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Changes of Epidemiological Characteristics of Japanese Encephalitis Viral Infection and Birds as a Potential Viral Transmitter in Korea

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ABSTRACT

Japanese encephalitis (JE) cases have been increasingly reported recently especially in Seoul and its vicinity. Pigs are known as amplifying host of JE virus (JEV), but do not play an important role in these recent events because pig-breeding is not common in Seoul. The distribution and the density of migratory birds are correlated with JE cases in cities and they might be highly potential hosts contributing to transmit JEV in metropolitan areas. JE genotype and sero-prevalence in birds should be determined for the verification of the transmission route of JEV in the recent sporadic occurrence of JE cases in Seoul.

Keywords: Japanese Encephalitis; Mosquito-Borne Diseases; Re-emerged Diseases; Potential Factor

Japanese encephalitis virus (JEV), member of Flaviviridae, causes Japanese encephalitis (JE) in human. JEV has transmission cycle including birds as reservoirs, pigs as amplifying hosts and *Culex* mosquitoes as vectors. Among *Culex* mosquitoes, *Culex tritaeniorhynchus* is primary vector, and other mosquitoes can transmit JEV between host vertebrates. JEV has only one serotype but it has I-V genotype depending on envelope gene sequences.¹ About 1,600 cases were reported annually until the 1970s but only 10 cases were reported annually after the introduction of JE vaccine and mandatory vaccination program for children 3–15 years of age in the 1980s in Korea.² However, average 20 cases were reported nation-widely and the trend of reporting number of JE cases are increasing since 2010.³ We analyzed changes of characteristics of reported JE cases in recent several years and suggest birds as potential factors affecting to epidemiology of JE cases.

In 2011 to 2016, total 131 cases including 17 deaths were reported in Korea. Seventy-five cases (57.3%) were male and 56 cases were female (42.7%). By age group, 38.9% were aged 50–59 years, 20.6% were aged 40–49 years, and 13.0% were aged 60–69 years. The number of JE cases showed seasonal pattern and most of cases were reported in August to November. In 2011 and 2014, 2 cases were reported in May and June, earlier than JE season, but they were imported cases from Laos and Thailand.³ By province, the number

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Author Contributions

Data curation: Bae W, Kim JH, Hwang ES. Formal analysis: Bae W, Kim JH, Kim J, Lee J, Hwang ES. Funding acquisition: Hwang ES. Investigation: Bae W, Kim JH, Kim J, Lee J, Hwang ES. Writing - original draft: Bae W, Kim JH, Hwang ES. Writing - review & editing: Hwang ES.

of recent JE cases showed totally different pattern compared to the 1980s. In the 1980s, 314 cases (20.5%) were reported from Jeonnam, 249 (16.2%) from Chungnam, and 232 (15.1%) from Seoul. In 2011–2016, 41 cases (31.3%) were reported from Seoul, 27 cases (20.6%) from Gyeonggi, and 12 cases (9.2%) from Daegu. The number of JE cases per 1,000,000 population showed high in metropolitan areas such as Daegu (0.81), Daejeon (0.76), and Seoul (0.69) (**Fig. 1A**).

According to quarterly survey of livestock in Korea, 5,536 pig farmhouses bred about 9,600,000 pigs in 2011–2016. The number of pig farmhouses was high in Chungnam (972.3 pig farmhouses), Jeonnam (944.8 pig farmhouses), and Gyeonggi (883.8 pig farmhouses). The number of pigs was high in Chungnam (1,981,187 pigs), Gyeonggi (1,567,357 pigs), and Jeonbuk (1,185,374 pigs) (**Fig. 1B**).⁴ Incidence rate of JE cases was higher in cities than in provinces, but less than 15,000 pigs were bred in the cities. In 2011 to 2016, 14,350 pigs were bred in Daegu and 990 pigs in Daejeon. In 2011 to 2014, average 43 pigs were bred in Seoul, and in 2015 and 2016, no pig was bred in Seoul. There is no available epidemiological information of each JE cases, however, from two Korea Centers for Disease Control and Prevention (KCDC) articles, the rate of proximity to swinery were 22.2% (10 cases among 45 cases) in 2007 to 2010, and 7.8% (8 cases among 103 cases) in 2011 to 2015.^{5,6} Eight provincial Public Institute of Health & Environment (PIHE) had monitored sero-positivity of JEV in domestic pigs from July to October annually. The sero-positive rate of JEV was 23.8% in 2011, 11.8% in 2012, 12.6% in 2013, and 10.2% in 2014.⁷ There is no significant relationship between sero-positivity of pigs and JE cases.

The discrepant relationship between the incidence rate of JE cases and pig breeding population in cities draw attention to find out other possible transmission factors. According to correlation analysis among number of JE cases per 1,000,000 population, pigs and herons by provincial, there was a positive correlation between the two variables, number of JE cases and herons ($r = 0.778$; $n = 10$; $P = 0.008$) but between number of JE cases and pigs, there

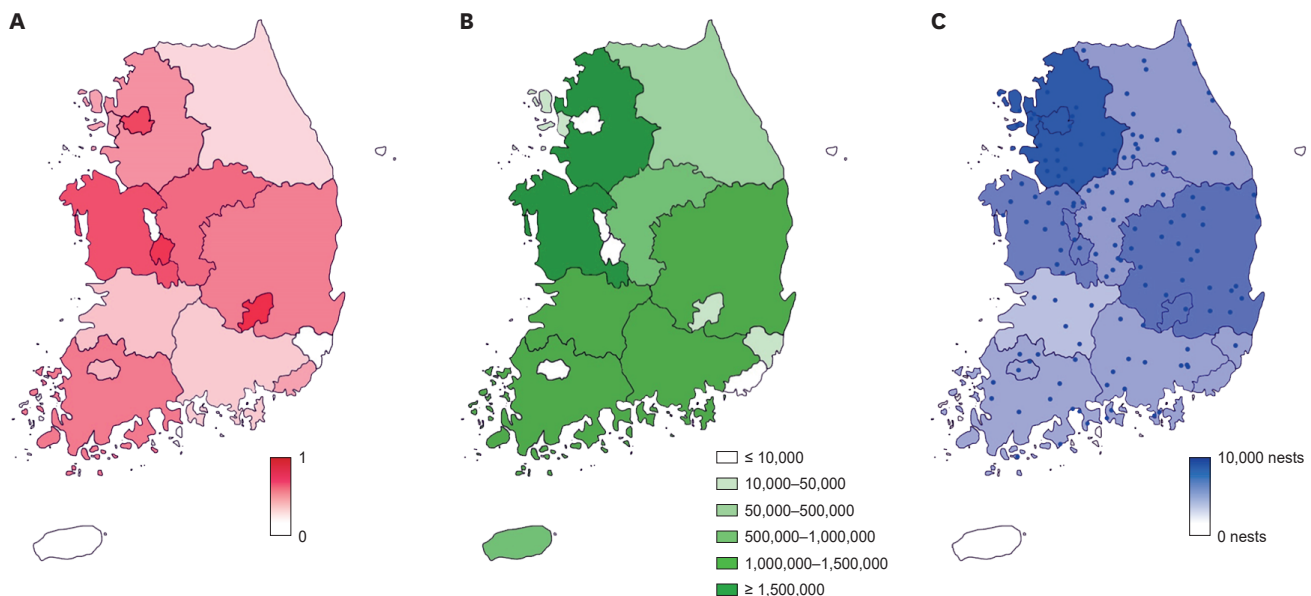


Fig. 1. Comparison of distribution of JE cases, pigs and herons in Korea. (A) Total number of JE cases per 1,000,000 population (2011–2016), (B) density of pigs (2011–2016), and (C) density of herons (2011–2012). In density of herons, dark blue dots indicate breeding place of herons investigated by NIER. JE = Japanese encephalitis, NIER = National Institute of Environmental Research.

was no significant correlation ($r = -0.005$; $n = 16$; $P = 0.986$). Wading birds, family Ardeidae including herons and egrets are considered JEV reservoir. National Institute of Environmental Research (NIER) investigated 148 breeding place of egrets and herons, and 8,290 nests were in Seoul·Gyeonggi·Incheon province, 5,719 nests in Gyeongbuk·Daegu province, 5,080 nests in Chungnam·Daejeon·Sejong province. The most frequent species were *Ardea cinerea* (13,422 nests), *Ardea alba* (7,835 nests), *Egretta garzetta* (5,810 nests).⁸ The distribution of wading birds and the incidence rate of JE cases are correlated well, especially in cities (Fig. 1C). However, there is no study for monitoring sero-positivity of JEV on herons and egrets in Korea. Recent studies reported wild birds can play a role in JEV reservoir and showed sero-positivity on JEV. In 2009, Saito et al.⁹ suggested wild ducks can play a role in JEV reservoir in Hokkaido, Japan. Wading birds are summer migratory birds flying to Korea in spring while ducks are winter migratory birds flying to Korea in autumn. Yang et al.¹⁰ reported that out of the 1,316 serum samples tested, 84.7% to 88.5% sero-prevalence in wild birds including ducks (*Anas formosa*, *Anas penelope*, *Anas acuta*, *Anas crecca*, *Anas platyrhynchos*, *Anas poecilorhyncha*), mandarin ducks (*Aix galericulata*), petrels (*Oceanodroma castro*), and Eurasian coots (*Fulica atra*). These reports may support birds as one of the possible factors of JEV transmitter in cities.

KCDC monitors population density of the JEV vector, *Culex tritaeniorhynchus* at 28 collection points in 10 provinces (Busan, Gyeonggi, Gangwon, Chungbuk, Chungnam, Jeonbuk, Jeonnam, Gyeongbuk, Gyeongnam, and Jeju) using black light traps and provides all data of vector surveillance at the web page of KCDC.¹¹ When compared of the reported number of JE cases to the number of collected *Culex tritaeniorhynchus*, there is no significant relationship between them. The patterns of weekly reported number of JE cases reached a peak after 3–4 week that the number of collected *Culex tritaeniorhynchus* reached a peak (Fig. 2). In 2005 and 2006, the high mosquito population in Seoul were *Culex pipiens* (83.1% in 2006), *Aedes vexans nipponii* (7.2%), and *Ochlerotatus koreicus* (2.8%).¹² The possibility of transmission of JEV by other species than *Culex tritaeniorhynchus* must be investigated in the near future.

In 1983, JE vaccine was included National Immunization Program (NIP) aimed at children 3–15 years of age.² Therefore, adults who were not included NIP, are considered at high-risk of JE infection. Epidemiology of JE cases in 2011 to 2015, 1 case (1.0%) had JE vaccine history, 27 cases (26.2%) had none and 75 cases (72.8%) was unknown.⁶ Lee et al.¹³ investigated the prevalence of neutralizing antibodies to JEV at high-risk group by plaque reduction neutralization test. Out of the 945 subjects aged 30–69 years, 927 (98.1%) exhibited antibodies against JEV with no significant differences between sex, age, or occupation. But this study had limitations of the test inclusion of small number of population and the application of simple positive or negative criteria. By pseudotyped virus test, the positive rate of age group 15–29 years was 95%, and the rate gradually decreased for 30–44, and 75.24% for ages 55–59.¹⁴ This result of the reducing tendency of neutralizing antibody titer with increasing ages represented well the shifting of JE case age to old ones year by year. For example, 9, 9, and 5 cases in age of 40–49, 50–59 and > 60, respectively, in 2010 and 5, 8, and 10 cases, respectively, in 2014.¹³

JEV are generally classified into five genotypes based on similarities of E gene.¹ Before 1951, genotype II, III were isolated and in the 1980s, genotype III was dominantly isolated. Since the 1990s, genotype I is frequently isolated from mosquitoes.¹⁵ After 2010, genotype V was isolated from *Culex bitaeniorhynchus*, *Culex orientalis*, and *Culex pipiens* (Table 1).^{16–19} Genotype V is a strain of JEV identified in Singapore in 1952 from a Malaysian patient and re-emerged at China in 2009.^{1,17,19}

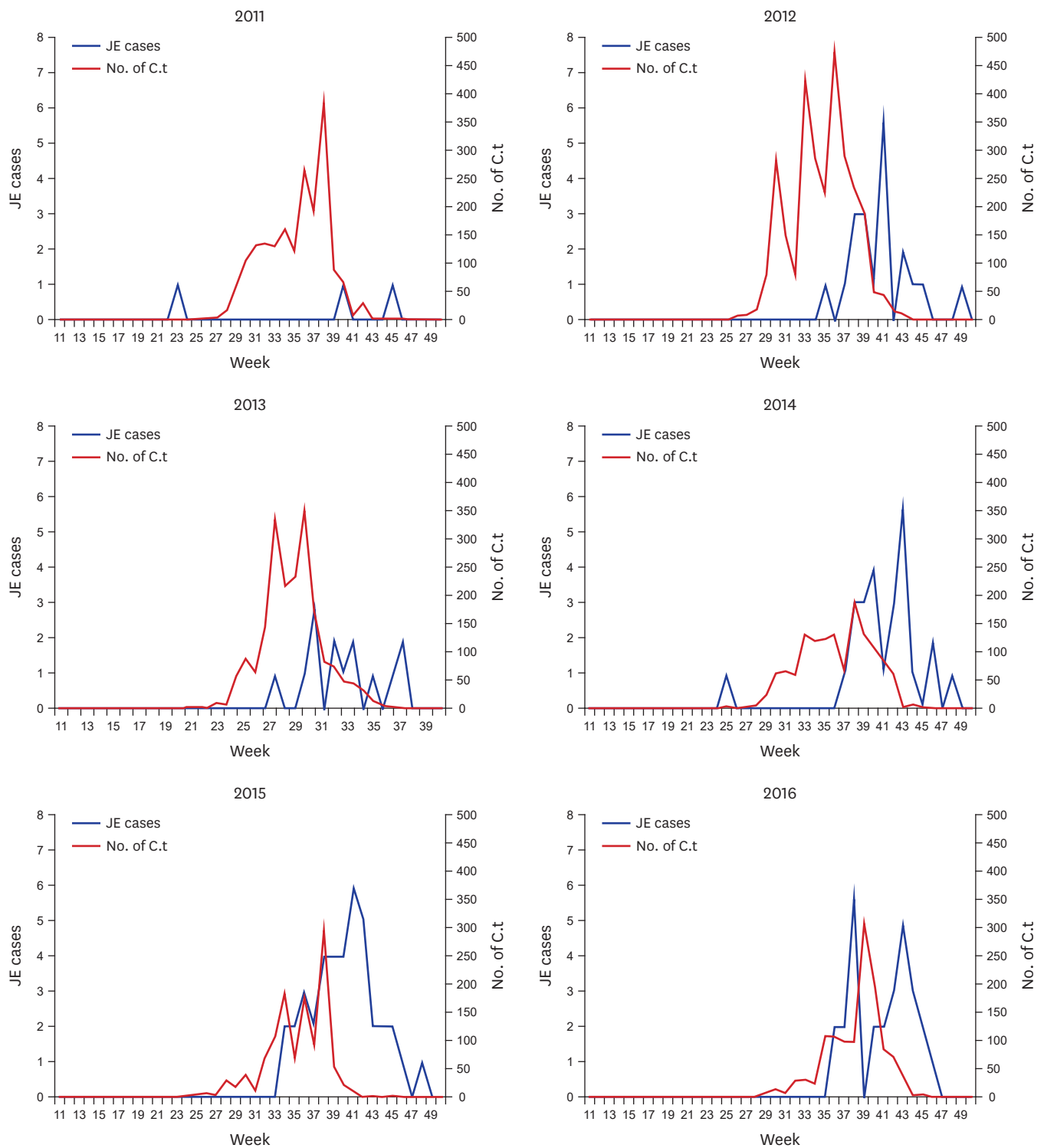


Fig. 2. Comparison of number between weekly reported JE cases and C.t (2011–2016). Number of JE cases and Number of C.t showed different seasonal pattern. JE = Japanese encephalitis, C.t = *Culex tritaeniorhynchus*.

After massive vaccination for JE in the 1980s, the number of JE cases decreased dramatically in Korea, in 1991 to 2009 less than 10 cases were reported annually. However, since 2010, the number of JE cases showed increasing and half of cases were aged 40–59 years. Pigs

Table 1. Reported strains and genotype of JEV in Korea

Strain	Year	Source	Location	Accession No.	Genotype
Roum	1946	Human	NA	FJ515922, FJ872377	3
K-29	1949	Human	NA	GQ415356	3
Korea Jap B	1950		NA	FJ515926, FJ872379	3
Bennett	1951	Homo sapiens	NA	HQ223285	2
JE-82	1982	Mosquito	NA	GQ415347	3
K82P01	1982	<i>Culex tritaeniorhynchus</i>	Jeonnam	U34926	-
JE-83	1983	Mosquito	NA	GQ415348	3
K83P34	1983	<i>Culex tritaeniorhynchus</i>	NA	FJ938231	3
K83P44	1983	<i>Culex tritaeniorhynchus</i>	NA	FJ938232	3
JE-84	1984	Mosquito	NA	GQ415349	3
K84A071	1984	<i>Culex tritaeniorhynchus</i>	NA	FJ938224	3
JE-85	1985	Mosquito	NA	GQ415350	3
JE-86	1986	Mosquito	NA	GQ415351	3
JE-87	1987	Mosquito	NA	GQ415352	3
K87A07	1987	<i>Culex tritaeniorhynchus</i>	NA	FJ938225	3
K87A071	1987	<i>Culex tritaeniorhynchus</i>	NA	FJ938226	3
K87P39	1987	<i>Culex tritaeniorhynchus</i>	Jeonnam	U34927	3
JE-88	1988	Mosquito	NA	GQ415353	3
K88A07	1988	<i>Culex tritaeniorhynchus</i>	NA	FJ938227	3
K88A071	1988	<i>Culex tritaeniorhynchus</i>	NA	FJ938228	3
JE-89	1989	Mosquito	NA	GQ415354	3
K89A07	1989	<i>Culex tritaeniorhynchus</i>	NA	FJ938229	3
JE-91	1991	Mosquito	NA	GQ415355	1
K91P55	1991	<i>Culex tritaeniorhynchus</i>	Jeonnam	U34928	-
K93A07	1993	<i>Culex tritaeniorhynchus</i>	NA	FJ938230	1
K94A07	1994	<i>Culex tritaeniorhynchus</i>	NA	FJ938216	1
K94A071	1994	<i>Culex tritaeniorhynchus</i>	NA	FJ938217	3
K94P05	1994	<i>Culex tritaeniorhynchus</i>	Jeonnam	U34929	1
K95A07	1995	<i>Culex tritaeniorhynchus</i>	NA	FJ938218	1
K96A07	1996	<i>Culex tritaeniorhynchus</i>	NA	FJ938219	1
KV1899	1999	Pig serum	Gyeonggi	AY316157	1
K01-GN	2001	<i>Culex tritaeniorhynchus</i>	Gyeongnam	FJ938220	1
K01-JB	2001	<i>Culex tritaeniorhynchus</i>	Jeonbuk	FJ938221	1
K01-JN	2001	<i>Culex tritaeniorhynchus</i>	Jeonnam	FJ938222	1
K05-GS	2005	<i>Culex tritaeniorhynchus</i>	Jeonbuk	FJ938223	1
A8.789	2008	<i>Culex tritaeniorhynchus</i>	Jeonnam	JN587257, JN587261	1
K10CT661	2010	<i>Culex tritaeniorhynchus</i>	NA	JX018150	1
K10CP372	2010	<i>Culex pipiens</i>	NA	JX018154	1
K10CT623	2010	<i>Culex tritaeniorhynchus</i>	NA	JX018160	1
K10CT621	2010	<i>Culex tritaeniorhynchus</i>	NA	JX018158	1
K10CT622	2010	<i>Culex tritaeniorhynchus</i>	NA	JX018159	1
K10CP371	2010	<i>Culex pipiens</i>	NA	JX018153	1
K10CT631	2010	<i>Culex tritaeniorhynchus</i>	NA	JX018161	1
K10CT632	2010	<i>Culex tritaeniorhynchus</i>	NA	JX018162	1
K10CT633	2010	<i>Culex tritaeniorhynchus</i>	NA	JX018163	1
K10CB662	2010	<i>Culex bitaeniorhynchus</i>	NA	JX018151	1
K10CB663	2010	<i>Culex bitaeniorhynchus</i>	NA	JX018152	1
K10CT611	2010	<i>Culex tritaeniorhynchus</i>	NA	JX018155	1
K10CT612	2010	<i>Culex tritaeniorhynchus</i>	NA	JX018156	1
K10CT613	2010	<i>Culex tritaeniorhynchus</i>	NA	JX018157	1
K10CP671	2010	<i>Culex pipiens</i>	NA	JX018164	1
K10CP672	2010	<i>Culex pipiens</i>	NA	JX018165	1
K10CP673	2010	<i>Culex pipiens</i>	NA	JX018166	1
K10CP674	2010	<i>Culex pipiens</i>	NA	JX018167	1
K10CP675	2010	<i>Culex pipiens</i>	NA	JX018168	1
A10.825	2010	<i>Culex tritaeniorhynchus</i>	Gyeongnam	JN587255, JN587259	1
A10.881	2010	<i>Culex tritaeniorhynchus</i>	Gyeongnam	JN587256, JN587260	1
10-1742	2010	<i>Culex tritaeniorhynchus</i>	Gyeonggi	JN587241	1

(continued to the next page)

Table 1. (Continued) Reported strains and genotype of JEV in Korea

Strain	Year	Source	Location	Accession No.	Genotype
10-1748	2010	<i>Culex tritaeniorhynchus</i>	Gyeonggi	JN587242	1
10-1728	2010	<i>Culex tritaeniorhynchus</i>	Gyeonggi	JN587240	1
10-1937	2010	<i>Culex tritaeniorhynchus</i>	Gyeonggi	JN587245	1
10-2044	2010	<i>Culex tritaeniorhynchus</i>	Gyeonggi	JN587248	1
10-2097	2010	<i>Culex tritaeniorhynchus</i>	Gyeonggi	JN587249	1
10-2130	2010	<i>Culex tritaeniorhynchus</i>	Gyeonggi	JN587250	1
10-2357	2010	<i>Culex tritaeniorhynchus</i>	Gyeonggi	JN587252	1
10-1827	2010	<i>Culex bitaeniorhynchus</i>	Gyeonggi	JN587243, JN587258	5
10-1835	2010	<i>Culex tritaeniorhynchus</i>	Gyeonggi	JN587244	1
10-1291	2010	<i>Culex tritaeniorhynchus</i>	Jeonbuk	JN587239	1
10-2204	2010	<i>Culex tritaeniorhynchus</i>	Jeonbuk	JN587251	1
10-1990	2010	<i>Culex tritaeniorhynchus</i>	Jeonnam	JN587246	1
10-1992	2010	<i>Culex tritaeniorhynchus</i>	Jeonnam	JN587247	1
10-2378	2010	<i>Culex tritaeniorhynchus</i>	Jeonnam	JN587253	1
10-2397	2010	<i>Culex tritaeniorhynchus</i>	Jeonnam	JN587254	1
K12HC959	2012	<i>Culex orientalis</i>	Gangwon	KJ420589	5
K12AS1148	2012	<i>Culex pipiens</i>	Gyeonggi	KJ420590	5
K12AS1151	2012	<i>Culex orientalis</i>	Gyeonggi	KJ420591	5
K12YJ1174	2012	<i>Culex orientalis</i>	Gyeonggi	KJ420593	-
K12YJ1182	2012	<i>Culex orientalis</i>	Gyeonggi	KJ420594	-
K12YJ1203	2012	<i>Culex orientalis</i>	Gyeonggi	KJ420592	5

Analyzed genotype data was collected from Schuh et al.¹⁶, Yun et al.¹⁵, Takhampunya et al.¹⁷, Seo et al.¹⁸, Kim et al.¹⁹

JEV = Japanese encephalitis virus, NA = not available.

play a less important role as amplifying hosts than past because of JEV vaccination and vector control in farms, but migratory birds including herons and ducks are potential hosts contributing to transmit JEV in metropolitan areas. Also, in Seoul, the population density of *Culex pipiens* was over 60% and *Culex tritaeniorhynchus* was less 1%. The prevalence of neutralizing antibody to JEV has been maintained at high levels in general population. Dominant genotype of JEV has changed from III to I around 1990, and newly isolated V since 2010. Cao et al.²⁰ suggested current JEV vaccine, SA14-14-2 live attenuated JE vaccine had low efficacy against genotype V. Although we could not reveal the direct evidence of recent transmission route of JEV by any host, we suggest that surveillance for JEV in migratory birds and other species of mosquitoes are needed. Also, investigating the genotype in reported cases and circulating JEV in environment is needed for supplement JEV control guideline which have been focused only on surveillance of pigs and *Culex tritaeniorhynchus*.

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