Evaluation of dogs (Canis familiaris) as an indicator of Japanese encephalitis (JE) outbreaks: A retrospective serological study in the Seoul metropolitan area around the 2010 resurgence of JE in the Republic of Korea

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\begin{abstract}
Japanese encephalitis (JE) is a mosquito-borne zoonotic neurological disease caused by Japanese encephalitis virus (JEV). JEV is the most common cause of viral encephalitis in Southeast Asia and the Western Pacific Rim. The fatality rate due to JE can reach 25% and up to 50% of the patients who survive develop permanent neurological damage. The annual incidence of human JE markedly increased in the Republic of Korea (ROK) in 2010 but the reason for the reemergence of JE in the ROK has not been established. This study aimed to assess the extent of exposure of domestic dogs to JEV in the Seoul metropolitan area, one of the most populated areas in the world. This cross-sectional study serologically investigated the spread of JEV in the dog population. Using serum neutralization test, we examined 3597 serum samples collected from pet, shelter, stray, and farmed dogs in the Seoul metropolitan area from 2006 to 2012. JEV-neutralizing antibodies found in shelter, stray, and farmed dogs sampled in approximately 2010 demonstrated increased JEV circulation in the dog population during the 2010 resurgence of JE reported among humans in the ROK. Five out of 1102 pet dogs (0.5%) were found to be neutralizing antibodies against JEV and 24 of 719 shelter dogs (3.1%) were positive with a peak of 13.0% in 2010. In addition, 25 of 690 stray dogs (3.6%) were positive with a peak of 9.7% in 2009, a year before the 2010 resurgence of JE. Furthermore, 39 of 1086 farmed dogs (3.6%) were seropositive with a peak of 9.2% in 2009. We therefore suggest that monitoring dog populations for seroconversion or seropositive dogs within JEV-active endemic areas may be useful for identifying risk areas for JE outbreaks and that JEV activity in dogs can be an indicator as the harbingers of JEV in humans.
\end{abstract}

\section{Introduction}

Japanese encephalitis (JE) is a mosquito-borne zoonotic neurological disease \cite{1,2} caused by the Japanese encephalitis virus (JEV) of the genus Flavivirus, family Flaviviridae. JEV is the most common cause of viral encephalitis in Southeast Asia and the Western Pacific Rim.

The fatality rate due to JE can reach 25%, and up to 50% of the patients who survive develop permanent neurological damage \cite{1,3}. In addition, chronic sequelae, including cognitive dysfunction and neurological deficits, affect children and are responsible for the high prevalence of JE disease worldwide \cite{4,5}. According to recent global estimates, approximately 67,900 JE cases occur annually in 24 JE-endemic countries, for an incidence of 1.8 per 100,000 people \cite{1}.

JE was one of the major public health concerns in the Republic of Korea (ROK) until the late 1960s, with several thousand cases reported annually. After introducing the mandatory immunization program, JE cases were markedly reduced, with <0.02 cases per 100,000 people annually during the past 4 decades \cite{6-8}. However, the annual incidence of JE increased markedly in 2010. Seven patients died in 2010, resulting in a case fatality rate of 26.9% (Appendix Fig. A.1) compared to 5 deaths in the past 25 years (1985–2009). The possible cause of this resurgence of JE in the ROK has not been revealed, and epidemiological information is limited \cite{7}.

The Seoul metropolitan area is one of the most populated areas in the
world, being the 10th most populated among 670 urban areas in Organization for Economic Co-operation and Development (OECD) countries [9]. In the Seoul metropolitan area, dogs are one of the closest animals to humans as companion pets and for guarding property. Previous studies reported that dogs bitten by mosquitoes may put humans at risk of becoming infected by JEV considering the vector competency of JEV [10,11], and it was also suggested that estimating dogs’ exposure to JEV infection could be a relevant indicator of the risk for humans to become infected [12]. Due to the very low level of viremia, dogs do not usually show any clinical signs of JEV infection or transmit the disease to humans, but JEV seroprevalences in dog populations may be valuable to evaluate risk factors for humans [13–16].

Therefore, dogs could be exposed to vector-borne pathogens such as JEV to the same extent as humans, and dog populations with JEV seroprevalence may be valuable as sentinels to evaluate the risk factors for JEV for humans [15,17].

The main objective of this study was to assess the extent of exposure to JEV among domestic dogs (Canis familiaris) as one of the animal species that is frequently in contact with humans. This cross-sectional study serologically investigated the level of JEV circulation among pet, shelter, stray, and farmed dogs in the Seoul metropolitan area (Seoul, Gyeonggi, and Incheon Provinces) in the 2010 resurgence of JE in the ROK. In addition, this retrospective study assessed domestic dogs as an indicator for detecting a JE outbreak or a sentinel to understand JEV activity.

2. Materials and methods

2.1. Samples

Archived serum samples collected from pet, shelter, stray, and farmed dogs in the Seoul metropolitan area from 2006 to 2012 before, during, and after the 2010 reemergence of JE were investigated in this study. Serum samples for this study were obtained from two separate sources: 1) the blood and serum bank of the Emerging Infectious Diseases Foreign Animal Disease Surveillance Program of the National Veterinary Research and Quarantine Service (NVRQS, Anyang, ROK) and 2) archived serum specimens from local animal hospitals located in Seoul, Gyeonggi, and Incheon Provinces. In addition, the purpose of this study was explained to dog owners by veterinarians of local animal hospitals to obtain informed consent to participate in the study before blood sampling. Samples of insufficient volume or poor quality were excluded from the analysis. Samples that were missing information needed for the risk analysis of this study were also excluded. A total of 3597 dogs sampled from the Seoul metropolitan area were used in this study. All animal handling and blood collection procedures were conducted in compliance with the regulations of the “Animal Care and Use Manual” of the NVRQS (No. 75/2006) and the “Animal Protection Law” of the Ministry of Agriculture, Food and Rural Affairs (No. 10310/2006). Ethical approval for this study was granted by the Animal Research Ethics Committee of Incheon National University (INU2015–029/ANIM-2022-09).

2.2. Serum neutralization test (SNT)

Serum samples were tested with SNTs using the JEV Anyang 300 strain [18,19]. The ability of serum samples to neutralize JEV was determined using a plaque reduction neutralization test (PRNT) according to a previously described method [20]. Samples were titrated in twofold serial dilutions that ranged from 1:10 to 1:80 to determine the end-point neutralization antibody titers. The number of plaques in each well was counted, and PRNT titers (PRNT_{50} values) were expressed as the reciprocal of the highest dilution that yielded a ≥ 90% reduction in the number of plaques compared with the counts in the virus-only control well. Serum samples with a PRNT titer of ≥1:10 were considered antibody positive.

2.3. Risk factor analysis

Risk factor information was obtained from animal owners using a questionnaire and from datasets archived in local animal hospitals. We investigated associations between seroprevalence and seropositivity risk factors, such as sex, habitat, flock size, vector control, area of residence, and province. The prevalence and Wilson’s 95% confidence intervals (Cls) [21] were calculated using Epitools Epidemiological Calculators (Ausvet, Canberra, Australia) [22]. A logistic regression model was used to identify potential risk factors that were correlated with animal seropositivity. The variables in the univariable analysis were evaluated for pairwise collinearity or associations using Pearson’s correlation coefficient or the chi-squared test for continuous or categorical variables, respectively. The strength of association was analyzed using odds ratios and 95% CIs. A p value < 0.05 was considered statistically significant. Statistical analyses in this study were performed using commercially available statistical software (SPSS Statistics for Windows, Version 25.0, IBM Corp., Armonk, NY, USA).

3. Results

We examined 3597 serum samples collected from pet, shelter, stray, and farmed dogs in the Seoul metropolitan area (Seoul, Gyeonggi, and Incheon, ROK) from 2006 to 2012. We found JEV-neutralizing antibodies in 93 serum samples by PRNT, with an overall prevalence of 2.6% (95% confidence interval, CI: 2.2–3.2%). During the investigation period in this study, the 2008–2010 JEV seroprevalence among shelter, stray, and farmed dogs increased, suggesting that the frequency of exposure to JEV among dogs in the Seoul metropolitan area was increased before the 2010 resurgence of JE reported among humans in the ROK.

From 2006 to 2012, five out of 1102 pet dogs (0.5%, 95% CI: 0.2–1.1%) were positive for neutralizing antibodies to JEV (Table 1). Furthermore, 24 of 719 shelter dogs (3.1%, 95% CI: 2.3–4.9%) showed antibodies against JEV, with a peak of 13.0% (95% CI: 7.8–21.0%) in 2010 (Fig. 1; Appendix Tables A.1–4). In addition, 25 of 690 stray dogs (3.6%, 95% CI: 2.5–5.3%) were positive for neutralizing antibodies against JEV, with a peak of 9.7% (95% CI: 4.5–19.6%) in 2009, a year before the 2010 resurgence of JE. Furthermore, 39 of 1086 farmed dogs (3.6%, 95% CI: 2.6–4.9%) were seropositive for JEV, with a peak of 9.2% (95% CI: 5.7–14.4%) in 2009 (Table 1). JEV-neutralizing antibodies found in shelter, stray, and farmed dogs sampled in approximately 2010 demonstrated increased JEV circulation in the dog population during the 2010 resurgence of JE reported among humans in the ROK.

The seroprevalences of male and female dogs were 2.9% (95% CI: 2.2–3.8%) and 2.3% (95% CI: 1.7–3.1%), respectively (Table 2). There was no significant difference in seroprevalence depending on the sex of the dogs. In contrast, our results revealed that seroprevalence among adult dogs was higher than that among puppies, suggesting that age markedly affected JEV seroprevalence among dogs (OR = 2.020, 95% CI = 1.173–3.478). The seroprevalence among dogs kept outdoors (3.0%, 95% CI: 2.3–4.0%) and free-ranging dogs (3.3%, 95% CI: 2.2–4.8%) was significantly higher than that among dogs kept indoors (1.4%, 95% CI: 0.9–2.3%). Population size was not associated with seropositivity for JEV. No significant difference was found in seropositivity between dogs under vector control practices, which included door/window screening, the use of repellents, mosquito nets, electric mosquito traps or rackets, covered water tanks, larvicides, and insecticides, and eliminating mosquito breeding sites (2.4%, 95% CI: 1.9–3.2%), and dogs not under vector control practices (2.8%, 95% CI: 2.1–3.7%). The seroprevalence was higher in rural districts (3.0%; 95% CI: 2.3–3.9%) than in urban districts (2.1%; 95% CI: 1.5–2.9%), but the difference was not statistically significant. There were significant differences in seroprevalence between geographic regions in the country. Dogs sampled in Seoul showed a markedly lower seroprevalence (1.4%;
95% CI: 0.7–2.6%) than those sampled in Gyeonggi (3.1%; 95% CI: 2.4–4.1%) and Incheon (2.5%; 95% CI: 1.7–3.5%).

4. Discussion

JE is considered a predominantly rural zoonosis with a wild cycle involving aquatic birds and Culex mosquitoes and a domestic cycle in which pigs are major hosts [23,24]. JEV is transmitted principally by Culex spp. mosquitoes that breed in rice paddies in an enzootic cycle involving wild birds as alternate JEV hosts and swine (domestic and feral) as the major hosts. However, some studies suggest that other epidemiological systems may exist. For example, a recent autochthonous human JEV case occurred in Seoul, ROK, where no pigs are reared in the city [25]. In addition, two studies from Singapore’s islands showed that JEV continued to circulate for decades after the abolition of pig farming [26,27]. Thus, we need to improve our knowledge of the existence of secondary JEV reservoir hosts to explain its transmission in areas with no or low pig density.

In this study, we found that there was a significant increase in JEV seroprevalence among dogs during the 2010 resurgence of JE in the ROK, although our estimates of JEV seropositivity among dogs were lower than those reported in Japan [16], Cambodia [12], and Vietnam [13], at 25%, 35%, and 71%, respectively.

This retrospective serosurvey demonstrated that detecting an increase in JEV seroprevalence among dogs is a good indicator of JEV infection pressure for humans, suggesting that changes are positively correlated with the proportion of dogs exposed to JEV and that the serological evidence of exposure to JEV among dogs can be an early indicator of the spread of JEV or JE resurgence among humans. In the present study, it is worth noting that a gradual increase in JEV

Table 1
Seroprevalence of Japanese encephalitis virus among domestic dogs (Canis familiaris) in the Seoul metropolitan area (Seoul, Gyeonggi, and Incheon), Republic of Korea, from 2006 to 2012, including before, during, and after the 2010 reemergence of Japanese encephalitis.

| Year | Positive/ Tested/ Prevalence | Pet dogs | Shelter dogs | Stray dogs | Farmed dogs | Total |
|------|-----------------------------|----------|--------------|-----------|-------------|-------|
| 2006 | Positive 0 Tested 126 | 0 97 | 0 91 | 1 207 | 2 521 |
|      | Prevalence 0.0 [0.0-3.0] | 1.0 [0.2-5.6] | 0.0 [0.0-4.1] | 0.5 [0.0-2.7] | 0.4 [0.1-1.4] |
| 2007 | Positive 0 Tested 135 | 0 99 | 0 127 | 1 183 | 1 544 |
|      | Prevalence 0.0 [0.0-2.8] | 0.0 [0.0-3.7] | 0.8 [0.1-4.3] | 0.0 [0.0-2.1] | 0.2 [0.0-1.0] |
| 2008 | Positive 1 Tested 150 | 1 114 | 1 102 | 3 155 | 13 |
|      | Prevalence 0.7 [0.1-3.7] | 3.5 [1.4-8.7] | 2.9 [0.1-8.3] | 3.2 [1.4-7.3] | 2.5 [1.5-4.2] |
| 2009 | Positive 1 Tested 163 | 3 129 | 6 119 | 10 143 | 20 |
|      | Prevalence 0.6 [0.1-3.4] | 2.3 [0.8-6.6] | 5.0 [2.3-10.6] | 7.0 [3.8-12.4] | 3.6 [2.4-5.5] |
| 2010 | Positive 1 Tested 204 | 13 100 | 6 62 | 174 | 36 |
|      | Prevalence 0.5 [0.0-2.7] | 13.0 [7.8-21.0] | 9.7 [4.5-19.6] | 9.2 [5.7-14.4] | 6.7 [4.9-9.1] |
| 2011 | Positive 2 Tested 167 | 3 89 | 7 87 | 114 | 457 |
|      | Prevalence 1.2 [0.3-4.3] | 3.4 [1.2-9.5] | 8.0 [4.0-15.7] | 5.3 [2.4-11.0] | 3.9 [2.5-6.1] |
| 2012 | Positive 0 Tested 157 | 0 91 | 2 102 | 110 | 460 |
|      | Prevalence 0.0 [0.0-2.4] | 0.0 [0.0-4.1] | 2.0 [0.5-6.9] | 0.9 [0.2-5.0] | 0.7 [0.2-2.0] |
| Total | Positive 5 Tested 1102 | 24 719 | 25 690 | 39 1086 | 93 |
|      | Prevalence 0.5 [0.2-1.1] | 3.1 [2.3-4.9] | 3.6 [2.5-5.3] | 3.6 [2.6-4.9] | 2.6 [2.2-3.2] |

† Apparent (estimated) prevalence (%) [95% confidence intervals].
seroprevalence among shelter, stray, and farmed dogs preceded the 2010 resurgence of JEV among humans, suggesting remarkable activity of the virus along with the upsurge in human JE cases. Although the results in the present study demonstrate the JEV seroprevalence in dogs correlated to the first wave of upsurge of human JE cases, our study was limited in that human JE cases in 2012 could not be synchronously matched, as the number of human JE cases in 2012 was higher than that in 2011, and JE seroprevalence among dogs was much lower than that in 2011. Considering that JEV seroprevalence among dogs did not seem to precisely match human JE cases every year during the period of the study, more studies and information are necessary to understand the correlation between JEV prevalence in a dog population and human JE cases at this moment. Future surveillance programs for JEV should extend a sentinel dog model to facilitate the prediction and response to a possible JEV outbreak or a long-term investigation of the correlation between dog JEV prevalence and human cases.

The univariable analysis identified significant correlations between seropositive dogs and age class, habitat, and geographic region (Table 2). The seroprevalence of JEV among dogs was markedly different among the different age classes, demonstrating that JEV prevalence was higher among adults than among puppies, which may represent more opportunities of exposure to JEV in their lifetime. Free-ranging dogs (OR = 1.494, 95% CI = 1.172–1.906) and dogs kept outdoors (OR = 1.264, 95% CI = 1.102–1.451) were shown to be most at risk; this may represent more opportunities for exposure to JEV by mosquito vectors because these dogs might not be protected under vector control practices. Our results also demonstrated that the prevalence of JEV antibodies was lower in Seoul Province than in other Seoul metropolitan areas, such as Gyeonggi and Incheon Provinces, probably because Seoul Province is the most urbanized area in the ROK with no metropolitan areas, such as Gyeonggi and Incheon Provinces, probably due to the first wave of upsurge of human JE cases, our study was limited in that human JE cases in 2012 could not be synchronously matched, as the number of human JE cases in 2012 was higher than that in 2011, and JE seroprevalence among dogs was much lower than that in 2011. Considering that JEV seroprevalence among dogs did not seem to precisely match human JE cases every year during the period of the study, more studies and information are necessary to understand the correlation between JEV prevalence in a dog population and human JE cases at this moment. Future surveillance programs for JEV should extend a sentinel dog model to facilitate the prediction and response to a possible JEV outbreak or a long-term investigation of the correlation between dog JEV prevalence and human cases.

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JEV genotype III (GIII), such as JEV 300, which was used in this study, has routinely and widely been used in JEV diagnostic laboratories in the ROK for JEV neutralization tests. JEV is classified into five genotypes, and GIII was the dominant genotype until the late 20th century in the ROK. JEV GI has replaced JEV GIII as the dominant genotype since the 1980s [28,29], and JEV GI and GV dominated during 2006–2011. However, it is generally accepted that all the JEV genotypes belong to the same serotype in that variability at either the nucleotide or peptide level does not support the existence of multiple serotypes [30,31]. No evidence of distinct serotypes based on genotyping has been presented, although distinct genotypic groupings of JEV may exist [31,32]. For this reason, all vaccines currently in use in the ROK are based on the JEV GIII strain, and it is well known that these vaccines can also prevent JEV infections caused by other genotypes [33,34]. In addition, a previous study also demonstrated that examination of virus-neutralizing antibody titers against GI-GIV viruses in humans after inoculation with GIII-inactivated vaccines showed no significant difference in seroconversion rates between genotypes [35]. Nevertheless, considering the opinion that antigenic differences between genotypes could affect immune responses including the vaccine’s efficacy [36,37], future study using GI or GV strains in neutralization tests might be beneficial to better elucidate seroprevalence for concurrently circulating JEV genotypes.

5. Conclusion

The results of this study provide an important and novel contribution to our understanding of the seroprevalence of JEV among companion animals associated with JE outbreaks in humans. The retrospective serosurvey in the present study demonstrated the potential of dogs as suitable JEV sentinels and suggested that the serological status of dogs can indicate the past and future levels of JE risk to humans and non-reservoir hosts. The results provide better JEV control information by indicating that surveillance of dogs for JEV antibodies could be useful to identify the spread of JEV in the Seoul metropolitan area. Furthermore, within JE-endemic areas, monitoring dog populations for seroconversion (negative to positive) or seropositive juvenile dogs could be useful for identifying risk areas for JE outbreaks in humans.

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CRedit authorship contribution statement

Jung-Yong Yeh: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

Declaration of Competing Interest

None of the above named authors is declaring any conflict of interest in relation to the above titled submitted manuscript.

Table 2
Univariable analysis of variables associated with Japanese encephalitis virus seropositivity outcomes in domestic dogs in the Republic of Korea. Seroprevalence was determined using a serum neutralization test.

| Exposure Variable | Label | Positive | Negative | Seroprevalence % (95% CI) | OR (95% confidence interval) | P value |
|------------------|-------|----------|----------|---------------------------|----------------------------|---------|
| Sex              | Male  | 51       | 1703     | 2.9 (2.2–3.8)             | 1.128 (0.935–1.361)        | 0.249   |
|                  | Female| 42       | 1801     | 2.3 (1.7–3.1)             | Reference                  |         |
| Age class        | Puppy | 16       | 1036     | 1.5 (0.9–2.5)             | Reference                  |         |
|                  | Adult | 77       | 2468     | 3.0 (2.4–3.8)             | 2.020 (1.173–3.478)        | 0.011   |
| Habitat          | Indoors| 16       | 1101     | 1.4 (0.9–2.3)             | Reference                  |         |
|                  | Kept outdoors | 51       | 1634     | 3.0 (2.3–4.0)             | 1.264 (1.102–1.451)        | 0.008   |
|                  | Free-ranging | 26       | 769      | 3.3 (2.2–4.8)             | 1.494 (1.172–1.906)        | 0.011   |
| Population size  | >50   | 27       | 928      | 2.8 (2.0–4.1)             | 1.085 (0.830–1.419)        | 0.580   |
|                  | 10–50 | 26       | 1049     | 2.4 (1.7–3.5)             | 1.022 (0.839–1.245)        | 0.899   |
|                  | <10   | 40       | 1527     | 2.6 (1.9–3.5)             | Reference                  |         |
| Vector control practices | No | 43       | 1496     | 2.8 (2.1–3.7)             | Reference                  |         |
|                  | Yes   | 50       | 2008     | 2.4 (1.9–3.2)             | 0.938 (0.775–1.135)        | 0.525   |
| Area             | Rural | 56       | 1798     | 3.0 (2.3–3.9)             | 1.173 (0.992–1.389)        | 0.093   |
|                  | Urban | 37       | 1706     | 2.2 (1.5–2.9)             | Reference                  |         |
| Province         | Gyeonggi| 55       | 1702     | 3.2 (2.4–4.1)             | Reference                  |         |
|                  | Incheon | 29       | 1153     | 2.5 (1.7–3.5)             | 0.855 (0.635–1.151)        | 0.311   |
|                  | Seoul | 9        | 649      | 1.4 (0.7–2.6)             | 0.509 (0.277–0.937)        | 0.015   |

(Apparent (estimated) prevalence (%) [95% confidence intervals).
Data availability

All personal information on pet owners is kept confidential by informed consent.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.onehlt.2022.100459.

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