Summary of dissertation

A study on the avian-to-swine transmission of influenza A viruses

(A 型インフルエンザウイルスの鳥豚間伝播に関する研究)

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The influenza A virus is a negative-sense single-stranded RNA virus that infects humans as well as a wide range of animals (Webster et al. 1992; Kuiken et al. 2004; Tong et al. 2012). Wild aquatic birds, such as wild ducks, geese, gulls, and shorebirds, are the natural reservoirs of the influenza A virus (Kida et al. 1988; Webster et al. 1992). Human influenza pandemics have historically been caused by genetic reassortment of human and avian influenza A viruses, and this reassortment typically occurs among viruses circulating in swine (Webster and Laver 1972; Scholtissek et al. 1978; Kawaoka et al. 1989; Yasuda et al. 1991; Smith et al. 2009). Experimental studies have suggested that swine are susceptible to both human (Kundin 1970) and avian viruses (Kida et al. 1994). Thus, the avian-to-swine transmission of influenza A viruses is an important factor contributing to the emergence of new pandemic strains.

Influenza A viruses are composed of eight gene segments, which encode at least seventeen viral proteins (Dubois et al. 2014). Of these, the polymerase complex consisting of PB2, PB1, and PA is responsible for viral replication in host cells. The PB2 protein is responsible for the cap binding of host's mRNA (Webster et al. 1992). The PB1 protein is associated with the catalytic activity of RNA synthesis (Kobayashi

et al. 1996; Neumann et al. 2004; Elton et al. 2006). The PA protein is involved in endonuclease activity of the polymerase complex for RNA replication (Dias A et al. 2009; Yuan P et al. 2009).

The amino acids at several positions on the polymerase complex have been known to determine the host range of influenza A viruses. The amino acid substitution from Glutamic acid (E) to Lysine (K) at position 627 on PB2 of avian viruses increases viral replication in mammalian hosts (Subbarao et al. 1993; Hatta et al. 2001; Shinya et al. 2004; Mok et al. 2014). Two simultaneous amino acid mutations from Valine (V) to Serine (S) at position 715 and from Isoleucine (I) to Serine (S) at position 750 in PB1 are known to reduce the number of cRNA and mRNA (Sugiyama et al. 2009). Several amino acid substitutions in PA were reported to affect viral replication in mammals (Yamayoshi et al. 2014).

Influenza A viruses have two glycoproteins. Hemagglutinin plays a role in attaching to the host cell of influenza A viruses whereas neuraminidase is associated with releasing of the virus from host cell (Webster, 1992). Hemagglutinin and neuraminidase are used to classify viruses into subtypes based on the antigenicity. HA and NA have 18 subtypes (H1-H18) and 11 subtypes (N1-N11), respectively (Tong, 2013). Only H17, H18, N10, and N11 are found to be circulating in bats (Tong et al. 2012, Tong et al. 2013).

Human influenza pandemics have historically been caused by genetic reassortment of human and avian influenza A viruses. Currently, H1 and H3 viruses are common subtypes of influenza A viruses circulating in swine population (Webster et al. 1992; Zhou et al. 1999). However, recently sporadic infections of H5N1 viruses and H9N2 viruses have been reported (Obadan et al. 2015). Therefore, the monitoring

of avian-to-swine transmission of H5N1 viruses and H9N2 viruses is getting more important.

The nucleotide sequence similarity provides important information to analyze the transmission of infectious disease pathogens. If an avian virus and a swine virus share the same nucleotide sequence, then this information suggests interspecies transmission of the virus. Phylogenetic analysis of nucleotide sequences can depicts the evolutionary relationships among viruses. However, phylogenetic analysis will face a problem regarding computational costs when a large number of nucleotide sequences are analyzed. For this reason, I needed to take a new approach to detecting interspecies transmission of influenza A viruses.

In chapter I, I introduced BLAST and reciprocal best hits technique to find pairs of avian and swine nucleotide sequences that may be associated with transmissions between avian and swine hosts.

In chapter II, I tracked viral transmission between avian and swine to investigate the evolution on polymerase genes associated with their hosts. I traced viral transmissions between avian and swine hosts by using nucleotide sequences of avian viruses and swine viruses registered in the NCBI GenBank. Using BLAST and the reciprocal best hits technique, I found 32, 33, and 30 pairs of avian and swine nucleotide sequences that may be associated with avian-to-swine transmissions for PB2, PB1, and PA genes, respectively. Then, I examined the amino acid substitutions involved in these sporadic transmissions. On average, avian-to-swine transmission pairs had 5.47, 3.73, and 5.13 amino acid substitutions on PB2, PB1, and PA, respectively. However, amino acid substitutions were distributed over the positions, and few positions showed common substitutions in the multiple transmission events. Statistical tests on the number of repeated amino acid substitutions suggested that no

specific positions on PB2 and PA were commonly observed in the transmission pairs for avian viruses to infect swine. I also found that avian viruses that transmitted to swine tend to possess I478V substitutions on PB2 before interspecies transmission events. Furthermore, most mutations occurred after the interspecies transmissions, possibly due to selective viral adaptation to swine.

In chapter III, I investigated interspecies transmission of influenza A viruses focusing on the HA gene. Using nucleotide sequences of HA gene of influenza A viruses isolated from avian and swine, my method detected 57 sequence pairs associated with interspecies transmissions between avian and swine. All of pair sequences had a BLAST E-value of zero and had more than 95% identities. Out of 28 H5 HA genes registered in NCBI database, 15 were associated with avian-to-swine transmissions, and others 13 genes were associated with the swine-to-swine transmissions. Out of 20 H9 HA genes registered in NCBI database, 11 were associated with avian-to-swine transmissions, and others 9 genes were associated with the swine-to-swine transmissions. Using this proportion, the reproduction number in swine population was estimated as 0.4643 for H5 viruses and 0.45 for H9 viruses. The 95% CI of reproduction number was estimated as [0.280, 0.649] and [0.232, 0.668] for H5 and H9, respectively. These results suggested that the transmissibility of H5 and H9 viruses among swine population is limited.

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