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学 位 論 文

Evaluating the risk of dengue importation and the control effect during the 2014 dengue outbreak in Japan

(わが国におけるデング熱の輸入リスクと 2014 年流行時の制御効果の評価)

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List of Publications

1. Yuan, B., Nishiura, H. (2018) Estimating the actual importation risk of dengue virus infection among Japanese travelers. PLoS One. *13*, e0198734.
2. Yuan, B., Lee, H. and Nishiura, H. (2019) Assessing dengue control in Tokyo, 2014. PLoS Negl Trop Dis *13*, e0007468.

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1. Yuan, B., Lee, H. & Nishiura, H. Assessing dengue control in Tokyo,2014. Epidemics 7-International Conference on Infectious Disease Dynamics. December3-6, 2019, Charleston, SC, USA.

2. Yuan, B. & Nishiura, H. Estimating the actual importation risk of dengue virus infection among Japanese travelers. The Fifth International Conference on Computational and Mathematical Population Dynamics. May19-24, 2019, Fort Lauderdale, Florida, USA.

3. Yuan, B., Lee, H. & Nishiura, H. Assessing the effectiveness of dengue control in Tokyo,2014. The 89th Annual Meeting of the Japanese Society for Hygiene. February1-3, 2019, Nagoya, Japan.

4. Yuan, B., Lee, H. & Nishiura, H. Assessing the dengue control in Tokyo,2014. Innovative Mathematical Modeling for the Analysis of Infectious Disease Data. January17-18, 2019, Hakodate, Japan.

5. Yuan, B. & Nishiura, H. Risk assessment of dengue virus infection among Japanese travelers visiting South and Southeast Asia. Epidemics 6-International Conference on Infectious Disease Dynamics. Novermber29-December1, 2017, Sitges, Spain.

6. Yuan, B. & Nishiura, H. The infection risk assessing of dengue for Japanese travelers visiting Southeast Asian countries. Japan Society for Mathematical Biology. October6-8, 2017, Sapporo, Japan.

Summary

Background and Purposes:

Dengue fever is one of the most common mosquito-borne infectious diseases worldwide. *Aedes aegypti* and *Aedes albopictus* are known as the two main vector species of dengue virus (DENV), which are composed of four antigenically related serotypes. While the primary infection with one serotype is often self-limiting, the secondary infection with a different serotype is more likely to cause severe clinical symptoms (WHO, 2020). The time from infection to illness onset ranges from 3 to 14 days (Gubler, D. J., 1998). At present, there is no specific treatment for the dengue infections and all the medical care are aimed at providing supportive treatment (WHO, 2020). Thereby the most effective measures to reduce the morbidity and mortality of dengue virus disease are to avoid infection. Dengue infections are seen mostly in tropical and subtropical countries, but nonendemic areas in temperate regions are also at increased risk due to the expansion of the *Aedes* species habitation areas and a growing volume of international travel. Japan is not an endemic country for the dengue virus, but the country has experienced a steady increase in the number of imported cases in the past decades, mainly from South and Southeast Asia. In 2014, an autochthonous dengue outbreak involving a total of 160 cases was observed in Tokyo. Once the outbreak has been recognized, the government took drastic mosquito control measures targeting both adults and the larvae, and timely news was disseminated. The subsequent park closure was a complement to containing the outbreak. This unexpected outbreak indicated that Japan is at risk of dengue during summer seasons. In this context, we first evaluate the risk of DENV infection among Japanese travelers to Asian countries, thereby obtaining an actual estimate of the number of DENV infections among travelers in the Chapter 1; then we conduct a modeling study to explore the transmission dynamics, evaluating the effectiveness of control measures which were implemented in the 2014 dengue outbreak in Tokyo in the Chapter 2.

Chapter 1: Estimating the importation risk of dengue infections among Japanese travelers to South and Southeast Asian countries

[Materials and Methods] For eight destination countries (Indonesia, the Philippines, Thailand, India, Malaysia, Vietnam, Sri Lanka, and Singapore), we collected age-dependent seroepidemiological data by conducting a systematic review. We also retrieved the number of imported cases, who were notified to the Japanese government, as well as the total number of

travelers to each destination from the year of 2006 to 2016. The catalytic model was used to describe the expected fraction of seropositive individuals by the age-independent force of infection. Assuming an identical infection risk between the Japanese travelers and the local population in the destinations, the expected number of dengue infections among Japanese travelers can be deduced. The likelihood function to estimate the force of infection and the reporting coverage of dengue infections can be obtained assuming that the observed number of imported cases follows a Poisson distribution and the seroprevalence data in each destination country follows Bernoulli sampling. The maximum likelihood technique gives the optimal estimation values of the country-specific force of infection and the reporting coverage of dengue infections among Japanese travelers. **[Results]** The Philippines, Sri Lanka and Indonesia were the three countries with the highest force of infection. The reporting coverage of dengue appeared to range from 0.6% to 4.3% through all eight selected countries. The risk of infection per journey was calculated ranging from 0.02% to 0.44%. **[Discussion]** We found that the actual number of imported cases of DENV infection among Japanese travelers could be more than 20 times the notified number of imported cases. This finding may be attributed to the substantial proportion of asymptomatic and under-ascertained infections.

Chapter 2: Assessing the intervention effectiveness in the dengue outbreak in Tokyo, 2014

[Materials and Methods] The case information in this epidemic including the visiting history to the parks, biting experience and the date of illness onset is publicly available on the official website of Tokyo Metropolitan Government. According to the date of exposure to parks at risk, we categorized all cases into three groups. Firstly, we partitioned the entire generation time of dengue infection as incubation period and the waiting time of infection. Using the derived distribution of generation time, we devised a generation-dependent epidemiological model to discretize the entire epidemic as a limited number of generation processes (we assume the unobserved primary case to be the generation zero, all the cases infected by the primary case to be the generation one, and so on). A piecewise function over time was integrated into the abovementioned model to describe the effectiveness of interventions taken at two time points. For each group of cases, the likelihood function to estimate unknown parameters was designed based on the convolution relationship between exposure time and incubation period. The maximum likelihood estimation technique is used to obtain the optimal parameter values. For each scenario with generation numbers as two, three or four, we identified the best fitted model with the lowest AIC by varying the unobserved

day of the primary infection. Finally, the effective reproduction number was calculated by the renewal equation. **[Results]** The mean incubation period was estimated as 5.8 days (95% CI: 5.5, 6.0). The mean generation time was estimated at 17.2 days, 16.1 days and 12.4 days for each scenario model with generation number to be two, three or four. The control measures including mosquito control and epidemic communication, which has been initiated from 28 August 2014, have reduced the transmission by 30%-70%. By estimating the effective reproduction number, we determined that the effect of these two measures was insufficient enough to lower the reproduction number to below the value of 1. However, once Yoyogi Park has been closed on 4 September, the value of the effective reproduction number began to fall below 1, and the associated relative reduction in the effective reproduction number was estimated as 20%–60%. **[Discussion]** In this modeling exercise, we cannot determine the exact generation number which occurred in the entire epidemic period, so three assumed generation numbers were considered independently and the according estimated parameters were analyzed. It seems natural that there was some interplay between the assumed generation numbers and the resulting estimates. However, regardless of the three different scenarios, the joint reduction effect of all interventions was estimated to be 44%-88% and the combined interventions were effective to control the outbreak.

Conclusion:

In Japan, the notified number of imported dengue cases truly represent the tip of iceberg that consists of substantial number of infected individuals, indicating a much higher importation risk than we directly see from observed datasets of confirmed cases. The 2014 dengue outbreak in Tokyo is thought to have represented the result of this increased risk. In this epidemic, all control measures that we explored including massive mosquito control, timely risk communication, and rigorous park closure appeared to be successful in interrupting the transmission chain. To avoid the next dengue outbreak in Japan, the epidemiological surveillance of imported cases and the routine monitoring of mosquitos especially in public green zone are considered to be essential.

List of Abbreviations

AIC	Akaike information criterion
AICc	Akaike information criterion with a correction for small sample size
CI	Confidence interval
DENV	Dengue virus
DF	Dengue fever
DHF	Dengue hemorrhagic fever
EIP	The extrinsic incubation period
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IP	The incubation period
JEV	Japanese encephalitis virus
MSE	Mean squared error
pmf	Probability mass functions
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
R_0	The basic reproduction number
RT-PCR	Reverse-transcription polymerase chain reaction
WNV	West Nile virus
YFV	Yellow fever virus
ZIKV	Zika virus

Introduction

History of dengue fever

Dengue fever disease has been known to have a long history. The earliest record of the first probable dengue case can date back to the Jin Dynasty of 265 – 420 AD. Plausible dengue epidemics have also been recorded in French West Indies in 1635 and in Panama in 1699 (McSherry, J. A., 1982). It is highly likely that the epidemic reported in Philadelphia in 1780 was also dengue outbreak (Carey, D. E., 1971). Until 1940, dengue-like epidemics were characterized occasionally and it is believed that around this time the virus colonized and become endemic in many tropical regions (Gubler, D. J., 1998). The huge ecologic disruption during the World War II as well as the improvements in transportation and rapid urbanization following the war have contributed to the extensive spread of dengue virus globally. The increased transmission of dengue caused the cocirculation of multiple serotypes in many Southeast Asian countries and the first recorded dengue hemorrhagic fever (DHF) cases have been reported in Manila, Philippines, in 1953 (Ooi, E. E. and Gubler, D. J., 2009; Bravo, L. et al., 2014). By the 1970s, the severe form of dengue, DHF had caused a large number of hospitalizations and death among children and dengue had reemerged in the Pacific Islands and Americas. In the 1980s and 1990s, an expanding geographical distribution of dengue outbreaks to new areas without epidemic history had been reported together with an increasing number of dengue incidences (Gubler, D. J., 1998). Whereas in the 1980s, Africa started to have the report about dengue epidemics caused by all the dengue serotypes, in the 1990s, the epidemics have become very common in East Africa and the Middle East.

Since the 21st century, dengue fever has continued to be the one of the most common mosquito-borne infectious diseases all over the world, which places a huge burden on social economy and health systems in most tropical and subtropical regions of the world. According to the World Health Organization (WHO), there are more than 1.2 million dengue cases to be reported across the Americas, South-East Asia and Western Pacific in 2009 and 3.3 million cases worldwide in 2016 (WHO, 2020). An aggregate annual cost of dengue of 2.1 billion USD from 2000-2007 in the Americas (Shepard, D. S. et al., 2011) and 950 million USD from 2001-2010 in South East Asia were estimated due to the economic and disease burden (Shepard, D. S. et al., 2013). In recent

years, many factors such as the rapid urbanization, population growth, frequent global travel, climate change, and virus evolution have been contributing to the further expansion of dengue fever to nonendemic countries in temperate regions at a higher speed. In Europe, the majority of dengue infections are related to the imported cases, but several local transmission events have been reported in some countries (ECDC, 2018) which indicate the threat of a possible outbreak. The same situation occurred in Japan, where an unexpected indigenous dengue outbreak was confirmed in 2014 summer.

Dengue virus

Dengue virus (DENV) is one member of the Flaviviridae family, genus Flavivirus, and its genome comprises the positive single-strand RNA encoding three structural proteins and seven non-structural proteins (Guzman, M. G., 2010). Unlike other flaviviruses including Zika virus (ZIKV), West Nile virus (WNV), Japanese encephalitis virus (JEV), tick-borne encephalitis virus and yellow fever virus (YFV), four genetically similar but antigenically distinct serotypes, i.e., DENV-1, DENV-2, DENV-3 and DENV-4, are distinguished by the ability to elicit antibodies to cross-neutralize (Perera, R. and Kuhn R. J., 2008; Muller, D. A. et al., 2017). Up to date, dengue epidemics caused by each or cocirculation of the four serotypes have been reported. Recovery from infection by any of the four serotypes can induce lifelong immunity against that virus, but it can only provide temporary or partial cross-protection to the other serotypes (CDC, 2009; Guerrant, R. L. et al., 2011). Thus, one person can be infected by DENV as many as four times all his life, each serotype for once.

Transmission

Human beings are the primary host of the dengue virus (Gould, E. A. and Solomon, T., 2008), and several species of *Aedes* mosquitos act as the transmission vector, including *A. aegypti*, *A. albopictus*, *A. polynesiensis* (Richards, V. et al., 2016) and *A. scutellaris* (Moore, P. R. et al., 2007). *A. aegypti* is the main vector causing the most epidemics which can be visually distinguished with its features, such as the small size, black-white bands on its legs and white-scale lyre on thorax. *A. aegypti* is widely distributed in the tropical and subtropical regions worldwide, especially Brazil,

Southeast Asia, the central Africa and India. In contrast, *A. albopictus* is the secondary vector of dengue virus in the endemic setting, but it has been responsible for the majority epidemics in emerging regions (Whitehorn, J. et al., 2015). Adult *A. albopictus* is very similar to *A. aegypti* in its color and white strip on their legs, however, some characteristic appearances are identified with smaller size and a single, silvery-white stripe down the middle of the top of the thorax. Owing to the greater ability to survive the low temperature, *A. albopictus* has extended to temperate areas including southern Europe, northern China, Japan and northern United States (Thomas, S. M. et al., 2012; Brady, O. J. et al., 2014; Kraemer, M. U. G. et al., 2015). The increasingly frequent human movement and shipping trade have accelerated a global expansion of these mosquitos. *A. aegypti* behaves a stronger preference to human living environment than *A. albopictus* to lay their eggs or search for a bloodmeal (Ponlawat, A. and Harrington, L. C., 2005; Jansen, C. C. and Beebe, N. W., 2010). The female mosquitos of these two *Aedes* need blood to produce eggs, thus they frequently bite humans. People can get infected by even one bite of an infective mosquito of such species which is known as horizontal transmission. Following the successful viral entry, the infected person will develop various symptoms after 3 to 14 days (most commonly 4-7 days), i.e., the incubation period (IP) (Gubler, D. J., 1998). If the infected person is bitten again during the febrile viral stage, it is possible to transmit virus to the uninfected mosquito. Subsequently, the mosquito develops infectiousness after the temperature-dependent extrinsic incubation period (EIP) (Chan, M. and Johansson, M.A., 2012). In addition, the vertical transmission may occur, i.e., dengue virus is transmitted from infected female or male parent mosquito to their offspring (Ferreira-de-Lima, V. H. and Lima-Camara, T. N., 2018), while the human-to-human transmission by birth or by sex has been reported but not common (Wiwanitkit, V., 2010; Liew, C. H., 2019).

Signs and symptoms

Infection by any serotype of dengue virus can result in a similar clinical spectrum of illness from asymptomatic (70-80%) (Reiter, S., 2010; Bhatt, S. et al., 2013) or mild fever to severe symptoms and only a small proportion of cases can develop life-threatening diseases with dengue hemorrhage fever (DHF) and dengue shock syndrome (DSS) (Whitehorn, J. and Farrar, J., 2010; Kularatne, S. A. M., 2015). The severity of clinical presentations is conditioned on some risk factors including the circulating DENV serotype and the immune status, age and genetic factors of the infected

person. The primary infection in young children with one serotype frequently causes inapparent outcomes. However, a significant increase in disease severity following the second heterotypic dengue infection has been observed among adults and children (Halstead, S., 2019). Note that the asymptomatic cases do not intuitively possess no infectiousness, but they may be more infectious to mosquito than the symptomatic case does (Duong, V. et al., 2015).

According to the guidelines for the clinical diagnosis of dengue published by WHO in 2009 (WHO, 2009), three phases are defined in the course of illness: febrile, critical and recovery. An acute infection starts with febrile phase which is featured with a sudden high fever (up to 40^{o}C) and other non-specific symptoms including headache, orbital pain, myalgias, nausea and vomiting. Around half of the symptomatic cases develop rash in the form from flushing skin or erythematous mottling to measles-like rash. The hemorrhagic manifestations are also common in this phase, ranging from skin hemorrhages to bleedings from gastrointestinal tract, nose, gums and other mucosal sites. A positive tourniquet test can be used to confirm this diagnosis. Normally in about 3-7 days after illness onset, most patient can recover from this phase, but other patient may proceed to the critical phase as fever resolves. The warning signs should be carefully monitored including abdominal pain, persistent vomiting, clinical fluid accumulation, mucosal bleed and liver enlargement. Especially, the comprehensive symptoms of plasma leakage can last 24 - 48 hours. Once the patient gets through this period, the recovery phase occurs with the reabsorption of the extravascular fluids. The physical conditions usually improve rapidly in two or three days. Another rash appearing as "white islands in a sea of red" is thought as the typical recovery manifestation. However, for a small proportion of patients, the persistent vascular leakage may result in severe symptom, which presents one of the three clinical features: severe plasma leakage, severe haemorrhage or severe organ impairment (WHO, 2009). Especially, the severe plasma leakage will lead to the shock, i.e., DSS.

Laboratory diagnosis

A reliable confirmation of dengue virus infection relies on the microbiological laboratory testing, targeting for the virus itself, antibody produced by immune system (Muller, D. A., 2017) and blood cell count (Chaloemwong, J. et al., 2018). Virus isolation is the traditional diagnostic method and also provides the most reliable evidence for the DENV infection (Gubler, D. J., 1998). Reverse

transcription polymerase chain reaction (RT-PCR) method has been widespread used in laboratory to detect the presence of the virus due to its operability (Muller, D. A., 2017). An elevated plasma level of the secreted dengue virus nonstructural protein (NS1) can be detected at the onset of symptoms, making the NS1 be one ideal diagnostic marker (Libraty, D. H., et al., 2002; Muller, D. A., 2017). In the primary infection, as the response to the viral invasion, Immunoglobulin M (IgM) is produced about 4 days after the symptom onset and remains detectable for one to three months. By contrast, Immunoglobulin G (IgG) was produced several days later than IgM, and it can remain detectable for as long as 60 years (Simmons, C. P. et al., 2012; Muller, D. A. et al., 2017). Antibody test with IgM and IgG provides the possibility to identify the primary infection and secondary infection or infection history (Lam, S. K. et al., 2000). In addition, complete blood count technique would be used to monitor the disease development (Chaloemwong, J. et al., 2018).

Treatment and prevention

Up to now, there is still no specific treatment for the infection and all the medical care are aimed at providing the symptomatic relief and supportive treatment. The most effective measures to reduce the morbidity and mortality of dengue infection are to avoid getting infectious bitten or receive dengue vaccine. Emphasis must be put on the vector control by integrating chemical methods, such as insecticide spraying and biological methods (gene-modified mosquitos) (Hoffmann, A. A. et al., 2011). The routine community hygiene management and active mosquito surveillance are crucial to achieve a sustainable mosquito suppression.

As of 2020, Dengvaxia® (CYD-TDV) developed by Sanofi Pasteur is the only approved dengue vaccine and it has been licensed in several countries with a reported 56 – 61 % vaccine efficacy against symptomatic dengue disease in Southeast Asia and Latin America (Capeding, M. R. et al., 2014; Villar, L. et al., 2015; Ferguson, N. M. et al., 2016). Due to the potential side effects of vaccination in low-endemicity settings, the vaccine is only recommended to those people who have experienced previous dengue infection or have been living in high-transmission setting (Ferguson, N. M. et al., 2016). To be specific, WHO suggests the vaccine receiver aged 9 years and above or in areas with a seroprevalence greater than 70% and three doses given six months apart (WHO, 2018). In 2019, an ongoing trial of another dengue vaccine candidate (TAK-003) developed by

Takeda Pharmaceutical Co.'s present 80% vaccine efficacy against dengue fever (Biswal, S. et al., 2019). A more vaccinee-friendly vaccine is expected.

Dengue in Japan

As the result of the acceleration of the process of globalization and global warming, the dengue virus has spread over a wider area in the past century. Japan is in a temperate zone and dengue is not endemic in the country. Despite only sporadic abundance of *Aedes aegypti*, the species *Aedes albopictus* is widespread across Honshu Island and all western parts of Japan, theoretically allowing for chains of dengue transmission to exist (Sukehiro, N. et al., 2013; Kobayashi, M. et al., 2002; Toma, T. et al., 2011; Hoshi, T. et al., 2014; Tsuda, Y. et al., 2014; Murakami, M. et al., 2017). Japan has experienced an increasing number of notified cases of imported DENV infection, mostly arising from Japanese travelers visiting South and Southeast Asian countries (Fukusumi, M. et al., 2016; Nakamura, N. et al., 2012; Yuan, B. and Nishiura, H., 2018). It is worth noting that a German traveler visiting Japan in the summer of 2013 was later diagnosed with DENV2 infection upon returning to Germany (Kobayashi, M. et al., 2014). In the past 80 years, two unusual dengue outbreaks occurred in Japan, i.e., the 1942 dengue outbreak in Nagasaki (Hotta, S., 1998) and the 2014 dengue outbreak in Tokyo (Yuan, B. et al., 2019). The former epidemic in Nagasaki recurred in summer until 1944 with a total recorded number of approximately 200,000 cases, while the latest epidemic in Tokyo was reported with a relatively small size of 160 confirmed cases. These reports have indicated that Japan is at risk of local transmission especially during the summer seasons.

In the remaining part of this dissertation, we employed mathematical modeling and statistical technique to analyze the epidemic situation of dengue fever in Japan. In the first chapter, we combined the data of imported dengue cases arriving Japan and the seroprevalence data of dengue infection in the visiting destinations for Japanese travelers to estimate the infection risk among Japanese travelers. In the second chapter, we retrospectively assess the effectiveness of multiple interventions implemented in the dengue outbreak in Tokyo in 2014.

Chapter 1. Estimating the importation risk of dengue infections among Japanese travelers to South and Southeast Asian countries

1.1 Background

The growing volume of international travel can contribute to the global spread of dengue virus. Japan has experienced an increasing number of notified cases of imported DENV infection, mostly arising from Japanese travelers visiting South and Southeast Asian countries. A published study analyzed the notification data of imported cases in Japan and calculated the destination country-specific incidence of imported dengue, showing that travelers visiting different Southeast Asian countries have different risks of infection (Nakamura, N. et al., 2006). By exploring not only data of imported cases but also of the volume of travelers, another study demonstrated that the incidence of imported cases reflects the local epidemiological dynamics in dengue-endemic countries (Fukusumi, M. et al., 2016). Despite these published studies analyzing imported case data, quantitative risk assessment of DENV infection among susceptible Japanese travelers has yet to be conducted. Owing to a large proportion of asymptomatic infections among individuals infected with DENV, it is possible that a greater number of travelers than reported cases are likely to be exposed to infection without recognition. However, many studies have looked into imported case data alone; as far as we understand, no study has statistically inferred the actual risk of infection among all travelers in endemic countries, thereby estimating the number of DENV infections among travelers. The present study aimed to statistically infer the risk of DENV infection among Japanese travelers to Asian countries to obtain an estimate of the number of DENV infections among travelers.

1.2 Methods

Data source

We investigated four datasets: (i) notification of imported dengue cases in Japan from 2006 to 2016; (ii) statistics of Japanese international travelers from 2006 to 2016; (iii) age-dependent seroprevalence of DENV in Asian countries; and (iv) average duration of stay among Japanese travelers in each Asian destination country.

Dengue fever is a notifiable disease, according to the Japanese Infectious Disease Control Law. Based on laboratory testing (i.e., virus isolation, polymerase chain reaction, detection of Nonstructural Protein 1(NS1)-antigen, detection of IgM antibody or neutralizing antibody), only confirmed cases are reported (IDSC, 2004; IDSC, 2015; NIID, 2017). Except for a fraction of cases during the 2014 outbreak, all confirmed cases were considered to have acquired infection overseas. We retrieved data of the number of cases and travel history (i.e., destination country) of each imported case from the National Institute of Infectious Diseases (IDSC, 2004; IDSC, 2015; Nakajima, K., 2005). From 2006 to 2016, dengue infections arose among travelers returning from a total of 63 countries or regions. In the present study, we focused on the following eight countries, which accounted for 83.5% of all notifications during the abovementioned period: Indonesia, Philippines, Thailand, India, Malaysia, Vietnam, Sri Lanka, and Singapore. The annual number of imported cases for these eight countries are given in Table 1.

We then obtained the volume of Japanese travelers from various sources. According to the National Tourism Organization of Japan (JTB, 2017a), the annual number of Japanese travelers was obtained for all eight countries, but only for the period 2011–2015. Traveler was defined as a Japanese having residential address in Japan who entered a foreign country as a visitor. We did not have access to the information of visit to multiple countries. Additionally, we collected the annual number of Japanese travelers to the Philippines, Thailand, Malaysia, Vietnam, and Singapore from 2006 to 2016 from the Japan Travel Bureau (JTB) Tourism Research & Consulting Co. (JTB, 2017b). For the remaining three countries (especially for datasets from 2006 to 2010 and in 2016), Japanese travelers' data were retrieved from Statistics Indonesia (2017) and the Ministry of Tourism, Indonesia (2017), Ministry of Tourism, India (2017), and Sri Lanka Tourism Development Authority (2017).

Table 1. Annual number of imported cases of dengue virus infection in Japan from 2006 to 2016

Country	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016
Indonesia	9	22	15	22	78	24	38	68	50	66	111
Philippines	15	14	11	9	34	22	60	43	32	75	63
Thailand	8	5	17	6	28	12	26	49	20	27	24
India	8	8	19	15	40	16	27	15	8	15	19
Malaysia	3	3	5	9	4	7	3	9	24	28	16
Vietnam	2	3	7	9	5	4	3	3	2	9	31
Singapore	0	1	3	1	1	1	2	3	3	4	5
Sri Lanka	0	1	0	3	2	2	5	5	7	7	7

Next, we systematically collected seroprevalence data of DENV infection in the abovementioned eight countries from 2006-16. The systematic review was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement. We searched MEDLINE and Web of Science databases, using the following search terms:

"Seroprevalence OR Seroepidemic OR Seropositive OR Serological OR Serosurvey OR IgG,

AND dengue OR DENV

AND Indonesia OR Philippines OR Thailand OR India OR Malaysia OR Vietnam OR Sri Lanka OR Singapore".

All titles identified by the search strategy were independently screened by two authors (BY and HN). Abstracts of potentially relevant titles were then reviewed for eligibility, and articles were selected for closer evaluation, if a description of seroepidemiological study of dengue was available. Multiple reports of the same country data were assessed, and the latest available data with the best precision of age band was included. Figure 1 shows the flow diagram of study selection. In addition to seroprevalence data, the year of sampling was retrieved; only datasets that were stratified by age group were analyzed. Table 2 shows a summary of seroprevalence data that were reanalyzed in the present study.

Finally, the average length of stay per journey to each country was also obtained from multiple data sources. Table 2 summarizes the length of stay data. While the year of seroepidemiological study may not be fully consistent with the years of observations of travelers' dengue, we impose an assumption of stationarity (i.e. the force of infection being a constant) in the mathematical model. Besides, we made the best effort to use the latest available evidence for seroprevalence data so that the mismatch between the time of importation and seroprevalence survey can be minimized.

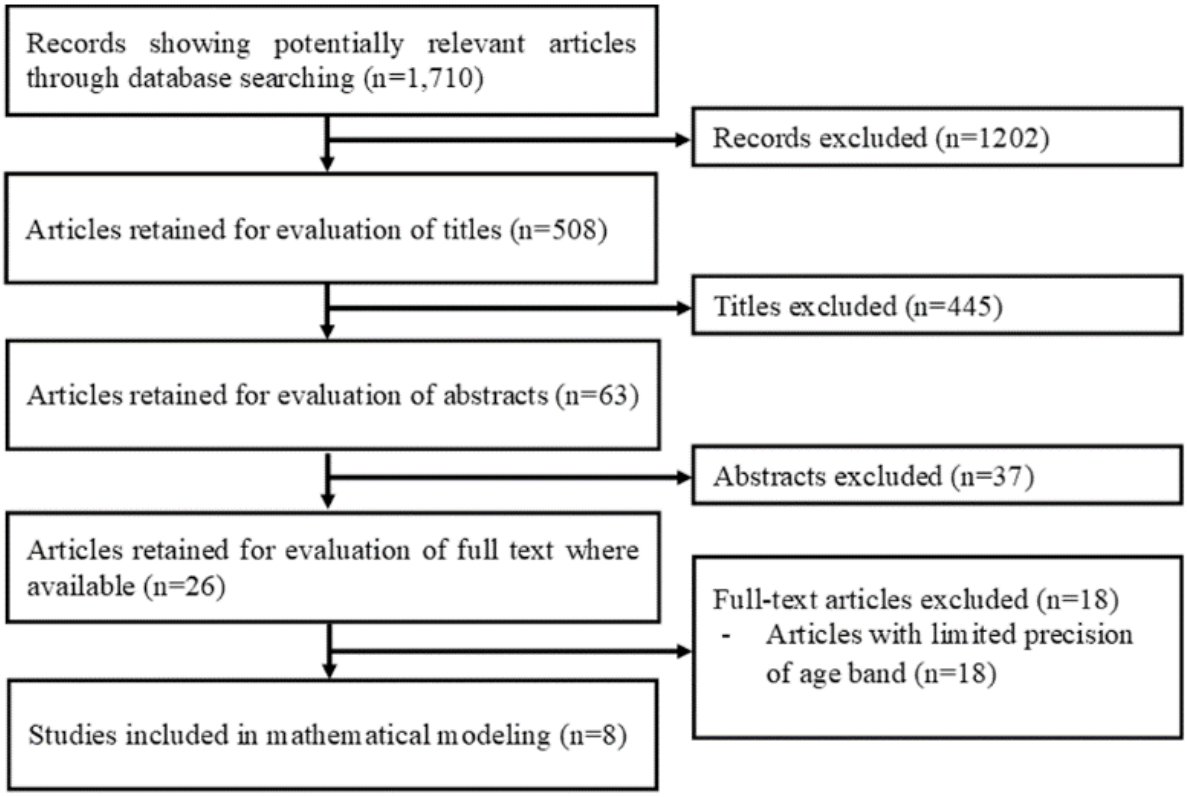

Fig 1. Flow diagram of study selection

Table 2. Average length of stay among Japanese travelers and sampling year of seroepidemio surveys in South and Southeast Asian countries

Country	Average length of stay [days][†]	Year of seroprevalence survey	References
Indonesia	8.1	2016	(OECD, 2014; Pravitno, A. et al, 2016; UN, 2015)
Philippines	9.6	2012	(Philippines, 2014; Alera, M. T. et al., 2016; UN, 2015)0
Thailand	6.0	2010	(Thailand, 2017; Rodríguez-Barraquer, I. et al., 2014; UN, 2015)
India	10.8	2012	(Garg, S. et al, 2017; SenGupta, A., 2012; UN, 2015)
Malaysia	5.9	2008	(Malaysia, 2013; Muhammad Azami, N. A. et al., 2011; UN, 2015)
Vietnam	7.0	2002	(Alera, M.T. et al., 2016; Thai, K. T. et al., 2005; UN, 2015)
Sri Lanka	9.2	2009	(Sri Lanka, 2017; Tam, 2013; UN, 2015)
Singapore	3.7	2010	(Singapore, 2015; Low, S. L. et al., 2015; UN, 2015)

[†]The average length of stay that was quoted in each source of information was used.

Mathematical model

We used a mathematical model to jointly estimate the local hazard (or force) of infection, i.e., the rate at which susceptible individuals experience infection, in each destination country and the coverage of reported DENV infections among Japanese travelers (Nishiura, H., 2006). We used the so-called "catalytic" model in which the fraction of exposed (and immune) individuals is described by the age-independent force of infection λ (Muench, H., 1959; Vynnycky, E. and White, R.G., 2010). Assuming that everyone is born susceptible, the expected seropositive fraction at age a is

$$i(a) = 1 - \exp(-\lambda a). \qquad (1)$$

Because dengue is frequently seen in urban settings, we assumed that travelers had a risk (hazard) of infection that was identical to that of the local population. N_k and D_k were the annual number of Japanese travelers to country k and their average duration of stay, respectively. The expected number of all DENV infections among travelers to country k is described as:

$$E(i_k) = N_k \left[1 - \exp\left(-\lambda_k \frac{D_k}{365}\right)\right]. \qquad (2)$$

Among E(i_k), only the fraction α_k was symptomatic, diagnosed, and notified. Thus, the expected number of imported DENV infections is

$$E(c_k) = \alpha_k N_k \left[1 - \exp\left(-\lambda_k \frac{D_k}{365}\right)\right]. \qquad (3)$$

What equations (2) and (3) indicate is that the risk of travelers is assumed as independent of their age. Assuming that the observed number of imported cases follow a Poisson distribution, and also considering that the seroprevalence data is the result of Bernoulli sampling with the expected fraction positive $i(a)$, the likelihood function to estimate the force of infection and the reporting coverage of DENV infections is

$$\mathrm{L}(\lambda_k, \alpha_k; A_k^a, B_k^a, c_k^t) \propto \prod_a i_k(a;\lambda_k)^{A_k^a} (1 - i_k(a;\lambda_k))^{B_k^a} \prod_t \frac{\mathrm{E}(c_k;\lambda_k,\alpha_k)^{c_k^t} \exp(-\mathrm{E}(c_k;\lambda_k,\alpha_k))}{c_k^t!} \qquad (4)$$

where A^a and B^a are the number of seropositive and seronegative individuals at median sampling age a, respectively. Maximum likelihood estimates of unknown parameters were obtained by minimizing the negative logarithm of equation (4). We calculated 95% confidence intervals using the profile likelihood method.

Travelers may experience a hazard of infection that differs from that of the local population. As part of sensitivity analysis, we explored the impact of having a different force of infection using the relative hazard ε, i.e.,

$$E(c_k) = \alpha_k N_k \left[1 - \exp\left(-\varepsilon\lambda_k \frac{D_k}{365}\right)\right]. \qquad (5)$$

Using the observed number of imported cases, yearly number of travelers, and estimated reporting coverage, the risk of infection among travelers to destination *k* in year *t* was calculated as

$$p_k^t = \frac{c_k^t}{\alpha_k N_k}, \qquad (6)$$

per journey. To jointly estimate the force of infection and reporting coverage, the following assumptions were made.

1. International travelers have a hazard of infection that is identical to that of the local population in each dengue-endemic country.

2. Two model parameters α and λ were assumed to be constant over time. Namely, dengue was approximately in a stationary state.

3. Seasonality was discarded owing to a shortage of finer scale data of travelers.

1.3 Ethical considerations

The present study analyzed only secondary datasets that were publicly available, all of which were de-identified before collection. As such, ethical approval by the Institutional Review Board was not required for the present study.

1.4 Results

From 2006 to 2016, there were a total of 2026 imported cases of DENV infection from 63 different countries that could potentially act as the source of exportation. Of these, 176 patients visited multiple countries and their place of infection was not determined. Of the remainder (1850 patients),

South and Southeast Asian countries accounted for the majority of cases. In descending order of incidence, imported cases came from Indonesia, Philippines, Thailand, India, Malaysia, Vietnam, Cambodia, Myanmar, Sri Lanka, Bangladesh, and Singapore. Owing to unavailability of seroepidemiological survey data, Cambodia, Myanmar, and Bangladesh were excluded and the remaining eight countries were included for further analyses (Fig 2). Imported cases from the eight selected countries accounted for 83.5% of the total notifications.

Table 3 shows the maximum likelihood estimate of the force of infection in each country. High estimates of the force of infection were obtained for the Philippines, Sri Lanka, and Indonesia followed by Vietnam and India. Assuming that travelers experienced an identical hazard of dengue infection during their journey, the reporting coverage α was estimated for each country (Fig 3). The reporting coverage of dengue ranged from 0.6% to 4.3%, implying that the actual number of DENV infections is 23.3–166.7 times greater than the reported number of cases. Malaysia and Sri Lanka had the highest estimate of reporting whereas Vietnam yielded the lowest value.

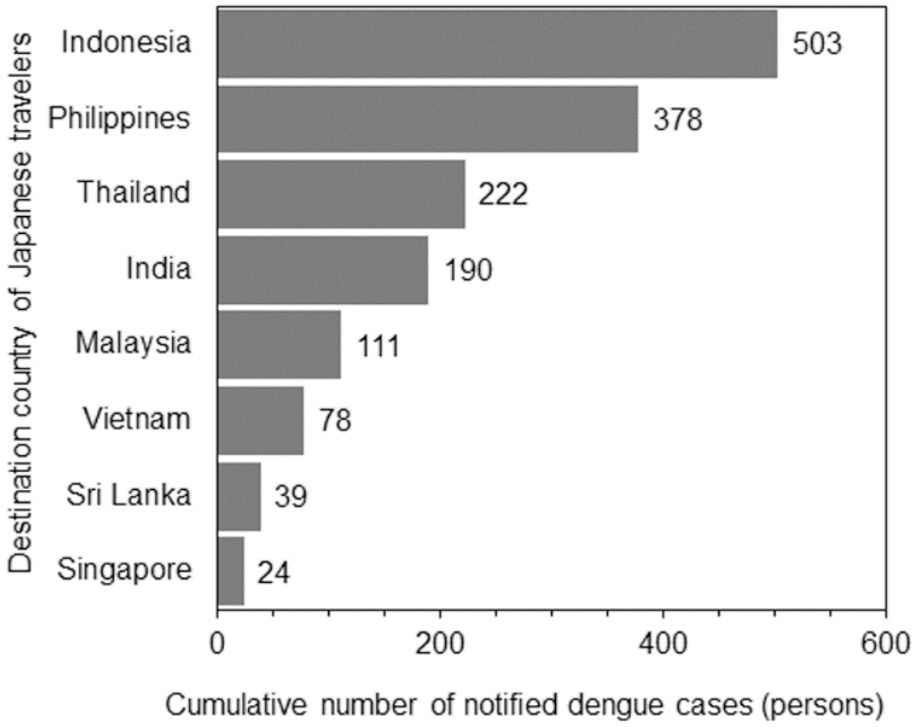

Fig 2. Cumulative number of imported cases of dengue virus infection from 2006 to 2016 by destination country. Eight countries are shown in descending order of cumulative number of cases. Only notified cases based on laboratory diagnosis are counted.

Table 3. Estimated force of infection of dengue virus infection in South and Southeast Asian countries. Force of infection in parenthesis shows 95% confidence intervals derived using the profile likelihood method.

Country	Force of infection, per year
Indonesia	0.15 (0.14, 0.17)
Philippines	0.17 (0.15, 0.19)
Thailand	0.08 (0.07, 0.08)
India	0.13 (0.12, 0.13)
Malaysia	0.05 (0.05, 0.05)
Vietnam	0.12 (0.11, 0.13)
Sri Lanka	0.14 (0.13, 0.15)
Singapore	0.02 (0.02, 0.02)

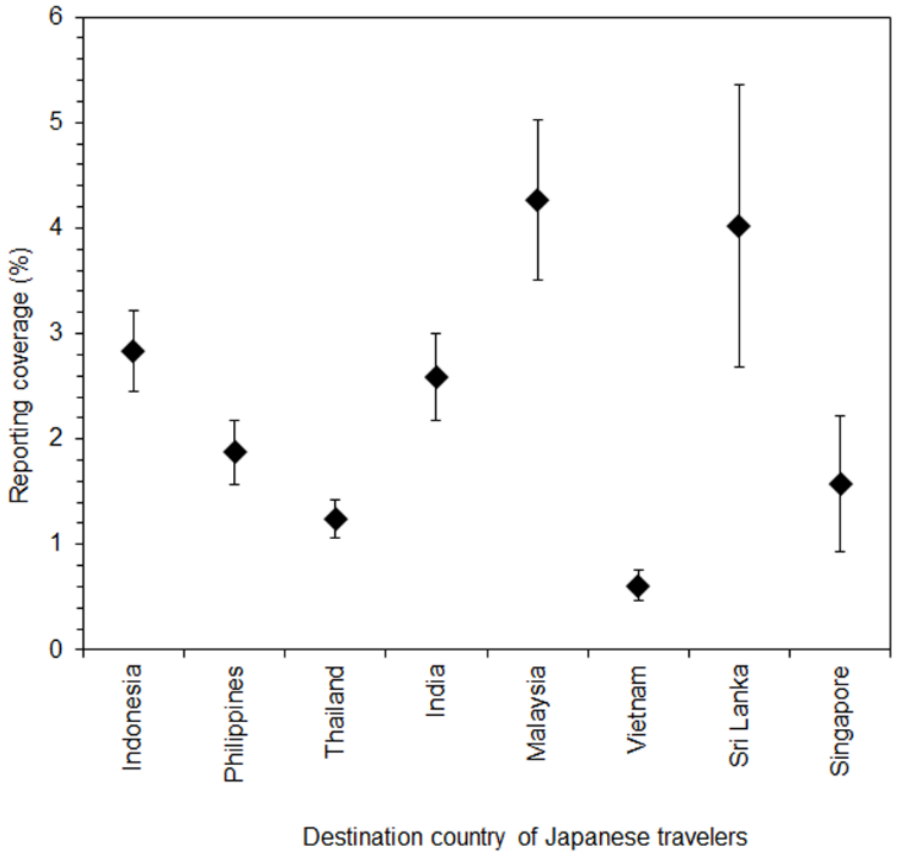

Fig 3. Estimated reporting coverage of dengue virus infection among travelers by destination country. Filled diamonds represent maximum likelihood estimates; whiskers extend to lower and upper 95% confidence intervals. Travelers were assumed to have experienced an identical force of infection of dengue virus infection to the local population.

The impact of seasonal force of infection on the estimate of α can be explored using equation (3). In our analysis, given observed number of imported cases c and travelers N, we have used the following equation:

$$\alpha_0 = \frac{c}{N[1-\exp(-\lambda_0 d)]}, \quad (7)$$

where d is the length of stay (years). However, it is possible that we missed the seasonal force of infection which may be h times greater than our assumed constant, i.e., $\lambda=h\lambda_0$ where $h>1.0$ during the summer season when the mosquito vector is active. Then the actual α that we should have estimated would be

$$\alpha = \frac{c}{N[1-\exp(-h\lambda_0 d)]}, \quad (8)$$

yielding the extent of bias by ignoring the seasonality as

$$\frac{\alpha}{\alpha_0} = \frac{1-\exp(-\lambda_0 d)}{1-\exp(-h\lambda_0 d)} \approx \frac{1}{h}. \quad (9)$$

That is, if the force of infection during the summer is h times greater than the assumed average, the estimated α was biased by $1/h$ times.

Using the observed number of imported cases, yearly number of travelers, and estimated reporting coverage, the risk of infection among travelers to destination k was calculated. The variation of estimated risk from 2006 to 2016 is shown in Fig 4. The risk of infection per travel ranged from 0.02% to 0.44%. The lowest estimate was obtained in Singapore and the highest from India.

Because travelers could have risk of infection that differed from that of the local population, we examined the sensitivity of reporting coverage to relative hazard $\mathcal{E}$. Fig 5 shows that the reporting coverage would be considerably increased if the hazard were lower than 1. Nevertheless, a dramatic increase of reporting coverage was seen only when $\mathcal{E}$ was substantially lower than 1 (e.g., lower than 0.5).

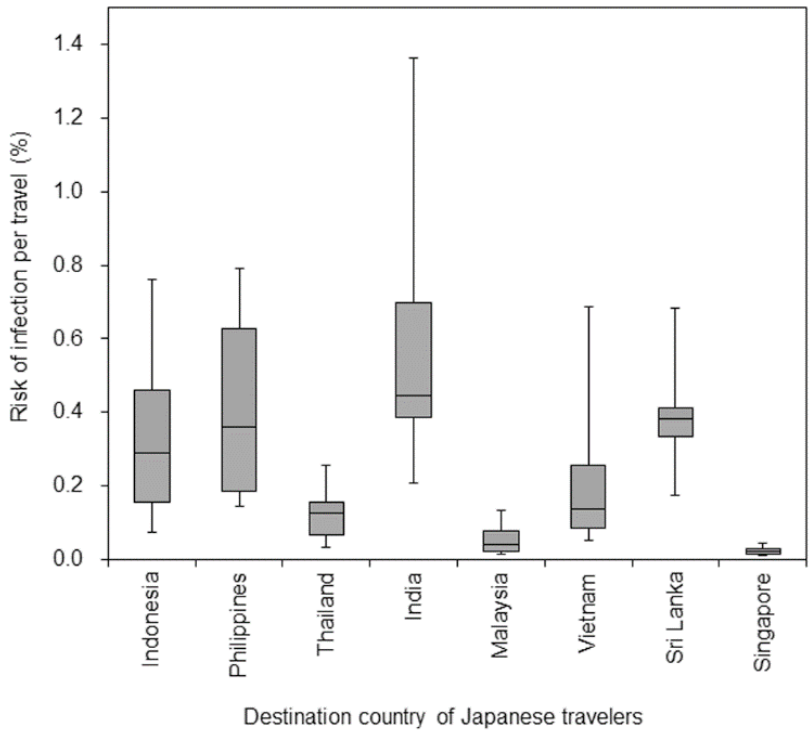

Fig 4. Estimated risk of dengue virus infection among Japanese travelers from 2006 to 2016 by destination country. Box whisker plot shows the range of estimated risk of infection per journey from 2006 to 2016. Middle line of box represents median, extending from lower to upper quartiles. Whiskers extend to lowest and highest risks.

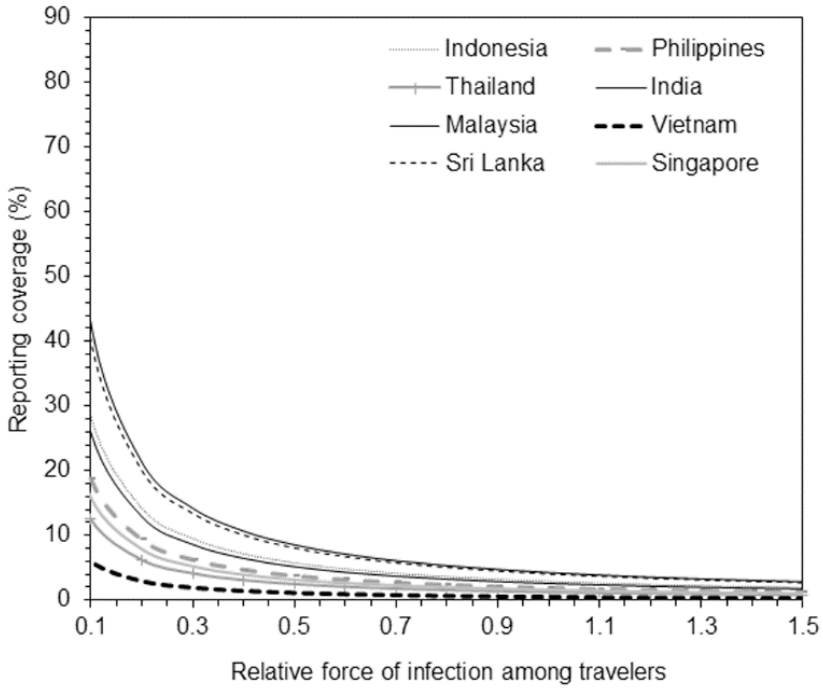

Fig 5. Sensitivity of reporting coverage to the relative hazard of travelers compared with local population. Relative hazard on horizontal axis shows the relative hazard, obtained as the ratio of the force of infection among Japanese travelers to that of the local population in each destination country. Vertical axis measures the estimated reporting coverage; only maximum likelihood estimates are shown.

1.5 Discussion

In the present study, we statistically inferred the risk of DENV infection among Japanese travelers to Asian countries, as the importation is recognized as increasing over time (Suzuki, T. et al., 2017). To accomplish this task, we used multiple pieces of data as a trick to estimate asymptomatically infected fraction, including seroprevalence data of travelers' destination country such that the force of infection could be explicitly estimated. Integrating the force of infection into the process of importation and assuming that travelers experienced a force of infection identical to that of local people during their travel, the reporting coverage of dengue was estimated to range from 0.6% to 4.3%. This finding implies that the actual importation of DENV infection among Japanese travelers may be more than 20 times the notified number of imported cases. Moreover, the risk of infection per travel ranged from 0.02% to 0.44%.

There are two take home messages from the results of our study. First, our modeling exercise allowed us to demonstrate that the observed number of imported cases truly represents the tip of the iceberg. Given that the Japanese government is annually notified of 200 imported cases per year (Fukusumi, M. et al., 2016; Takasaki, T., 2011; Taniguchi, K. et al., 2008), one would expect that 4,000 (or even more) imported DENV infections could have occurred; however, the majority of infections remain unrecognized. While the finding is subject to validation through seroprevalence survey among all travelers, including healthy individuals, coming back from Southeast Asia, the present study is the first to explicitly show how DENV infections are under-ascertained among Japanese travelers who are asymptomatic or mildly infected.

Second, the risk of infection among travelers was explicitly estimated to range from 0.02% to 0.44% per journey. These figures indicate that there have been an estimated 20–440 DENV infections per 100,000 travelers visiting South and Southeast Asian countries. Of course, this estimated risk of infection was greater than the published estimate (Fukusumi, M. et al., 2016), which depends on reported cases alone. It is critical to consider the undetected proportion of infected individuals when determining the risk of local outbreaks in Japan because these individuals can contribute to secondary transmission despite being asymptomatic or undiagnosed (Duong, V., et al., 2015). For instance, undiagnosed individuals might be bitten by *Aedes* species during their infectious period while being unaware of their own risk of causing a dengue outbreak (Thai, K. T. D., et al., 2011; Egger, J. R. and Coleman, P. G., 2007).

Three technical problems in this study must be noted. First, we ignored spatial heterogeneity. The seroprevalence data that we analyzed could have relied on observations in a particular geographic locality, and representativeness of the sample may not be fully guaranteed. The risk of dengue would undoubtedly vary with geographic space (Salje, H., 2017), but travelers' data were only classified by the destination country to which they traveled. In the future, precise estimation of the risk of dengue among travelers should ideally rest on more detailed information with respect to travel patterns (e.g. volume and risky behavior of travelers by different destinations) and seroprevalence data in each geographic locality.

Second, the present study ignored more precise temporal resolution. Monthly dengue incidence data were unavailable for all eight destination countries, and we had to compromise by using yearly numbers of cases as well as travelers. Not only dengue transmission but also tourism pattern involves seasonality, but we had to ignore this aspect, which we aim to improve in the future study. At least, we have mathematically clarified the impact of seasonal force of infection on the estimate of ascertainment probability. Moreover, it should be noted that there was a mismatch of timing in the datasets, e.g., Vietnam in 2002 and 2006-2016 for seroepidemiological and case data, respectively. Whereas estimates in Vietnam did not appear to be considerably different from others, such mismatch should be filled by updated seroepidemiological data in the future.

Third, we made an inherent assumption that the force of infection among travelers was identical to that of the local population during travel; we were unable to fully validate this assumption. Especially, it is critical to focus on travelers' behavior during the stay: the extent of risky behaviors would greatly differ by the purpose of visit. We believe that the assumption of identical risk was qualitatively sound because most travelers' tourist destinations were capital cities (e.g., Bangkok); the main spatial hotspot of a dengue epidemic is frequently found in urban settings. While the issue of spatial representativeness still remains (e.g. mismatch between travelers' destination and seroprevalence survey area; Binh Thuan province in Vietnam), we believe that the assumption holds better for dengue with urban foci than malaria which requires biting events during night time in rural area. At the very least, it should be remembered that the extent of underestimation that we have shown might have been overemphasized if the force of infection among travelers was lower than that of the local population.

While all these limitations cannot be immediately overcome, we believe that we have successfully quantified the reporting coverage of DENV infection among travelers returning to Japan, yielding the estimate of the risk of infection per travel. Evaluation relying only on reported cases can result in remarkable underestimation of the risk of infection. The existence of a substantial number of unrecognized infections could trigger local outbreaks of dengue in Japan; therefore, more precise data collection and monitoring of travelers is required.

Chapter 2. Assessing the intervention effectiveness in the dengue outbreak in Tokyo, 2014

1.1 Background

Japan is in a temperate zone and dengue is not endemic in the country. However, Japan has experienced a steady increase in the number of imported cases, mainly from South and Southeast Asian countries (Fukusumi, M. et al., 2016; Nakamura, N. et al., 2012; Yuan, B. and Nishiura, H., 2018). In 2014, autochthonous transmission was confirmed in metropolitan Tokyo (Seki, N. et al., 2015; Arima, Y. et al., 2014; Kutsuna, S. et al., 2015; Quam, M.B. et al., 2016; Ishikawa, H. et al., 2017; Furuya, H., 2015), resulting in a large outbreak involving a total of 160 confirmed cases, a shockingly high incidence for a previously dengue-free nation. It is now recognized that Japan is indeed at risk of dengue outbreaks during the summer season, indicating that a certain risk exists for the summer Olympic Games in 2020. This threat requires concrete planning for possible countermeasures in the event of another outbreak.

The 2014 dengue outbreak in central Tokyo was caused by a single serotype, DENV1, which showed high homology with the predominant circulating serotype in Southeast Asia (Seki, N. et al., 2015). There were two notable characteristics of this outbreak. First, of the total 160 people with a confirmed dengue diagnosis, 129 had visited or worked near Yoyogi Park, a national park that belongs to Shibuya Ward. Shibuya is a special ward that is a major commercial and business center. Shibuya has one of the busiest railway stations in the city, Shibuya Station, about 1 km from the park. Dengue transmission was concentrated in the park, where relatively high vector competence (mean biting rate 7.1 bites per person per 8 minutes) was observed (Tsuda, Y. et al., 2016). Second, a few local residents seemed to remain for extended periods in Yoyogi Park (i.e., perhaps homeless people), and these people appeared to have been infected at a higher frequency than other individuals (IDSC, 2015); however, the role of these individuals in amplifying transmission as primary cases has not been verified. Once the outbreak was recognized in late August 2014, concerted efforts were made to contain spread of the virus, including mosquito control targeting both adults and larvae, disseminating news of the outbreak via mass media, communication of dengue risk by experts to raise public awareness, and even a total ban on entering the park. Descriptive documents with details of the outbreak are available but are mostly limited to Japanese language (Seki, N. et al., 2015; Kutsuna, S. et al., 2015; Ishikawa, H. et al., 2017; IDSC,

2015; BSWPH, 2014). However, the effectiveness of interventions during the outbreak remains an important epidemiological question.

Mathematical modeling techniques are powerful tools for retrospective assessment of disease outbreaks. These methods include objective measurement of transmission such as the effective reproduction number, i.e., the actual average number of secondary cases generated by a single primary case, sometimes in the presence of interventions (Wallinga, J. and Teunis, P., 2004; Cowling, B.J. et al., 2008). In the present study, we formulated a mathematical model and derived a likelihood function, aiming to estimate the effectiveness of interventions during the 2014 outbreak. Because several countermeasures were implemented on different dates during the outbreak, we calculated the effective reproduction numbers to assess the effectiveness of these interventions.

1.2 Methods

Epidemiological data

In Japan, DENV infection is categorized as a category IV disease, according to the Infectious Disease Law; thus, all physicians are required to notify diagnosed cases to the government via local health centers upon diagnosis (IDSC, 2004). The clinical characteristics of infection include (i) high-grade fever, which is typically biphasic, following an incubation period of 2–14 days (Chan, M. and Johansson, M. A., 2012; Nishiura, H., 2007); (ii) headache, reddish face and/or conjunctivitis; (iii) general fatigue; (iv) muscle and joint pain, followed by (v) generalized rash that starts on the chest and abdomen. For patients with these characteristics, a physician must confirm the diagnosis via virus isolation, PCR method, detection of nonstructural protein 1, elevated IgM antibodies against DENV, or plaque reduction neutralization testing.

During the 2014 outbreak, notifications as well as details of the outbreak and interventions were summarized in an official report by the Tokyo metropolitan government (BSWPH, 2014). In the present study, we retrieved the dates of exposure and illness onset from this report. The date of exposure was calculable because many cases were associated with exposure at Yoyogi Park. Individuals who did not have a history of visiting Yoyogi Park had a history of being bitten by

mosquitoes in one of several other parks in the nearby Kanto region. However, the date of exposure was partly censored. Of the total 160 reported cases, four were excluded owing to the absence of information about illness onset (i.e., people who were serologically diagnosed, including local residents of the park). The remaining reported cases were statistically categorized into one of the following three groups (Fig 1):

(i) cases exposed to DENV on a single day with a known date of illness onset (i.e., complete observation; Group 1, n=79),

(ii) cases exposed to DENV for a certain number of days with a known date of illness onset (i.e., interval-censored observation of exposure; Group 2, n=47),

(iii) cases without information of the date of exposure but with a known date of illness onset (i.e., missing observation of exposure; Group 3, n=30).

Individuals in Group 2 visited Yoyogi Park on two or more consecutive days. Accordingly, the reported cases were categorized into Groups 1, 2, and 3 (Fig 2). The details of each individual cases including the availability of their date of exposure and illness onset were presented in Table 1.

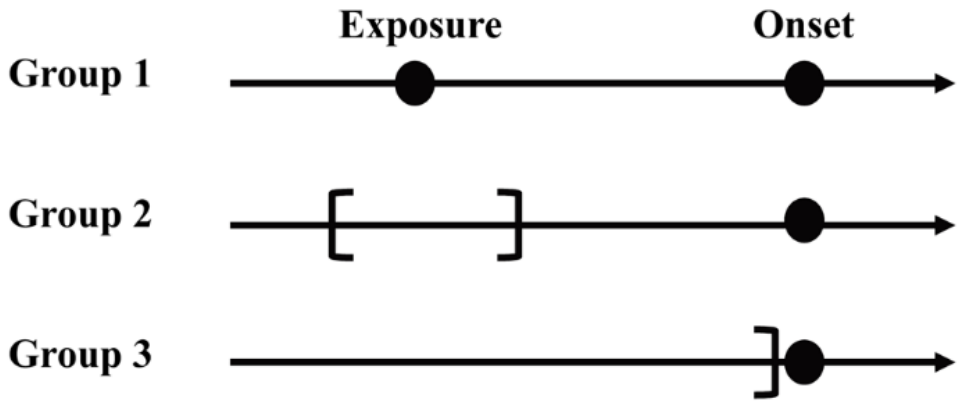

Fig 1. Illustrated time of exposure and classification of cases. In the present study, diagnosed cases of dengue virus fell into one of three groups. In Group 1, the exact date of exposure was known. In Group 2, exposure dates were interval censored, calling for interval-censored likelihood. In Group 3, no information was available with respect to time of exposure. Depending on this grouping, we used slightly different likelihood functions.

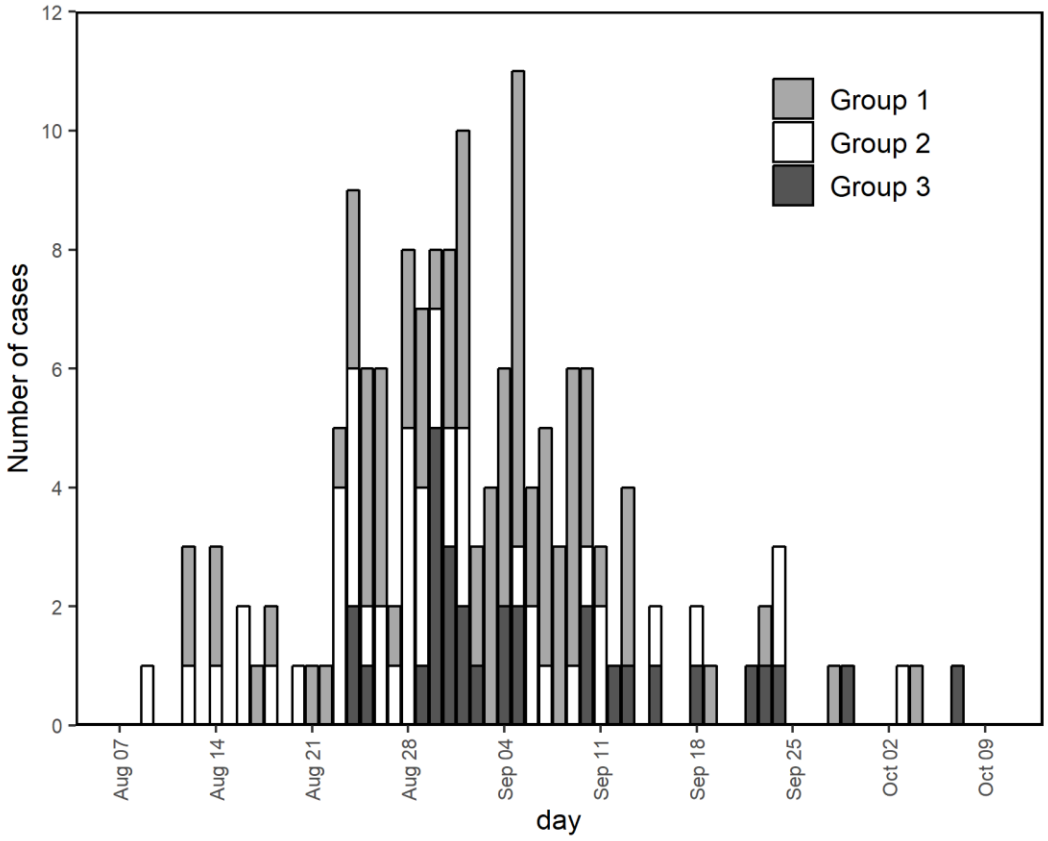

Fig 2. Temporal distribution of dengue by three classification groups of datasets. According to statistical information of the date of exposure, cases were classified into three groups: Group 1, exact date of exposure was known; Group 2, exposure dates were interval censored; and Group 3, no information was available. In the figure, the date of illness onset is shown for these three groups. Light grey, white, and dark grey bars represent cases in Groups 1, 2, and 3, respectively.

Table 1. Dates of exposure and illness onset among a total of 156 dengue fever cases in Tokyo, Japan, 2014

Time of illness onset	Exposure time	Group	Case ID (BSWPH, 2014)	Time of illness onset	Exposure time	Group	Case ID (BSWPH, 2014)
12 Aug	4 Aug	1	52	14 Aug	(5 Aug, 13 Aug)	2	35
12 Aug	7 Aug	1	78	16 Aug	(9 Aug, 10 Aug)	2	4
14 Aug	10 Aug	1	25	16 Aug	(9 Aug, 10 Aug)	2	16
14 Aug	9 Aug	1	50	18 Aug	(1 Aug, 17 Aug)	2	3
17 Aug	10 Aug	1	10	20 Aug	(11 Aug, 18 Aug)	2	1
18 Aug	11 Aug	1	12	23 Aug	(16 Aug, 18 Aug)	2	19
21 Aug	16 Aug	1	11	23 Aug	(16 Aug, 18 Aug)	2	20
22 Aug	17 Aug	1	15	23 Aug	(16 Aug, 18 Aug)	2	31
23 Aug	10 Aug	1	13	23 Aug	(16 Aug, 18 Aug)	2	71
24 Aug	20 Aug	1	7	24 Aug	(16 Aug, 17 Aug)	2	24
24 Aug	17 Aug	1	18	24 Aug	(14 Aug, 17 Aug)	2	36
24 Aug	20 Aug	1	22	24 Aug	(16 Aug, 18 Aug)	2	37
25 Aug	20 Aug	1	17	9 Aug	(20 Jul, 8 Aug)	2	160
25 Aug	18 Aug	1	21	12 Aug	(20 Jul, 11 Aug)	2	143
25 Aug	18 Aug	1	29	24 Aug	(20 Jul, 23 Aug)	2	9
25 Aug	19 Aug	1	30	25 Aug	(20 Jul, 24 Aug)	2	6
26 Aug	21 Aug	1	23	27 Aug	(15 Aug, 25 Aug)	2	5
26 Aug	19 Aug	1	38	28 Aug	(17 Aug, 24 Aug)	2	49
26 Aug	21 Aug	1	39	28 Aug	(20 Aug, 24 Aug)	2	63
26 Aug	22 Aug	1	57	28 Aug	(17 Aug, 21 Aug)	2	72
27 Aug	25 Aug	1	59	29 Aug	(21 Aug, 24 Aug)	2	45
28 Aug	20 Aug	1	14	30 Aug	(25 Aug, 26 Aug)	2	32
28 Aug	22 Aug	1	51	31 Aug	(25 Aug, 26 Aug)	2	33
28 Aug	23 Aug	1	77	31 Aug	(25 Aug, 29 Aug)	2	98
29 Aug	22 Aug	1	47	1 Sep	(25 Aug, 26 Aug)	2	34
29 Aug	23 Aug	1	56	1 Sep	(22 Aug, 29 Aug)	2	68
29 Aug	23 Aug	1	75	26 Aug	(20 Jul, 25 Aug)	2	44
30 Aug	23 Aug	1	87	26 Aug	(20 Jul, 25 Aug)	2	55
31 Aug	22 Aug	1	58	28 Aug	(20 Jul, 27 Aug)	2	40
31 Aug	26 Aug	1	69	28 Aug	(20 Jul, 27 Aug)	2	61
31 Aug	25 Aug	1	94	29 Aug	(20 Jul, 28 Aug)	2	54
1 Sep	27 Aug	1	43	29 Aug	(20 Jul, 28 Aug)	2	28
1 Sep	26 Aug	1	46	30 Aug	(20 Jul, 29 Aug)	2	41
1 Sep	22 Aug	1	62	1 Sep	(20 Jul, 31 Aug)	2	65
1 Sep	23 Aug	1	70	5 Sep	(28 Aug, 29 Aug)	2	111
1 Sep	21 Aug	1	83	6 Sep	(27 Jul, 4 Sep)	2	93
2 Sep	27 Aug	1	53	6 Sep	(30 Aug, 1 Sep)	2	113
2 Sep	26 Aug	1	60	9 Sep	(2 Sep, 5 Sep)	2	105
3 Sep	28 Aug	1	64	10 Sep	(4 Sep, 5 Sep)	2	138
3 Sep	28 Aug	1	74	11 Sep	(2 Sep, 9 Sep)	2	139
3 Sep	30 Aug	1	97	7 Sep	(20 Jul, 6 Sep)	2	128
3 Sep	28 Aug	1	101	11 Sep	(20 Jul, 10 Sep)	2	130
4 Sep	27 Aug	1	73	15 Sep	(20 Jul, 14 Sep)	2	148
4 Sep	31 Aug	1	100	18 Sep	(20 Jul, 17 Sep)	2	147
4 Sep	28 Aug	1	110	24 Sep	(20 Jul, 23 Sep)	2	155
4 Sep	30 Aug	1	121	24 Sep	(20 Jul, 23 Sep)	2	150
5 Sep	30 Aug	1	81	3 Oct	(20 Jul, 2 Oct)	2	158
5 Sep	27 Aug	1	85	24 Aug	-	3	2
5 Sep	31 Aug	1	90	24 Aug	-	3	48
5 Sep	27 Aug	1	112	25 Aug	-	3	26
5 Sep	30 Aug	1	119	29 Aug	-	3	118
5 Sep	31 Aug	1	123	30 Aug	-	3	27
5 Sep	31 Aug	1	124	30 Aug	-	3	67
5 Sep	2 Sep	1	127	30 Aug	-	3	82
6 Sep	3 Sep	1	88	30 Aug	-	3	95
6 Sep	27 Aug	1	89	30 Aug	-	3	102
7 Sep	2 Sep	1	103	31 Aug	-	3	76
7 Sep	4 Sep	1	107	31 Aug	-	3	80
7 Sep	30 Aug	1	108	31 Aug	-	3	86
7 Sep	30 Aug	1	115	1 Sep	-	3	42

8 Sep	2 Sep	1	91	1 Sep	-	3	66
8 Sep	31 Aug	1	104	2 Sep	-	3	96
8 Sep	3 Sep	1	133	4 Sep	-	3	92
9 Sep	31 Aug	1	109	4 Sep	-	3	99
9 Sep	5 Sep	1	116	5 Sep	-	3	84
9 Sep	4 Sep	1	120	5 Sep	-	3	129
9 Sep	2 Sep	1	126	10 Sep	-	3	132
9 Sep	3 Sep	1	137	10 Sep	-	3	135
10 Sep	4 Sep	1	114	12 Sep	-	3	131
10 Sep	2 Sep	1	117	13 Sep	-	3	145
10 Sep	3 Sep	1	122	15 Sep	-	3	146
11 Sep	7 Sep	1	142	18 Sep	-	3	141
13 Sep	4 Sep	1	125	22 Sep	-	3	151
13 Sep	7 Sep	1	134	23 Sep	-	3	149
13 Sep	5 Sep	1	140	24 Sep	-	3	153
19 Sep	14 Sep	1	144	29 Sep	-	3	154
23 Sep	15 Sep	1	152	7 Oct	-	3	159
28 Sep	22 Sep	1	156				
4 Oct	28 Sep	1	157				

In this outbreak, the actual primary case, which must be an imported case, was not identified. We defined the initial calendar day as t_0, i.e., day 0 of the epidemic, which was not empirically observed. We assumed that exposure among secondary and subsequent generations of human cases began to occur after t_0. The earliest observed date of exposure was 4 August 2014, and we denoted d_0 as the gap number of days between t_0 and 4 August (i.e., d_0=4 August minus t_0).

Table 2 shows the timeline of the outbreak. The index case, i.e., the first identified clinical case, had illness onset on 9 August and the confirmatory diagnosis was made on 26 August. Mosquito control and public notification of the outbreak started on 28 August; because infected adult *Aedes* mosquitos continued to be detected in Yoyogi Park, the government decided to close the park on 4 September.

Table 2. Event log of the 2014 dengue outbreak in Tokyo, Japan.

Date	Events
9 August 2014	Illness onset in the index case (with the earliest date of illness onset)
25 August 2014	Medical attendance of index case (10-year-old girl)
26 August 2014	Confirmed diagnosis of the index case and confirmation of the autochthonous transmission in Tokyo
†28 August 2014	Interventions against adult and larva of *Aedes albopictus* started in Yoyogi park along with public dissemination of the outbreak as a news
†4 September 2014	Ban on entrance to Yoyogi park
25 September 2014	Absence of virus detection in *Aedes albopictus* in Yoyogi park
7 October 2014	Illness onset in the last case

Epidemic curve as the probability distribution

Here, we describe the transmission dynamics of DENV in Tokyo using a mathematical model. First, we decomposed the generation time of DENV infection into two parts, i.e., (i) the time from illness onset in an infected human to secondary transmission in another human via a mosquito, denoted as the random variable, t_{Trans}, and (ii) the time from infection in a human to their illness onset, again denoted by the random variable, t_{IP}, corresponding to the intrinsic incubation period. Let w_t and f_s be the probability mass functions (pmf) to which random variables t_{Trans} and t_{IP} follow, respectively, and we assumed that both functions would be derived from the cumulative distribution functions of gamma distribution, $G(s)$, i.e., $f_s=G(s;\ \mu_{IP},\ \sigma_{IP})-G(s-1;\ \mu_{IP},\ \sigma_{IP})$ for $s>0$. The parameters μ_{IP} and σ_{IP} are the mean and standard deviation of the (intrinsic) incubation period in humans. Similarly, we assume that the parameters μ_{Trans} and σ_{Trans} would determine the mean and standard deviation of the pmf w_t. Then, the pmf of the generation time, g_t, defined as the time from infection in a human to infection in its secondary human case via a mosquito, can be modeled by convolution,

$$g_t = \sum_{\tau=0}^{t} f_{t-\tau} w_\tau. \qquad (1)$$

As we did not know the parameters that govern f_s and w_t, we jointly estimated them using other epidemiological parameters (see below). The abovementioned model does not explicitly account for the lifespan of the female *Aedes* species, which is considered to be 6 weeks or longer (VDCI). For simplicity, we ignored this matter, because the time scope of the Tokyo epidemic in 2014 was from 9 August to 7 October 2014, consistent with the average lifespan; thus, the empirically estimated generation time was sufficiently shorter than the lifespan.

Using gt, we devised a generation-dependent epidemiological model, which has been described elsewhere (Akhmetzhanov, A.R. et al., 2018). The following part gives the derivation of the generation-dependent model. To introduce the generation-dependent model, we first use the renewal equation

$$i(\mathrm{t}) = R(\mathrm{t}) \int_0^\infty i(t-\tau) g(\tau) d\tau, \qquad (2)$$

where $i(\mathrm{t})$ represents the incidence at time *t*, $R(\mathrm{t})$ is the effective reproduction number, and $g(\tau)$ is the probability density function of generation time. Let $i_m(t)$ be the incidence of generation *m* at time *t*, $i(\mathrm{t})$ can be the total number of cases over different generations *m* at time *t* as

$$i(\mathrm{t}) = \sum_{m=1}^{n} i_m(t), \qquad (3)$$

where *n* represents the maximum number of generations.

Moreover, $i_m(t)$ can be rewritten from equation (2) as

$$i_m(t) = R_{m-1} \int_0^{\infty} i_{m-1}(t-\tau) g(\tau) d\tau, \qquad (4)$$

where R_{m-1} is the reproduction number of generation *m*. If we substitute equation (4) into (3) and discretize it, $i(\mathrm{t})$ with $n = 4$ can be derived as

$$i(t) = \ R_0(g_t + R_1(g * g)_t + R_2R_1(g * g * g)_t + R_3R_2R_1(g * g * g * g)_t). \qquad (5)$$

In this model, we assumed that the epidemiological dynamics described by the generation-dependent model are what is expected in the absence of interventions. We defined the unobserved primary case as generation 0. The primary case produces generation 1, and the size of generation 1 is R_0 cases with the relative timing of infection following g_t (i.e., following the infection time of the primary case, there would be $R_0 g_t$ cases on day *t*). Subsequently, generation 1 produces R_1 cases of generation 2, where R_1 is the reproduction number of generation 1, and there would be $R_0R_1(g * g)_t$ cases as a function of time since primary case *t*, where * is the convolution operator. If there are only two generations (excluding generation zero), the expected value of the incidence at *t* days since infection in the primary case is $R_0 g_t + R_0R_1(g * g)_t$. Continuing this procedure through generation 4, and normalizing the quantity by the cumulative number of cases, we obtain the probability density function of infection, $h(t)$, as

$$h(t) = \frac{R_0(g_t + R_1(g * g)_t + R_2R_1(g * g * g)_t + R_3R_2R_1(g * g * g * g)_t)}{R_0 + R_1R_0 + R_2R_1R_0 + R_3R_2R_1R_0}, \qquad (6)$$

where $R_{\mathrm{m\text{-}1}}$ denotes the reproduction number of generation *m*, describing the average number of secondary cases in generation *m* produced by a single primary case in generation ($m-1$), in the absence of interventions. $R_0 + R_1R_0 + R_2R_1R_0 + R_3R_2R_1R_0$ represents the total number of cases, considering up to the fourth generation. R_0 is cancelled out and we have

$$h(t) = \frac{g_t + R_1(g*g)_t + R_2R_1(g*g*g)_t + R_3R_2R_1(g*g*g*g)_t}{1 + R_1 + R_2R_1 + R_3R_2R_1}. \quad (7)$$

Equation (7) describes the epidemic curve of infection as the probability distribution in the absence of interventions. It should be noted that $h(t)$ is a function of the time of infection, not illness onset.

Though only arithmetically, R_0 can be calculated by dividing the observed cumulative number of cases by $(1 + R_1 + R_2R_1 + R_3R_2R_1)$. Additionally, we incorporated the effectiveness of the interventions implemented during the 2014 outbreak. As Table 1 shows, mosquito control started on 28 August (T_1); subsequently, Yoyogi Park was closed on 4 September (T_2). We wished to assess the effectiveness of these two interventions separately. We used relative reduction in the reproduction number, $\varepsilon(t)$, defined as follows:

$$\varepsilon(t) = \begin{cases} 1 & t < T_1 \\ \varepsilon_1 & T_1 \le t < T_2. \\ \varepsilon_1\varepsilon_2 & T_2 \le t. \end{cases} \quad (8)$$

Using $h(t)\varepsilon(t)$, we described the observed epidemic dynamics. Normalizing the product, $h(t)\varepsilon(t)$, we obtained the probability density of the epidemic curve of infection,

$$u(t) = \frac{h(t)\varepsilon(t)}{\sum_{\tau=0}^{T} h(\tau)\varepsilon(\tau)}, \quad (9)$$

where T is the last date of the outbreak. Using the parameterized incidence function, $u(t)$, the effective (or instantaneous) reproduction number $R(t)$ is calculated as the estimator of the renewal equation (Wallinga, J. and Lipsitch, M., 2007; Nishiura, H., 2010), i.e.,

$$R(t) = \frac{u_t}{\sum_{\tau=0}^{t} u_{t-\tau}g_\tau}. \quad (10)$$

Note that $u(t)$ is now described on a daily basis; thus, u_t represents the daily probability on day t.

Likelihood function

We did not know the first day of exposure, t_0; therefore, we varied d_0 within a plausible range, using 3 to 9 days as a theoretically possible range (BSWPH, 2014). Assuming that the incubation period was independently and identically distributed, our mathematical model was formulated using the convolution of infection probability, u_t, and distribution of the incubation period, f_s. We let t^e be the date of exposure and t^s be the date of illness onset. Unknown parameters $\boldsymbol{\theta_n} = \{\mu_{IP}, \sigma_{IP}, \mu_{Trans}, \sigma_{Trans}, R_1, R_2, \dots, R_{n-1}, \varepsilon_1, \varepsilon_2\}$ were estimated using a maximum likelihood

method. The exact number of generations was unknown; thus, we fit three different models with a variable number of generations (i.e., n=2, 3, and 4 excluding generation 0) and later compared the Akaike information criterion values with a correction for small sample size (AICc) and mean squared error (MSE). We did not consider additional generations, because more generations unrealistically required shorter duration of the extrinsic incubation period (EIP). As a sensitivity analysis, we compared models with and without ε_1 and ε_2, i.e., models in which (i) two effectiveness measures are jointly estimated (i.e., $\varepsilon_1 \neq 1$ and $\varepsilon_2 \neq 1$), (ii) only the park closure effect is estimated ($\varepsilon_1=1$ and $\varepsilon_2 \neq 1$), (iii) only mosquito control and public awareness campaigns are factored in ($\varepsilon_1 \neq 1$ and $\varepsilon_2=1$), and (iv) no effect of control measures are taken into account ($\varepsilon_1=1$ and $\varepsilon_2=1$). To this end, we fixed the generation time distribution, using parameters informed from the best model with $\varepsilon_1 \neq 1$ and $\varepsilon_2 \neq 1$ and jointly estimated only R_{i} and these effectiveness parameters, comparing AICc values across different models.

For case $i \in \text{Group 1}$ where the case has exact dates of exposure t_i^e and symptom onset t_i^s [44], the likelihood of observation is

$$L_i^1(\boldsymbol{\theta}_{\mathrm{n}}; t_i^e, t_i^s, d_0) = u_{\mathrm{z}(t_i^e, \mathrm{d}_0)} f_{t_i^s - t_i^e,} \qquad (11)$$

where $\mathrm{z}(\tau, d_0) = \tau - t^* + d_0 + 1$ and t^*represents the first exposure time (4 Aug 2014). For case j in Group 2, the case has an interval-censored observation for exposure date t_j^e, ranging from the first date of visit E_j^L to the last date of visit E_j^R (i.e., $t_j^e \in \left[E_j^L, E_j^R\right]$) (Reich, N. G. et al., 2009; Cowling, B. J. et al., 2007). The likelihood function for case j in Group 2 is

$$L_j^2\left(\boldsymbol{\theta}_{\mathrm{n}}; E_j^L, E_j^R, t_j^s, d_0\right) = \sum_{\tau=E_j^L}^{E_j^R} u_{\mathrm{z}(\tau, \mathrm{d}_0)} f_{t_j^s - \tau.} \qquad (12)$$

For case k in Group 3, the exposure can take place from time 0 to the onset of symptoms (i.e., $t_k^e \in [t_0, t_k^s]$),

$$L_k^3(\boldsymbol{\theta}_{\mathrm{n}}; t_k^s, d_0) = \sum_{\tau=t_0}^{t_k^s} u_{\mathrm{z}(\tau, \mathrm{d}_0)} f_{t_k^s - \tau.} \qquad (13)$$

In addition, cases in Group 1 were used to estimate the incubation period, i.e.,

$$L^{IP}(\mu_{IP}, \sigma_{IP}; t^e, t^s) = \prod_{i=1}^{n_1} f_{t_i^s - t_i^e,} \qquad (14)$$

where n_m is the total number of cases in Group m = {1, 2, 3}. In Eq. (14), we did not consider interval-censored data because these are already reflected in Eq. (12) with exposure distribution $u(t)$ during the interval. The total likelihood function is

$$\mathrm{L}(\boldsymbol{\theta}_\mathrm{n}; t^e, t^s, E^L, E^R, d_0) = \prod_{i=1}^{n_1} L_i^1 \prod_{j=1}^{n_2} L_j^2 \prod_{k=1}^{n_3} L_k^3 \prod_{i=1}^{n_1} L_i^{IP}. \quad (15)$$

For the estimation of unknown parameters $\boldsymbol{\theta}_\mathrm{n}$, we used the method of maximum likelihood estimation to minimize the negative likelihood $\mathrm{L}(\boldsymbol{\theta}_\mathrm{n}; t^e, t^s, E^L, E^R, d_0)$. We did not impose any constraints for the range of parameters.

The 95% confidence interval (CI) of the effective reproduction number was computed with a parametric bootstrap method. We let $H(\theta^*)$ be the Hessian matrix for estimated values θ^*. The 100 sets of parametric bootstrap samples were generated from the multivariate normal distribution with the mean and covariance, the latter of which was obtained with $diag(H^{-1}(\theta^*))$. Simulating 100 times, 2.5th and 97.5th percentile values of the resampled distribution were used to calculate the 95% CI. All statistical analyses were conducted using R 3.5.1 (R Core Team, 2018).

1.3 Ethical considerations

In the present study, we analyzed data that are publicly available (BSWPH, 2014). As such, the datasets used in this study were de-identified and fully anonymized in advance; the analysis of publicly available data with no identifying information does not require ethical approval.

1.4 Results

Figure 2 shows the incubation period distribution, confirming that the observed and estimated frequencies agreed well. The mean and standard deviation of the incubation period were estimated at 5.8 (95% CI: 5.5, 6.0) days and 1.8 (95% CI: 1.6, 2.1) days, respectively.

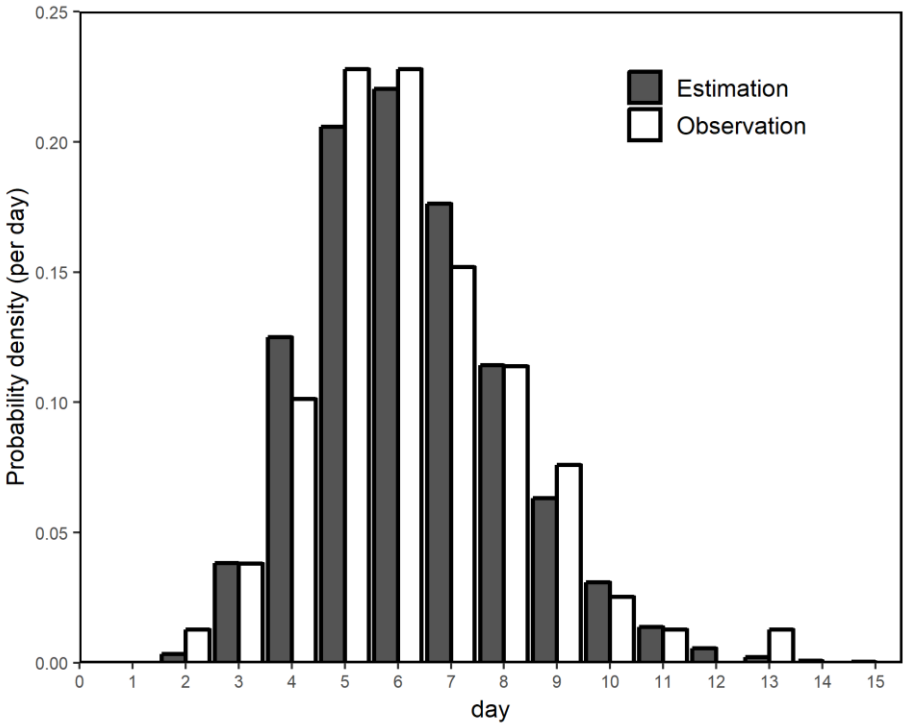

Fig 2. Comparison between observed and estimated distributions of the incubation period. Horizontal axis represents the incubation period of dengue virus infection, i.e., the time from mosquito exposure to illness onset. Grey bars represent observed frequency of the incubation period; white bars represent the estimated probability distribution.

Fitting three different models with a different assumed number of generations, the observed temporal patterns were well captured overall (Fig 3A). Identifying a few best-fit models with a different number of generations, the most likely first date of exposure was in the range 26–28 July 2014, dating back 7–9 days from 4 August 2014 (Table 3). Regardless of the assumed number of generations, the first generation (i.e., generation 1) coincided with a small peak in the epidemic curve on 14 August; generation 2 was considered to be primarily responsible for the highest peak at the end of August (Fig 3B–3D). Only when generation 4 was assumed to have existed, generation 3 was considered responsible for a small peak on about 7 September 2014.

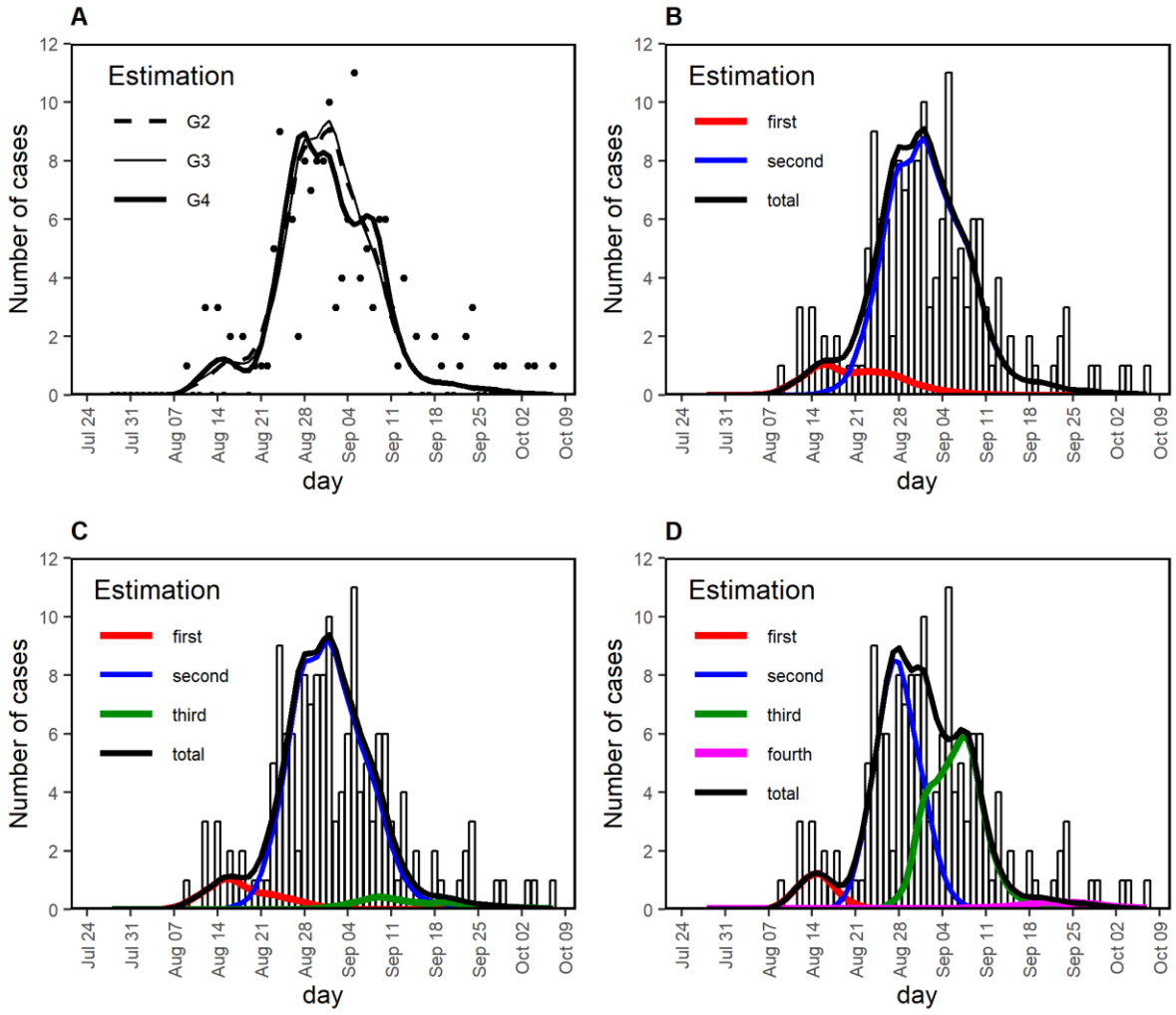

Fig 3. Comparison between observed and estimated epidemic curves of the 2014 dengue outbreak in Tokyo. (A) Total number of cases with time of illness onset. Black dots show the observed number of cases; lines are derived from mathematical models with different numbers of assumed generations of infection, G2, G3, and G4, representing a total number of generations of 2, 3, and 4, excluding generation zero. (B–D) Comparison between observed and estimated cases by different numbers of assumed generations of infection, G2, G3, and G4, corresponding to panels B, C, and D, respectively. The observed number of cases is shown as bars. Red, blue, green, and magenta lines depict the estimated cases generated by first, second, third, fourth generation cases, respectively. Black line represents the estimated total number of cases.

Table 3. Comparison of model fit by the number of generations and time lag between infection in the unobserved primary case and observed first exposure (4 August 2014). n represents the number of generations for generation-dependent model (i.e., there are in total n+1 generations including generation zero). d_0 defines lag days required from the actual start of exposure to the first reported date of exposure on 4 August 2014. Mean μ_{IP} and standard deviation σ_{IP} of the incubation period were assumed as the parameters governing the gamma distribution. Mean μ_{Trans} and standard deviation σ_{Trans} of the waiting time for the generation time other than incubation period were also assumed to follow a gamma distribution. R_{n-1} stands for the reproduction number of the n^{th}-generation infection in the absence of interventions. To account for the effectiveness of interventions, ε_i is factored in our mathematical model to represent the relative reduction in the reproduction number due to mosquito control (ε_1) and park closure (ε_2). AIC stands for the Akaike information criterion.

n	d_0	μ_{IP}	σ_{IP}	μ_{Trans}	σ_{Trans}	R_1	R_2	R_3	ε_1	ε_2	AIC
2	3	5.8	1.9	8.7	7.4	7.0	-	-	0.9	0.7	1858.7
2	4	5.8	1.9	9.4	7.4	7.5	-	-	0.8	0.7	1856.1
2	5	5.8	1.8	10.0	7.3	8.1	-	-	0.8	0.7	1854.9
2	6	5.8	1.8	10.8	7.3	8.7	-	-	0.7	0.6	1854.4
2	7	5.8	1.8	11.3	7.2	9.3	-	-	0.7	0.6	1854.3
2	8	5.8	1.8	11.9	7.2	10.0	-	-	0.6	0.6	1854.5
2	9	5.8	1.8	12.5	7.2	10.7	-	-	0.6	0.6	1854.8
3	3	5.8	1.9	5.5	5.4	3.8	2.8	-	0.5	0.5	1860.0
3	4	5.8	1.9	8.6	6.6	7.2	0.1	-	0.9	0.7	1858.1
3	5	5.8	1.8	8.7	5.9	7.3	0.2	-	0.8	0.7	1856.6
3	6	5.8	1.8	9.0	5.5	7.7	0.2	-	0.8	0.7	1855.7
3	7	5.8	1.8	9.3	5.2	8.0	0.2	-	0.8	0.8	1855.3
3	8	5.8	1.8	9.8	5.0	8.3	0.2	-	0.8	0.8	1855.0
3	9	5.8	1.8	10.2	4.8	8.7	0.2	-	0.8	0.8	1855.0
4	3	5.8	1.9	5.0	4.2	4.3	2.1	0.2	0.5	0.5	1861.8

4	4	5.8	1.9	5.3	3.3	5.0	1.8	0.3	0.5	0.5	1859.2
4	5	5.8	1.8	5.6	2.7	5.3	2.0	0.4	0.5	0.4	1857.0
4	6	5.8	1.8	5.9	2.3	5.3	2.5	0.4	0.4	0.4	1855.2
4	7	5.8	1.9	6.2	2.1	5.3	3.4	0.4	0.3	0.4	1854.1
4	8	5.8	1.9	6.6	2.0	5.3	4.4	0.4	0.3	0.4	1854.0
4	9	5.8	1.8	7.6	2.3	6.6	1.1	0.3	0.9	0.3	1855.7

Table 4 summarizes the parameter estimates derived from the identified best model, given the assumed number of generations. The mean generation time from DENV infection in a human to another DENV infection in a human via a mosquito bite (i.e., $\mu_{IP} + \mu_{Trans}$) was estimated at 17.2 days, 16.1 days, and 12.4 days for the assumed number of generations 2, 3, and 4, respectively. As the assumed number of generations increased, the estimated mean and standard deviation became significantly smaller, reflecting that the explainable transmission dynamics greatly varied by the assumed number of generations. The reproduction numbers in the absence of interventions were greater than the value of 1 by generation 2 (i.e., R_1) for the three models, and by generation 3 (i.e., R_2) if the assumed number of generations was 4. It should be noted that if the number of generations was 2, then the reproduction number of generation 3 (i.e., R_2) was not estimated but must have been zero (owing to the absence of generation 3). It should be noted that the latest estimate of R_i was below the value of 1 and this was not surprising, because the epidemic came to an end in October 2014.

The mosquito control and public awareness campaigns from 28 August 2014 were considered to have definitely reduced transmission, with secondary transmission reduced by an estimated 30%–70%, with a wide confidence interval (Table 4). Using the AIC value as the weight, the ensemble estimates of ε_1 and ε_2 following model averaging over different assumed numbers of generations were 0.6 and 0.6, respectively. Despite these reductions, it appeared that the estimated effective reproduction number did not decline below the threshold value of 1 (Fig 4). However, park closure in combination with the mosquito control and awareness campaigns successfully reduced the reproduction number. The relative reduction in the effective reproduction number owing to the closure of Yoyogi Park was estimated to be 20%–60%, again with a wide confidence interval (Table 4); however, the combined effect was estimated to be as large as a 44%–88% reduction in the reproduction number. These findings were robust for the assumed number of generations.

Table 4. Estimates of the best models by number of generations.

Parameters	Two-generation model (G2)	Three-generation model (G3)	Four-generation model (G4)
d_0	7	9	8
μ_{IP}	5.8 (5.5, 6.0)	5.8 (5.5, 6.0)	5.8 (5.5, 6.1)
σ_{IP}	1.8 (1.6, 2.1)	1.8 (1.6, 2.1)	1.9 (1.6, 2.1)
μ_{Trans}	11.4 (9.1, 14.3)	10.3 (8.4, 12.6)	6.6 (5.8, 7.5)
σ_{Trans}	7.3 (5.5, 9.6)	4.9 (3.3, 7.2)	2.0 (1.2, 3.4)
R_0	15.1	13.6	4.0
R_1	9.3 (4.7, 18.6)	8.8 (4.5, 17.1)	5.4 (2.7, 10.5)
R_2	-	0.2 (0.1, 0.8)	4.3 (1.1, 16.8)
R_3	-	-	0.4 (0.2, 1.0)
ε_1	0.7 (0.3, 0.9)	0.7 (0.2, 1.0)	0.3 (0.1, 0.7)
ε_2	0.6 (0.2, 0.9)	0.8 (0.1, 1)	0.4 (0.1, 0.7)
MSE	2.9	2.8	2.6
AICc	1855.0	1856.0	1855.2

Note: Two-generation, three-generation, and four-generation models indicated that there were a total two, three, and four generations, excluding generation zero. Mean μ_{IP} and standard deviation σ_{IP} of the incubation period were assumed as the parameters governing the gamma distribution. Mean μ_{Trans} and standard deviation σ_{Trans} of the duration of the generation times, other than incubation period, were also assumed to follow a gamma distribution. R_{n-1} is the reproduction number of the n^{th}-generation infection in the absence of interventions. To account for the effectiveness of interventions, ε_i was factored into the model as the relative reduction in the reproduction number owing to mosquito control (ε_1) and park closure (ε_2). MSE represents the mean squared error. AICc is the Akaike information criterion with a correction for small sample size, calculated as AICc= AIC+ $(2k(k+1))/(n-k-1)$, where n is the number of data points (i.e., n=156) and AIC= $2NLL+2k$ where NLL is the negative log-likelihood and k is the number of parameters. The model average is the average of three former modes with weight as a function of AIC, which was calculated as 0.35, 0.25, and 0.40.

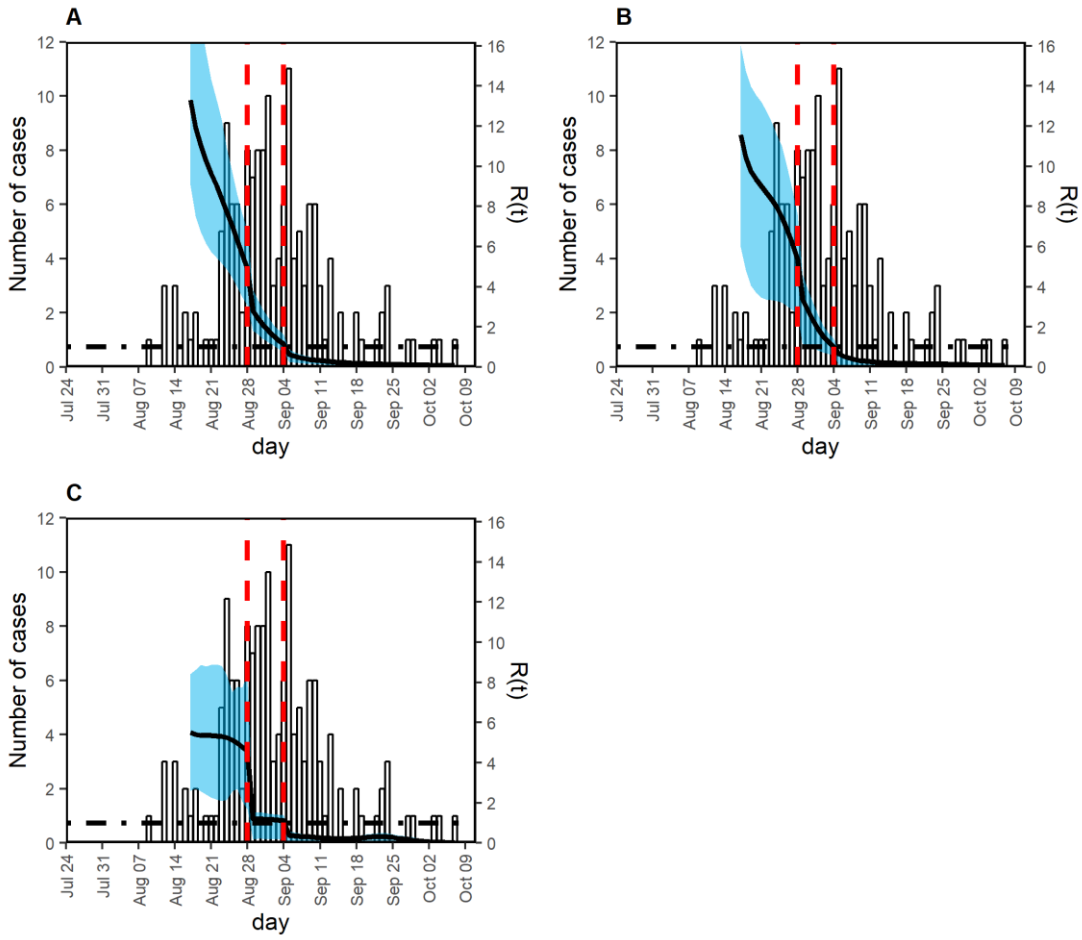

Fig 4. Effective reproduction number in three models with a different number of generations. Left vertical axis shows the observed number of cases (i.e., incidence as a function of the date of illness onset), and the right vertical axis shows the effective reproduction number, illustrated using a black solid line. Two red vertical dashed lines indicate times at which interventions started. The earlier one (from 28 August) included mosquito control and dissemination of outbreak information via mass media. The later vertical line indicates the date on which Yoyogi Park was closed. Black dot-dashed horizontal line indicates the threshold value 1 for the effective reproduction number, below which the outbreak will eventually be controlled. The effective reproduction number was estimated, assuming three different numbers of generations of infection, i.e., (A) two, (B) three, and (C) four generations excluding generation zero. Owing to the uncertainty of estimation during the very early stage of the epidemic, the effective reproduction number was plotted from 17 August. The shaded cyan area represents the 95% confidence interval (CI) of the effective reproduction number, calculated using 100 bootstrap samples.

We conducted model comparisons to assess the importance of accounting for the effectiveness of the abovementioned control measures. The sensitivity results are summarized in Table 5. It appeared that the latest estimate of the reproduction number was sensitive to the presence of effectiveness parameters (ε_1 and ε_2). Regardless of whether the assumed number of generations was 2, 3, or 4, AICc values of the model with both ε_1 and ε_2 were minimal (AICc=1855.1, 1856.0, and 1855.2 for models with 2, 3, and 4 generations, respectively), outperforming models without one or both effectiveness parameters. Figure 5 compares the reproduction number for two-generation model over time, hypothetically examining counterfactual scenarios in which the relative reduction in the reproduction number during park closure, ε_2 (and mosquito control, measured by ε_1) is assumed at 1. If ε_2 was 1, the time at which the reproduction number declines would be delayed, indicating that the park closure has played an important role in reducing the transmissibility.

Table 5. Comparison of model fit by the number of generations and four settings of hypothesized effectiveness measures.

	Parameters	Setting 1 ($\varepsilon_1 \neq 1$ and $\varepsilon_2 \neq 1$)	Setting 2 ($\varepsilon_1 = 1$ and $\varepsilon_2 \neq 1$)	Setting 3 ($\varepsilon_1 \neq 1$ and $\varepsilon_2 = 1$)	Setting 4 ($\varepsilon_1 = 1$ and $\varepsilon_2 = 1$)
Two-generation model (G2)	d_0	7	7	7	7
	μ_{IP}	5.8	5.8	5.8	5.8
	σ_{IP}	1.8	1.8	1.8	1.8
	μ_{Trans}	11.4	11.4	11.4	11.4
	σ_{Trans}	7.3	7.3	7.3	7.3
	R_0	15.1	15.1	15.1	15.1
	R_1	9.4	7.2	9.3	4.8
	R_2	-	-	-	-
	R_3	-	-	-	-
	ε_1	0.7	1.0	0.5	1.0
	ε_2	0.6	0.5	1.0	1.0
	AICc	1855.1	1858.7	1858.5	1871.6
Three-generation model (G3)	d_0	9	9	9	9
	μ_{IP}	5.8	5.8	5.8	5.8
	σ_{IP}	1.8	1.8	1.8	1.8
	μ_{Trans}	10.3	10.3	10.3	10.3
	σ_{Trans}	4.9	4.9	4.9	4.9
	R_0	13.6	13.6	13.6	13.6
	R_1	8.8	7.6	8.8	7.3
	R_2	0.2	0.2	0.1	0.1
	R_3	-	-	-	-
	ε_1	0.7	1.0	0.7	1.0
	ε_2	0.8	0.7	1.0	1.0
	AICc	1856.0	1858.0	1856.4	1859.5
	d_0	8	8	8	8
	μ_{IP}	5.8	5.8	5.8	5.8
	σ_{IP}	1.9	1.9	1.9	1.9
	μ_{Trans}	6.6	6.6	6.6	6.6
	σ_{Trans}	2.0	2.0	2.0	2.0
	R_0	4.0	4.0	4.0	4.0
	R_1	5.4	6.1	5.5	6.5

Four-	R_2	4.3	1.0	4.0	0.6
generation	R_3	0.4	0.4	0.2	0.2
model (G4)	ε_1	0.3	1.0	0.2	1.0
	ε_2	0.4	0.4	1.0	1.0
	AICc	1855.2	1858.0	1865.1	1864.9

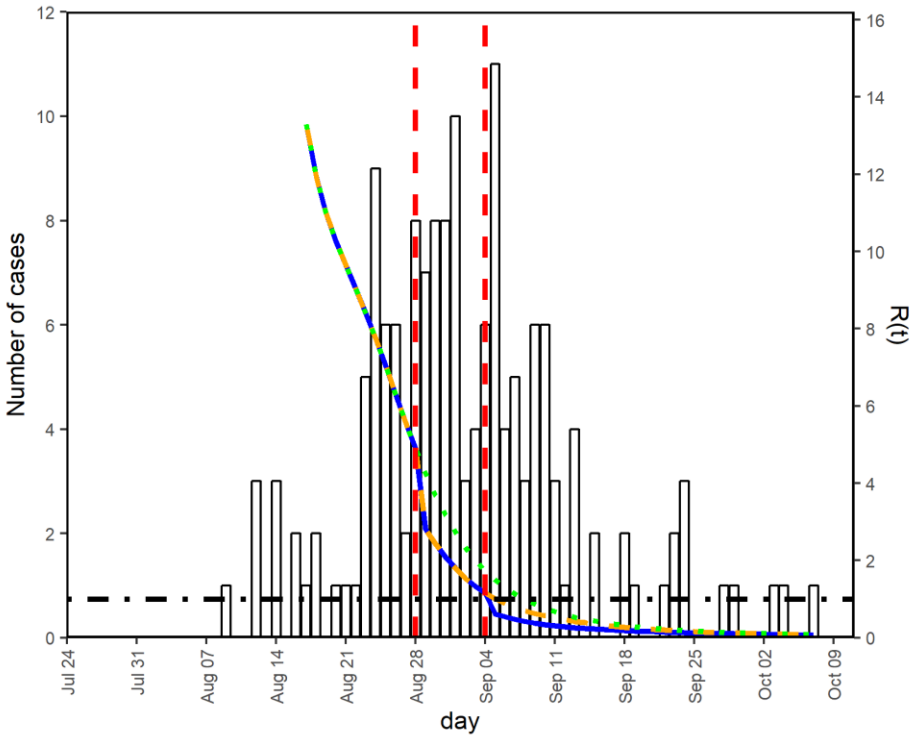

Figure 5. Effective reproduction number with two-generation model. Left vertical axis shows the observed number of cases (i.e., incidence as a function of the date of illness onset), and the right vertical axis shows the effective reproduction number, illustrated using a black solid line. Two red vertical dashed lines indicate times at which interventions started. The earlier one (from 28 August) included mosquito control and dissemination of outbreak information via mass media. The later vertical line indicates the date on which Yoyogi Park was closed. Black dot-dashed horizontal line indicates the threshold value 1 for the effective reproduction number, below which the outbreak will eventually be controlled. The effective reproduction number was estimated, assuming two generations of infection and using the estimated effectiveness values ε_1 and ε_2. Blue line shows the best fit, while dashed orange shows when ε_2 was artificially assumed as 1. Similarly, dashed light green line shows when both ε_1 and ε_2 were artificially assumed as 1.

1.5 Discussion

In the present study, we performed a retrospective epidemiological assessment of interventions to control the 2014 dengue outbreak in Tokyo, using a limited number of confirmed cases (n=160). Because the anticipated number of generations of infection was limited, and also because we sought to conduct careful evaluation of the causal association between the timing of interventions and the effective reproduction number R_t, we did not use the structured compartmental modeling approach (e.g., using ordinary differential equations). Instead, we developed a novel method to directly parameterize the incidence of infection, by convoluting the incidence of infection with the incubation period, allowing us to precisely incorporate the timing of interventions and observe their effect on virus transmission dynamics on a given day. The proposed method suitable for application (i) when the generation structure is imaginable from a published estimate of the mean generation time or visually identifiable from the observed epidemic curve, (ii) when the time of infection needs to be modeled in relation to the timing of interventions, and (iii) when the observed data include doubly interval-censored data. As a consequence of our analyses, the effectiveness of mosquito control, dengue risk communication to elevate public awareness, and closure of the focal area of transmission were objectively evaluated in relation to the effective reproduction number of DENV infection.

In practical terms, what the present study adds to the literature is that in the case of the 2014 dengue outbreak in Tokyo, all control measures that we explored (i.e., mosquito control, public awareness campaigns, and park closure) acted as essential factors governing the observed patterns of the epidemic. This notion is supported by our model comparisons in which both ε_1 and ε_2 were required to act as free parameters, to better describe the observed epidemic dynamics. Of these interventions, mosquito control and raising public awareness were not sufficiently effective to break the chain of transmission, as they maintained R_t>1, although the reproduction number exhibited a decreasing trend due to decrease in the observed incidence. Although mosquito control from 28 August 2014 was very intensive, DENV-positive *Aedes* were detected after these measures had been implemented (BSWPH, 2014). To fully halt virus transmission, the combined effect of mosquito control, public awareness campaigns, and park closure was needed for a substantial reduction in R_t; this joint reduction effect was estimated to be 44%–88%. Therefore, according to our model results, we can conclude that control of the dengue outbreak at local level in Tokyo was

essential to describe the empirically observed data and successful in reducing transmissions. It should be noted that the park closure effect includes not only the prevention of exposure among susceptible visitors but also removal of infectious hosts, including local residents of the park, from the focal area of transmission.

In developing the generation-based modeling approach, the proposed system was quantified with estimated mean generation time from 12 to 17 days. Considering the published length of the EIP (Chan, M. and Johansson, M.A., 2012; Nishiura, H., 2007; Tjaden, N.B. et al., 2013), with summer temperatures in metropolitan Tokyo above 30 ^{o}C in August, a mean generation time of about 2 weeks is regarded as a reasonable length or slightly shorter than the temperature-dependent estimate. Our estimate was consistent with an empirical estimate by Siraj et al. (Siraj, A. S. et al., 2017), indicating that a mean generation interval of 17 days occurs with the highest probability at 30 ^{o}C, although the estimate was obtained for *Aedes aegypti* and not for *Aedes albopictus*, the latter of which is abundant in Japan. The mean incubation period of 5.8 days is also consistent with the literature (IDSC, 2004), and we attained a finer estimation than that of Ishikawa et al. (VDCI); those authors used only known 67 intrinsic incubation periods for the estimation (with an estimated mean of 6.3 days), potentially resulting in a biased estimate of the variance owing to small sample size.

Although we imposed three different assumptions, i.e., three different numbers of generations, the resulting AIC values were comparable, and we were unable to select the best model. However, the model with two generations alone indicated that the outbreak came to an end with the latest estimate of a generation-dependent reproduction number (of generation 1) as large as 9.3; if this model result were true, the reproduction number of the subsequent generation (generation 2) had to abruptly drop to 0. As mentioned in the Results, regardless of the assumed number of generations, the latest estimate of R_i was below the value of 1 because the epidemic came to an end in October 2014. However, using the model with two generations, this had to happen quite abruptly, with a drop from 9.3 to 0. Using the model with four generations, the mean generation time had to be 12 days, allowing only about 6 to 7 days from illness onset in an infected person to EIP in a mosquito to biting a susceptible person. Thus, there was some interplay between the assumed number of generations and the resulting estimates, i.e., as the number of generations increased, the

generation time was estimated to be shorter. As long as we cannot specify the exact number of generations, the only approach to address relevant uncertainty is to use multiple models with different numbers of generations, to verify that our practical conclusions about the effectiveness of interventions would not change drastically by varying the number of generations.

The present study was not free from limitations. First, this study rested on confirmed dengue cases in patients with symptomatic illness who undertook testing. Febrile patients who had visited Yoyogi Park were advised to seek medical attention during the outbreak; however, a substantial number of asymptomatic infections would have been missed (Duong, V. et al., 2015), and the present evaluation was made based only on diagnosed cases. Thus far, we have failed to explicitly estimate the ascertainment rate or number of asymptomatic infections using the observed empirical data. Second, Yoyogi Park was undoubtedly the focal area of transmission, but later transmission occurred in other parks in the Kanto region. In addition to spatial heterogeneity, the exposure behaviors in those parks were not rigorously traced, leading to substantial uncertainty in the empirical data (i.e., many cases belonging to Group 3 in our likelihood), thus making later epidemic data difficult to be captured by our simple model. Third, qualitatively, local residents of Yoyogi Park were suspected of being amplifiers of transmission. However, it was not possible to trace the behavior of these infected individuals, e.g., when they left Yoyogi Park and where they went after leaving the park, including whether they moved to another park as a next destination, thus being responsible for causing subsequent cases. It must be remembered that our high estimate of the reproduction number in the early stage of the epidemic could have reflected the existence of superspreaders. Fourth, we ignored environmental and ecological factors in our model (Shang, C. S. et al., 2010; Zhang, Y. et al., 2016; Codeco, C.T. et al., 2018). The temperature mostly remained stable during the course of the 2014 outbreak; thus, the EIP can be assumed to be approximately stable. However, we ignored rainfall, which could have altered the population dynamics of *Aedes* species. Fifth, we examined only a limited number of assumed generations; theoretically, there could be six, seven, or an even greater number of generations explaining the observed epidemiological dynamics. However, considering the published EIP, it was implausible that there were six or more generations during the course of the 2014 outbreak.

Despite these limitations, we strongly believe that among the existing models, our model provides the most meticulous approach to account for the exact impact of the intervention start date in changing the dynamics of dengue infection over time, because we directly modeled the time of infection in relation to the timing of interventions while maximally using interval-censored data of exposure and illness onset among cases. We successfully captured that impact using convolution of the incidence and the incubation period. By combining mosquito control, public awareness campaigns, and park closure, dengue control during the 2014 outbreak in Tokyo was highly successful. Should a similar event happen in the future, concerted efforts including similarly combined interventions, accompanied by identification of the location of exposure, should be implemented.

Conclusions and Prospects

To understand the current epidemic risk of dengue fever in Japan, two pieces of work were done. Chapter 1 evaluated the importation risk of dengue cases in Japan from the year 2006 to 2016. Chapter 2 retrospectively assessed the effectiveness of interventions implemented in the epidemic. All the resulting findings are from mathematical modeling or statistical analysis under reasonable assumptions and I believe the results contribute to improve understandings of the epidemiological parameters of dengue virus disease.

i. The force of infection was estimated for eight countries which accounts for more than 80% of all imported cases in Japan: Indonesia, the Philippines, Thailand, India, Malaysia, Vietnam, Sri Lanka and Singapore. The presented value ranging from 0.02 to 0.17 help us understand the relative risk of dengue infection in these countries. The local transmission level in the Philippines and Indonesia are the highest, so that Japanese travelers should consider self-protection (e.g., to use mosquito repellent) when they visit these high-risk countries.

ii. The country-specific reporting coverages vary greatly from 0.6% to 4.3%. This number tells us the reported number of imported cases is just the tip of the iceberg, with many unnotified infections among Japanese travelers. The real importation risk of dengue infections is much larger than what can be observed. Thereby, policymakers must be cautious in interpreting such case data.

iii. Two interval distributions of epidemiological significance were obtained by maximizing data use. The incubation period was estimated at mean 5.8 days (SD: 1.8 days). The mean generation time ranges from 12.4 days to 17.2 days if the total generation number is assumed to be two, three or four. These two parameters are of great significance to describe the transmission dynamics in epidemiology and the values here are in line with other estimates (Chan, M. and Johansson, M.A., 2012).

iv. Evaluating the interventions implemented during an outbreak of mosquito-borne disease is of utmost importance, offering lessons for future control strategies. In the Tokyo dengue outbreak, three combined interventions took place, i.e., mosquito control and news dissemination at early time and the subsequent closure of Yoyogi park. We revealed that the first-phase actions alone could not interrupt the chain of transmission; however, adding park

closure to these interventions was substantially effective in reducing the number of transmissions.

To minimize the probability of the next autochthonous dengue outbreak, a systematic epidemiological surveillance is very necessary. This involves the effective management of imported dengue cases (e.g. carrying out the epidemiological survey for returning Japanese or inbound foreigners from high-risk areas during high-incidence season of dengue fever) and a routine monitoring of Aedes mosquitos in urban green space. Identifying the geographical distribution of dengue-related risk in central Tokyo is our ongoing project. The hypothesis for doing this exercise is that the foreign travelers are likely to bring dengue virus into the dengue-free area if the infectious people have experienced being bitten by mosquito during their stay. By using the mobile phone data which provides information about the visited locations of foreign travelers in Tokyo, we aim to explore the movement pattern of travelers from various countries at different risk of dengue fever and so as to identify the area in Tokyo with highest risk of dengue outbreak.

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