

CHAPTER 1 INTRODUCTION

1.1 Background

Avian influenza is a highly infectious, contagious, zoonotic viral disease that belongs to the family “Orthomyxoviridae”. AIV was first identified in 1878 in Italy, because of its pathogenicity from other diseases of poultry and causes high mortality (Alexander *et al.*, 2009), first, it was called fowl plague that related to fowl cholera. In 1880, it was called Typhus exudatious gallinarum. In 1918, it was called Spanish flu and caused the death of more than 50 million people around the globe. In 1955, it was confirmed classical fowl plague (Lupiani *et al.*, 2009). In 1957, it was called Asian flu, and in 1968 it was called Hong Kong flu (Horimoto and Kawaoka, 2005). During the First International Symposium on Avian Influenza, which took place in 1981, the term "fowl plague" was eventually replaced with the more accurate term "avian influenza."

The avian influenza A virus subtype H5N1 is highly pathogenic for humans and poultry that causes huge economic losses in Indonesia and the rest of the world while the low pathogenic avian influenza A virus subtype H9N2 causes low fatality as compared to H5N1. The burden of this disease has resulted in the death of millions of poultry all over the world (Liu *et al.*, 2020) including Indonesia (Lam *et al.*, 2008; Karo-Karo *et al.*, 2019).

The disease is endemic in Indonesia continuously presenting a latent threat to both poultry industry and public health due to its zoonotic nature in the form of economic losses and medication (Dharmayanti *et al.*, 2018). Many temporal and spatial risk factors are associated with the outbreak of the disease, virulence of

different strains of AI viruses and frequent mutation leading in ineffective prophylactic and therapeutic approaches in the control of AI in Indonesia (Henning *et al.*, 2019).

The first outbreak of the HPAI virus subtype H5N1 was identified in Indonesia in December 2003 (Li *et al.*, 2004), affecting all provinces of Indonesia except one province. The H5N1 virus was first introduced to Java and soon spread to other parts of the country (Amin, 2005). So far, AIV is a big problem in Indonesia's poultry industry. The outbreak was so fatal that it killed more than 16 million poultry and damaged Indonesia's poultry industry (Oner *et al.*, 2006; Lam *et al.*, 2008). Thirty-two (32) provinces out of thirty-four (34) have confirmed the outbreak, which has resulted in the death of millions of birds (Karo-Karo *et al.*, 2019) and the closure of many farms in high-incidence areas (Basuno *et al.*, 2010).

The highly populated areas (Gempol, Jombang, Pasuruan, Batu, Singosari, Sukorejo, Mojokerto, Lamongan, and Madiun) in East Java that are used for poultry and duck farming were the cause of the spread of AIV infection. Backyard poultry in Java lacks basic hygiene measures, mixes of different domestic poultry species and ducks scavenged on the harvested paddy. All these factors may greatly increase the risk and spread of avian influenza infection (Sumiarto and Arifin, 2008).

In April 2011, Gorontalo announced a new outbreak, leaving only one province free of disease (OIE, 2011). In September 2012, many Central Java duck farms recorded increased mortality due to AIV subtype H5. All H5N1 viruses that were found in Indonesia until the year 2008 were grouped into clade 2.1. Within this

clade, there are three virus sub lineages: 2.1.1, 2.1.2, and 2.1.3 (WHO, 2008). The viruses belonging to clade 2.1.1 were predominantly isolated from HPAI-infected chickens during outbreaks that occurred between 2003 and 2005 (Takano *et al.*, 2009).

Between 2004 and 2007, the viruses belonging to clade 2.1.2 were isolated from both birds and humans in Sumatra, while in 2004, viruses belonging to clade 2.1.3 were identified in either birds or humans. Whereas the clade 2.1.3 viruses have become prevalent in a number of Indonesian areas, a novel sub lineage virus has arisen since 2004. In succeeding decades, clade 2.1 viruses spread throughout Indonesia and became enzootic (Dharmayanti *et al.*, 2014).

Furthermore, a novel HPAI H5N1 clade 2.3 virus has been identified in poultry outbreaks from the year 2012. It was reported in September 2012 that the AIV H5 subtype caused severe mortality on numerous duck farms in Central Java. Surprisingly, the duck isolates' HA genes were most similar to those of the recently discovered clade 2.3.2.1 viruses, which were circulating in Vietnam, China, and Hong Kong, rather than the long-established Indonesian clade 2.1 isolates (Dharmayanti *et al.*, 2014).

The Clade 2.3.2.1c was discovered during an outbreak in September 2013 at a turkey farm in East Java, Indonesia. Clade 2.1.3.2b was discovered in February 2014 when a sick chicken was found at a live-poultry market. Clade 2.3.2.1c was designated as Eurasian lineage, while clade 2.1.3.2b was designated as Indonesian lineage based on the place of emergence (Shimizu *et al.* 2016). According to the findings of Karo-karo *et al.* (2022), clades 2.3.2.1c and 2.3.2.1b have a common

ancestor, which is clade 1. This suggests that clade 2.3.2.1 emerged and diversified from China and other Asian countries (Karo karo *et al.*, 2022). The last human death was reported in Bali because of the HPAI H5N1 virus clade 2.3.2.1 on September 26, 2017 (Esfandiari *et al.*, 2018).

According to a study by Shimizu *et al.* (2016), there was a higher seroprevalence of avian influenza H5N1 Eurasian lineage (84%) than Indonesian lineage (34%), among workers in live-poultry marketplaces in Indonesia (Shimizu *et al.* 2016). The collection of genetic and antigenic data is essential in order to gain a deeper comprehension of the epidemiology of HPAI in Indonesia. Scavenging ducks are potentially an important source of H5 virus for local hens, according to the findings of a recent epidemiological investigation conducted in smallholder farms in central Java, Indonesia (Lam *et al.*, 2008; Takano *et al.*, 2009).

In late 2016 and early 2017, LPAIV H9N2 was first identified by chicken farm managers from different production sectors, known as sectors 1, 2, and 3, complaining of increased mortality and decreased production of eggs. Some viral diseases, such as NDV, IBV, and H5N1 which have not been observed in all cases, have been identified. It has been estimated that more than 50% of cases were positive for H9N2. The introduction of the enzootic LPAI subtype H9N2 in Indonesia was officially announced by the government in 2017, but it is still poorly documented.

The introduction of this LPAI as an endemic zoonotic may endanger the function of H9N2 as an incubator to promote the emergence of new zoonotic strains by the Chinese center for disease control and prevention (Jin *et al.*, 2014). Since 2004, to monitor AIV subtype H5N1 infection, the Indonesian Government has been

introducing vaccination and enhancing biosecurity in poultry farms, performing intensive surveillance of pathogens, implementing endemic and comprehensive stamping-out policies in newly infected areas, and improving public awareness of the disease (Dharmayanti *et al.*, 2018).

A novel H9N2 genotype, expressed by A/Chicken/Sidrap/07161511-1/2016, A/Chicken/Sidrap/07161511-61/2016, A/Chicken/Sidrap/07170094-44OA/2017 A/chicken/West-Java/BBLitvet-RI/2017, A/chicken/East Java/Spq147/2018, A/chicken/East Java/BLi25Ut/2018, and A/chicken/Central Java/SLO.105/2018 were isolated from Indonesian poultry birds, and these replaced by Y280 or G1 Lineage (Muflihanah *et al.*, 2017; Dharmayanti *et al.*, 2020; Nugroho *et al.*, 2021). Inter-and intra-subtype genotype genomic recombination between LPAIV subtype H9N2 (G1-like), HPAIV subtype H5N1 (clade 2.2), and H7N9 viruses resulted in these novel reassortants.

The genotype of H9N2 in Indonesia represented 98% sequence identity with that of A/Muscovy duck/Vietnam/LBM719/2014(H9N2) was isolated from chicken in a study conducted by (Jonas *et al.*, 2018). The characteristics of circulating AIVs of the subtype H9N2 in Indonesia are poorly understood due to the paucity of available evidence. The vast majority of these virus sequences have been assigned the G1 (clade h9.4.1) or Y280 designation (h9.4.2 of clade).

Viruses from India, Iran, Israel, and Pakistan are part of the h9.4.1 lineage, whereas the h9.4.2 lineage was composed entirely of strains that were discovered in China. Before the year 2007, H9N2 was categorized as belonging to the clade h9.4.2.1–h9.4.2.4. After 2010, the clade H9.4.2.5 represented by Guangxi/55/2005

became well established and quickly began spreading throughout the country. In addition to this, every single H9.4.2.5 virus that was found between 2013 and 2015 had human-like receptor specificity, which suggests that there is a chance of transmission between species (Shen *et al.*, 2015).

The live bird markets are an important factor in the endemicity of the avian influenza virus in Indonesia (Karo-Karo *et al.*, 2019). An LBM was defined as an open space in which > 2 poultry stalls sell live poultry at least once a week, and only those selling >400 poultry per day were considered eligible for this study. Live bird markets (LBMs) are an important component of traditional poultry marketing systems in many developing countries.

The characteristics of LBMs have not been particularly well studied but are known to vary from region to region depending upon the needs of the local customers (Senne *et al.*, 2003; Kung *et al.*, 2007). Such live bird retail stalls (LBRS) can serve as an AIV reservoir, a major threat to animal and public health, and local risk factors that are important to recognize (Chaudhry *et al.*, 2018).

The most important known risk factors are poultry trading patterns (Kung *et al.*, 2007), mixing newly arrived birds in cages with leftover birds from previous batches (Indriani *et al.*, 2010), mixing species in the same cages, chicken purchasing source, sharing of equipment used for slaughtering, eating, watering and weighing birds, the number of birds sold per day (Senne *et al.*, 2003; Garber *et al.*, 2007), and movement of poultry through LBMs (Kung *et al.*, 2007).

The findings of early surveillance programs carried out in Indonesia showed a much higher prevalence of HPAI H5N1 in these LBMs when compared to poultry-

producing areas, suggesting that the HPAI virus would spread extensively during the trading process (Joerg Henning *et al.*, 2019). However, there are major variations in the supply chain of backyard poultry (e.g., Kampung or indigenous chickens) and commercial poultry (e.g., broilers and layers) sold in urban LBMs in Indonesia (Joerg Henning *et al.*, 2019).

The current research study was carried out to investigate the following risk factors [LBMs trading category (Desvaux *et al.*, 2011), the chicken population in LBMs, how many days operational per week? are you keeping birds outside cages? are you having chicken breeds other than broilers in the cages? are you see rodents in the stall? are you share the equipment used for slaughtering, feeding, watering, and weighing the birds (Indriani *et.al.*, 2019)? are you mixing the poultry arriving on different days in the same cages? an inspection team from authorities come to visit LBMs, use of detergent during cleaning mixing poultry arriving on different days in the same cages, the average length of poultry stays in LBMs, mixing of different species in the same cage, use of detergent during cleaning the wooden tables, are wild birds present around stall? presence of sick birds in the stall, are you dispose of the dead birds? presence of Guinea fowl in the stall, presence of Pheasants in the stall, presence of Chukars/partridges in the stall, presence of pet birds, stray cats accessing the stall, market vehicle picking up birds from the farm] (Sims *et al.*, 2003), by filling out a standardised predesigned questionnaire. Furthermore, the study was important in estimating the prevalence, identifying the molecular characteristics, and phylogenetic analysis of AIV subtypes H5N1 and H9N2 in poultry of live bird markets of East Java province of Indonesia.

A comprehensive study is needed to explore the exact epidemiology and pathogenesis of the disease and molecular identification and characterization of the virus and risk factors associated with the transmission of the AIV. Therefore, present study was carried out to explore the prevalence, molecular identification and characterization and risk factors associated with AIV.

The recent survey was conducted in live bird markets of four cities of East Java province namely Surabaya, Pasuruan, Sidoarjo and Malang. These cities are located in areas where a large number of ducks and chickens are traded daily coming from different areas. People travel from all corners of the province to participate in these markets. These areas were selected based on human and poultry density, highly populated LBMs, trading patterns and previous history of AIV in LBMs.

In addition, considering that a significant proportion of the land in this region is comprised of paddy fields and water bodies, the presence of ducks and chickens that are allowed to roam freely reduces the effectiveness of prevention and control measures. This ultimately results in the continuous spread of the virus (Loth *et al.*, 2011; Henning *et al.*, 2013).

1.2 Formulation of the problem

1. Are avian influenza A virus subtypes H5N1 and H9N2 prevalent in the study area of East Java Indonesia?
2. What type of molecular characteristics and mutations of AI A virus subtypes H5N1 and H9N2 in the study area of East Java?

3. What kinds of factors are associated with the prevalence and transmission of AIV subtype H5N1 in poultry of live bird markets?
4. What kinds of factors are associated with the prevalence and transmission of AIV subtype H9N2 in poultry of live bird markets?

1.3 Research objectives

The findings of early surveillance programs of AI carried out in Indonesia showed a much higher prevalence of HPAI A virus subtype H5N1 in these LBMs when compared to poultry producing areas, suggesting that the HPAI virus would spread extensively during the trading process. Besides, it is very important to report the newly circulating strains and to understand their pandemic potential.

Prior characterization, efficient isolation, and detection of AI A virus subtypes H5N1 and H9N2 from surveillance samples are major challenges. Thus, the molecular characterization of the circulating influenza viruses is important to know the host range, pathogenesis, and receptor specificities which might lead to successful vaccine production or renovation. Therefore, the current study was designed for four primary goals with some additional specific.

1.3.1 General objectives

The general objective of the study was to estimate the prevalence, investigate molecular characterization, perform a phylogenetic analysis, and determine the associated risk factor of avian influenza A virus subtypes H5 and H9 in poultry of live bird markets in East Java Province of Indonesia.

1.3.2 Specific objectives

The study was conducted to achieve the following specific objectives:

1. To estimate the prevalence of avian influenza A virus subtypes H5N1 and H9N2 in poultry of live bird markets of East Java province of Indonesia.
2. To determine the molecular characterization (sequencing, homology) and phylogenetic analysis of avian influenza A virus subtypes H5N1, and H9N2 circulating in poultry of live bird markets in East Java.
3. To identify the associated risk factors responsible for the prevalence and transmission of avian influenza A virus subtype H5N1 in poultry of live bird markets in East Java.
4. To identify the associated risk factors responsible for the prevalence and transmission of avian influenza A virus subtype H9N2 in poultry of live bird markets in East Java.

1.4 Benefits of research

The first case of avian influenza (bird flu) traces back to 1878 in northern Italy, where it was called "fowl plague". "Fowl plague" was caused by a virus at the turn of the 20th century, but it wasn't identified as a type A influenza virus until 1955. During the First International Symposium on Avian Influenza, which took place in 1981, the term "fowl plague" was eventually replaced with the more accurate term "avian influenza."

Over the years globalization and increasing growth of international trade in livestock, poultry, and their products have increased the importance of viable

reporting of international disease. For contagious and zoonotic diseases, a permanent and compact surveillance system is needed because without surveillance it is impossible to determine the disease conditions, and implementation of proper control, and eradication programs. So, the current study was important in terms of surveillance for the prevalence of avian influenza subtypes H5N1, and H9N2 because of its widespread zoonotic and economic impacts on the human and poultry industry.

1.4.1 Theory Benefits

1. The proposed study estimated the prevalence of AIV subtypes H5N1 and H9N2 in poultry of live bird markets of East Java Indonesia.
2. The proposed study depicted the new knowledge about the molecular characterization, phylogenetic analysis and mutations of these viruses in the study area.
3. The study helped to investigate the poorly documented newly emerging AIV subtype H9N2 in the study area of the country.
4. Furthermore, the study identified the associated risk factors that help in the transmission of the disease in poultry of live bird markets.

1.4.2 Practical Benefits

1. The current work provided new findings of avian influenza A virus subtypes H5N1 and H9N2 prevalence and their associated risk factors in the live bird markets of East Java.

2. The current study contributed new information on the molecular characterization and phylogenetic analysis of the AI viruses, which was an indication of a novel genotype of the virus. This information would be beneficial for the development of vaccines.
3. This study provided more precise data that can be used in the development of future policies for the prevention, control, and eradication of AI A virus subtypes H5N1 and H9N2 in Indonesia.