IOP Conference Series: Earth and Environmental Science

Molecular Epidemiology of Avian Influenza Virus (H5N1), Sumatera Indonesia

To cite this article: S Juariah *et al* 2020 *IOP Conf. Ser.: Earth Environ. Sci.* **430** 012019

IOP Conf. Series: Earth and Environmental Science **430** (2020) 012019 doi:10.1088/1755-1315/430/1/012019

Molecular Epidemiology of Avian Influenza Virus (H5N1), Sumatera Indonesia

S Juariah[1], M P Irawan[1], A Surya[1], Darmadi[1], D P Mahardika[2]*

[1] Department of Medical Laboratory Technology, Universitas Abdurrab, Indonesia
[2] Department of Biomedical Sciences, Universitas Abdurrab, Indonesia

*Corresponding author: dimas.pm@univrab.ac.id

Abstract. The highly pathogenic H5N1 avian influenza virus has been causing outbreaks in several regions in Indonesia, which is followed by high human casualties. It is panzootic virus in poultry and continues to spread and pose a major challenge to health in both animal and human. The H5N1 virus is considered as a serious pandemic threat which has potentially to re-endemic over time. This is proven by mutation of the H5N1 avian influenza virus in the globe. This study aims to investigate the distribution, prevalence, and transmission of avian influenza virus (H5N1) in Sumatera. In a retrospective analysis of 136 samples of H5N1 HA (Hemagglutinin) cds between 2004 and 2016. We present a phylogenetic approach with Neighbor-joining (NJ) tree analysis and Bayesian molecular clock-like-lines, to reconstruct the molecular epidemiology of DNA sequences of H5N1 virus isolated from Sumatera. Overall, the distribution was distinguished into 7 clades, with prevalence detected at 9 out of 13 regions in Sumatera, and all the transmission was originated from outside the island. The spread of H5N1 avian influenza on Sumatera is not linear and homogeneous. Therefore, it has shown a significant change in the variant that currently exists with the original species.

1. Introduction

Phylogenetics is increasingly popular due to the abundance of available genetic sequences, and as a result phylogenetic analysis has been shown to be effective in getting estimates of transmission and death rates in pathogenic sequences, such as influenza viruses [1]. Phylogenetic trees are fast, simple and flexible 'way' to distinguish communicable disease outbreaks with a super-spreader, homogeneous and chains of influenzas transmission patterns in different host population. It has proven to be an essential tool in the monitoring genetic origins, selection pressures, evolutionary rates, reassortment histories of influenza virus infections in birds, pigs, and humans [2].

The basis of this phylogenetic analysis is an evolutionary tree reconstructed from the same pathogenic DNA [3]. If the evolution rate of organisms is high, it can be assumed that the evolutionary and epidemiological of a pathogen is relatively the same, thus the phylogenetic trees is possibly used to describe the dynamics of disease transmission [4], [5]. This phylogenetic method is statistical in nature and assumes a basic model that describes the dynamics of evolution and the population of genetic sequences [6]. The observations and predictions of epidemiological processes are based on patterns of infection transmission within a population. The role of phylogenetic analysis is to reconstruct the transmission chain and the evolutionary history base on the quantity of changes in pathogen sequence data DNA [7].

Previous research shown that there were ten protein tagging commonly used in the characterization of avian influenza virus. The 10 proteins are: polymerase proteins PB1, PB2, and PA, nonstructural proteins NS1 and NS2, nucleocapsids, matrix proteins M1 and M1, Neuraminidase (NA) proteins and Hemagglutinin (HA) proteins. The last two proteins are the most commonly used in the process of identifying and analyzing H5N1. Influenza type A subtypes based on HA and NA antigens are differentiated based on the findings of surface proteins in viral envelopes [8], [9]. Markers of potential pathogenicity commonly used in Avian the H5N1 virus is hemagglutinin (HA), given the structure and specifications of hemagglutinin receptors that are unique to influenza viruses making it an effective marker [10], [11].

2. Methods

Phylogenetic analysis was carried out using MEGA X [12] with a series of 32 sequences (all samples from 2004 to 2016) from previous studies conducted in Sumatera and compared with 106 reference strains from GenBank representing all genotypes from Indonesia. The sequence analyzed was all restricted to the same 1658 cds of HA nucleotide from viral polymerase which did not contain cracks, deletions or insertions. Alignment was built using clustalW2 software Phylogenetic trees were calculated by the neighboring unification method implemented in the BioNJ program as part of the MEGA platform, bootstrap analysis was performed 1000 times to confirm the reliability of the structure produced [13].

3. Results and Discussion

3.1 H5N1 avian influenza virus distribution

The evolutionary history was inferred using the Neighbor-joining method [14]. The optimal tree with the sum of branch length = 0,26012817 is shown. Phylogenetic trees are aligned with the existing scale as a reference, where the length of the branch is in units equal to the evolutionary distance of the pathogen. Evolutionary distances are calculated using the Tamura-Nei method [15] and are in units of the number of basic substitutions per site. The rate variation between sites is modeled by the gamma distribution (parameter form = 1)

Pathogen sequence data provide richer information than strain type as a sequence accumulate mutations over time. The biased composition between sequences is considered, in this analysis 32 DNA sequences from H5N1 Avian Influenza Viruses found in Sumatera are used as a comparison in the comparison of pathogenic evolution. Where all ambiguous positions have been deleted for each sequence pair, a total of 1658 positions in the last dataset.

As shown in Figure 1, Phylogenetic analysis has been carried out on 136 H5N1 HA (Hemagglutinin) cds samples throughout Indonesia during last 13 years and dividing H5N1 viruses in Indonesia into 11 different clades. Where of the eleven variants, only 7 of them were found to have spread in 9 out of 13 provinces on the island of Sumatera. The regions which includes Aceh, North and West Sumatera, Riau and Kepulauan Riau, South Sumatera, Bangka Belitung, Jambi and Lampung.

3.2 H5N1 avian influenza virus prevalence

During 2004 to 2016 there were 12 times of the H5N1 virus pandemic in Indonesia. As seen in Figure 2, in Sumatera alone, there were only 5 times of the H5N1 virus pandemics. Which was happened during 2004, 2005, 2006, 2010 and 2016 respectively.

After the first H5N1 avian virus clade 3 pandemics on the island of Sumatera in 2004, the next mutation, the H5N1 clade 4 variant appeared in 2005. It was followed by the appearance of the H5N1 clade 7, clade 8 and clade 6 variants in 2006 and 2010 respectively. Phylogenetic analysis detected the emergence of the clade 9 and clade 11 H5N1 Avian Virus mutations on the island of Sumatera in the 2016 pandemic.

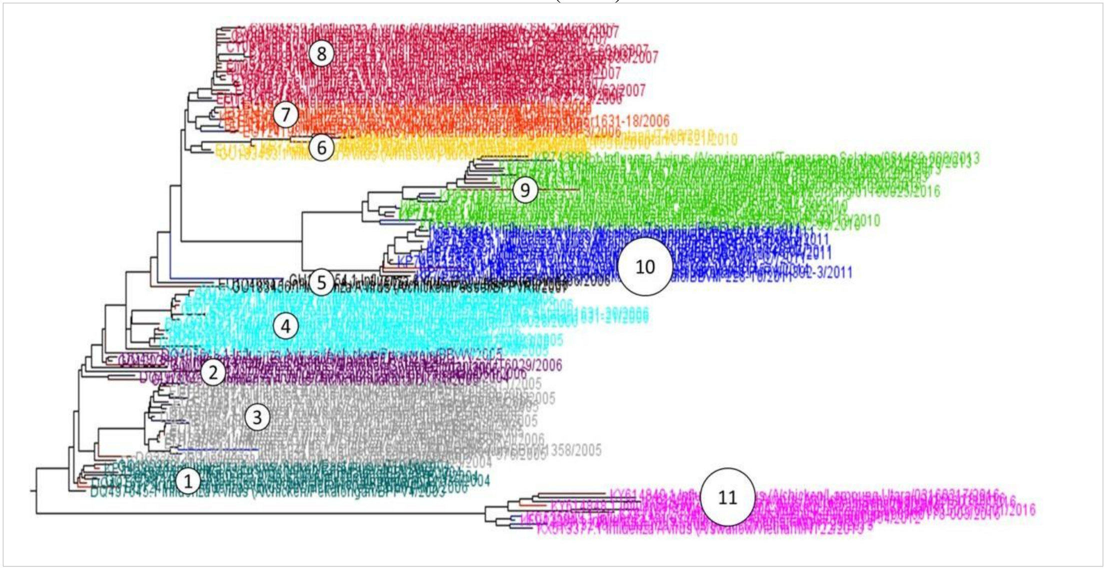

Figure 1. H5N1 avian influenza virus genotypes in Indonesia between 2004-2016. The colors represent the evolutionary distances of the H5N1 genotypes.

This is becoming increasingly interesting, because since 2010, the variants of H5N1 clade 3, 4, 7 and clade 8 did not appear again. And this continues into the last H5N1 an influenza virus pandemic recorded in 2016. As seen in Figure 2 below, from 2010 to 2016, the variant of the H5N1 virus that spreads on the island of Sumatera is completely different compared to the first spread.

3.3 H5N1 avian influenza virus transmission

In 2004 there were 7 pandemics in Indonesia involving 4 types of H5N1 virus, but nothing was seen to transmitted into the Sumatera region. As shown in Figure 4a below, during 2004 the phylogenetic analysis of H5N1 virus pandemic in Bangka Belitung was genotypically different from the other 6 regions outside Sumatera.

Meanwhile, in 2005 two new clades of the H5N1 virus were introduced in Sumatera, and spread throughout Riau, Jambi, Palembang and Lampung. They have a variant that are very similar to the H5N1 variant found in West and East Java, Bali, and NTB at almost the same time. Therefore, the possibility of an inter-transmission between these areas is very high.

In 2006, the H5N1 variants that caused of the pandemic in the Sumatera was mostly different from the variants on other islands, for instance Java, Kalimantan and Papua. During this period, the transmission seems to be local. As seen in Figure 4c, Kepulauan Riau, Riau and North Sumatera share the same variant of virus. Bangka Belitung and Aceh in other groups. While only Lampung is seen to share similarities of the H5N1 virus variant from outside Sumatera.

In the 2010 pandemic, only Riau was exposed to the spread of H5N1 viruses in the Sumatera. The H5N1 variant was identified as clade 6 and is estimated to be phylogenetically transmitted from the western and southern Kalimantan regions. As shown in Figure 4d, otherwise geographically between several other pandemics which occur almost simultaneously in Jakarta and West Java is relatively close. Genotyping analysis of existing sequence data proved to be very different.

Conversely in the 2016 pandemic, none of the other regions had similarities with the H5N1 Virus variant which was spread in The North Sumatera. However, based on the phylogeny dataset of the Avian Influenza Virus H5N1 transmission between 2004 and 2016 in Indonesia, the H5N1 Virus in the North Sumatera was genotypically the same with variant that had spread in Jakarta and West Java during 2010 and 2013.

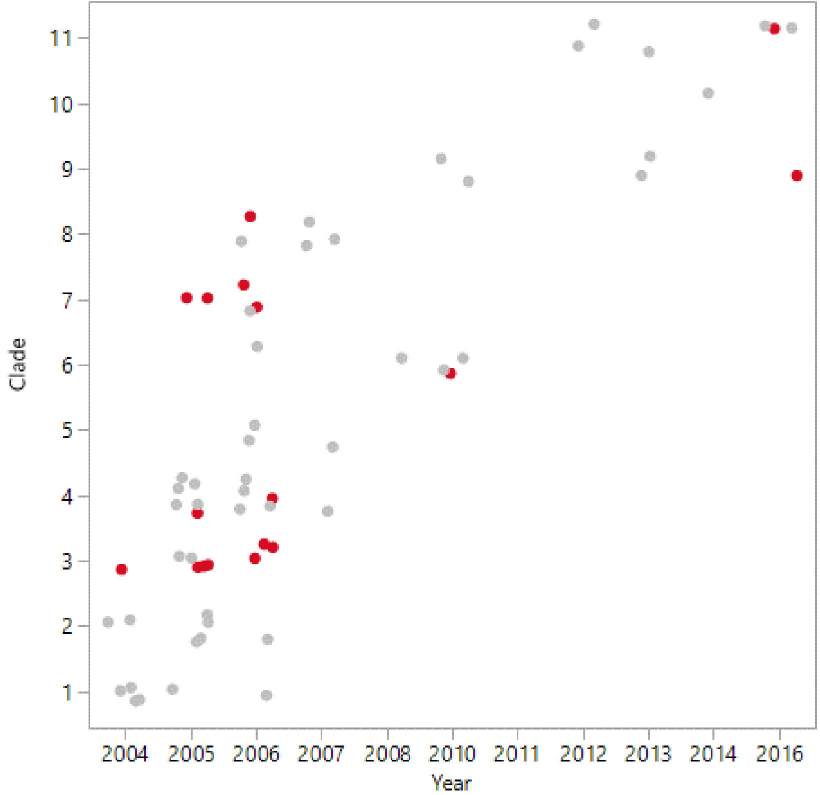

Figure 2. Distribution of H5N1 avian influenza virus clades across the regions. The red dots represent the variation of H5N1 viruses during 2004-2016 in Sumatera.

Figure 3. Prevalence of H5N1 avian influenza virus clades in Indonesia between 2004-2016.

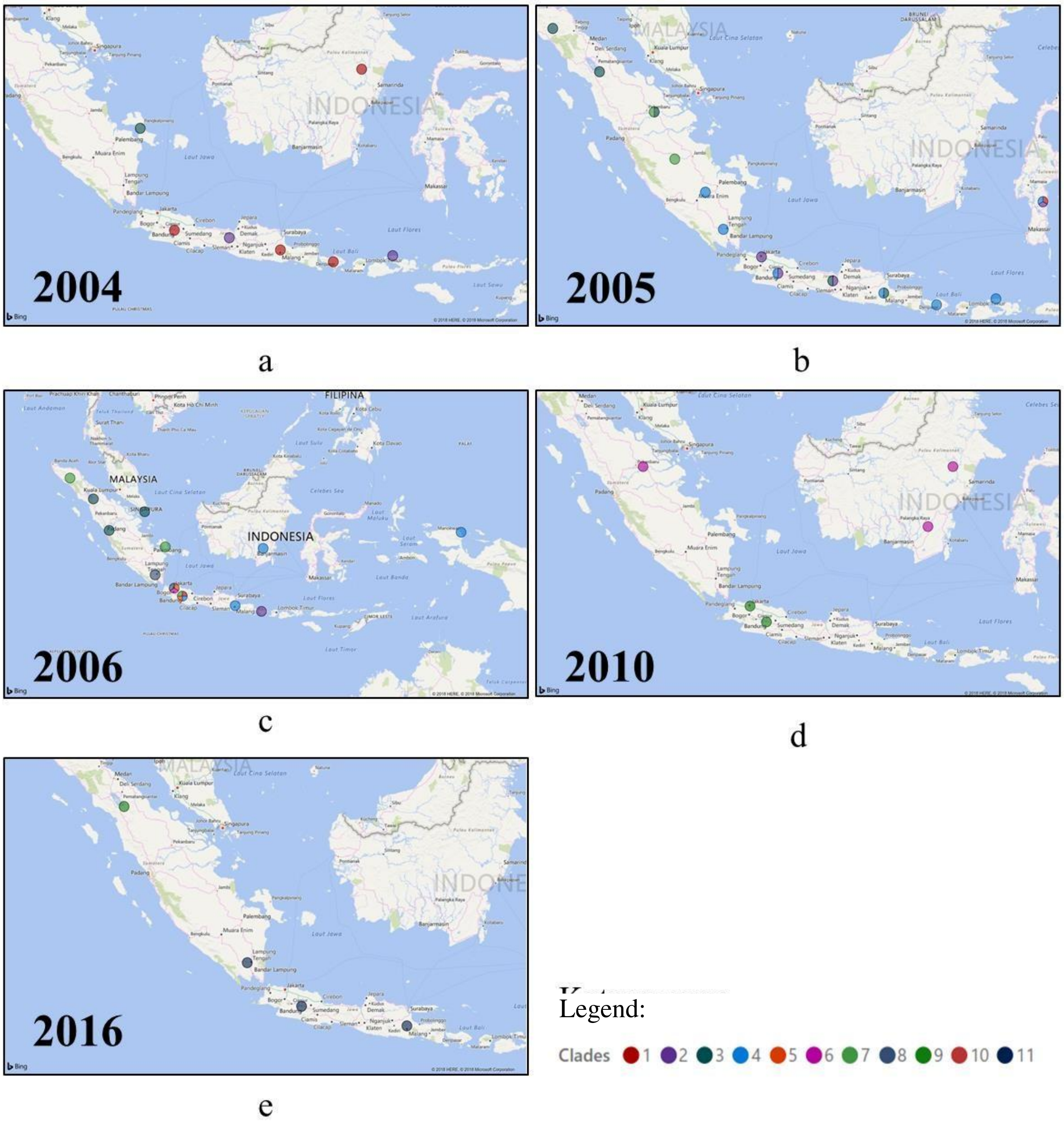

Figure 4. Transmission of H5N1 Avian Influenza Virus clades across Sumatera regions between 2004 and 2016.

4. Conclusion

Phylogenetic analysis of 136 samples of H5N1 HA (Hemagglutinin) cds throughout Indonesia over the past 13 years, has divided the H5N1 virus in Indonesia into 11 different clades. Of all the variants, only 7 of them have been found to have been infected in 9 of the 13 provinces on the island of Sumatera. The area includes Aceh, North and West Sumatera, Riau and Riau Islands, South Sumatera, Bangka Belitung, Jambi, and Lampung.

Overall, there have been 12 pandemics of the H5N1 virus in Indonesia during 2004 to 2016, only 5 times which have spread to areas on Sumatera, namely in 2004, 2005, 2006, 2010 and 2016. Where during the 13 years period, there has been a significant change in the H5N1 Virus genotype from the original variant that first contracted in Sumatera. This is believed to occur due to mutations of local variants and transmissions from variants outside of Sumatera which were detected in 2010.

The spread of H5N1 Avian Influenza on Sumatera is not linear and homogeneous. This research proves that not all variants which have infected Indonesia have proven to be pathogens in the Sumatera regions which have experienced a pandemic in almost the same time. Phylogenetic analysis between 2004-2016, has shown a significant change in the variant of the H5N1 virus that currently exists with the original species.

Molecular epidemiology can be considered as one of the prevention instruments and monitors the movement of people and goods related to the transmission of pathogens such as H5N1 virus, as well as being a reference for increasing the effectiveness of antibiotics to be used in future outbreaks

References

[1] O. G. Pybus *et al.*, "Unifying the spatial epidemiology and molecular evolution of emerging epidemics," *Proc. Natl. Acad. Sci.*, vol. 109, no. 37, pp. 15066–15071, 2012.

[2] S. R. Amit Roy, "Molecular Markers in Phylogenetic Studies-A Review," *J. Phylogenetics Evol. Biol.*, vol. 02, no. 02, 2014.

[3] P. Ajawatanawong, "Molecular Phylogenetics : Concepts for a Newcomer," 2016.

[4] M. A. Suchard, P. Lemey, G. Baele, D. L. Ayres, A. J. Drummond, and A. Rambaut, "Bayesian phylogenetic and phylodynamic data integration using BEAST 1.10," *Virus Evol.*, vol. 4, no. 1, pp. 1–5, 2018.

[5] H. Tian *et al.*, "Correction for Tian et al., Avian influenza H5N1 viral and bird migration networks in Asia," *Proc. Natl. Acad. Sci.*, vol. 112, no. 22, pp. E2980–E2980, 2015.

[6] M. A. Suchard and A. Rambaut, "Many-core algorithms for statistical phylogenetics," *Bioinformatics*, vol. 25, no. 11, pp. 1370–1376, 2009.

[7] G. E. Leventhal, H. F. Günthard, S. Bonhoeffer, and T. Stadler, "Using an epidemiological model for phylogenetic inference reveals density dependence in hiv transmission," *Mol. Biol. Evol.*, vol. 31, no. 1, pp. 6–17, 2014.

[8] J. S. M. Peiris, M. D. de Jong, and Y. Guan, "Avian Influenza Virus (H5N1): a Threat to Human Health," *Clin. Microbiol. Rev.*, vol. 20, no. 2, pp. 243–267, 2007.

[9] B. J. Smith, J. L. McKimm-Breshkin, M. McDonald, R. T. Fernley, J. N. Varghese, and P. M. Colman, "Structural studies of the resistance of influenza. Virus neuramindase to inhibitors," *J. Med. Chem.*, vol. 45, no. 11, pp. 2207–2212, 2002.

[10] M. C. Lawrence *et al.*, "Structure of the Haemagglutinin-neuraminidase from Human Parainfluenza Virus Type III," *J. Mol. Biol.*, vol. 335, no. 5, pp. 1343–1357, 2004.

[11] J. Stevens, O. Blixt, T. M. Tumpey, J. K. Taubenberger, J. C. Paulson, and I. A. Wilson, "Structure and receptor specificity of the hemagglutinin from an H5N1 influenza virus," *Science (80-.).*, vol. 312, no. 5772, pp. 404–410, 2006.

[12] S. Kumar, G. Stecher, M. Li, C. Knyaz, and K. Tamura, "MEGA X : Molecular Evolutionary Genetics Analysis across Computing Platforms," vol. 35, no. 6, pp. 1547–1549, 2018.

[13] O. Gascuel, "BIONJ: an improved version of the NJ algorithm based on a simple model of sequence data.," *Mol. Biol. Evol.*, vol. 14, no. 7, pp. 685–695, 1997.

[14] N. Saitou and M. Nei, "The Neighbor-joining Method: A New Methode for Reconstructing Phylogenetic Trees." The University of Chicago, pp. 406–425, 1987.

[15] K. Tamura and M. Nei, "Estimation of the Number of Nucleotide Substitutions in the Control Region of Mitochondrial DNA in Humans and," vol. 10, no. 3, 1993.