Prevalence of avian influenza in humans and different bird species in Indonesia: A review

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Abstract
Avian influenza, commonly called bird flu, is highly contagious and pandemic zoonosis of global importance, primarily affecting birds and other mammals, including humans. The present review is intended to highlight a report on the prevalence of avian influenza in humans and different bird species of Indonesia. The study is based on 27 scientific articles from 2004 to May 2021 in which the prevalence of avian influenza is determined mainly by the following molecular, virological, and serological tests: polymerase chain reaction (PCR), hemagglutination inhibition (HI), enzyme-linked immunosorbent assay (ELISA), and Rapid antigen detection test. A vast divergence in the prevalence of avian influenza was observed due to the diversity in sensitivity and specificity of the tests applied. The prevalence of avian influenza varies due to spatial and temporal factors, bird species, and breed differences. An average maximum prevalence (25%) was found in poultry (domestic and commercial birds) as compared to ducks (20.13%) and other birds (10.66%). An average documented prevalence (16.3%) was found in humans. Birds sold in live bird markets showed maximum prevalence due to different geographical distribution. The already published studies dissection illustrates that avian influenza infects all types of birds and humans in Indonesia. A proper surveillance system, effective vaccination plan, and segregation and culling strategy regarding avian influenza-infected birds are desperately needed to eradicate avian influenza in Indonesia.

Introduction
Avian influenza (AI) is a highly contagious viral disease prevalent in many wild water bird reservoir populations and is periodically introduced into the backyard poultry sector. Due to their zoonotic potential, AI viruses have been considered a severe veterinary and public health concern in the poultry industry for the last three decades (1,2). The H5N1 subtype of the highly pathogenic avian influenza (HPAI) virus has been an animal and human health threat since its spread from China to several continents in 2003 (3). As of 2012, the virus has been reported in poultry and wild birds in 63 countries across Asia, Europe, and Africa (4). By January 2014, 650 human infections and 386 fatalities had occurred around the world (5). HPAI H5N1 is currently considered endemic in China, Bangladesh, Eastern India, Indonesia, Vietnam, and Egypt. Intermittent outbreaks have occurred periodically in other countries in Asia, including the Lao People's Democratic Republic, Cambodia, Myanmar, and Nepal (6). Viruses of avian influenza (AI)
were isolated from various avian organisms comprising different orders (7,8). However, the isolation of AI viruses was mainly documented from the order of Anseriformes (6) in particular from ducks (subfamily Anatinae) which were detected to carry several H3, H4, and H6 subtype viruses, but less commonly H5, H7, and H9 viruses (9,10).

The Government of Indonesia first recorded the presence of a highly pathogenic avian influenza virus subtype H5N1 to the World Organization for Animal Health in February 2004. Since the first outbreak in 2003 in poultry, the HPAI subtype H5N1 was confirmed in 31 out of 33 provinces of Indonesia (11). The FAO sought to provide resources to the organization starting in 2004 to improve the disease control system in Indonesia that was weakened by policy interventions (including decentralization) implemented in the wake of the financial crisis in Asia (11).

The findings of early surveillance programs in Indonesia showed a much higher prevalence of HPAI H5N1 in live birds Markets (LBMs) compared to poultry-producing areas, suggesting that the HPAI virus would spread extensively during the trading process. However, there are significant differences in the supply chains of backyard poultry (e.g., Kampung or indigenous chickens) and commercial poultry (e.g., broilers and layers) sold in Indonesian urban LBMs. Few broiler flock owners sell their chickens directly to LBMs; they are typically flocking owners with flock sizes of less than 5,000 birds. It is believed that contract farms provide 70% of Indonesia total broiler meat, independent farms offer 20%, and large and integrated farms provide 10%. Backyard chicken is often purchased by middle-class or small-volume poultry traders who ride their motorcycles to many villages to trade with farmers or purchase birds from local village marketplaces. They then sell these birds in rural areas at LBMs, from where the birds are shipped to smaller wholesale poultry markets (6). Improper surveillance, underreporting, a lack of knowledge, the informal market places trading poultry and poultry products, and the lack of effective regulation about avian influenza prevalent in birds and humans are the leading causes of avian influenza spreading in Indonesia.

The current review focuses on the prevalence of AI in humans and different bird species in Indonesia, where the disease is endemic, provides an overview of the current prevalence of avian influenza, and extracts conceptual ideas for the key policymakers and stakeholders to manage eradicate AI in Indonesia.

Materials and methods

A comprehensive and detailed search was conducted to find related research and data published between 2004 and May 2021 using critical databases including Google Scholar, ResearchGate, PubMed, Science direct, and Google search engine. A plethora of parameters, including epidemiology, seroprevalence, range of hosts, risk factors, and appropriate combinations of those mentioned above, were used to ensure that relevant studies were found.

Inclusion and Exclusion Criteria

The following molecular virological and serological assays were used to select articles: polymerase chain reaction (PCR), hemagglutination inhibition (HI), Rapid antigen detection strips, and enzyme-linked immunosorbent assay (ELISA). The current analysis also considered peer-reviewed literature on humans, poultry, ducks, and other birds published in English. The study excluded articles published in languages other than English, conference papers, and those not peer-reviewed.

Study Characteristics

Initially, 3825 published article titles were reviewed, with 80 articles being chosen for further consideration. The abstracts of 60 publications were selected for screening after duplicates were removed. After screening, the abstracts of 20 papers were eliminated, and the remaining 40 articles were chosen for full paper assessment. Another 13 studies were eliminated after a thorough review of the articles because they did not meet our inclusion criteria. The remaining 27 publications were included in the study to ensure that the data was of high quality. The majority of the papers in the collected literature were on birds, with only five articles about humans.

Results

The results of an overview of epidemiology, including seroprevalence, time of infection, location, range of hosts, and the diagnostic tests used for detection of AI from 2004 and May 2021 in different parts of Indonesia, have been shown in the following comprehensive (Table 1). Figure 1 graphically shows the overall prevalence of avian influenza in all birds and humans in many provinces and islands of Indonesia. AIV is more prevalent on Java island as compared to others, while the study figure 2 shows the methodology of the review article.

Avian influenza epidemiology and seroprevalence in Indonesia

Epidemiology is the study and analysis of the distribution, patterns and determinants of health and disease conditions in defined populations. The current analysis discovered that many studies on poultry and ducks were conducted using proper selection criteria and used non-randomization approaches for their studies or surveys. According to several published researches, the leading cause of avian influenza persistence in Indonesia is a lack of biosecurity measures and mutations in the virus strains. That is why the disease is still prevalent in the country. Because
of regional and temperature differences, the disease incidence and prevalence differ between chickens and ducks (12,3).

Figure 1: Avian influenza in Indonesia.

The 2nd-largest island in Indonesia is Java, divided into four administrative provinces: Banten, West Java, Central Java, and East Java, and two particular regions, Jakarta and Yogyakarta. This island represents 60% of humans and 70% of Indonesia poultry and duck population. More than 620 million chickens and ducks are estimated to be present in Java Island [https://www.thecrazytourist.com/25-best-things-java-indonesia] (11). Avian influenza reports are highest on this island due to many conventional and intensive farming systems and the comparatively better veterinary diagnosis and surveillance systems. Average estimates of avian influenza from this island (Banten, West Java, Central Java, and East Java, and two unique regions, Jakarta and Yogyakarta) are highly variable depending upon the type of diagnostic test used for bird species, farming system, and environmental factors. The documented range of prevalence of AIV among different bird species in Java Island was as follows [e.g., 1-69.38% in poultry, 1-59.28% in ducks, and 1-23% in other birds]. The province wise overall prevalence of avian influenza in different species of West part of Java Island are in poultry 27.5%, ducks 16.25%, human 22.5%, and other birds 17.5%, in Central Java in poultry 24.73%, ducks 31.61%, humans 2.5% and other birds 15.6%. In comparison, in East Java it was depicted in poultry 22.23%, ducks 5.2%, other birds 11.2%, moreover prevalence of AIV in Banten found in poultry 35%, duck 14.65%, in Jakarta, it was reported in poultry 13.86%, ducks 2.45%, human 15%, and other birds 3.04% and in Yogyakarta reported in poultry 27.45%, other birds 13.5% respectively (Table 1). Avian influenza has been reported in all types of poultry and duck farming systems (i.e., conventional, intensive, and smallholdings). Higher prevalence was reported in the free-ranging farming system as compared to control. Backyard poultry was more affected as compared to other poultry and ducks. The reported subtypes in this island are H5N1 (11-28).

In the present review, three studies were selected from Bali Island, a province of Indonesia and the westernmost of the Lesser Sunda islands. Bali has a pretty constant climate throughout the year due to its location barely 8 degrees south of the equator. The average year-round temperature is around 30 °C, with an average humidity of around 85%. More than 15 million chickens and ducks are estimated to be present in Bali Island. On this island, avian influenza is reported predominantly in poultry, ducks, and other species of birds. Maximum prevalence is reported in poultry as compared to ducks and other birds. The seroprevalence ranged between 10-35.5% in poultry, 17.7-25% in ducks, and 6.66-9.40% in other birds. LBMs showed a high prevalence of AIV as compared to villages in Bali. The average prevalence of AIV in LBMs in different species: Poultry 20.46%, and ducks 21.35%, while at villages prevalence reported only in other birds like Petulu heron 9.40% and Serangan 6.66% respectively (28-30).

In the current review, two studies were selected from West Timor (Indonesian: Timor Barat). Except for the district of Oecussi-Ambeno, West Timor (Indonesian: Timor Barat) covers the western part of the island of Timor. West Timor total area, including the offshore islands, is 14,732.35 km² (5,688.19 sq mi). West Timor is administratively part of Indonesia East Nusa Tenggara Province. The island is home to 35.5% of the province population. Small poultry farmers make a major contribution to the national population (chicken 93.38 %). In this province, avian influenza is
reported predominantly in chickens as compared to ducks. No case reports in other birds. The reported seroprevalence was in backyard poultry 72.2%, commercial poultry 13.2%, and ducks 18%, respectively (4,31,32).

Avian influenza in human

To the best of our knowledge, the first case of AIV in a human was reported in 2005. The outbreak peaked in 2006 with 55 cases and 45 deaths. Concerns about human-to-human transmission were also raised at that time (33). Two hundred thirty-five human cases of avian influenza H5N1 have been confirmed so far in numerous countries, including Indonesia. 105 (44.7%) of the total number of victims have been reported in Indonesia. Maximum prevalence and fatality were reported in West Java 80% (25/30) at the study time. Most human infections have occurred due to direct contact between humans and diseased poultry or polluted settings (34).

The frequency of avian influenza in humans was found in 5 out of 27 reviewed articles, and three studies revealed occupational risks. The selected articles also explained the incidence of avian influenza at the national level. The overall prevalence of avian influenza ranges from 1-44% in selected articles from different parts of the country. A study was conducted among the rural farmers in 12 farms of Sukabumi district of West Java Indonesia by Van Beest et al. (35) to determine the seroprevalence of AIV. The researchers recorded an overall 3-5% avian influenza prevalence in the farmworkers in this study. Another study reported a 44% seroprevalence of avian influenza A virus (H5N1) among market workers by HI assays. It indicates that LBMs workers were at a high risk of infection as compared to poultry farmworkers. LBMs workers who touched the poultry were at high risk compared to others (36).

An epidemiological study was conducted between July 2005 -June 2006, among 598 suspected cases of H5N1 virus infection in all provinces of Indonesia among sporadic and family clusters cases. Out of 598 cases, 54 were confirmed H5N1 positive from 8 provinces. Serum samples were tested through HI assays while RT-PCR identified the respiratory specimen. In 2005 the reported prevalence was 35%, while in 2006, it was 65%. High prevalence was reported among sporadic and family clusters cases of H5N1 virus infection (22). A study was conducted in East Jakarta among outpatients with influenza-like illnesses and in patients with a severe acute respiratory infection. This study reported maximum case prevalence for seasonal influenza (31%) compared to avian influenza (15%). The avian influenza subtype H5N1 was reported only in one patient. According to the findings of this study, seasonal influenza was found to be more prevalent than avian influenza (37).

A study conducted by Agyrdzadana Frisa et al. (20) described the prevalence of AIV at three sentinel parts (Malang, Yogyakarta, and Semarang) of Java Island. PCR was performed to detect influenza A, and influenza B virus. Maximum prevalence was reported for influenza A 8.5% as compared to influenza B 2.8%. The Malang area was more affected than Yogyakarta and Semarang because of the climatic change. PCR was used as a primary diagnostic tool for the human avian influenza virus in only two studies, but mostly the researcher applied HI as a screening test (Table 1).

Diagnosis of Avian Influenza

Serological, molecular, and virological tests have been the preferred choices for diagnosing avian influenza in Indonesia. The most common serological test used in Indonesia by frequency is HI. The use of PCR techniques was found more frequently at molecular detection laboratories of AIV. HI is commonly used for screening sera because it is less expensive compared to ELISA (Table 1). Both conventional and real-time PCR-based AIV detection and differentiation methods have been used in Indonesia. The use of isolation and microbiological characterization of AIV is increasingly applied, that reported in the current review. Most of the studies reported incidence of AIV was confirmed in seropositive poultry and ducks by using real-time (RT) PCR and Reverse Transcriptase PCR (14). Meanwhile, in another study, the researchers reported 2.1.1 clade, 2.1.3 clade, and IDN/6/05 from ducks, while 2.1.3 was isolated from chickens using real-time PCR (16).

Effects of Avian Influenza on species of birds

Since 2003, the widespread H5N1 highly pathogenic avian influenza in chickens have caused devastation in Indonesia compared to other species like ducks. Although human cases of avian influenza may be significantly reduced, the situation in poultry remains unsettled (38). The highest prevalence and mortality were reported in backyard chickens (average 59%, range: 49–69%), compared to ducks (average 32%, range: 19–45%) and other birds (average 28%, range: 16–40%) (11, 12). Another study conducted by R. Damayanti et al. (39) to determine the effect and pathogenicity of AIV H5N1 HPAI virus found clade 2.3.2 in 30-day old infected ducks and indigenous chickens by rearing together. The results of the investigation revealed that all chickens have died after 48 hours, whereas ducks just displayed clinical indications (39). In comparison to ducks, chickens have a higher infection and incidence rate of AIV.

Effect of Avian influenza on Breeds

Susanti et al. (40) investigated the Mx gene's potential as an avian influenza virus resistance marker in three different chicken breeds: backyard chickens, Hy-Line Brown laying hens, and White Leghorn broilers. Compared to backyard and layer chickens, most White Leghorn broilers demonstrated GG genotype resistance.
Table 1: Describes the prevalence of avian influenza from 2004-2021 based on different diagnostic tests like ELISA, HI, ®Rapid antigen, and PCR in humans and different species of birds from different provinces and islands of Indonesia.

<table>
<thead>
<tr>
<th>References</th>
<th>Year of study</th>
<th>Island</th>
<th>Location</th>
<th>Host</th>
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<td>ELISA= Enzyme-linked immunosorbent assay, HI= Hemagglutination inhibition, PCR= Polymerase Chain Reaction.</td>
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Effects of Management Systems on the occurrence of Avian Influenza

Joerg Henning et al. (18) investigated the seroprevalence of AIV in 'stationary' duck management system and 'moving' duck management system and reported a higher prevalence and spreading rate in 'moving' duck management system as compared to stationary duck management system. Desniwaty Karo et al. investigated that prevalence and mortality of AIV is higher in the open management system (backyard poultry) as compared to the controlled system (commercial poultry and duck) (41).

Geographical and Seasonal Effects on Distribution of Avian Influenza

Henning et al. (42) described a significantly higher prevalence in Java Island than in other parts of Indonesia. More than 620 million chickens and ducks are estimated to be present on Java Island. A substantial number of both conventional and intensive farming systems have been observed on this island, resulting in a high number of avian influenza infections. The incidence and prevalence of avian influenza disease are high from March to May and peak in July. After that, it decreases before rising again in January to March. Infection incidence appeared to have a seasonal pattern (15).

Risk Factors associated with Avian Influenza

Joerg Henning et al. (21) recorded that live bird markets in Indonesia are essential for the prevalence and endemicity of AIV. From the survey data, a total of 22 risk factors possibly influencing HPAI H5 virus prevalence were identified, including chicken cages, stacking systems, display table materials, and slaughter surfaces areas. A group of researchers in a study found that the density of poultry, human density, the environmental factors, road density, percentage of paddy field, and percentage of water sources had a statistically significant relationship with the prevalence and outbreaks of HPAI in poultry and human in Indonesia (43). Another study conducted by Karo et al. reported that the type of poultry, type and size of the farm, and incoming contacts were significantly associated with HPAI prevalence in the country. One study in Indonesia showed that 76% of human HPAI cases were proven to be associated with poultry contact.

Economic impacts of Avian Influenza

A study conducted by Basuno et al. (44) on the socio-economic impacts of avian influenza outbreaks on Small-scale Producers in Indonesia reported that the HPAI outbreak did not destroy the social systems in rural areas. However, it damaged the rural economy by ruining the existing economic system, increasing unemployment and migration. These losses occurred due to increased mortality, lower productivity, decreased demand for poultry products, and low price. Highly pathogenic avian influenza subtype H5N1 was first reported in Indonesia in 2003-2004 that spread in many provinces and caused the death of more than 16 million poultry at the end of 2007. AIV has been a significant problem in the poultry industry till today (22). HI, the test was applied in 16 out of 27 studies and was reconfirmed through PCR in 3 studies. Overall PCR was used in 16 studies, while ELISA in one and Rapid antigen in one study. The maximum seroprevalence of avian influenza was confirmed with HI compared to ELISA, while PCR was used to confirm the prevalence and molecular characterization of AIV (Table 1).

AI, specifically in humans. In the published literature, there is only limited information on disease economic losses.

Furthermore, the current research discovered minimal validity and scope of studies by determining a realistic seroprevalence of AI from 2004 to May 2021. Most prevalence articles have minimal and ambiguous diagnostic designs and non-randomized sampling procedures or tiny sample sizes reflecting limited inauthentic seroprevalence. There is also a lack of defined methodologies for AI diagnosis in specific articles, which leads to erroneous prevalence estimates. All serological tests used for incidence are practical but have limited specifications and sensitivity. It is regarded as highly important to isolate and characterize the prevalent species-specific strains of the AI virus for correct estimation and knowledge of its epidemiology. Only minimal information on the incidence of AI in human and birds other than chickens and ducks have been reported. As a result, precise data on the presence of AI in different bird species is critical for future control and eradication efforts. In humans, poultry, and ducks, most studies about the
incidence of AI are carried out with molecular, serological, and virological tests, i.e., HI, and PCR (Table 1).

ELISA and @Rapid antigen have been used as confirmatory diagnostic tests for AI in 2 studies. The HI test was applied in 16 out of 27 studies in maximum studies and was reconfirmed through PCR in 3 studies (Table 1). PCR was used in 16 studies and proved a more sensitive, accurate, reliable, and specific diagnostic test than others. No researcher described any other serological and virological tests for the diagnosis of AI. The highest prevalence of 59.28% and a minimum of 1% was reported in ducks through swab samples, 44% in poultry, and 23% in other birds’ species. The prevalence of AIV in Indonesia demonstrated a statistically significant association with different environmental factors road density, percentage of paddy field, and water sources. In this review, collectively, poultry had the highest prevalence of AIV 25%, followed by ducks (20.13%) and other birds (10.66%) (Table 1) (12, 43). Previously varying seroprevalence of AIV in poultry has been reported from other countries, for example, Nigeria 52.9%, Egypt 61.6%, South Korea 77.2%, and China 57%, which were higher than the results of our review (46-49).

Based on seasonal and environmental parameters, the total prevalence of avian influenza type A in swab samples were 22.05% in an earlier study conducted in Egypt, which is close to our findings (50). The present study results showed similarities with those in Pakistan (51) and other countries like Iran, where a similar seroprevalence of 23-50% was reported (52). Wilcox et al. (53) conducted a study on AIV in Ducks in Northwestern Minnesota from July – October in 2007 and 2008. The reported prevalence of AIV was 9.1% in 2007 while 17.9% in 2008. The prevalence of AIV was affected by the climatic change that was peaked in late summer. These findings are similar to ours.

Five out of the 29 reviewed articles revealed AI incidence in humans and explained the association between birds and humans. Three studies were conducted about the prevalence of AI in humans in close contact with birds like LBMs and poultry farm workers, while two studies revealed the H5N1 strain in the diseased people who were already admitted to the hospital. In these studies, the average reported prevalence of AIV in humans was 16.3%. The reported prevalence ranged in these studies 1-44% (35-37). A prior study among poultry workers in Iran found a 17% prevalence of avian influenza in exposed individuals and a 3% prevalence in non-exposed individuals, which differed from our findings (54). A previous study among poultry professionals in Pakistan was undertaken to investigate the prevalence of AIV. The overall prevalence across different poultry professionals was 50.3%. The results of this study revealed similarities to our findings (55), and the prevalence of AI was highly variable. It could be related to geographical factors, differences in farming techniques, or seasonal or environmental influences, as several studies have shown that the prevalence rate varies from area to area and changes with changing environmental conditions (16,42). A variation in the incidence of AI was also discovered in the same region or area. Three studies conducted in Central Java revealed differences in AI incidence in different bird species, which could be attributable to differences in diagnostic techniques used (17,43). A previous study in LBMs in Bangladesh conducted by Kim et al. indicated that the prevalence of AIV was impacted by the type of poultry, environmental site, and trading, which is comparable to our findings (56). A prior investigation was undertaken in Bangladesh during three winter seasons among semi-scavenging ducks. The overall prevalence measured was 39.76%. The highest frequency 43.89% was reported from December 2009 to February 2010, followed by two winters in a row. This study found a seasonal effect on the prevalence of AIV, which is consistent with our findings (50).

Most of the studies were conducted about the incidence of AI in poultry and ducks than humans and other birds’ species. There is a dearth of large-scale, randomized seroprevalence research of AI in other bird species that can explain its actual prevalence in all birds in Indonesia. Prevention, control, and eradication of AI from Indonesia depend on the successful control strategies of diseased birds, especially in poultry and ducks. As a result, in order to control this disease, a “One Health” strategy is required.

The authors highlighted the following primary barriers and challenges in AI prevention, control, and eradication strategies in Indonesia: (i) strict biosecurity and standard operating procedures (SOPs) are not being followed at LBMs and poultry farms, (ii) in some areas, there is inadequate disease surveillance and access to diagnostic laboratories, (iii) handling of infected birds, carcasses, or diagnostic specimens without adequate personal protection, (iv) a poor communication about disease symptoms between human patients and medical staff, (v) a failure to keep data records on poultry sales, migrations, disease status, and vaccination on farms, (vi) incorrect knowledge on the effects of immunization, treatment and culling procedures/policies by farmers, and (vii) a scarcity of facilities capable of isolating, identifying, and typing isolates. Some developed countries like New Zealand, Australia, and Iceland have succeeded in eradicating the disease by implementing the following measures: regular vaccination policies, ongoing surveillance, slaughtering or culling of cheerful birds, and strict biosecurity and biosafety practices in farm and live bird markets.

**Conclusion**

AI is more common in poultry and ducks than in other species in Indonesia, but a large-scale systematic and randomized study program and approaches are needed to determine its accuracy and actual prevalence. It has been
discovered that vaccination and culling programs and the stamping of seropositive flocks are underutilized. There is an intensive need to expand surveillance and disease reporting systems and improve the diagnostic capacity of laboratories in all provinces at the district level in Indonesia. Campaigns should be initiated among poultry farmers, the public, policymakers, and other stakeholders to educate them about the hazards of AI and its ultimate consequences on the country national economy.

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Conflict of interest

All authors declare no conflict of interest.

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5. WHO. Cumulative number of confirmed human cases of avian influenza A/H5N1) reported to WHO.


انتشار أنفلونزا الطيور بسبب العوامل المكانية والزمانية وأنواع الطيور والاختلافات في سلالاتها.

انطلق أنفلونزا الطيور بسبب العوامل المكانية والزمانية وأنواع الطيور والاختلافات في سلالاتها. بلغ أعلى متوسط انتشار (25%) في الدواجن (الطيور الداجنة والتجارية) مقابلة بالبط (20.13%)، بينما بلغ في الطيور الأخرى (10.66%)، كما متوسط معدل الانتشار (16.3%) في الإنسان. ظهرت الطيور الحية والتي يتم بيعها في الأسواق أعلى نسبة لانتشار الفايروس بسبب التوزيع الجغرافي المختلف. وتتحدث الدراسات المنشورة بأن أنفلونزا الطيور تسبب جميع أنواع الطيور والانسان في إندونيسيا، فضلًا عن الحاجة الماسة إلى نظام مراقبة وقضية فاعلة لتحقيق الاستراتيجية عزل واستعداد فيما يتعلق بالطيور المصابة القضاء على أنفلونزا الطيور في إندونيسيا.