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Studies on the Spread of H5N1 Influenza Viruses in Egypt

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The H5N1 avian influenza viruses raised the concern to the public health in the world. As the WHO reported more than 50% case fatality rate from clinically diagnosed as H5N1 infections by the 24th of June 2019. Sporadic human infections of H5N1 viruses causing severe respiratory disorders leading to death have been reported. Although the human-to-human transmission of the viruses is limited, family case clusters of H5N1 infections were reported from Sumatra, Cambodia, Thailand, China and Pakistan.

In 2006 H5N1 influenza viruses were first isolated from poultry in Egypt and declared to be enzootic in 2008. The number of human cases in Egypt has been increasing dramatically since 2014. Two-thirds of the human H5N1 cases were reported in the world by 2019 were from Egypt. The increase in 2014 might be related to either increase of the number of outbreaks among poultry or due to increase in the transmission potential of the H5N1 viruses among human populations. In this study we aim to estimate the transmission potential of H5N1 viruses in humans to improve the control measures for the viral spread in Egypt. One way to estimate transmissibility of infectious diseases is to measure R_0 , which is defined as the average number of secondary cases originating from a primary infected case in a whole susceptible population. To estimate R_0 of avian influenza virus infections in a human population, the number of human cases and/or cases with history of bird contact are frequently used.

To elucidate the cause of the rapid increase in human cases of H5N1 influenza virus infections had increased from 2014 to 2015 in Egypt, I have estimated the R_0 of H5N1 infections in human population in Egypt using candidate transmission pairs identified from the nucleotide sequences, infection time, and geographic location of viruses. Sensitivity analysis shows that R_0 is below unity, suggesting that major outbreak will not occur, with broad range of threshold values of genetic distance, sampling time interval, and geographical distance. Using genetic distance, sampling time interval, and geographical distance, I estimated R_0 of 0.05 (95% CI; 0.01, 0.13) assuming that human-to-human transmissions occurred within a city, 0.23 (95% CI; 0.14, 0.35) assuming human-to-human transmissions among cities, suggesting that human-to-human transmission of H5N1 viruses is rare in Egypt.

R_0 of H5N1 infections in human population in Egypt was estimated by using candidate transmission pairs identified from the nucleotide sequences, infection time, and geographic location of viruses. The nucleotide sequences of H5N1 influenza viruses were used to obtain candidate transmission pairs in two different ways. The first approach makes clusters of human viruses using genetic distances with respect to a given threshold value. This approach does not use nucleotide sequences of avian viruses. The second approach use a phylogenetic tree of influenza viruses constructed from nucleotide sequences of humans and birds and use genetic distance to divide clusters into transmission chains. The second approach reduces a possibility that two avian-to-human transmissions are clustered together. In

fact, the estimated values of R_0 were smaller when I analyzed sequences with phylogeny than when without phylogeny. There is a possibility that the R_0 would further decrease if I had more avian sequences similar to human viruses. In sensitivity analysis, the effect of threshold values on R_0 were smaller when with a phylogenetic tree than without a phylogenetic tree. ²² Sensitivity analysis of sampling time interval showed a large effect on R_0 between 0 days and 30 days. R_0 was estimated as 0.22, 0.28, and 0.42 when the sampling time interval threshold was 10, 20, and 30 days, respectively. It is known that the sampling time interval of transmission period is less than 30 days. Sensitivity analysis of geographical distance showed a large effect on R_0 between 0 km and 150 km. R_0 was estimated as 0.067, 0.12, 0.22, 0.27 when the geographical distance threshold was 0, 50, 100, and 150 km, respectively. These results indicate that the threshold of sampling time interval and geographical distance are important variables to estimate R_0 using our data. I estimated R_0 with combinations of different threshold values for genetic distance, sampling time difference and spatial difference as sensitivity analyses. In all analyses, the upper bound of the 95% CI of R_0 was below unity.

Although sensitivity of R_0 against geographical distance is also high, the setting of geographical distance to detect clusters of human cases is not straightforward. Since several studies reported that most human-to-human transmissions of H5N1 occurred within their household, the threshold of geographical distance might be suitable to set 0 km, i.e., human-to-human transmissions occurred only within the same city. If this is the case, the R_0 estimate is 0.05 (95% CI: 0.01, 0.13). However, the human cases of H5N1 in Egypt have not been well understood whether they occur within or between household so far, and this assumption may underestimate R_0 . If I do not set any threshold of geographical distance, the R_0 estimate is 0.23 (95% CI: 0.14, 0.35). However, this setting may overestimate R_0 . This setting assumes that genetic distance and sampling time difference are enough to determine the cluster of human cases formed by only human-to-human transmission. If multiple introductions of H5N1 viruses from avian to human occur, this setting may lead to misinterpret them as human-to-human transmission events. To estimate an accurate R_0 , a detailed surveillance, e.g., contact tracing, ²³ is required. Regardless of the settings for threshold of geographical distance, the upper bound of the 95% CI of R_0 was below unity.

Human cases of the highly pathogenic avian influenza (HPAI) H5N1 had increased in 2014–2015). The upper bounds of 95% CI of our estimates of R_0 using various methods were below unity, suggesting that major outbreak will not occur shortly. Using genetic distance and sampling time, R_0 was estimated to be 0.23 with 95% CI from 0.14 to 0.35. Furthermore, the distribution of geographical distance between human cases shows unclear, meanwhile, the distribution should be right-skewed if most human cases are attributed to human-to-human transmission. ²⁴ Sensitivity of R_0 against geographical distance suggests that the outbreak of H5N1 among birds in Egypt occurs widely (diameter of the area is 200 km hypothesized from the saturation of R_0 estimate with assuming that human-to-human transmission is rare. Our results supported that most human cases should be attributed to avian-to-human transmissions. The dramatic increase in human cases in Egypt would be attributed to the high prevalence

of H5N1 among avian species, and avian-to-human transmissions in wide regions of Egypt may explain the unclear trend in the distribution of geographical distance between human cases. Vaccines against H5N1 viruses have been used in Egypt since 2006 and this can be efficient in back yard settings in Egypt. Despite the intensive use of H5N1 vaccines, the virus became endemic in 2008 and the number of human cases has increased. vaccination of poultry makes it difficult to monitor the spread of viruses. Moreover, the immune pressure of the vaccinated poultry accelerated the virus evolution in Egypt. Therefore, to reduce the risk of H5N1 infections in humans I need to implement a more effective control measures other than vaccinations in poultry, because the control of avian influenza using vaccination is difficult.

This study proposed a method to estimate human-to-human transmissibility of zoonotic pathogens using nucleotide sequences as well as temporal and geographic information of infections. The integration of multiple types of data to the analyses can lead a more accurate estimation than analysis using a single type of data. However, there are some limitations in this study. First, I did not include exposure history to birds in our analysis. The World Health Organization reported exposure history of 37 laboratory-confirmed human cases of avian 25 influenza A (H5N1) virus infection in Egypt during the period from the 3rd of March to the 31st of March 2015. Of these, 36 cases had a history of contact with poultry or visiting poultry markets. One case was under investigation (World Health Organization, 2015). The inclusion of bird contact information may reduce the estimates of R_0 . There is no link between the sequence data used in this study and exposure history data so far, the improvement of surveillance system that can link them is required to estimate an accurate R_0 . Second, the number of available sequences in the database is limited. There would be difference in the sampling probability between human and avian sequences. Assuming that the sampling probability in human sequences is higher than that of avian sequences, I may have overestimated the R_0 . Surveillance of both human and avian viruses is important to correctly estimate R_0 by our method. In this study, I estimated R_0 of H5N1 viruses in human population using nucleotide sequences, sampling date, and sampling location of viruses. Taking into account the phylogeny, genetic distance, sampling time difference among viruses, R_0 was estimated to be 0.05 (95% CI; 0.01, 0.13) assuming that human-to-human transmissions occurred within a city, 0.23 (95% CI; 0.14, 0.35) assuming human-to-human transmissions among cities. Sensitivity analysis confirmed that R_0 is below unity, suggesting that major outbreak will not occur, with broad range of threshold values of genetic distance, sampling time interval, and geographical distance. In conclusion human-to-human transmission of H5N1 viruses in Egypt is still limited. The large increase in human cases is most probably attributed to the increase in avian cases. Monitoring both avian and human populations is required to prevent major outbreaks of H5N1 infections among the human population.

Understanding spread of the H5N1 influenza virus is crucial for surveillance and vaccine strain selection. We analyzed the geographic spread of the virus using epidemiological and virological data over a period of 10 years from Egypt and some other Mediterranean countries. In this study we analyzed

the spatial dynamics of H5N1 virus spread and transmission. We identified the evolutionary tempo and mode among Egypt and the other countries that were affected by the same outbreak between 2006 and 2016. This study highlighted the complex history of the H5N1 virus and the countrywide spread during a small time period. The introduction of the virus into Egypt seems to be happened in 2005, while the virus was introduced into Gaza and Israel in 2006, to Jenin in late 2010, and Qatanna in late 2014. The spread of the virus seems to be started from Egypt towards the other 4 regions in the neighboring countries near by the eastern borders of Egypt. Clade 2.2.1.1 appeared in 2007 in Egypt and circulated in Egypt, Gaza, Israel.

Phylogeographic analysis suggested that human viruses in the phylogenetic tree were distributed into clades 2.2, 2.2.1, 2.2.1.2, and 2.2.1.1, however clade 2.2.1.1a which hypothesized to be a vaccine skip mutant does not show any human viruses. Clade 2.2.1.1 evolved from clade 2.2.1 in 2008. To examine the evolution and spatial dispersion of H5N1 virus, we used HA gene of H5N1 viruses sampled from Egypt, and four neighboring regions. Assuming the number of genetic sequences is indicator for H5N1 viruses' evolution, I analyzed the yearly change in the number of H5N1 influenza virus available in the NCBI influenza virus resources. From 2006 to 2016 the number of available sequences appears as waves of increases and decreases either due to virus evolution, outbreak occurrence or missing bias. In 2006, H5N1 virus was first introduced into Egypt causing outbreak among poultry with a certain number of avian to human transmissions in 2006-2007. In 2008 the H5N1 virus became enzootic among poultry represented as increased number of avian sequences (cases), but fewer human cases were reported. 2009 showed increase in both human and avian cases, and this could be related to either increased human susceptibility to the virus or increased viruses' evolution in avian population. Between 2009 and 2011, the number of avian sequences increased as clade 2.2.1.1. The large increase in poultry cases was due to the mass vaccination strategies that had started since late 2006 and three years on their efficiency became limited. From 2011 to 2013 both avian and human cases were decreased as there were no reports about new virus strains. By late 2014, further evolution of these clades resulted in the new clade 2.2.1.2, which was circulating in 2015 and caused increase in the number of avian cases, but interestingly the human cases reported were low. This decrease in human cases could be revealed to the lower pathogenicity of the new virus strain toward humans.

Analyzing H5N1 virus introductions in Egypt and neighboring countries supported that the Nile Delta region in Egypt is the origin of the outbreak since it represents the first introduction of the virus in 2005. Phylogeographic analysis of the virus introductions supported that the Nile Delta region is the geographic origin of the virus in Gaza, Jenin, Israel and Qatanna. We found well-supported migration paths between Egypt and Jenin in 2011, and Egypt and Qatanna in 2015 as shown in Figure 5. Such long distances transition most probably occurred through viral transmission the migratory birds or due to poultry trade between countries.

In our study, we confirmed that most of the introduction of H5N1 virus in Egypt were from the most populated region, the Nile Delta. By including more data from the countries that were affected by the

same outbreak, Gaza, Israel, Syria, we also confirmed that the Nile Delta region was the source of the virus introductions from 2005 to 2016.

In conclusion, to clarify the cause of the increase in H5N1 human cases, I investigate the transmissibility of H5N1 viruses among humans via estimating the basic reproduction number R_0 using nucleotide sequences and sampling dates of viruses. To this end, full-length hemagglutinin gene sequences of human and avian H5N1 influenza viruses isolated from 2006 to 2016 in Egypt were obtained from the NCBI influenza virus resource. Taking into account the phylogeny, genetic distance, sampling time difference among viruses, R_0 was estimated to be 0.05 (95% CI: 0.01, 0.13) assuming that human-to-human transmissions occurred within a city, 0.23(95% CI: 0.14, 0.35) assuming human-to-human transmissions among cities. These results indicate that human-to-human transmission of H5N1 viruses in Egypt is limited, and the large increase in human cases is likely attributed to other factor than increase in human-to-human transmission potential. Moreover, spatio-temporal distributions of H5N1 was inferred through phylogeographic analysis to investigate the transmission of the H5N1 viruses in Egypt and its neighboring countries from 2006 to 2016. I conducted a discrete states phylogeographical analysis with Bayesian statistical framework using time-stamped, geographically referenced sequences of H5N1 viruses. Analysis of the phylogeographic tree revealed that Egyptian viruses were the main source of the outbreaks that occurred in the neighboring countries including Gaza, Israel, and Qatana. Spatial projection of the H5N1 viruses detected long-range migration events among Egypt, Gaza, and Qatana. Statistical analysis of the phylogeographic tree's trunk supported the Nile delta region as the common ancestor of the viruses circulating in poultry in Egypt. These results suggested that a strategy containing virus transmissions in avian populations in Egypt, especially in Nile delta region is a key to control H5N1 infections among both human and avian populations in Egypt and its neighboring countries.