

CHAPTER 1 INTRODUCTION

1.1 Background

Avian influenza refers to the disease caused by infection with avian (bird) influenza (flu) viruses. There are three types of the Influenza virus; A, B, and C type. These type A viruses occur naturally among wild aquatic birds worldwide and can infect domestic poultry and other bird and animal species (CDC, 2014). The disease is also classified as zoonosis because it can infect mammals including humans. Influenza A virus is further classified on the basis of the surface glycoproteins, hemagglutinin (HA) and neuraminidase (NA). Sixteen HA subtypes and nine NA subtypes of influenza A virus have been identified (USDA, 2014). Most influenza A viruses circulate in waterfowl, but those that infect mammalian hosts are thought to pose the greatest risk for zoonotic spread to humans and the generation of pandemic or panzootic viruses (Tong *et al.*, 2012).

Avian Influenza Viruses subtype H5 are maintained as low pathogenic avian influenza (LPAI) forms in many *anseriform* species (Sharp *et al.*, 1997, 1993; Süß *et al.*, 1994 in Bryan *et al.*, 2013). In 1996 the antecedent HPAI H5N1 virus A/Goose/Guangdong/1/96 was detected on a goose farm in Southern China causing an outbreak in the summer and early fall with 40% morbidity in birds (Xu *et al.*, 1999 in Bryan *et al.*, 2013). Reassortants of this virus were next detected in domestic poultry, ducks, and geese in Hong Kong in 1997 (Shortridge *et al.*, 1998 in Bryan *et al.*, 2013) with associated human cases resulting from direct contact with infected birds. H5N1 virus continued to circulate in the wider region in at

least ducks and geese (Guan *et al.*, 2002a in Bryan *et al.*, 2013) generating a number of reassortants. In 2002, H5N1 virus were detected in resident waterfowl and flamingos as well as wild little egrets and gray herons and other wild migratory birds; of particular note was the fact that the infection was lethal in some of the aquatic birds, an atypical presentation in these hosts this fact is the evidence of wild birds infection with the highly pathogenic viruses (Bryan *et al.*, 2013).

The migratory nature of the aquatic fowl reservoir resulting the wide geographic spread and distribution of most circulating subtypes. A consequence of this geographic distribution in migratory birds is the potential contact of infected bird with domestic avian and mammalian species, including humans. Close contact of terrestrial bird or mammalian species with infected poultry or waterfowl or their biological products is the major route for interspecies transmission. (Webster *et al.*, 1992 in Bryan *et al.*, 2013).

Indonesia is one of the five countries where highly pathogenic avian influenza viruses of the H5N1 subtype (H5N1 HPAI) remain endemic in poultry (Daniels *et al.*, 2012). There is only one province that claimed as free region of avian influenza infection in Indonesia. North Maluku is the only province that not reported a case of this disease (Okezone, 2011). In Kalimantan, the first case of avian influenza reported in 2004, since that, a surveillance undertaken to monitor this virus. The samples collected from four provinces in the island (West Kalimantan, Central Kalimantan, South Kalimantan, East Kalimantan) consist of 4629 serum samples and 2426 cloacal swab samples. Samples species consist of

chicken both broiler and layer, quail, and whistling duck. Approximately 11,43% of serum sample positive to H5N1 antibody test through HA/HI assay, and the cloacal swab samples showed 11,72% positive to this virus by RT-PCR. Samples from West Kalimantan showed 232 from 1675 positive for avian influenza in HA/HI assay. This positive test result continued to RT-PCR test and resulting 4 positive number of samples (Figri *et al.*, 2011).

Formerly, West Kalimantan declared and certified as free zone of avian influenza infection. Since February 2004 until May 2010 there are no cases found in this province (Ditjennak, 2010). Furthermore, avian influenza cases in West Kalimantan from 2012-2014 found and recorded 3 cases infect poultry (Ditjennak, 2014). In human cases, until 15th December 2014, there were total 197 human cases with 163 death in Indonesia, while there were total 676 cases and 398 death in the world (CDC, 2014). The lack of success in controlling the H5N1 threat in Indonesia was probably attributable to the widespread endemicity that had been established by the time the threat was recognised and, among other things, the decentralised nature of government control (Daniels *et al.*, 2013).

Bats may now be added to the list of mammalian hosts of influenza A viruses. Base on study by Tong *et al.* (2012), they capture many bats from eight locations in southern Guatemala in two consecutive years (180 bats in May, 2009, and 136 bats in September, 2010), the genetic sequence databases using eight bat virus gene segments identified influenza type A sequences as the most significant matches and reported that H17N10 as the new genome that did not represent a 'true' influenza virus, and it should be renamed influenza-like virus. In the further

study, Tong *et al.* (2013), by using consensus degenerate RT-PCR, they identified a novel influenza A virus, designated as H18N11, in a flat-faced fruit bat (*Artibeus planirostris*) from Peru.

Bats (order *Chiroptera*) are of particular interest, because they comprise nearly 1,200 species worldwide, accounting for approximately one-fourth of all mammal species, and their global distribution, abundance, ability to fly and migrate over large distances, and sociality favor the acquisition and spread of viruses (Turmelle, 2009). Globally bats comprise around 20% of all mammal species, but this percentage rises in Southeast Asia where, at least locally, bats may comprise around 40% of all mammals (Ecology Asia, 2014). The Flying foxes are potentially effective transmitters of EIDs because they are capable of foraging over long distances (more than 80 km) in a single night reported for *Pteropus vampyrus* in Malaysia, (Epstein *et al.*, 2009 in Harrison *et al.*, 2011), and may forage in farms and commercial orchards that are populated by domestic animals, which can serve as amplifier hosts to people (Breed *et al.*, 2006 in Harrison *et al.*, 2011). This harmful potential should be controlled or monitored routinely to prevent an outbreak or detect a new survival virus such as Avian Influenza Virus.

Bats in Indonesia not only as a biodiversity wealth, a reservoir of many viruses (reviewed by Pavri *et al.*, 1971; Sendow *et al.*, 2006, 2010 in Clayton *et al.*, 2012), they also have an advantage to become a culinary dish. The survey result The Tomohon traditional market, Langowan, and Kawangkoan North Minahasa District, Sulawesi in March 2011, data showed that the average sales

bat is 150 kg per day. Based on surveys and interviews conducted the bat seller communities in the Market Bersehati Manado in October 2011 during the study data showed that the bat average sales is 100 head per day or every day meat bat contribute the meat supply approximately 30 kg -50 kg (Ransaleh et al., 2014). Study conducted by Harrison et al. (2011) conclude that people bought flying foxes for nutritional reason (80%), though a substantial proportion (29%) also believed people purchased these bats due to alleged medical properties for chest ailments one vendor stated that flying foxes were “obat meruyan”, medicine used to treat sick women after giving birth. There is also increasing realisation that human contact with flying foxes through activities such as hunting could lead to adverse effects on public health via the transfer of emerging infectious diseases (EIDs) (LeBreton *et al.*, 2006 in Harrison *et. al.*, 2011).

The rapid detection and diagnosis of AI virus in poultry, wild birds, and other species are critical to controlling the virus (Spackman, 2008). Furthermore, if this genome (H17N10 and H18N11) can produce a real influenza virus that can cause human or animal infections, then the situation for influenza virus-caused diseases would be more complicated because bats are known to harbor many viruses and are regarded as a reservoir host for many human- and animal-infecting viruses, including the SARS (severe acute respiratory syndrome) coronavirus (Shi, 2013). Therefore, some research to detect any risk or threat to animal and human health related to avian influenza should be undertaken in the near future. Encouraged by this background, the research entitled “Antigenic Detection of Avian Influenza Subtype H5 in Bats Collected from West Kalimantan” need to be

conducted. The detection of influenza virus A subtype H5 using Hemagglutination Assay (HA) is the chosen method, then continuing to the antisera assay to detect antigen to specific hemagglutinin subtype.

1.2 Problem Formulation

Based on the background above, the problem can be formulated: “Is there avian influenza virus subtype H5 presence in bats collected from West Kalimantan using Hemagglutination Inhibition assay?”

1.3 Theoretical Basis

Avian Influenza (H5N1) was initially confined to poultry, but in recent years it has emerged as a highly fatal infectious disease in the human population. In 1997, the avian influenza A virus subtype H5N1 crossed the avian-human species barrier for the first time. Eighteen individuals were infected, six of whom died. In January 2003, avian influenza re-emerged among humans in Hong Kong (Korteweg, 2008). In 2004, the remains of a domestic dog (*Canis familiaris*) tested positive by RT-PCR for H5N1 days after feeding on the carcass of ducks from H5N1 infected areas (Songserm *et al.*, 2006 in Bryan *et al.*, 2013). In Indonesia, avian influenza outbreak confirmed in the poultry farm in the middle year of 2003 and officially announced in 25 January 2004. Even the official data of place and time uncertain confirmed, some party believe that the initial case emerged in Pekalongan, Central Java (Nidom, 2010a). This Influenza type A viruses affect humans and other animals and cause significant morbidity, mortality and economic impact (Peiris and Yen, 2014).

Influenza A virus (H5N1) have been transmitted multiple times to pig population in Indonesia and that one virus has acquired the ability to recognize human type receptors, if the regulation of pig transportation in Indonesia uncontrolled by its goverment, the opportunities for this avian virus to adapt to mammals will increase, as will the risk for emergence of a new pandemic influenza virus (Nidom *et al.*, 2010). Human case of avian influenza infection in West Kalimantan reported in 2010. The patient was a 9-year-old girl who had direct contact with birds before she developed symptoms. She suspected human case of H5N1 Avian Influenza and was admitted to the hospital on 13 September and recovered (reviewed by ProMED-mail, 2010 in TravelHealth.gov.hk, 2010).

Influenza viruses are enveloped viruses containing a segmented negative-sense RNA genome, and belong to the *Orthomyxoviridae* family. They can be classified into three types: A, B, and C, among which influenza A viruses have the most genetic variation and the broadest host range (Zhang *et al.*, 2013). These viruses are further subtyped on the basis of their viral surface proteins, the haemagglutinin (HA) and neuraminidase (NA). Until recently, HA subtypes 1–16 and NA subtypes 1–9 were recognised in aquatic wild bird reservoirs; only a few were established in mammalian species, including humans. More recently, H17 and H18, and N10 and N11, have been described in South and Central American bat species (Peiris and Yen, 2014).

The Mutations of influenza viruses are important for adaption to new host environments, escape from immune pressure, and the emergence of drug-resistant viruses. Mutations can also lower the barrier to cross-species transmission. For

example, point mutations in avian viral genes circulating in pigs have partially adapted these proteins for replication in humans. More details, the Influenza A Virus evolution proceeds via reassortment and mutation (Mehle *et al.*, 2012). The mutation process can occur through antigenic shift and antigenic drift. Antigenic drift is a minor mutation and slow mutation through point mutation, this mutation can lead to influenza epidemic. Meanwhile, antigenic shift occur due to genetic reassortment between different influenza virus subtype the two distinct viruses coinfect the same cell and exchange one or more of their gene segments to produce progeny possessing a new genome constellation (Wibawa *et al.*, 2013).

Bats are being increasingly recognized as an important reservoir of zoonotic viruses of different families, including SARS coronavirus, Nipah virus, Hendra virus and Ebola virus. Several recent studies hypothesized that bats, an ancient group of flying mammals, are the major reservoir of several important RNA virus families from which other mammalian viruses of livestock and humans were derived (Smith and Wang, 2013). Two completely new influenza A virus subtypes (H17N10 and H18N11) were recently discovered in bats, dramatically expanding the host range of the virus. The found genomes were reconstructed by RT-PCR. Each of the bat genomes maintains the same genomic architecture and coding potential of other influenza A viruses (Mehle, 2014).

Two main diagnostic pathways can be used to achieve detection, subtyping and pathotyping this avian influenza. The conventional pathway includes virus isolation in eggs and HA test and HI test for subtyping. The hemagglutination (HA) assay is a tool used to screen cell culture or

aminoallantoic fluid harvested from embryonating chicken eggs for hemagglutinating agent, such as influenza A virus. Common HA test cannot distinguish between AI and NDV. HI test is then necessary to tell them apart (Lennström, 2009). In this research Hemagglutination Inhibition Assay were used to identify the virus presence in the sample.

1.4 The Aim of Research

The aim of this research is to detect the antigenic evidence of Avian Influenza Virus in bats collected from West Kalimantan using standardized antisera to detect the subtype of the virus.

1.5 Outcome of Research

The result of this study will give information about the evidence of Avian Influenza Virus circulation or track in bats, thus, bats considered as a reservoir of Avian Influenza Virus and potentially as a new mammalian host of Avian Influenza Virus. This result will be included in the consideration factor while arranging a policy toward implementation of control measures correlated to Avian Influenza pandemic lead by wildlife.