may assist to explain why influenza virus outbreaks differ in their seasonality between temperate areas in the Northern and Southern Hemisphere (Weber *et al.,* 2008). If this is the claim, more people are exposed to contact transmission in cold conditions, where the beginning of epidemics has also been linked to fluctuations in temperatures (Deyle *et al.,* 2016).

Additionally the effects of temperature on HPAI H_5N_1 outbreaks, nevertheless majority of research have highlighted the significance of bird migration, poultry transportation, chicken products, vegetation zones, and human activities (Cox *et al.,* 2000). Because the cold weather and temperature fluctuations are the main reasons why the viral avian flu tends to peak in the winter. In contrast to hot climates, the virus persists better in cool temps. In other countries, avian influenza epidemics also rise throughout the winter months (Liang *et al.,* 2020). In Germany, the HPAI H5N1 virus in poultry has been noted twice, once in the middle of winter 2006 and once in the middle of summer 2007, as a result, changes in the distribution of climate change brought on by more frequent droughts or floods will indirectly alter the distribution and abundance of the millions of birds raised and may have a significant effect on the distribution of the risk of HPAI persistence (Gilbert *et al.,* 2008).

However, at high temperatures, absolute humidity positively affects influenza, which means wetter conditions improve the survival of the virus when it is warm. This was the case during the avian flu outbreak in India, where thousands of birds were being slaughtered in Kerala and zoos and other states were closed. (Wood *et al .,* 2010).However, the bird flu viruses first case of a pandemic was discovered in Hong Kong during the cold season (Cox *et al.,* 2000; Zhang *et al.,* 2014), when chicken immunity could rise due to the cooler temperatures and mostly due to the rainy seasons. There were several outbreaks in South Africa during the cold of April and May 2021 of the extremely contagious avian influenza (H5N1) at various chicken farms

(Uwishema *et al.,* 2021). The virus spread to Kenya, a tropical country, influenza Virus incidence often peaks in the wet months of March-April, October-November, and the chilly month of July, which broadly coincides with the southern hemisphere winter (Matheka *et al.,* 2013).

2.4.5 Impact of Management system

The influenza A virus load may vary depending on environmental risk factors for each bird (Abdelmohsen, 2012). Most recent avian flu outbreaks have mostly been attributed to the restricted practices employed in chicken rearing (Sims *et al.,* 2008). Infection outbreaks are made easier to spread by the frequent overcrowding of hens in comparably smaller cages and lengthy sheds that can house thousands of chickens. The chicken are exposed to the ammonia fumes from the collected decomposing feces all day (Lam *et al.,* 2008). The hens' natural immune system is weakened by the constant stress of the rearing procedures, leaving them extremely susceptible to disease (Jin *et al.,* 2011). Intensive production practices, hens are kept in overcrowding conditions, which limits their capacity to exercise, forage for food, and dust-bathe (Hemsworth *et al.,* 2021).Due to this, hens experience severe physical and emotional distress, which increases their risk of contracting a disease (Weeks *et al.,* 2017).

Since the HPAIV discovered in free-range chickens is typically linked to the spread of the poorly pathogenic avian influenza virus from wild birds, rearing practices in confined and free-range farms have been regarded a risk factor that causes influenza A in chickens (Wang *et al.,* 2013). Commercial chicken farming has made bird flu more virulent as a result of overcrowding. For instance, in the production of modern broilers, 20,000–30,000 day-old chicks are placed on the litter material, and as they become bigger, the crowding gets worse. The likelihood of a devastating AI epidemic is therefore very high (Greger, 2007; Anderson *et al.,* 2014).

When investigations of the spread of H_5N_1 epidemics in Asia were done, they coincided with areas that had the most chicken, therefore intensive chicken production is condusive for highly deadly influenza viruses (Perry, 2005). Free-range chickens are rather ineffective at transmitting the AI because their dropping is largely shed outside in the sunny places where it can easily dry up but can persist in wet faeces for weeks during rainy conditions. However, at ambient temperatures, once the feces have dried, the AI is deactivated (Kurscheid *et al.,* 2015).

The large proportion of egg-laying chickens are housed in enclosures worldwide, which are made of bare wire and are so small that each hen is given less room than one of a conventional size (Pym *et al*., 2012). This creates the perfect environment for viruses to propagate either orally or by excreta, which invariably causes food to be contaminated in the confined spaces and causes an increase in HPAIV. Mainly because of the conditions in which chicken are kept, caged together in enormous warehouses, makes the H_5N_1 virus become so virulent in hens (Greger, 2007). Because chicken immune systems are overworked under stress and modern poultry breeds have consistently low immunological competence, groups of chicken kept in cramped, crowded, unclean settings spread viruses more quickly (Ritchie *et al.,* 1995). In 2004, HPAIVs (H5N1) outbreak hit Lao, affecting 90 percent of commercial chicken farms rather than free-range chicken enterprises (USDA, 2006).

On the other hand, hens that have access to the outdoors are always more likely to come into touch, either directly or indirectly, with wild aquatic fowl like ducks, geese, and swans that may carry the avian influenza virus, mainly in the faeces, nasal discharge, and eye discharge that could result in virus transmission (Ssematimba *et al.,* 2013). The highly virulent avian influenza epidemics that the Nigerian poultry industry experienced were most devastating to the commercial layer-type chicken produced under that sector's commercial practices. With a total flock size of 939, 620 lost in 127 farms with proven outbreaks, the commercial layer-type chicken experienced a significant economic loss. This was explained by the high proportion of

laying chickens in each flock compared to other chicken breeds in these sectors. Additionally, it was shown that deep litter reared broilers had the greatest average death rate whereas commercial layers had the lowest (Akanbi *et al.,* 2014).

Small flocks with low densities and genetic variety have lower viral loads because fewer chickens cannot produce enough virus to cause mutations. A disease that has evolved into a highly deadly form in a small population of hens swiftly disappears after killing all of its hosts. The virus can quickly move from one chicken to another in high-density areas and can change from a low pathogenic form to a high pathogenic one there (Swayne *et al.,* 2011). Compared to commercial poultry flocks, Free-range flocks have been found to be more likely to come into contact with wild birds bearing the LPAI strains, providing them with a constant challenge and maintaining their immunity (Fasanmi *et al.,* 2017).

2.4.6 Impact of restocking source

Periodic market rest days, forthrightly chicken depopulation, or sale bans to communities through market closure and restrictions on restocking poultry from other markets will dramatically minimize the circulation of AIVs (Offeddu *et al.,* 2016). H_5N_1 positivity rates in chicken gathered from other farms were greater, and it was found that they had also higher H_7N_9 positivity rates than the main home restocking (Offeddu *et al.,* 2016). The majority of farmer customers buy live birds at neighbourhood markets and bring them home as new stocks (Wang *et al,.* 2017). Additionally, for restocking, farmers purchase their bird for restocking from other counties in Kenya, and birds purchased from market places are frequently ones that have AIVs (Kariithi *et al,.* 2020).The frequency of restocking by farmers in Kenya is determined by demand, and it can happen a single time or multiple and before being bought by traders or sold to consumers in various places, these birds are not tested for disease. , and this, along with the custom of mingling birds that have recently arrived with those already in the homes, makes it ideal for the mixing and spreading of influenza (Kariithi *et al.,* 2020).

2.4.7 Impact of vaccination status

In endemic countries, vaccination is an essential tool for management and prevention of H_5N_1 HPAI in poultry (Swayne *et al.,* 2012). The Haemagglutinin (HA) since 2004, various gene changes have been made to the immunization strains used in China to achieve an antigenic match between the vaccinations and the prevalent emerging strains (Li, Bu, and Chen, 2014). Inactivated vaccines are widely used in China to protect poultry. Since 2012, the clade of AIVs has been mostly controlled in China by the inactivated H_5N_1 vaccination developed from the vaccine strain Re-6, which was created using reverse genetics and contains the HA and NA genes of the virus (ZENG *et al.,* 2020).

Influenza education for chicken farmers, employees, veterinarians, and the government officials is required. If the media fails to provide information or provides misleading information, the public's confidence in the safety of all chicken products intended for human consumption would decline, which will have a significant negative economic impact on the nation (Swayne *et al.,* 2000).

However, there are a few fundamental controls that apply to all situations. To stop the spread of the disease, one such approach is the humane and safe culling of diseased birds and those in touch with them (Ramey *et al.,* 2022). Human euthanasia slows the spread of disease by reducing the amount of virus shed from any one spot, but it seldom totally stops it because some virus will have already been discharged before euthanasia begins and frequently before the condition is discovered (Hill *et al.,* 2018). Lack of vaccination, poor management, and inadequate biosecurity lead to the spread of AI in birds, however in China, poultry that has received immunization is totally protected from H_5N_8 virus infection (Cui *et al.,* 2020). According to the findings of the impact evaluation, moderate levels of HPAI vaccination in Indonesia were adequate to dramatically lower the occurrence of HPAI-compatible events in mixed populations of semi-commercial and backyard chicken, however, mass vaccination of

backyard and small-scale commercial poultry is unlikely to be a sustainable control measure against HPAI in the medium to long term due to the significant investment necessary to attain these effects (Bett *et al.,* 2015).

Evaluation of vaccination programs should be essential to achieve the required immunity targets for the highly pathogenic avian influenza subtype H5N1 (HPAI) vaccine in developing countries. The vaccination is typically implemented as periodic campaigns, and the vaccination must always be adjusted to local poultry production systems and socioeconomic contexts (Lesnoff *et al.,* 2009).

Africa commercial flocks of layers and broilers are regularly immunized against avian influenza infections prevalent in the nations. The majority of native hens are not vaccinated, with the exception of areas where special programs have been put in place to convince farmers of the benefits of protecting birds from disease and the ensuing increases in output (Nyaga *et al.,* 2007). Partnerships between county governments at the local level, vaccine manufacturers, and suppliers of agro-veterinary services are required in Africa as well to facilitate the production of affordable vaccines and their prompt delivery to smallholder widespread chicken farmers (Ipara *et al.,* 2021). Quality certified vaccines are successful in limiting the introduction of AI viruses and their spread when used properly and in conjunction with other disease management methods (Ntsomboh-Ntsefong *et al.,* 2017). In order to help the vaccine's spread and achieve the goal of increasing productivity in smallholder farming, governments or non-governmental organizations frequently launch vaccination programs. Vaccines against viral diseases are available in Kenya, and additional support for immunizing chickens against the virus has been provided through projects and non-governmental organizations (Otiang *et al.,* 2021).

CHAPTER THREE

MATERIALS AND METHODS

3.1 The Study site

The study was based in Uasin Gishu County, Kenya. Uasin Gishu County is made up of counties; Moiben, Kapseret, Soy, Kesses, Ainabkoi and Turbo. There are two distinct rainy seasons with an average yearly rainfall of 900 to 1200 mm, Uasin Gishu County in Kenya. Due to its plateau location at 2085m ASL, the county experiences cold, temperate weather, with average yearly temperatures ranging from 8.4 °C to 27 °C. The months of April and June, are wet, while February and March mark its driest period.

A large population is involved in Agriculture including poultry and livestock farming; most of the women and youth are engaged in poultry farming as their source of income. Due to rapidly increasing human population because of urbanization, the demand for poultry products is continuously increasing and thus small and large scale poultry farming has also increased significantly. Most of the commercial poultry farmers and some small scale farmers are keen on flock health and they often present suspected diseased for screening at RVIL, Eldoret. Therefore, adequate sampling of chicken from catchment areas in Uasin Gishu was implemented at the RVIL facility.

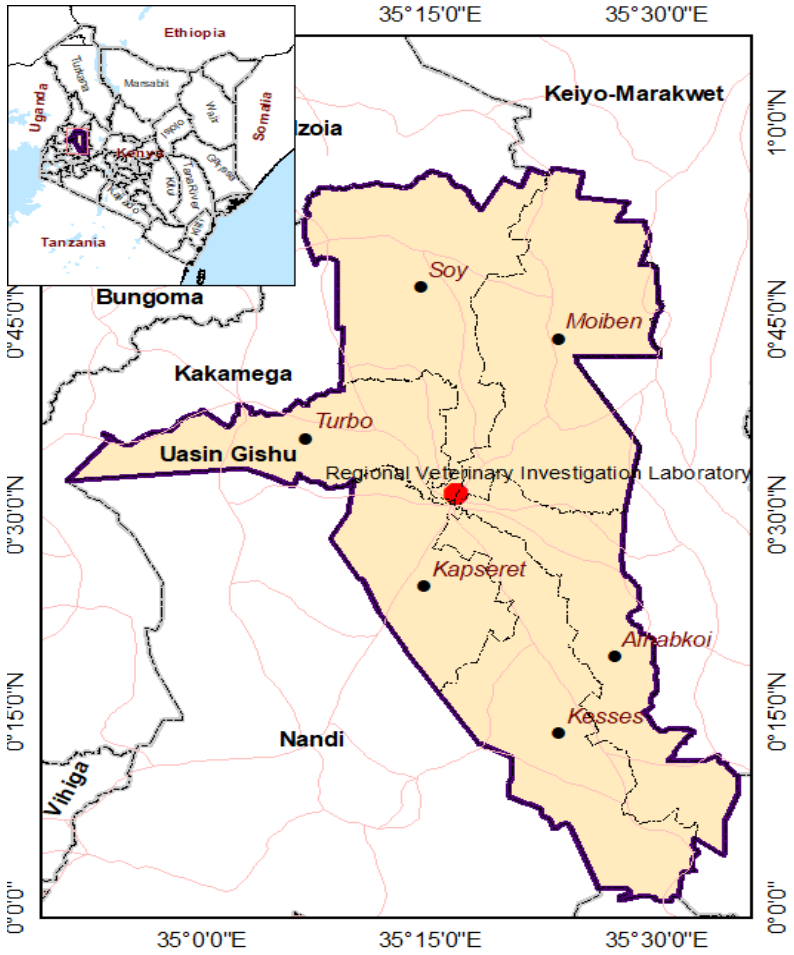


Figure 3.1: Study area: Map of Uasin Gishu County showing location of six Sub County for sources of chicken screened at Regional veterinary investigation laboratory (Source; Municipal council of Uasin Gishu County)

3.2 Inclusion criteria

Poultry farmers who were living in Uasin Gishu County and brought their chicken for screening at RVIL at the time of study and gave an informed consent to participate in the study.

3.3 Exclusion criteria

1. Poultry farmers who were not living in Uasin Gishu County and brought their chicken for screening at RVIL at the time of data collection.
2. Farmers who brought chicken for screening but did not consent to participate in the study.

3.4 Sampling techniques

This was a cross sectional study involving chicken farmers from whole of Uasin Gishu, County whose chicken were screened at Regional Veterinary Investigation Laboratory (RVIL) for suspected zoonotic diseases. The study involved cluster sampling design, in each cluster of ten chicken farmers who signed the consent form (Appendix ii) for their chicken to be screened for avian influenza A virus, five chicken were randomly selected from the population. Consecutive sampling was employed among the population in the random selection until the sample of 305 chicken was achieved. The farmers who did not consent to the study were given normal services with no punitive measures as consent was voluntary. The study was spread through the rainy and dry seasons during the month of May and June 2020 and February and March 2021.

3.4 Sample Size Determination

This study used the sampling formula described by Naing *et al.* (2006)

$$n = \frac{(Z_\alpha)^2 p(1-p)}{d^2}$$

Where;

n = required sample size
z = Critical value for 95% confidence level (1.96)
p = prevalence of influenza A virus in chicken (0.8% based on Munyua *et al*., 2013)
d = margin of error (1%), thus;

$$n = \frac{(1.96)^2 0.008(1-0.008)}{0.01^2}$$

n=304.87

This study collected samples from 305 chicken and satisfied the margin of error (d) (1%)

Table 3.1: Distribution of sampled chicken from different region of Uasin Gishu County based on parameters (intrinsic and extrinsic risk factors)

Parameters	Attributes	Frequency	% frequency
Age	Young<4Mnths	201	65.90
	Old>4Mnths	104	34.10
	Total	305	100.00
Breed	Indigenous	260	85.25
	Hybrid	45	14.75
	Total	305	100.00
Sex	Male	141	46.23
	Female	164	53.77
	Total	305	100.00
Season	Wet	168	55.08
	Dry	137	44.92
	Total	305	100.00
Vaccination	Vaccinated	93	30.49
	Unvaccinated	212	69.51
	Total	305	100.00
Management system	Free range	167	54.75
	Confined	138	45.25
	Total	305	100.00
Restocking	Market	28	9.18
	Home village	171	56.07
	Own chicken	106	34.75
	Total	305	100.00

3.5 Sample collection, handling/storage and administration of questionnaire

Oropharyngeal samples were collected from the chickens by swabbing the oropharyngeal area near the opening of the trachea using plastic shafted polyester tipped swabs. All swabs were then kept in 2 ml cryo-vials containing viral transport media. Each cryovial sample were labeled with the date, sex and age of the chicken and then stored in a refrigerator at 4°C at RVIL for five days, awaiting transportation in liquid nitrogen (-70°C) to KEMRI /CDC, Kisumu for diagnostic testing of influenza A virus using real time PCR method once every week until all samples were tested.

Face to face interviews with farmers were conducted to gather information on possible risk which were recorded using a researchers designed structured questionnaire (Appendex1). Sex was recorded as male or female, age was categorized as young (below 4 months) and old (above 4 months) best on the look of their combs, legs ,vent wattles and feathers, breed was categorized as indigenous and hybrid breeds, management was categorized as free range and confined and season was divided in wet and dry seasons

3.6 Sample Processing Procedures for Laboratory RNA Extraction and Detection of Influenza A Virus in Samples

The 305 samples were tested by real-time reverse transcription polymerase chain reaction (rt RT-PCR) (Qiu *et al.,* 2009). RNA was extracted from the oropharyngeal specimens using a MagMax viral RNA isolation kit (Ambion Inc, Applied Biosystems, CA, USA) on a Kingfisher Flex system following the manufacturer's instructions by use of lysis buffer ,wash1, wash 2, elusion buffer and master mix reagents . The prepared samples containing unknown RNA materials were placed in a 96 well plate with one well containing positive control of a known synthesised RNA template of influenza A virus and another well with a negative control of Nuclease free water which are contained in a MagMax kit. Controls were used to validate the

extraction steps of PCR. The sample were loaded and analyzed in rt-PCR machine (Figure 3.1, 3.2, 3.3 and 3.4). The PCR 7500 machine was set to run at 10 minutes at 45^0C for reverse transcription, 10 minutes at 95^0C to activate the Taq polymerase. At 45 cycle PCR with denaturation at 94^0C for 20 seconds and annealing/ extension at 80^0C for 15 seconds and final extension at 70^0C for 5 minutes. After a complete rtPCR, results were analyzed and read at the annealing and extension step and recorded as cycle threshold (CT) values.

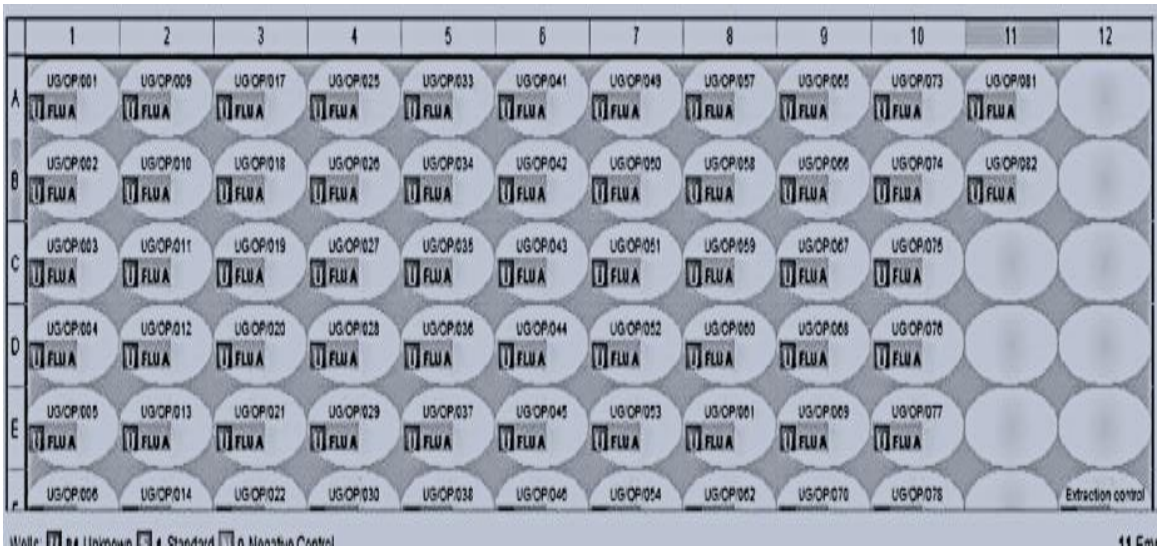


Figure 3.1: Plate 1 layout showing arrangement of oropharyngeal samples in a 96 well plate (sample 1-82) Loaded in the rtpcr machine for detection of influenza A virus

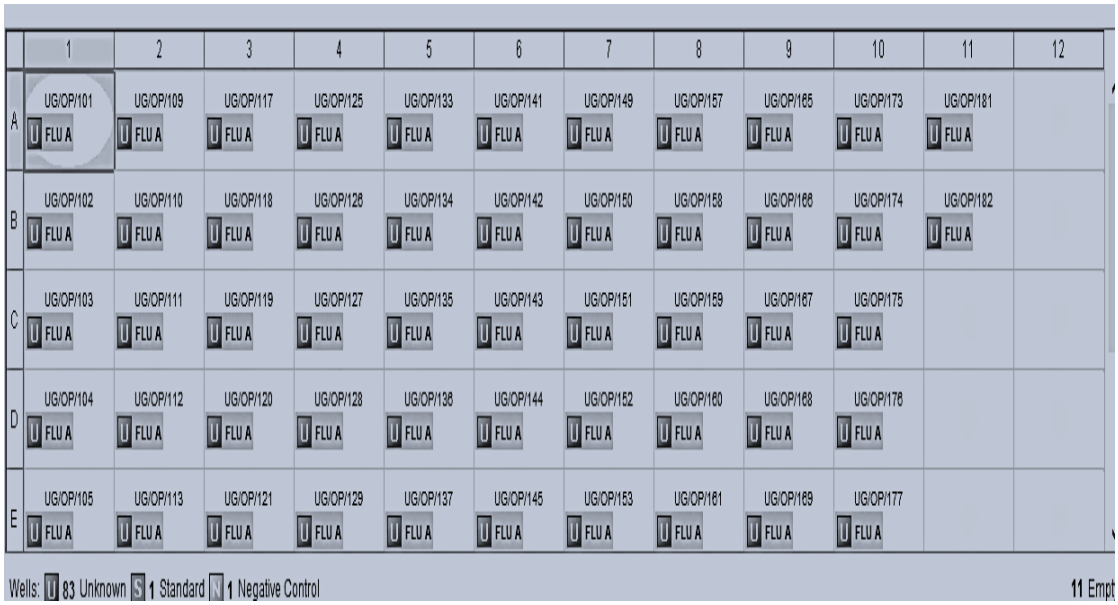


Figure 3.2: Plate 2 layout showing arrangement of oropharyngeal samples in a 96 well plate (sample 101-182). Loaded in the rtpcr machine for detection of influenza A virus.

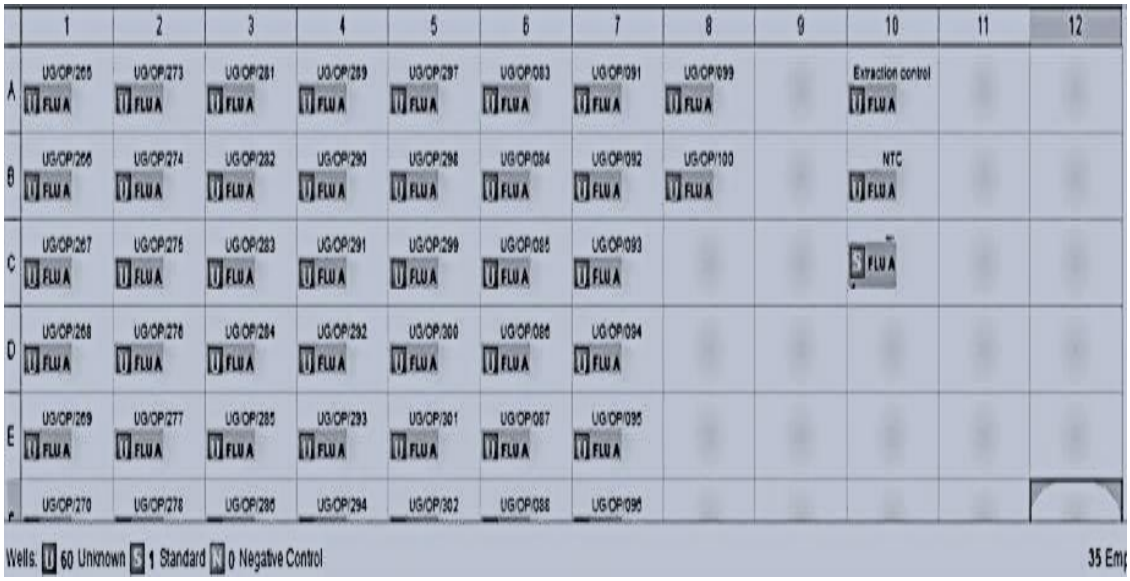


Figure 3.3: Plate 3 layout showing arrangement of oropharyngeal samples in a 96 well plate (sample 183-264). Loaded in the rtpcr machine for detection of influenza

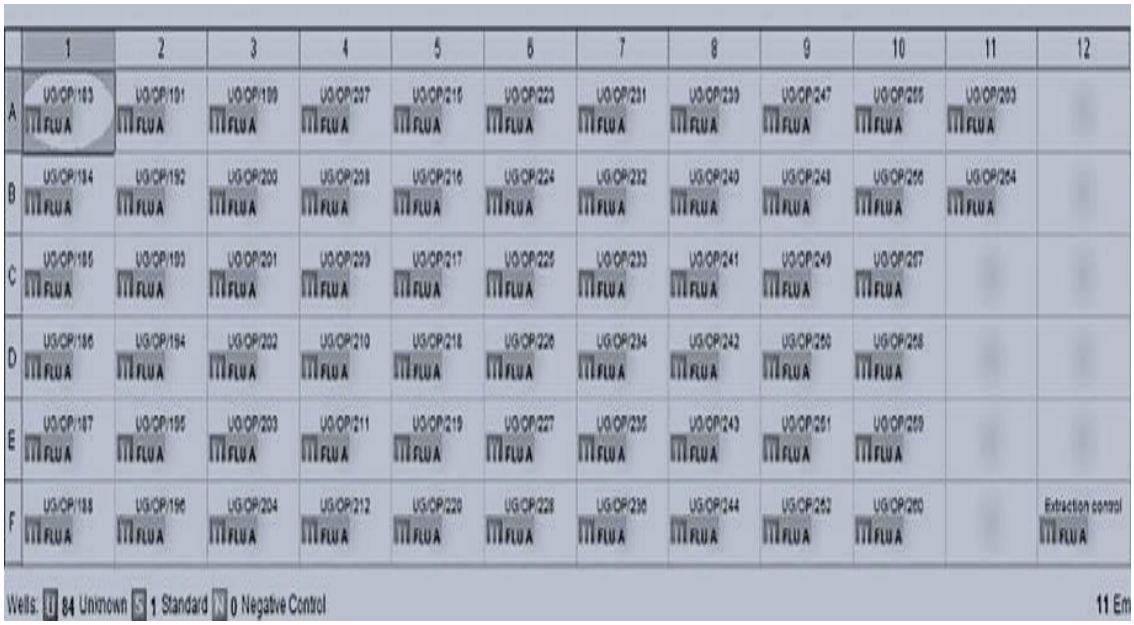


Figure 3.4 Plate 4 layout showing prepared oropharyngeal samples in a 96 well plate (sample 265-305, 83-100). Loaded in the rtpcr machine for detection of influenza A virus.

3.7 Study variables

The study variables were independent and dependent variables. The independent variables are those that manipulate the problem in the study and they were the risk factors associated with the prevalence of influenza A virus. Dependent variables rely on the independent variables in order for it to occur which is the outcomes of the cause and it was the occurrence of influenza A virus in chicken in Uasin Gishu County.

3.7 Data analysis

Data was entered into spread sheets in Microsoft Excel 2019 and analyzed using Statistical Package for Social Sciences (SPSS) version 20. Descriptive statistics was used to calculate prevalence. Chi-square test and odds ratio values were used to compare paired data sets for the independent and dependent variables. . Tables and graphs were used to present the results.

CHAPTER FOUR

RESULTS

4.1 Detection of Influenza A Virus and Prevalence of influenza A virus in chicken in Uasin Gishu County

Four samples out of 305 sampled were found positive and 301 were negative for influenza A virus as shown in Fig 4.1, 4.2, 4.3 and 4.4.

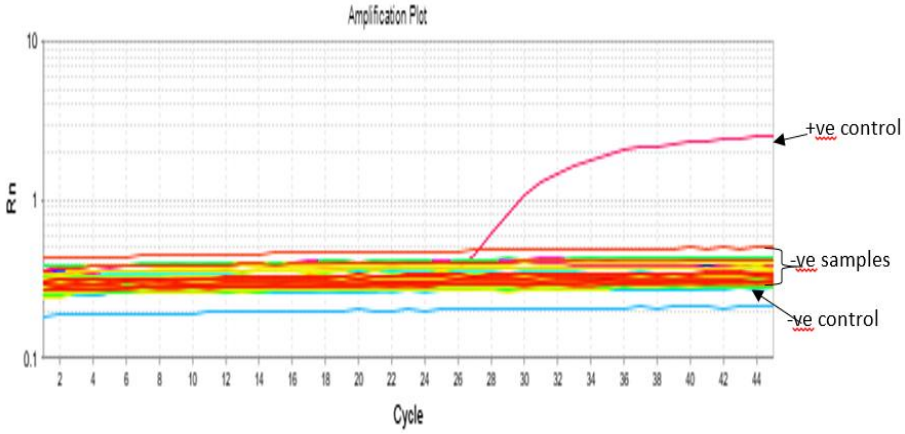


Figure 4.1: The amplification plot with negative detection of influenza A virus at Cycle Threshold (Ct) >40.0 in sample (1-82).

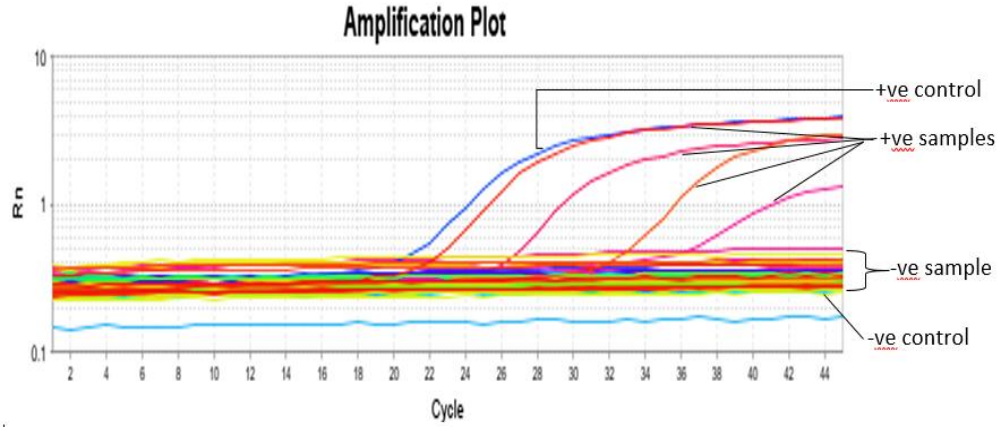


Figure 4.2: The amplification plot positive for Influenza A virus at Cycle Threshold (Ct) value of 21.146, 22.373, 36.998 and 32.546 in sample (101-182) of a 96 well plate.

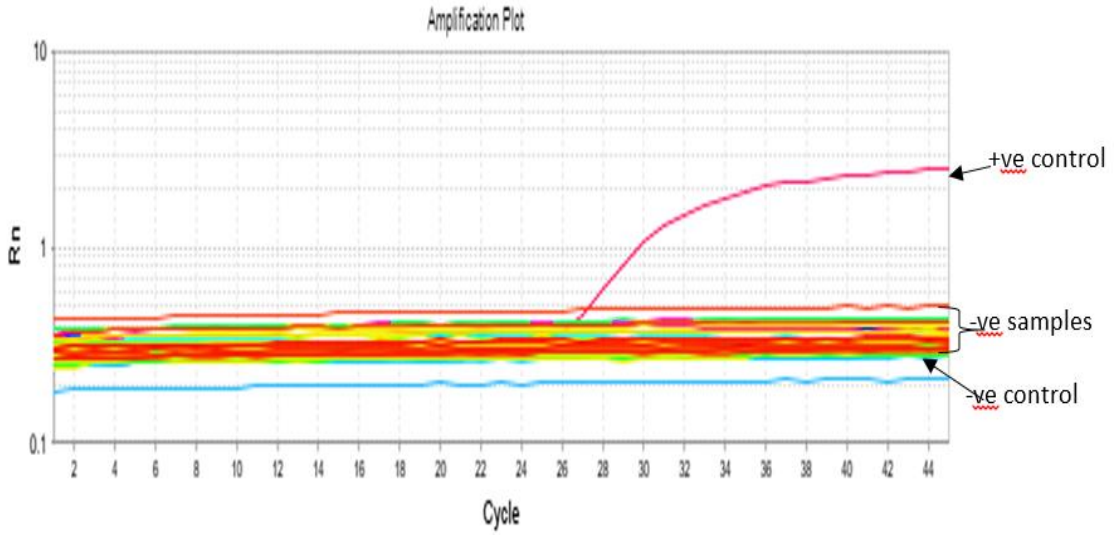


Figure 4.3: The amplification plot with negative detection of influenza A virus at Cycle Threshold (Ct) >40.0 in (sample 183-264).

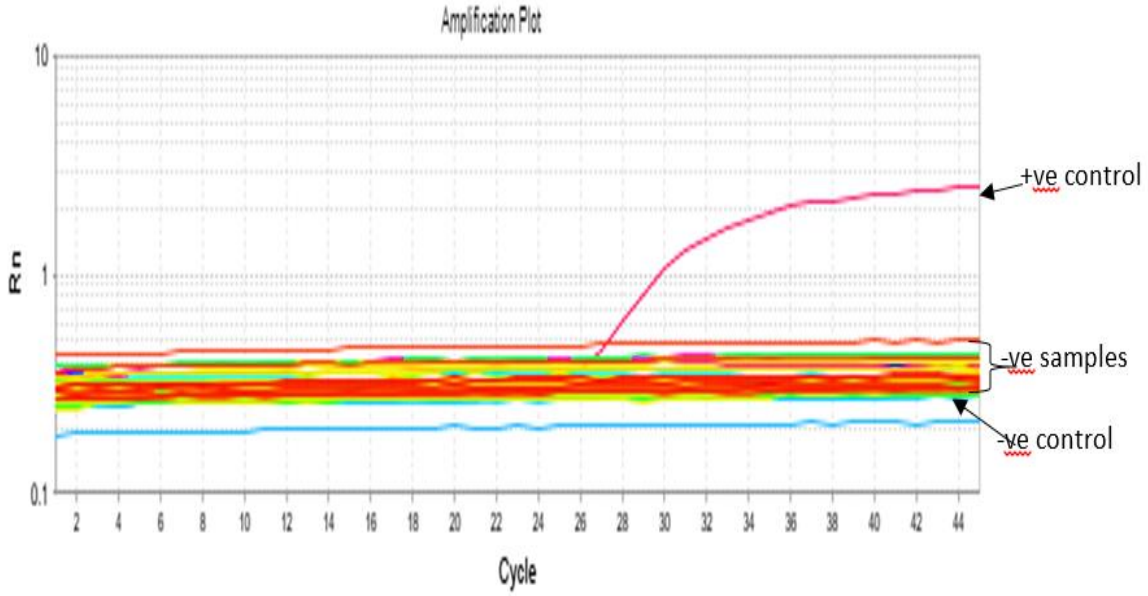


Figure 4.4: The amplification plot with negative detection of influenza A virus at Cycle Threshold (Ct) >40.0 in sample (265-305, 83-100).

Out of the total three hundred and five (305) chicken that were screened, only four (1.31%) tested positive for influenza A virus while 301 (98.69%) tested negative. In terms of Sub-county, none of the positive cases were reported in Ainabkoi, Kapseret and Soy Sub-county. In Moiben, out of 113 screened chicken, two (2) were positive for influenza A virus giving a prevalence rate of 1.80%. In Kesses, out of 37 screened chicken, one (1) was positive for influenza a virus giving a prevalence rate of 2.70% while in Turbo, out of 42 screened chicken, one (1) was positive for influenza a virus giving a prevalence rate of 2.38% (Table 4.1).

Table 4.1 Prevalence Rate of Influenza A virus in chicken in the six sub County of Uasin Gishu County

Sub-County	Total	Negative	Positive	Prevalence (%)
Kesses	37	36	1	2.7
Turbo	42	41	1	2.4
Kapseret	58	58	0	0
Moiben	113	111	2	1.8
Ainabkoi	30	30	0	0
Soy	25	25	0	0
Overall Prevalence	305	301	4	1.31

4.3 Association between Influenza A virus infection prevalence in chicken and assessed Intrinsic Risk Factors

4.3.1 Breed

Out of the 305 sampled chicken, specimens from three indigenous breed and one hybrid tested positive for Influenza A virus. Chi-square and odds ratio (OR) analyses shows that breed type significantly influences the prevalence of the influenza A virus infecting chicken in Uasin Gishu County ($p = 0.0000$; OR =0.52) (Table 4.3 and Table 4.4).

4.3.2 Age

Three of the four confirmed cases of influenza A virus were in young chicks under the age of four months and only one of the confirmed case was an adult above the age of four months. The chi-square test suggested that there was no significant (p-value = 0.6992) association between age and the frequency of the influenza A virus infection, although the odds ratio shows that there was 1.55 more times chance for chicks (less than 4 months old) to test positive for influenza A virus compared to adult (more than 4 months old chickens (Table 4.3 and Table 4.4). This indicated that age was not a significant risk factor that influenced the positivity of the viral infection in this study.

4.3.3 Sex

The influenza A virus infection was examined in 104 male and 201 female chicken. Each gender had two positive outcomes for influenza A virus. The Chi-square test yielded a p-value of 0.8790, and the odds ratio was 1.16 indicating that gender was not significantly associated with the viral infection (Table 4.2 and Table 4.3). This shows that the study found no evidence linking the sex of the chicken to the frequency of their infection with influenza A virus in Uasin Gishu County.

Table 4.2: Influenza A virus infection associated with the intrinsic risk factors (age, breed and sex) of chicken

Factor	Type	No. Examined	Positive cases	Frequency %	P – values
Age	Old (< 4 months)	104	1	0.96	0.6992
	Young(<4months)	201	3	1.49	
	Total	305	4		
Breed	Hybrid	45	1	2.22	0.0000
	Indigenous	260	3	1.15	
	Total	305	4		
Gender	Female	104	2	1.92	o.8790
	Male	201	2	0.99	
	Total	305	4	1.31	

Table 4.3: Odds ratio relationships between influenza A infection with the assessed intrinsic risk factors (age, breed and sex) of chicken

Risk factors	Type	No. Examined	Positive cases	Frequency %	OR
Age	Old (< 4 months)	104	1	0.96	1.55
	Young(< 4 months)	201	3	1.49	
	Total	305	4		
Breed	Hybrid	45	1	2.22	0.52
	Indigenous	260	3	1.15	
	Total	305	4		
Gender	Female	104	2	1.92	1.16
	Male	201	2	0.99	
	Total	305	4	1.31	

4.4 Association between influenza A virus infection Prevalence in chicken and Extrinsic Environmental Risk Factors

4.4.1 Seasonal Weather Variation

The influence of seasonal weather variation revealed 3 (1.79%) positive cases out of 168 (55%) sampled chickens during the rainy season compared to only 1(0.73%) positive case out of 137(39%) sampled during the dry season. Despite the rainy season having a higher number of positive cases, the viral infection did not change significantly between the dry and wet seasons (Table 4.5; $p = 0.42$)

4.4.2 Vaccination Status

Out of the 305 chicken, 93 (30.5%) were vaccinated against avian flu disease whereas 212 (69.5%) were not vaccinated. Three (1.4%) of the unvaccinated chicken tested positive for influenza A virus while only one (1,1%) chicken of the population that had received vaccination tested positive for influenza A virus (Table 4.4). Chi-square test result revealed that there was no significant difference in for influenza A virus infection rates in chicken that had received vaccinations compared to chicken that had not been vaccinated, ($p=0.8100$). Despite the observation that more positive cases of Influenza A virus occurred in the unvaccinated chickens compared to the vaccinated chickens in Uasin Gishu County, the Chi-square test result ($p = 0.81$) indicates that both vaccinated and unvaccinated chickens still had an equal likelihood of getting the virus infection and thus vaccination did not significantly protect the chicken from the infection (Table 4.4).

4.4.3 Management System

A total of 3(1.8%) of the positive cases of chicken with the influenza A virus were from free range management system while only 1(0.7%) case was from confined management system. Chi-square test result ($p = 0.5747$) showed that despite free range management system having more positive cases compared to confined management system, management systems did not

significantly influence the frequency of Influenza A virus amongst the chicken in Uasin Gishu County (Table 4.4).

4.4.4 Restocking Source

Chickens were restocked from the market, the farmers' own stock, and the home village. There was no significant difference between the various sources from where the farmers obtained supply to restock their chicken as evidenced the Chi-square test result ($p = 0.549$) indicating that chicken may be infected with influenza A viruses, regardless of where the new restocking supply came from (Table 4.4).

Table 4.4: Association between Influenza A Virus infection in Chicken and Extrinsic Environmental Risk Factors

Risk factors	Type	No. Examined	Positive cases	Frequency %	P-value
Season	Dry	137	1	0.72	0.4200
	Wet	168	3	1.78	
	Total	305	4		
Vaccination(avian flu vaccine)	Vaccinated	93	1	1.07	0.8100
	Unvaccinated	212	3	1.41	
	Total	305	4		
Management System	Confined	138	1	0.72	0.5477
	Free Range	167	3	1.79	
	Total	305	4		
Restocking	Market	28	0	0	0.5490
	Home Village	171	2	1.16	
	Own Chicken	106	2	1.88	
	Total	305	4	1.31	

Chi square test at 95% confidence interval

CHAPTER FIVE

DISCUSSIONS

5.1 Prevalence of influenza A virus in chicken in Uasin Gishu County

During the study, Influenza A virus was detected at 1.3% and scrutiny of the previous data surveillance findings show that this was the first time influenza A virus has been detected in Uasin Gishu County in Kenya. Studies on influenza viruses elsewhere have recorded variable prevalence rates of AIVs in chicken in different countries around the world. In a study conducted in Nairobi Kenya the virus was detected at 0.8% in chicken sampled in live bird markets (Munyua *et al*., 2013). The small variation between these results showing slightly higher prevalence in the current study may be explained by spatial and temporal differences between the studies. Munyua *et al.* (2013), investigated the presence of avian influenza in birds traded in live markets in Nairobi (LBMS) while the current study screened chicken brought to the laboratory by farmers in Uasin Gishu County.

Sick chicken brought to the laboratory are more likely to be infected with AIV and this may explain the slightly higher rate of infection. It is also possible that the infection is increasing over time due to cross border trade in the East Africa region.

5.2 Association between Intrinsic and Extrinsic Suspected Risk Factors and the Infection of Chicken with Influenza A Virus

5.2.1 Association between Chicken Breed and Influenza A Virus Infection

Results of the current study show that local chicken breeds had significantly higher rate of influenza A virus infection. Since the layers were not infected, it is possible that various breeds of chicken have varying levels of resistance to the influenza A virus infection than has previously been documented (Sarwar *et al.,* 2013). A p-value of 0.0000 from statistical analysis supported the finding that breed type had a statistically significant impact on the prevalence of the influenza A virus infection. According to a study conducted in Nigeria between the years

of 2006 and 2008, layer and broiler chicken breeds were both infected with the highly pathogenic avian influenza (HPAI)H_5N_1, with mortality rates of 11.11 percent for layers, 45.51 percent for cockerels, and 73.92 percent for broiler breeds, however, the findings of the current study contradict with these and those of Sarwar *et al*., (2013), who also found that Pakistan had multiple outbreaks of highly virulent avian influenza between 1995 and 2003, killing 3.2 million laying chickens by 1999. The local breed of chicken are mostly reared by free range scavenger management system which exposes chicken to make contacts with wild bird populations in surrounding habitats. This increases the chances of chicken to contract diseases from wild birds through shared contaminated food/water sources and contact with excreta.

5.2.2 Association between Chicken sex and Influenza A Virus Infection

Chicken of both sexes showed similar infection results in the current study, with each sex having only two infection cases. The Chi-square test indicates that there is no significant relationship in influenza A virus infection between the male and female sex of the chicken. The results of this study contradict those of Morgan & Klein's (2019), who suggested that biological differences between male and female influence how influenza infections and treatments differ between the sexes, with females developing stronger immune responses and thus having better resistance to the virus. Since the current study was cross-sectional, young and older chicken were screened. Immunity differences between male and female are more expressed at reproductive maturity and thus young chicken are equally susceptible to infections because their immune status are not different at young age.

5.2.3 Association between Chicken Age and Influenza A Virus Infection in Chicken

The current study found a higher rate of infection in young chicken below 4 months of age compared to older chicken above 4 months although the difference was not statistically significant. However, findings of Cheema *et al*., (2011), suggested that older chicken become more vulnerable to Avian Influenza A virus (AIV) compared to younger chicken, contradicting

the current findings. This may be due to the general lack of sanitation in chicken houses (increased crowded exposure with increasing age) and the fragility of young chicks whose immune system has not fully matured to respond against infections including influenza A virus infection. The outcome also differs with that of Nooruddin *et al*., (2006), who found that the frequency of infection was higher in birds 34 weeks of age and lower in birds 8 to 12 weeks of age. This discrepancy may also be the result of inadequate sanitation in the majority of chicken homes and the susceptibility of chicks to the influenza A virus as immunity is not completely established. However, lack of stock records kept by the majority of farmers made it challenging to get the correct age of chicks or grown adult chickens. Therefore, a mistake in the classification of age might be the cause of the differences in these findings. Nearly double the number (201) of chicken were classified as young compared to old (104) and thus the difference in sample size between young and old is another source the difference in outcome.

5.2.4 Association between Chicken Management System and Influenza A Virus Infection in Chicken

Despite more Influenza A virus cases being detected in free ranging management system than in confined management system, statistical analysis showed that there was no significant difference between the two management systems with respect to Influenza A virus infection. This finding agrees with that of Wang *et al.* (2013) who found that there was no difference between free ranging and confined farming methods as they both contribute to the infection of Influenza A viruses in equal measure. The low prevalence observed during the current study may indicate that there was no major outbreak of the disease in the study region in both free range and confined chicken rearing systems. However, free range reared chicken are more likely to come in contact with wild birds and their excreta increasing chances of infection with the virus if the wild birds are infected. Majority of farmers rearing local chicken breeds practice free range management system which may be the reason for the observed higher prevalence of

Influenza A virus among the local breeds compared to the exotic breeds (layers and broilers usually raised under confined commercial systems).

5.2.5 Association between Seasonal Weather Variation and Influenza A Virus Infection in Chicken

There was higher number of positive cases of Influenza A virus infection in chicken during the wet season compared to during dry season. However, despite the higher number of cases observed during the wet season, statistical analysis found no significant difference in infection between the seasons. This means that seasonal weather variation had no effect on the incidence of influenza during the wet and the dry seasons which correspond to the cold and hot seasons respectively in the region. These findings agree with those of Liang *et al.,* (2020) who found little evidence on impact of temperature on HPAI H_5N_1 outbreaks. However, it disagrees with Zhang *et al.* (2014) who documented that viruses that caused the influenza A pandemic in Hong Kong were first isolated during the cold-weather season (Si *et al.*,2010). Among the factors that are likely encourage influenza transmission include lower temperature, absolute and relative humidity, less ultraviolet radiation from the Sun and crowding of hosts. During the wet season, temperatures in Uasin Gishu are low causing chicken to stay close to each other and thus encouraging easy spread of infections if some are exposed.

5.2.6 Association between Vaccination Status and Influenza A Virus Infection in Chicken

The current study found that vaccinated and unvaccinated chicken had equal chances of contracting influenza A virus. This suggests that the available vaccine may not be effective in protecting the chicken against the virus probably due to development of variants that are resistant to the elicited immune response to the antigens in the vaccine (Swayne *et al.*, 2000).Vaccine failure may also be the cause of non-protection. This may be due to improper handling and administration of the vaccine which is more probable when farmers do not utilize professional services offered by veterinary personnel due to the costs involved. This result also

shows that the vaccine used or the method of administration may not be effective in protecting the chicken (Swayne *et al.,* 2000).

5.2.7 Association between the Source of Supply/Restocking Chicken and Influenza A Virus Infection in chicken

The findings of this study on chicken supply and re-stocking sources indicate higher positive cases from own chicken and home village sources for chicken supply. However, re-stocking sources of chicken by farmers had statistically insignificant differences between all sources of supply meaning that the chicken can contract Influenza A virus irrespective of the source of the new stock suggesting that transmission of the virus can occur at any point along the supply chain. This study result contradicts that of Hasan *et al*., (2019), whose finding indicated that most of the infected cases of chicken stock in Pakistan is in purchased live bird markets and from hawkers. This suggests that the handling conditions by hawkers and in market places are ideal for the transmission of the viruses.

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATION

6.1 Conclusions

1. The occurrence of influenza A virus in chicken in Uasin Gishu County, Kenya was established at a prevalence rate of 1.3%, indicating the potential of influenza A virus, a contagious avian flu disease in chicken to spread among the population.

2. Out of all the intrinsic risk factors studied, only the breed of chicken demonstrated a statistically significant effect on the occurrence of Influenza A virus. Extrinsic risk factors like vaccination status, restocking source, management systems of chicken and seasonal weather variations did not affect the prevalence of avian influenza virus in chicken in Uasin Gishu County, Kenya.

6.2 Recommendation

1. The study has shed light the prevalence of Influenza A virus and there is need to create awareness on the virus among poultry farmers where both the national and county government policymakers to consider complete implementation of effective measures to control AIVs since it causes a contagious avian flu disease.

2. This study has established the risk factor of chicken breed types on the infection of AIVs in Uasin Gishu County, Kenya and recommends that proper control measures should be put in place by ministry of agriculture in the county to reduce the economic loss to poultry farmers.

3. The study used rtPCR to determine whether chicken were positive or negative with influenza A virus and recommends a future study to identify the strains of avian influenza

A virus targeting the highly pathogenic(HPAI) viruses and low pathogenic avian influenza(LPAI) viruses to enable development of effective vaccines.

4. Further research to involve field survey observations in poultry farms by researchers to clearly capture the possible risk factors on infection A virus in chicken. This will ensure proper programme sensitization among poultry farmers on symptoms, transmission, control and prevention of the virus.

REFERENCES

Abdelmohsen, M. S. (2012) Evaluation of different methods for isolation of avian influenza virus.*cu Theses*.

Abolnik, C., Gerdes, G. H., Sinclair, M., Ganzevoort, B. W., Kitching, J. P., Burger, C. E., ... & Olivier, A. J. (2010). Phylogenetic analysis of influenza A viruses (H6N8, H1N8, H4N2, H9N2, H10N7) isolated from wild birds, ducks, and ostriches in South Africa from 2007 to 2009. *Avian diseases*, *54*(s1), 313-322.

Acland, H. M., Bachin, L. S., & Eckroade, R. J. (1984). Lesions in broiler and layer chickens in an outbreak of highly pathogenic avian influenza virus infection. *Veterinary Pathology*, *21*(6), 564-569.

Ahrens, A. K., Selinka, H. C., Mettenleiter, T. C., Beer, M., & Harder, T. C. (2022). Exploring surface water as a transmission medium of avian influenza viruses–systematic infection studies in mallards. *Emerging microbes & infections*, (just-accepted), 1-30.

Akanbi, O. B., & Taiwo, V. O. (2014). Mortality and pathology associated with highly pathogenic avian influenza H5N1 outbreaks in commercial poultry production systems in Nigeria. *International Scholarly Research Notices*, *2014*.

Alexander, D. J. (2007). An overview of the epidemiology of avian influenza. *Vaccine*, *25*(30),

Anderson, L., Auster, M., Caster, T., Charoni, J., Clinkenbeard, P., Fitzpatrick, M. ... & Young, W. (2014). Playing Chicken with Big Ag: Advocating Regionally Sensitive Food Sovereignty to Address Pandemic Influenza.

Asante, I. A., Bertram, S., Awuni, J., Commey, A. N. O., Aniwa, B., Ampofo, W. K., & Gabriel, G. (2016). Highly pathogenic avian influenza A (H5N1) virus among poultry, Ghana, 2015. *Emerging infectious diseases*, *22*(12), 2209.

Awuni, J. A., Bianco, A., Dogbey, O. J., Fusaro, A., Yingar, D. T., Salviato, A. ... & Monne, I. (2019). Avian influenza H9N2 subtype in Ghana: virus characterization and evidence of co-infection. *Avian Pathology*, *48*(5), 470-476.

Beato, M. S., Capua, I., & Alexander, D. J. (2009). Avian influenza viruses in poultry products: a review. *Avian Pathology*. Doi: 10.1080/03079450902912200

Bereket, A., Desalew, T., & Shigdaf, M. (2014). Study on major causes of chicken mortality and associated risk factors in Bahir Dar Zuria District, Ethiopia. *African Journal of Agricultural Research*, *9*(48), 3465-3472.

Bett, B., McLaws, M., Jost, C., Schoonman, L., Unger, F., Poole, J., ... & Mariner, J. (2015). The effectiveness of preventative mass vaccination regimes against the incidence of highly pathogenic avian influenza on Java Island, Indonesia. *Transboundary and Emerging Diseases*, *62*(2), 163-173.

Bueckert D. (2004) Avian flu outbreak raises concerns about factory farms. Daily Herald-Tribune (Grande Prairie, Alberta), April 8, p. Retrieved from http://cp.org/english/online/full/agricu...040407/a040730A.html.

Cameroon, A.(2012,August). Avian influenza. Retrieved from http://agriculture.vic.gov.au/agriculture/pests-diseases-and-weeds/animal-diseases/poultry/avian-influenza/avian-influenza-bird-flu

Capua, I., & Marangon, S. (2006). Control of avian influenza in poultry. *Emerging Infectious Diseases*, *12*(9), 1319.

Chaharaein, B., Omar, A. R., Aini, I., Yusoff, K., & Hassan, S. S. (2009). Detection of H5, H7 and H9 subtypes of avian influenza viruses by multiplex reverse transcription-polymerase chain reaction. *Microbiological research*, *164*(2), 174-179.

Chaka, H., Goutard, F., Bisschop, S. P., & Thompson, P. N. (2012). Seroprevalence of Newcastle disease and other infectious diseases in backyard chickens at markets in Eastern Shewa zone, Ethiopia. *Poultry Science*, *91*(4), 862-869.

Cheema, B. F., Siddique, M., Sharif, A., Mansoor, M. K., & Iqbal, Z. (2011). Sero-prevalence of avian influenza in broiler flocks in district Gujranwala (Pakistan). *International Journal of Agriculture and Biology*, *13*(6).

Cox, N. J., & Subbarao, K. (2000). Global epidemiology of influenza: past and present. *Annual review of medicine*, *51*(1), 407-421

Cui, P., Zeng, X., Li, X., Li, Y., Shi, J., Zhao, C. ... & Chen, H. (2022). Genetic and biological characteristics of the globally circulating H5N8 avian influenza viruses and the protective efficacy offered by the poultry vaccine currently used in China. *Science China Life Sciences*, *65*(4), 795-808.

Deyle, E. R., Maher, M. C., Hernandez, R. D., Basu, S. & Sugihara, G. Global environmental drivers of influenza. Proc. Natl Acad. Sci. USA 113, 13081–13086 (2016).

Duan, L., Bahl, J., Smith, G. J. D., Wang, J., Vijaykrishna, D., Zhang, L. J., ... & Guan, Y. (2008). The development and genetic diversity of H5N1 influenza virus in China, 1996–2006. *Virology*, *380*(2), 243-254.

Dudo, A. D., Dahlstrom, M. F., & Brossard, D. (2007). Reporting a potential pandemic: A risk-related assessment of avian influenza coverage in US newspapers. *Science communication*, *28*(4), 429-454.

Farnsworth, M. L., Miller, R. S., Pedersen, K., Lutman, M. W., Swafford, S. R., Riggs, P. D., & Webb, C. T. (2012). Environmental and demographic determinants of avian influenza viruses in waterfowl across the contiguous United States. *PLoS One*, *7*(3), e32729.

Fasanmi, O. G., Odetokun, I. A., Balogun, F. A., & Fasina, F. O. (2017). Public health concerns of highly pathogenic avian influenza H5N1 endemicity in Africa. *Veterinary world*, *10*(10), 1194.

Fasina, F. O., Rivas, A. L., Bisschop, S. P., Stegeman, A. J., & Hernandez, J. A. (2011). Identification of risk factors associated with highly pathogenic avian influenza H5N1 virus infection in poultry farms, in Nigeria during the epidemic of 2006–2007. *Preventive veterinary medicine*, *98*(2-3), 204-208.

Firger, J. (2015, May 29). Avian Flu Threatening U.S. Poultry, Eggs Supply. *Newsweek* [USA]. Retrieved from http://www.newsweek.com/avian-flu-threatening-us-poultry-eggs-supply-337123

Fodor, E., & Te Velthuis, A. J. (2020). Structure and function of the influenza virus transcription and replication machinery. *Cold Spring Harbor perspectives in medicine*, *10*(9), a038398.

Fouchier, R. A., Bestebroer, T. M., Herfst, S., Van Der Kemp, L., Rimmelzwaan, G. F., & Osterhaus, A. D. (2000). Detection of influenza A viruses from different species by PCR amplification of conserved sequences in the matrix gene. *Journal of clinical microbiology*, *38*(11), 4096-4101.

Fournie, G. et al. Interventions for avian influenza A (H5N1) risk management in live bird market networks. Proc. Natl Acad. Sci. USA 110, 9177–9182 (2013).

Gharieb, R., Mohamed, M., Khalil, A., & Ali, A. (2019). Influenza A viruses in birds and humans: Prevalence, molecular characterization, zoonotic significance and risk factors' assessment in poultry farms. *Comparative Immunology, Microbiology and Infectious Diseases*, *63*, 51-57.

Gilbert, M., Slingenbergh, J., & Xiao, X. (2008). Climate change and avian influenza. *Revue scientifique et technique (International Office of Epizootics)*, *27*(2), 459.

Globig, A., Staubach, C., Sauter-Louis, C., Dietze, K., Homeier-Bachmann, T., Probst, C. ... & Conraths, F. J. (2018). Highly pathogenic avian influenza H5N8 clade 2.3. 4.4 b in Germany in 2016/2017. *Frontiers in Veterinary Science*, 240.

Gonzales, J. L., Stegeman, J. A., Koch, G., de Wit, S. J., & Elbers, A. R. (2013). Rate of introduction of a low pathogenic avian influenza virus infection in different poultry production sectors in the Netherlands. *Influenza and other respiratory viruses*, *7*(1), 6-10.

Greger, M. (2007). Bird Flu: A Virus of Our Own Hatching. *NY: Lantern Books*.

Hasan, R. B. (2019). *Temporal and Spatial Pattern of Avian Influenza in Ducks in the Major Wetlands in Bangladesh* (Doctoral dissertation, A thesis submitted in the partial fulfilment of the requirements for the degree of Master of Science in Epidemiology Department of Medicine and Surgery Faculty of Veterinary Medicine).

Hasni, M. S., Chaudhry, M., Mushtaq, M. H., Durrani, A. Z., Bin Rashid, H., Gill, S. S. ... & Sattar, H. (2021). Prevalence and Associated Risk Factors of Avian Influenza H9 in Backyard Poultry Populations of Two Agroecological Zones of Pakistan. *Pakistan Veterinary Journal*, *41*(1).

Hemsworth, P. H. (2021). Cage production and laying hen welfare. *Animal Production Science*, *61*(10), 821-836.

Hill, S. C., Manvell, R. J., Schulenburg, B., Shell, W., Wikramaratna, P. S., Perrins, C., ... & Pybus, O. G. (2016). Antibody responses to avian influenza viruses in wild birds broaden with age. *Proceedings of the Royal Society B: Biological Sciences*, *283*(1845), 20162159.

Hinchliffe, S. (2015). More than one world, more than one health: Re-configuring interspecies health. *Social science & medicine*, *129*, 28-35.

Hirve, S., Newman, L. P., Paget, J., Azziz-Baumgartner, E., Fitzner, J., Bhat, N. ... & Zhang, W. (2016). Influenza seasonality in the tropics and subtropics–when to vaccinate? *PloS one*, *11*(4), e0153003.

Hulse-Post, D. J. (2005). Role of domestic ducks in the propagation and biological evolution of highly pathogenic H5N1 influenza viruses in Asia. Proc. Natl Acad. Sci. USA 102, 10682–10687.

Hurt, A. C., Vijaykrishna, D., Butler, J., Baas, C., Maurer-Stroh, S., Silva-de-la-Fuente, M. C. ... & González-Acuña, D. (2014). Detection of evolutionarily distinct avian influenza A viruses in Antarctica. *MBio*, *5*(3), e01098-14.

Ipara, B. O., Otieno, D. J., Nyikal, R., & Makokha, N. S. (2021). The contribution of extensive chicken production systems and practices to Newcastle disease outbreaks in Kenya. *Tropical Animal Health and Production*, *53*(1), 1-13.

Isoda, N., Twabela, A. T., Bazarragchaa, E., Ogasawara, K., Hayashi, H., Wang, Z. J., ... & Sakoda, Y. (2020). Re-invasion of H5N8 high pathogenicity avian influenza virus clade 2.3. 4.4 b in Hokkaido, Japan, 2020. *Viruses*, *12*(12), 1439.

Jin, Y., Hu, Y., Han, D., & Wang, M. (2011). Chronic heat stress weakened the innate immunity and increased the virulence of highly pathogenic avian influenza virus H5N1 in mice. *Journal of Biomedicine and Biotechnology*, *2011*.

Kang, Y. M., Cho, H. K., Kim, J. H., Lee, S. J., Park, S. J., Kim, D. Y., ... & Kang, H. M. (2021). Single dose of multi-clade virus-like particle vaccine protects chickens against clade 2.3.

2.1 and clade 2.3. 4.4 highly pathogenic avian influenza viruses. *Scientific Reports*, *11*(1), 1-10.

Kariithi, H. M., Welch, C. N., Ferreira, H. L., Pusch, E. A., Ateya, L. O., Binepal, Y. S., ... & Suarez, D. L. (2020). Genetic characterization and pathogenesis of the first H9N2 low pathogenic avian influenza viruses isolated from chickens in Kenyan live bird markets. *Infection, Genetics and Evolution*, *78*, 104074.

Kausar, A., Anwar, S., Siddique, N., Ahmed, S., & Dasti, J. I. (2018). Prevalence of avian influenza H9N2 virus among wild and domesticated bird species across Pakistan. *Pakistan Journal of Zoology*, *50*(4), 1347-1354.

Kemboi, D. C., Chegeh, H. W., Bebora, L. C., Maingi, N., Nyaga, P. N., Mbuthia, P. G., ... & Githinji, J. M. (2013). Seasonal Newcastle disease antibody titer dynamics in village chickens of Mbeere District, Eastern Province, Kenya. *Livestock Research for Rural Development*, *25*(10), 181.

Kilpatrick, A. M., Chmura, A. A., Gibbons, D. W., Fleischer, R. C., Marra, P. P., & Daszak, P. (2006). Predicting the global spread of H5N1 avian influenza. *Proceedings of the National Academy of Sciences*, *103*(51), 19368-19373.

Kirunda, H., Kabi, F., Muwereza, N., Kabuuka, T., Kerfua, S. D., Kasaija, P. D. ... & Wabwire-Mangen, F. (2015). Seroprevalence and risk factors for exposure of free-range poultry to avian influenza viruses in important bird areas in Uganda. *Avian Diseases*, *59*(1), 64-70.

Koutsos, E. A., & Klasing, K. C. (2014). Factors modulating the avian immune system. In *Avian Immunology* (pp. 299-313). Academic Press.

Kuchipudi, S. V., Tellabati, M., Sebastian, S., Londt, B. Z., Jansen, C., Vervelde, L. ... & Chang, K. C. (2014). Highly pathogenic avian influenza virus infection in chickens but not ducks is associated with elevated host immune and pro-inflammatory responses. *Veterinary research*, *45*(1), 1-18.

Kurscheid, J. (2015). *Investigation into the role of live bird markets in the transmission and spread of highly pathogenic avian influenza in Bali and Lombok, Indonesia* (Doctoral dissertation, Murdoch University).

Lakspere, T. (2014). Genomic analysis of A (H1N1) pdm09 influenza virus in Finland.

Lam, P. Y. (2008). Avian influenza and pandemic influenza preparedness in Hong Kong. *Annals academy of medicine Singapore*, *37*(6), 489.

Lawes-Wickwar, S., Ghio, D., Tang, M. Y., Keyworth, C., Stanescu, S., Westbrook, J. ... & Epton, T. (2021). A rapid systematic review of public responses to health messages encouraging vaccination against infectious diseases in a pandemic or epidemic. *Vaccines*, *9*(2), 72.

Le, T. B., Le, V. P., Lee, J. E., Kang, J. A., Trinh, T. B. N., Lee, H. W., ... & Yoon, S. W. (2021). Reassortant highly pathogenic H5N6 avian influenza virus containing low pathogenic viral genes in a local live poultry market, Vietnam. *Current microbiology*, *78*(11), 3835-3842.

Lee, D. H., Bertran, K., Kwon, J. H., & Swayne, D. E. (2017). Evolution, global spread, and pathogenicity of highly pathogenic avian influenza H5Nx clade 2.3. 4.4. *Journal of veterinary science*, *18*(S1), 269-280.

Lesnoff, M., Peyre, M., Duarte, P. C., Renard, J. F., & Mariner, J. C. (2009). A simple model for simulating immunity rate dynamics in a tropical free-range poultry population after avian influenza vaccination. *Epidemiology & Infection*, *137*(10), 1405-1413.

Li, C., Bu, Z., & Chen, H. (2014). Avian influenza vaccines against H5N1 'bird flu'. *Trends in biotechnology*, *32*(3), 147-156.

Li, X., Lv, X., Li, Y., Xie, L., Peng, P., An, Q. ... & Chai, H. (2022). Emergence, prevalence, and evolution of H5N8 avian influenza viruses in central China, 2020. *Emerging microbes & infections*, *11*(1), 73-82.

Li, X., Sun, J., Lv, X., Wang, Y., Li, Y., Li, M. ... & Chai, H. (2020). Novel reassortants avian influenza A (H9N2) virus isolate in migratory waterfowl in Hubei province, China. *Frontiers in microbiology*, *11*, 220.

Liang, W. S., He, Y. C., Wu, H. D., Li, Y. T., Shih, T. H., Kao, G. S., ... & Chao, D. Y. (2020). Ecological factors associated with persistent circulation of multiple highly pathogenic avian influenza viruses among poultry farms in Taiwan during 2015-17. *PloS one*, *15*(8), e0236581.

Lierz, M., & Heffels-Redmann, U. (2019). Respiratory disorders. In *BSAVA Manual of Backyard Poultry Medicine and Surgery* (pp. 160-177). BSAVA Library.

Lowen, A. C., Mubareka, S., Steel, J., & Palese, P. (2007). Influenza virus transmission is dependent on relative humidity and temperature. *PLoS pathogens*, *3*(10), e151.

Lycett, S. J., Pohlmann, A., Staubach, C., Caliendo, V., Woolhouse, M., Beer, M. ... & Global Consortium for H5N8 and Related Influenza Viruses. (2020). Genesis and spread of multiple reassortants during the 2016/2017 H5 avian influenza epidemic in Eurasia. *Proceedings of the National Academy of Sciences*, *117*(34), 20814-20825.

Madsen, J. J., Grime, J. M., Rossman, J. S., & Voth, G. A. (2018). Entropic forces drive clustering and spatial localization of influenza A M2 during viral budding. *Proceedings of the National Academy of Sciences*, *115*(37), E8595-E8603.

Martin, V., Zhou, X., Marshall, E., Jia, B., Fusheng, G., FrancoDixon, M. A. ... & Gilbert, M. (2011). Risk-based surveillance for avian influenza control along poultry market chains in South China: the value of social network analysis. *Preventive veterinary medicine*, *102*(3), 196-205.

Matheka, D. M., Mokaya, J., & Maritim, M. (2013). Overview of influenza virus infections in Kenya: past, present and future. *The Pan African Medical Journal*, *14*.

Metras, R., Stevens, K. B., Abdu, P., Okike, I., Randolph, T., Grace, D. ... & Costard, S. (2013). Identification of potential risk factors associated with highly pathogenic avian influenza subtype H5N1 outbreak occurrence in Lagos and Kano States, Nigeria, during the 2006–2007 epidemics. *Transboundary and emerging diseases*, *60*(1), 87-96.

Miriam, K. N., Gowland, M. J., & Nkurumwa, A. (2014). Indigenous Chicken Rearing Systems and Their Influence on Household Income among Small-Scale Farmers in Mau-Narok Division of Njoro District, Nakuru County, Kenya. *International Journal of Advanced Research*, *2*(5), 638-650.

Mohammed, S. E. A. (2008). Prevalence of avian influenza virus (h5n1) antibodies in chickens in Khartoum north, Sudan (doctoral dissertation, university of Khartoum).

Monke, J., & Corn, M. L. (2007, January). Avian influenza in poultry and wild birds. Congressional Research Service, Library of Congress.

Monne Isabella; Tony M. Joannis; Alice Fusaro; Paola De Benedictis; Lami H., Lombin; Husseini Ularamu; Anthony Egbuji; Poman Solomon; Tim U. Obi; Giovanni Cattoli; Ilaria Capua (2008) Reassortant Avian Influenza Virus (H5N1) in Poultry, Nigeria, 2007; *Emerging Infectious Diseases*.;14(4):637-640.

Morgan, R., & Klein, S. L. (2019). The intersection of sex and gender in the treatment of influenza. *Current opinion in virology*, 35, 35-41.

Moyen, N., Hoque, M., Mahmud, R., Hasan, M., Sarkar, S., Biswas, P. K., ... & Fournié, G. (2021). Avian influenza transmission risk along live poultry trading networks in Bangladesh. *Scientific reports*, *11*(1), 1-11.

Munyua, P. M. (2015). *Identification and characterization of influenza a viruses among domestic animals in selected sites in Kenya* (Doctoral dissertation, University of Nairobi).

Munyua, P. M., Githinji, J. W., Waiboci, L. W., Njagi, L. M., Arunga, G., Mwasi, L., ... & Katz, M. A. (2013). Detection of influenza A virus in live bird markets in Kenya, 2009–2011. *Influenza and other respiratory viruses*, *7*(2), 113-119.

Naguib, M. M., Verhagen, J. H., Mostafa, A., Wille, M., Li, R., Graaf, A. ... & Olsen, B. (2019). Global patterns of avian influenza A (H7): virus evolution and zoonotic threats. *FEMS Microbiology Reviews*, *43*(6), 608-621.

Naing, Lin & Winn, Than & Nordin, Rusli. (2006). Practical Issues in Calculating the Sample Size for Prevalence Studies. Archives of Orofacial Sciences. 1.

Napp, S., Majó, N., Sánchez- Gónzalez, R., & Vergara- Alert, J. (2018). Emergence and spread of highly pathogenic avian influenza A (H5N8) in Europe in 2016- 2017. *Transboundary and Emerging Diseases*, *65*(5), 1217-1226.

Ngotho, A. (2014, January 28). KENYA AT RISK OF POULTRY VIRAL DISEASE. *THE STAR*. http://allafrica.com/stories/201401280872.htm

Nguyen, D. C., Uyeki, T. M., Jadhao, S., Maines, T., Shaw, M., Matsuoka, Y. ...& Katz, J. M. (2005). Isolation and characterization of avian influenza viruses, including highly pathogenic H5N1, from poultry in live bird markets in Hanoi, Vietnam, in 2001. *Journal of virology*, *79*(7), 4201-4212..

Nili, H., & Asasi, K. (2002). Natural cases and an experimental study of H9N2 avian influenza in commercial broiler chickens of Iran. *Avian Pathology*, *31*(3), 247-252.

Nooruddin, G. M., Hossain, M. T., Mohammad, M., & Rahman, M. M. (2006). Sero-epidemiology of avian influenza virus in native chicken in Bangladesh. *Int J Poult Sci*, *5*, 1029-1033.

Ntsomboh-Ntsefong, G., Shariati, M. A., Khan, M. U., & Karapetkovska-Hristova, V. (2017). Incidence of avian flu shocks on poor household livelihoods of poultry farmers in Africa. *International Journal of Avian & Wildlife Biology (IJAWB)*, *2*(1), 7-11.

Nyaga, P. (2007). Good biosecurity practices in small scale commercial and scavenging production systems in Kenya.

Offeddu, V., Cowling, B. J., & Peiris, J. M. (2016). Interventions in live poultry markets for the control of avian influenza: A systematic review. *One Health*, *2*, 55-64.

Ofula, V. O., Franklin, A. B., Root, J. J., Sullivan, H. J., Gichuki, P., Makio, A., ... & Schnabel, D. (2013). Detection of avian influenza viruses in wild waterbirds in the Rift Valley of Kenya using fecal sampling. *Vector-Borne and Zoonotic Diseases*, *13*(6), 394-40

OIE. (2015). Two More Avian Flu Outbreaks Found in Nigeria. Poultry news [Nigeria].

Olabisi, L. S., Osuntade, O., Liverpool-Tasie, L. S. O., & Adebiyi, J. (2021). Participatory modelling for climate change adaptation: the poultry sector in Nigeria. *Climate policy*, *21*(5), 666-677.

Omiti John, M., & Okuthe, S. O. (2008). An overview of the poultry sector and status of highly pathogenic avian influenza (HPAI) in Kenya. *Draft Report. International Food Policy Research Institute/International Livestock Research Institute*.

Omiti, J. M., & Okuthe, S. O. (2009). *An overview of the poultry sector and status of Highly Pathogenic Avian Influenza (HPAI) in Kenya: Background Paper*. International Food Policy Research Institute (IFPRI).

Otiang, E., Thumbi, S. M., Campbell, Z. A., Njagi, L. W., Nyaga, P. N., & Palmer, G. H. (2021). Impact of routine Newcastle disease vaccination on chicken flock size in smallholder farms in western Kenya. *Plos one*, *16*(3), e0248596.

Otte, J., Pfeiffer, D., & Roland-Holst, D. (2007). Avian Influenza, Public Health and Smallholder Livelihoods in the Greater Mekong Sub-Region.

Pa'gina principal.Retrieved from Track 2015 avian influenza outbreaks in North America. (n.d.). Retrieved from http://www.wattagnet.com/articles/22140-track-2015-avian-influenza-

outbreaks-in-north-america Pathobiology of avian influenza virus infections in birds and mammals. *Avian influenza*, *1*.

Pabbaraju, K., Wong, S., Lee, B., Tellier, R., Fonseca, K., Louie, M., & Drews, S. J. (2011). Comparison of a singleplex real- time RT- PCR assay and multiplex respiratory viral panel assay for detection of influenza "A" in respiratory specimens. *Influenza and Other Respiratory Viruses*, *5*(2), 99-103.

Paul, M. C., Vergne, T., Mulatti, P., Tiensin, T., & Iglesias, I. (2019). Epidemiology of avian influenza viruses. Frontiers in veterinary science, 6, 150.

Peacock, T. H. P., James, J., Sealy, J. E., & Iqbal, M. (2019). A global perspective on H9N2 avian influenza virus. *Viruses*, *11*(7), 620.

Perry M. 2005. Asia must change age-old farming to stop disease. Reuters, September 22

Petrova, V. N., & Russell, C. A. (2018). The evolution of seasonal influenza viruses. *Nature Reviews Microbiology*, *16*(1), 47-60.

Pfeiffer, J., Suarez, D. L., Sarmento, L., To, T. L., Nguyen, T., & Pantin-Jackwood, M. J. (2010). Efficacy of commercial vaccines in protecting chickens and ducks against H5N1 highly pathogenic avian influenza viruses from Vietnam. *Avian diseases*, *54*(s1), 262-271

Poovorawan, Y., Pyungporn, S., Prachayangprecha, S., & Makkoch, J. (2013). Global alert to avian influenza virus infection: from H5N1 to H7N9. *Pathogens and global health*, *107*(5), 217-223.

Pusch, E. A., & Suarez, D. L. (2018). The multifaceted zoonotic risk of H9N2 avian influenza. *Veterinary sciences*, *5*(4), 82.

Pym, R. A., & Alders, R. G. (2012). Introduction to village and backyard poultry production. *Altern. Syst. Poult. Health Welf. Product*, 97-109.

Qiu, B. F., Liu, W. J., Peng, D. X., Hu, S. L., Tang, Y. H., & Liu, X. F. (2009). A reverse transcription-PCR for subtyping of the neuraminidase of avian influenza viruses. *Journal of virological methods*, *155*(2), 193-198.

Ramey, A. M., Hill, N. J., DeLiberto, T. J., Gibbs, S. E., Camille Hopkins, M., Lang, A. S. ... & Wan, X. F. (2022). Highly pathogenic avian influenza is an emerging disease threat to wild birds in North America. *The Journal of Wildlife Management*, *86*(2), e22171.

Rebel, J. M., Peeters, B., Fijten, H., Post, J., Cornelissen, J., & Vervelde, L. (2011). Highly pathogenic or low pathogenic avian influenza virus subtype H7N1 infection in chicken lungs: small differences in general acute responses. *Veterinary research*, *42*(1), 1-11.

Reemers, S. S., van Leenen, D., Koerkamp, M. J. G., van Haarlem, D., van de Haar, P., van Eden, W., & Vervelde, L. (2010). Early host responses to avian influenza A virus are prolonged and enhanced at transcriptional level depending on maturation of the immune system. *Molecular Immunology*, *47*(9), 1675-1685.

Ritchie BW. (1995). *Avian Viruses: Function and Control* (Lake Worth, FL: Wingers Publishing).

Robertson, I. D. (2020). Disease control, prevention and on-farm biosecurity: the role of veterinary epidemiology. *Engineering*, *6*(1), 20-25.

Roche, X., Fredrick, K., Kamata, A., Okuthe, S., Kone, P., Wiersma, L. ... & Lubroth, J. 2016–2018 *Spread of H5N8 highly pathogenic avian influenza (HPAI) in sub-Saharan Africa* (Lake Worth, FL: Wingers Publishing).

Sampathkumar, P., & Maki, D. G. (2005, December). Avian H5N1 Influenza—Are We Inching Closer to a Global Pandemic? In *Mayo Clinic Proceedings* (Vol. 80, No. 12, pp. 1552-1555). Elsevier.

Sandman, P. M., & Lanard, J. (2006). Bird flu, pandemic flu, and poultry markets: playing Ostrich or talking Turkey *Retrieved March*, *1*, 2008.

Sarwar, M., Muhammad, K., Rabbani, M., Younus, M., Sarwar, N., Ali, M. A., & Ahad, A. (2013). Prevalence of avian influenza viruses in live bird markets of Lahore. *JAPS, Journal of Animal and Plant Sciences*, *23*(2), 388-392.

Selam, M., & Kelay, B. (2013). Causes of village chicken mortality and interventions by farmers in Adaa District, Ethiopia. *International Journal of Livestock Production*, *4*(6), 88-94.

Si, Y., Wang, T., Skidmore, A. K., de Boer, W. F., Li, L., & Prins, H. H. (2010). Environmental factors influencing the spread of the highly pathogenic avian influenza H5N1 virus in wild birds in Europe. *Ecology and Society*, *15*(3).

Sims, L. D. (2008, November). Risks associated with poultry production systems. In *International conference poultry in the twenty-first century* (Vol. 1, p. 24). Bangkok: Thailand: Avian Influenza and beyond: 5–7 November 2008.

Sims, L., Harder, T. C., Brown, I. H., Gaidet, N., Belot, G., von Dobschuetz, S. ... & Lubroth, J. (2017). *Highly pathogenic H5 avian influenza in 2016 and early 2017-observations and future perspectives*. (Lake Worth, FL: Wingers Publishing).

Sonaiya, E. B. (2007). Family poultry, food security and the impact of HPAI. *World's Poultry Science Journal*, *63*(1), 132-138.

Spackman, E., Senne, D. A., Myers, T. J., Bulaga, L. L., Garber, L. P., Perdue, M. L., ... & Suarez, D. L. (2002). Development of a real-time reverse transcriptase PCR assay for type A influenza virus and the avian H5 and H7 hemagglutinin subtypes. *Journal of clinical microbiology*, *40*(9), 3256-3260.

Ssematimba, A., Hagenaars, T. J., De Wit, J. J., Ruiterkamp, F., Fabri, T. H., Stegeman, J. A., & De Jong, M. C. M. (2013). Avian influenza transmission risks: analysis of biosecurity measures and contact structure in Dutch poultry farming. *Preventive veterinary medicine*, *109*(1-2), 106-115.

Stevens, K. B., Gilbert, M., & Pfeiffer, D. U. (2013). Modelling habitat suitability for occurrence of highly pathogenic avian influenza virus H5N1 in domestic poultry in Asia: a spatial multi criteria decision analysis approach. *Spatial and spatial-temporal epidemiology*, *4*, 1-14.

Stokstad, E. (2022). Wrestling with bird flu, Europe considers once-taboo vaccines. *Science (New York, NY)*, *376*(6594), 682-683.

Suarez, D. L. (2005). Overview of avian influenza DIVA test strategies. *Biologicals*, *33*(4), 221-226.

Sulaiman, L., Shittu, I., Fusaro, A., Inuwa, B., Zecchin, B., Gado, D. ... & Meseko, C. (2021). Live bird markets in Nigeria: A potential reservoir for H9N2 Avian Influenza Viruses. *Viruses*, *13*(8), 1445.

Swayne, D. E., & Kapczynski, D. R. (2016). Vaccines and vaccination for avian influenza in poultry. *Animal influenza*, 378-434.

Swayne, D. E., & Suarez, D. L. (2000). Highly pathogenic avian influenza. *Revue Scientifique technique-office international des epizooties*, *19*(2), 463-475.

Swayne, D. E., Suarez, D. L., & Sims, L. (2020). Influenza, p 210–256. *Diseases of poultry*. Wiley, Ames, IA, USA. *Scientific reports*,

Tan, K. X., Jacob, S. A., Chan, K. G., & Lee, L. H. (2015). An overview of the characteristics of the novel avian influenza A H7N9 virus. *Frontiers in microbiology*, *6*, 140.

Tehrani, F., Shoushtari, A., Ghalyanchilangeroudi, A., & Amirhajloo, S. (2021). Outbreak Investigation of Officially Reported and Highly Pathogenic Avian Influenza (H5N8 Subtype) in Iran during 2016. *Archives of Razi Institute*, *76*(1), 17.

Thurlow, J. (2010). *Implications of avian flu for economic development in Kenya* (No. 951). International Food Policy Research Institute (IFPRI).

Tombari, W., Paul, M., Bettaieb, J., Larbi, I., Nsiri, J., Elbehi, I. ... & Ghram, A. (2013). Risk factors and characteristics of low pathogenic avian influenza virus isolated from commercial poultry in Tunisia. *PLoS One*, *8*(1), e53524.

Trampuz, A., Prabhu, R. M., Smith, T. F., & Baddour, L. M. (2004, April). Avian influenza: a new pandemic threat. In *Mayo Clinic Proceedings* (Vol. 79, No. 4, pp. 523-530).

USDA. 2006. *Draft Summary of the National Highly Pathogenic Avian Influenza (HPAI) Response Plan. USDA, APHIS, Veterinary Services, Emergency Management.* Washington, DC. 72 p.

Uwishema, O., Adriano, L. F., Chalhoub, E., Onyeaka, H., Mhanna, M., David, S. C. ... & Berjaoui, C. (2021). Bird flu outbreak amidst COVID‐ 19 pandemic in South Africa: efforts and challenges at hand. *Journal of Medical Virology*, *93*(10), 5676-5679.

Van Kerkhove, M. D., Vandemaele, K. A., Shinde, V., Jaramillo-Gutierrez, G., Koukounari, A., Donnelly, C. A., ... & WHO Working Group for Risk Factors for Severe H1N1pdm Infection. (2011). Risk factors for severe outcomes following 2009 influenza A (H1N1) infection: a global pooled analysis. *PLoS medicine*, *8*(7), e1001053.

Wade, A., Jumbo, S. D., Zecchin, B., Fusaro, A., Taiga, T., Bianco, A., ... & Monne, I. (2018). Highly pathogenic avian influenza A (H5N8) virus, Cameroon, 2017. *Emerging Infectious Diseases*, *24*(7), 1367.

Wang, X., Wang, Q., Cheng, W., Yu, Z., Ling, F., Mao, H., & Chen, E. (2017). Risk factors for avian influenza virus contamination of live poultry markets in Zhejiang, China during the 2015–2016 human influenza season. *Scientific Reports*, *7*(1), 1-9.

Wang, Y., Jiang, Z., Jin, Z., Tan, H., & Xu, B. (2013). Risk factors for infectious diseases in backyard poultry farms in the Poyang Lake area, China. *PloS one*, *8*(6), e67366.

Weber, T. P., & Stilianakis, N. I. (2008). Inactivation of influenza A viruses in the environment and modes of transmission: a critical review. *Journal of infection*, *57*(5), 361-373.

Weeks, C. A., & Duncan, I. J. (2017). How Can We Sustain the Demand for Eggs?. In *The Meat Crisis* (pp. 139-154). Routledge.

Wegdan H. Ali and M.A.S. Kheir, 2007. Isolation of Highly Pathogenic avian Influenza Virus (H5N1) from Poultry in Sudan in 2006. *Journal of Animal and Veterinary Advances, 6: 61-63.*

Wilson, H. M., Hall, J. S., Flint, P. L., Franson, J. C., Ely, C. R., Schmutz, J. A., & Samuel, M. D. (2013). High seroprevalence of antibodies to avian influenza viruses among wild waterfowl in Alaska: implications for surveillance. *PLoS One, 8*(3), e58308.

Wood, J. P., Choi, Y. W., Chappie, D. J., Rogers, J. V., & Kaye, J. Z. (2010). Environmental persistence of a highly pathogenic avian influenza (H5N1) virus. *Environmental science & technology, 44*(19), 7515-7520.

Yang, J., Xie, D., Nie, Z., Xu, B., & Drummond, A. J. (2019). Inferring host roles in bayesian phylodynamics of global avian influenza A virus H9N2. *Virology, 538*, 86-96.

Yoon, S. W., Webby, R. J., & Webster, R. G. (2014). Evolution and ecology of influenza A viruses. *Influenza pathogenesis and control-volume I*, 359-375.

ZENG, X. Y., CHEN, X. H., WU, J. J., BAO, H. M., PAN, S. X., LIU, Y. J., ... & CHEN, H. L. (2020). Protective efficacy of an H5/H7 trivalent inactivated vaccine produced from Re-11, Re-12, and H7-Re2 strains against challenge with different H5 and H7 viruses in chickens. *Journal of Integrative Agriculture, 19*(9), 2294-2300.

Zhang, Z., Chen, D., Chen, Y., Wang, B., Hu, Y., Gao, J. ... & Xiong, C. (2014). Evaluating the impact of environmental temperature on global highly pathogenic avian influenza (HPAI) H5N1 outbreaks in domestic poultry. *International journal of environmental research and public health*, *11*(6), 6388-6399.

Zhao, Y., Richardson, B., Takle, E., Chai, L., Schmitt, D., & Xin, H. (2019). Airborne transmission may have played a role in the spread of 2015 highly pathogenic avian influenza outbreaks in the United States. *Scientific reports*, *9*(1), 1-10.

APPENDICES

Appendix I: Questionnaire

Questionnaire For data collection on chicken age, breed, sex, management system, restocking system and vaccination from the farmers bringing their chicken to be screened at the regional veterinary laboratory Eldoret, Uasin Gishu County, Kenya. During the study the researcher used both English and Kiswahili.

Questionnaire No… Date of interview..... …

Name of interviewer.....

A. Background information

1. Subcounty.....location.....

B. Breed of chicken sampled

2. Type of chicken breed sampled? (a) Layer breeds (b) broiler breeds (c) local breeds.

C. Age and sex of chicken sampled

3. ☐ old (above 4 months) .....☐male..... ☐ female.

☐ Young (below 3 months) .....☐male.....☐ Female

D. Management system of chicken

4. What type of management system do you practice?

(a) Free-range management system

(b) Confined commercial management system.

5. Where do you get your chicken stock and restocking source? (a) Purchase from the market (b) from Neighbors /from your home village (d) hatched from your own chickens.

6. Do you vaccinate your chicken? (a) Yes (b) No. if yes

7. Are newly introduced chicken initially isolated from the other chickens?

(a) Yes (b) No

C. Avian flu disease and its control

8. Are you aware of a disease known as avian flu disease? (1) Yes (2) No

If yes, answer the following questions.

i) Does sick chicken(s) show the following signs……

Paralysis..... (Yes/No/Don't know)

Swelling..... (Yes/No/Don't know)

Discoloration of the head..... (Yes/No/Don't know)

Diarrhoea..... (Yes/No/Don't know)

Nasal discharge..... (Yes/No/Don't know)

Coughing..... (Yes/No/Don't know)

Difficulty breathing..... (Yes/No/Don't know)

9. Is there season or part of the year for the occurrence of avian influenza?

Yes

No

If yes, specify the season

Dry

Rainy

10. Which chicken are mostly affected?

☐ Adults (Above 4 months) ☐Male ☐Female

☐ Chicks below 4 months months) ☐Male ☐ Female

11. How many of the chicken get sick? (a) All (b) few (c) none

12. How many of the sick chicken die? (a) All (b) (c) few (d) none

13. How do you think the disease is spread among chicken(s)?

Direct contact with sick poultry

Indirect contact (faecal matter, respiratory discharge, intestinal content, feathers

Appendix II: Questionnaire consent form

I (respondents name)……..hereby give my permission to Carozone Sitati (researcher) to allow me to respond to questionnaire and quote my response in the research paper. I understand the thesis title is **"PREVALENCE OF INFLUENZA A VIRUS IN CHICKEN AND DETERMINATION OF RISK FACTORS OF ITS PRESENCE AND SPREAD IN UASIN GISHU COUNTY, KENYA"**

I understand that the researchers will maintain my anonymity with regard to my responses to questionnaire items.

I hereby give my permission.

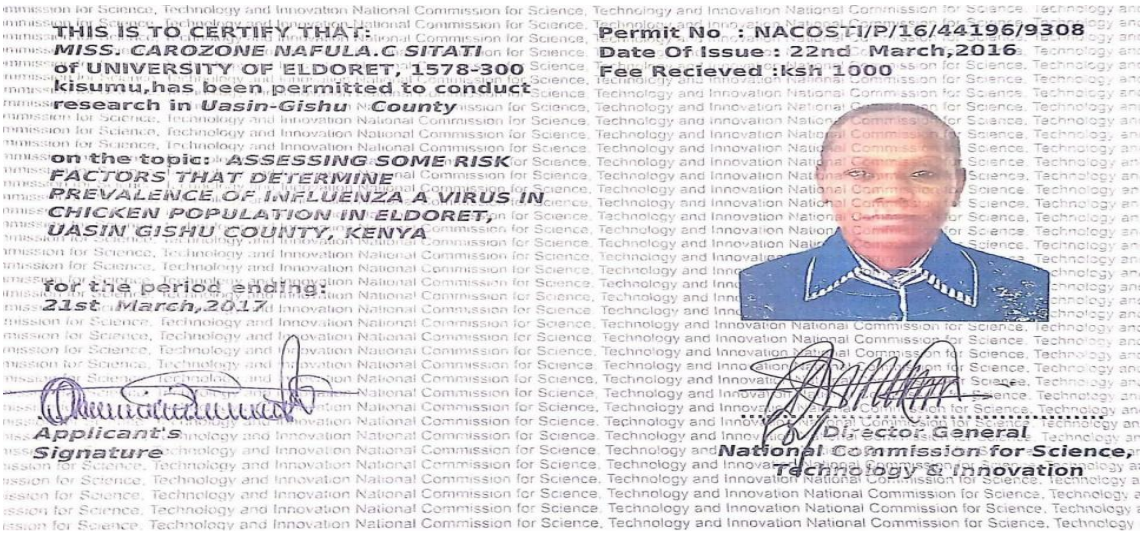
Signature…… Date…….

Appendix III: List of reagents

- MagMax Kit
- Pipette tips, 200ul (RT 250)
- Pipette tips, 1000ul
- Pipette tips, 20ul
- Pipette tips, 10ul
- Absolute Ethanol
- Absolute Isopropanol
- Cryogenic vials 1.8ml
- Microcentrifuge tubes 1.5ml
- Sterile gloves
- Disposable Masks (N 95)
- Kingfisher combo kits
- Oralphargeal swabs
- RNase-free (PCR-grade) water
- Standardized probes &primers
- Agpath ID one step PCR Kit
- Viral transportation media

Appendix IV: Research Approval

The research study was approved by National commission for science, technology and Innovation (NACOSTI). Permit number NACOSTI/P/16/44196/9308



Appendix V: Similarity Report

Turnitin Originality Report

PREVALENCE OF INFLUENZA A VIRUS IN CHICKEN AND DETERMINATION OF RISK FACTORS OF ITS PRESENCE AND SPREAD IN UASIN GISHU COUNTY, KENYA by Carozone Sitati

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