

Monitoring and Distribution of Mosquitoes in the Downtown Areas and Migratory Bird Habitats in the Jeonbuk Region, 2021

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Abstract: This survey was performed to monitor the spread of specific mosquito-borne pathogens at Jeonbuk. The frequency of occurrence of mosquito-borne pathogens including Japanese encephalitis virus, West Nile virus, Zika virus, and yellow fever virus was assessed by collecting mosquitoes twice a month from March to December 2021 from various areas in Jeonbuk. A total of 15,975 mosquitoes from 15 species and 7 genera were collected. The highest number of 9,116 mosquitoes (trap index: TI, 506.4) were collected in the Wanju cowshed, followed by the habitat for migratory birds and the downtown area in Jeonju. In the Gunsan habitat for migratory birds, 3,217 mosquitoes (TI, 178.7) were collected in the reed fields, 356 (TI, 19.7) in the men's toilets, and 1,948 (TI, 108.2) in the women's toilets. In Jeonju, 677 mosquitoes (TI, 37.6) were collected in Deokjin Park, 358 (TI, 19.8) at Deokjin-gu Office, and 303 (TI, 16.8) at Jeonbuk National University. The largest population of mosquitoes was collected in the men's toilets in Gunsan and Deokjin Park in downtown Jeonju. RT-PCR was performed to determine the pathogen infection of the collected mosquitoes. The results showed that the mosquitoes were negative for pathogen infection. These results provide a basis for tackling mosquito-borne diseases in the Jeonbuk region.

Keywords: Flavivirus; Japanese encephalitis; Malaria; Mosquitoes

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1. Introduction

There are 3,209 species of mosquitoes in the world, and 54 species of mosquitoes are reported in Korea ^[1]. Flavivirus refers to a genus, a sub-biological classification of the Flaviviridae family, and representative species of flaviviruses include Zika virus, Dengue virus, Japanese encephalitis virus, yellow fever virus, West Nile virus, and St. Louis encephalitis virus), among others ^[2,3].

The hosts of flaviviruses are mosquitoes and humans, which means that humans can be infected by the bite of a flavivirus-infected mosquito. Recent studies have shown that flaviviruses can be transmitted to humans not only from mosquitoes, but also through blood transfusions and sexual intercourse ^[4,5].

In recent years, the increase in temperature of subtropical areas around the world due to global warming has expanded the habitat of the *Aedes albopictus* and *Aedes aegypti* mosquitoes, the host mosquitoes of Zika and dengue viruses, respectively [6,7]. In addition, the habitat of the *Culex tritaeniorhynchus* mosquito, the host mosquito of Japanese encephalitis virus, is also expanding [8].

As the distribution of vectors is expanding due to climate change, the incidence of endemic diseases such as Japanese encephalitis is increasing, and the possibility of introducing overseas mosquito-borne infectious diseases (dengue fever, Zika virus infection, West Nile fever, etc.) is also gradually increasing due to the rapid increase in overseas travel and international exchanges.

The major mosquito-borne infectious diseases in Korea are malaria transmitted by *Anopheles spp.* and Japanese encephalitis transmitted by *Culex tritaeniorhynchus*. While there have not been any instances of domestic infections, the number of cases of dengue fever, Zika virus infection, and chikungunya fever in Korea has been steadily rising each year among individuals who contracted these diseases abroad [9]. In addition, the possibility of domestic outbreaks of these infectious diseases is always present because mosquitoes capable of transmitting these diseases live in Korea. Therefore, preemptive surveillance of the seasonal occurrence of mosquitoes and pathogen infection is very important [10].

In this study, we investigated the seasonal occurrence of mosquitoes and the infection rates of representative mosquito-borne infectious viruses (Japanese encephalitis virus, yellow fever virus, dengue virus, West Nile virus, and other flaviviruses) by collecting mosquitoes from urban centers and migratory sites in three districts (Jeonju, Gunsan, and Wanju counties) of Jeollabuk-do, which operates a regional center in Jeollabuk-do, to provide basic data for efficient epidemic prediction and control measures for mosquito-borne infectious diseases.

2. Materials and methods

2.1. When and where to collect

Infectious disease-carrying mosquitoes were collected for 9 months from March to November 2020 in Jeollabuk-do. The mosquitoes were collected from a total of 7 sites in Hwasan-myeon in Wanju-gun, Geumcheolbird Observatory in Gunsan-si, and Deokjin-gu Office, Deokjin Park, and Chonbuk National University in Jeonju. Black light traps and BG sentinel traps were installed at each site to attract and collect mosquitoes, and they were collected from before dusk to dawn (6 p.m. to 6 a.m. the next day). Mosquitoes were collected once a week in the barns, twice a month in the city center, and during the migratory season in bird migratory areas (Table 1).

Table 1. Area and environment of collecting mosquitoes

Sites	Environments	Trapping method	Latitude and longitude
Barn	Cowshed	BL	N36.001138°, E127.223671°
Toilet (Men's)	Bird sanctuary	BL	N36.019929°, E126.765432°
Reed forest of migratory birds		BG	N36.019929°, E126.765432°
Toilet (Women's)		BL	N36.019929°, E126.765432°
Jeonbuk National University	City	BL	N35.844434°, E127.129235°
Deokjin-gu Office		BL	N35.829629°, E127.134519°
Deokjin Park		BG	N35.847955°, E127.121865°

2.2. Classification and identification of mosquitoes

The collected mosquitoes were brought to the laboratory intact and frozen until classification and identification. The mosquitoes were classified and identified through observation under a stereomicroscope (SZ61, Olympus, Japan) based on a morphological classification table for mosquito species ^[11,12]. The trap index (TI) of the collected mosquitoes was calculated by dividing the number of collected mosquitoes by the number of days and traps.

2.3. Identification of the pathogen of the mosquitoes

The collected mosquitoes were morphologically sorted and identified, and for pathogen identification, female mosquitoes separated by species were placed in 2 mL sterile lysis tubes (MK28 Precellys[®] Lysing kits, Bertin Technologies SAS, Montigny-Le-Bretonneux, France) with 1 to 50 mosquitoes and 1.2 mL of phosphate-buffered saline (PBS) in each tube. The contents were then homogenized using an automated homogenizer (Precellys24, Bertin Technologies SAS) at 5,000 rpm for 30 seconds, twice. Subsequently, the tube was removed and placed on ice for 5 minutes. Then, the tubes were placed in a refrigerated centrifuge and centrifuged at 4°C, 13,000 rpm for 1 minute. The supernatant was separated and the genetic material was extracted.

RNA isolation was performed using Clear-S[™] total RNA extraction kit (Invirustech, Gwangju, Korea), and flavivirus detection was performed using Clear-MD[™] flavivirus RT-PCR detection kit (Invirustech, Gwangju, Korea). The primary reagent was composed of 10 µL 2xqRT-PCR Green Master Mix, 2 µL primer mix, 5 µL template RNA, and 3 µL water to a total volume of 20 µL. The reagents were mixed well and centrifuged (spin-down). PCR was then performed according to the conditions in **Table 2**, and the amplification curve and melting curve were read by analyzing the positive control. If a melting curve was not observed around 80–84°C, it was considered as negative, and if it was positive, the positive band was confirmed by electrophoresis after amplification with primers and sequenced (**Table 3**).

Table 2. RT-PCR conditions and melt curve for the diagnosis of flaviviruses

Cycle	Step	Temperature	Time
1 cycle	Reverse transcription	50°C	15 min
	Enzyme activation	95°C	10 min
40 cycles	Denaturation	95°C	10 sec
	Annealing	60°C	15 sec
	Extension	72°C	10 sec
	Signal reading	80°C	30 sec
	Denaturation	95°C	30 sec
	Melting*	60–95°C	15 sec
	Cooling	25°C	30 min

Table 3. Primer sequence of 2nd PCR for diagnosis of flavivirus

Name	Sequence (5' to 3')
M13 Forward	TGTAACGACGGCCAGT
M13 Reverse	CAGGAAACAGCTATGAC

3. Result

3.1. Mosquito collection results

The total number of mosquitoes collected was 15,975 with 7 genera and 15 species. By environment, 9,116 mosquitoes (Trap Index: TI.506.4) were collected in the Wanju barn, followed by the migratory bird arrival site and Jeonju city center. In Gunsan, 3,217 birds (TI. 178.7) were collected in the reed forest of the migratory bird sanctuary, 356 birds (TI.19.7) in the men’s restroom, and 1,948 birds (TI.108.2) in the women’s restroom. In Jeonju, 677 birds (TI 37.6) were collected at Deokjin Park, 358 birds (TI. 19.8) at Deokjin-gu Office, and 303 birds (TI. 16.8) at Chonbuk National University. The highest number of individuals were collected at the women’s restroom in the Gunsan migratory flyway and at Deokjin Park in Jeonju city center (**Table 4**).

Table 4. Total number of female mosquitoes collected and trap indices at cattle sheds, bird sanctuaries, and downtown areas in the Jeonbuk region

	Locations	Trap	Number of female mosquitoes	Duration of trap placement (nights)	TI
Gunsan	Reed forest of migratory bird sanctuary	BG	3,217	18	178.7
	Toilet (Men’s)	BL	356	18	19.7
	Toilet (Women’s)	BL	1,948	18	108.2
Wanju	Cowshed	BL	9,116	18	506.4
Jeonju	Deokjin Park	BG	677	18	37.6
	Deokjin-gu Office	BL	358	18	19.8
	Jeonbuk National University	BL	303	18	16.8
	Cowshed		15,975	126	126.7

Based on **Table 4**, speckled-winged mosquitoes (*Anopheles spp.*) were the dominant species with 10,364 individuals (64.9%), followed by red house mosquitoes (*Culex pipiens pallens*) with 2,572 individuals (16.1%), golden forest mosquitoes (*Aedes vexans*) with 1,189 individuals (7.5%), speckled-winged swamp mosquitoes (*Mansonia uniformis*) with 604 individuals (3.8%), big black mosquitoes (*Armigeres subalbatus*) with 516 individuals (3.3%), little red house mosquitoes (*Culex tritaeniorhynchus*) with 225 individuals (1.4%), white-banded forest mosquitoes (*Aedes albopictus*) with 167 individuals (1.1%), yellow swamp mosquitoes (*Coquillettidia ochracea*) with 98 individuals (0.6%), spotted winged house mosquitoes (*Culex bitaeniorhynchus*) with 83 individuals (0.5%), Korean forest mosquito (*Ochlerotatus koreicus*) with 82 individuals (0.5%), oriental house mosquito (*Culex orientalis*) 50 (0.3%), golden shoulder forest mosquito (*Aedes lineatopennis*) with 22 individuals (0%), dorsal forest mosquito (*Ochlerotatus dorsalis*) with 3 individuals (0%), white back forest mosquito (*Aedes alboscuteallatus*) with 3 individuals (0%), and Hatori forest mosquito (*Ochlerotatus hatorii*) with only 1 individual (0%).

The dominant species, *Anopheles spp.* began to be collected in March and peaked in July with 8,127 individuals (TI. 580.5), followed by 1,434 individuals (TI. 102.4) in August, and 735 individuals (TI. 52.5) in June. The next most abundant species, *Culex pipiens pallens*, was collected from March through November, with a high abundance of 479 (TI. 34.2) in July, followed by a gradual increase in abundance to 575 (TI. 41.1) in August and 683 (TI. 48.8) in September (**Tables 5 and 6**).

Table 5. Total number of female mosquitoes collected at Jeonbuk region with black light trap and BG from March to November 2021

Species collected	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Total
<i>Ae. albopictus</i>	0	0	0	6	48	45	58	9	1	167 (1.1%)
<i>Ae. alboscuteallatus</i>	0	0	0	0	1	0	2	0	0	3 (0%)
<i>Ae. lineatopennis</i>	0	0	0	0	18	0	4	0	0	22 (0%)
<i>Ae. vexans</i>	0	0	4	440	455	74	213	3	0	1,189 (7.5%)
<i>Anopheles spp.</i>	2	14	5	735	8,127	1,434	5	2	0	10,364 (64.9%)
<i>Ar. subaltatus</i>	0	0	0	3	17	145	173	173	5	516 (3.3%)
<i>Coq. ochracea</i>	0	0	0	1	26	35	36	0	0	98 (0.6%)
<i>Cx. bitaeniorhynchus</i>	0	0	0	22	33	6	20	2	0	83 (0.5%)
<i>Cx. orientalis</i>	0	0	3	4	22	7	13	1	0	50 (0.3%)
<i>Cx. pipiens pallens</i>	2	36	214	397	479	575	683	1010	85	2,572 (16.1%)
<i>Cx. tritaeniorhynchus</i>	0	0	0	0	0	80	141	4	0	225 (1.4%)
<i>Man. uniformis</i>	0	0	0	317	54	108	125	0	0	604 (3.8%)
<i>Och. dorsalis</i>	0	0	0	1	0	0	1	1	0	3 (0%)
<i>Och. hatorii</i>	0	0	0	1	0	0	0	0	0	1 (0%)
<i>Och. koreicus</i>	0	2	6	8	32	16	12	5	1	82 (0.5%)
Total	4	52	228	1,935	9,312	2,525	1,526	301	92	15,975 (100%)

Table 6. Trap index I(TI) of female mosquitoes collected at Jeonbuk region with black light trap and BG from March to November 2021

Species collected	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	TI
<i>Ae. albopictus</i>	0	0	0	0.4	3.4	3.2	4.1	0.6	0.1	11.9
<i>Ae. vexans</i>	0	0	0.3	31.4	32.5	5.3	15.2	0.2	0	84.9
<i>Ae. alboscuteallatus</i>	0	0	0	0	0.1	0	0.1	0	0	0.2
<i>Ae. lineatopennis</i>	0	0	0	0	1.3	0	0.3	0	0	1.6
<i>Anopheles spp.</i>	0.1	1	0.4	52.5	580.5	102.4	3.2	0.1	0	740.3
<i>Ar. subalbatus</i>	0	0	0	0.2	1.2	10.4	12.4	12.4	0.4	36.9
<i>Coq. ochracea</i>	0	0	0	0.1	1.9	2.5	2.6	0	0	7
<i>Cx. orientalis</i>	0	0	0.2	0.3	1.6	0.5	0.9	0.1	0	3.6
<i>Cx. pipiens pallens</i>	0.1	2.6	15.3	28.4	34.2	41.1	48.8	7.2	6.1	183.7
<i>Cx. tritaeniorhynchus</i>	0	0	0	0	0	5.7	10.1	0.3	0	16.1
<i>Cx. bitaeniorhynchus</i>	0	0	0	1.6	2.4	0.4	1.4	0.1	0	5.9
<i>Man. uniformis</i>	0	0	0	22.6	3.9	7.7	8.9	0	0	43.1
<i>Och. dorsalis</i>	0	0	0	0.1	0	0	0.1	0.1	0	0.2
<i>Och. koreicus</i>	0	0.1	0.4	2.6	2.3	1.1	0.9	0.4	0.1	5.9
<i>Och. hatorii</i>	0	0	0	0.1	0	0	0	0	0	0.1
TI	0.3	3.7	16.3	138	665	180	109	21.5	6.6	0

3.2. Pathogen surveillance

15,975 mosquitoes (527 pools) collected from March through November 2021 were pooled and tested for flavivirus carriage and were all negative (**Figure 1**).

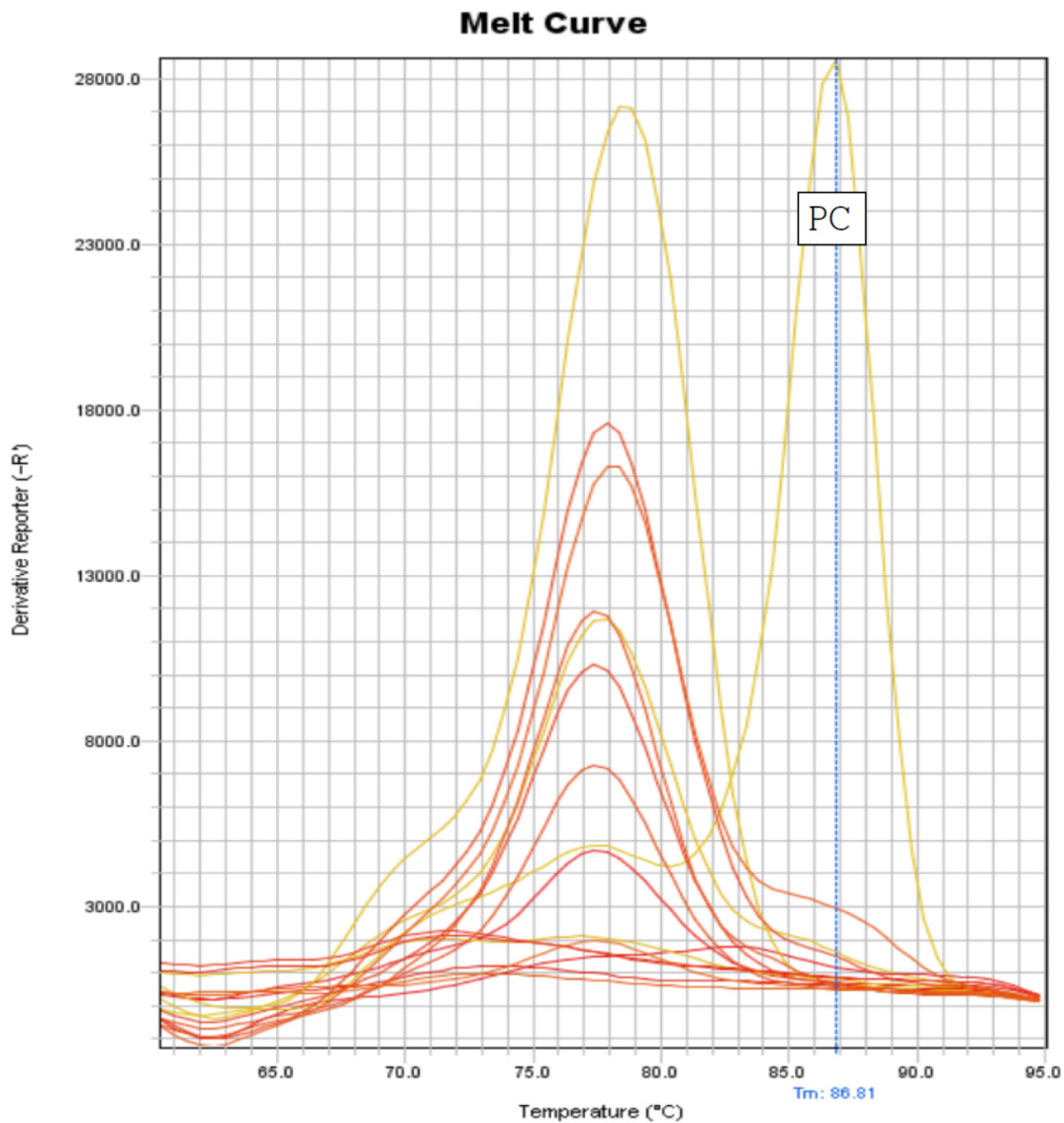


Figure 1. Melting curve peak of RT-PCR for flavivirus detection. Peaks above 84°C indicate positivity. All specimens were confirmed negative as the peaks were all below 84°C. Abbreviation: PC, positive control.

4. Discussion

Global warming is expected to increase the survival rate of overwintering mosquitoes and mosquito eggs in the winter. This leads to the early emergence of overwintering mosquitoes in spring, which increases the likelihood of vector-borne diseases occurring earlier due to climate change. Furthermore, concerns have been raised about the increase in mosquito populations due to issues such as changes in ecosystems in areas where Japanese encephalitis and malaria vector mosquitoes are prevalent, also influenced by climate change^[13].

With climate change, the distribution of vectors is expanding, leading to the increasing occurrence of endemic diseases such as Japanese encephalitis, and the possibility of introducing overseas mosquito-borne infectious diseases (dengue, Zika virus infection, West Nile fever, etc.) is also increasing due to the rapid

increase in international travel and exchange^[14]. A study reported that 3.9 billion people in 128 countries are at risk of dengue virus infection^[15].

A total of 15,975 female mosquitoes were collected from seven locations in Jeonbuk Province from March to November 2021. There were seven genera and 15 species of mosquitoes collected. The number of mosquitoes is much higher compared to the 8,234 mosquitoes collected in 2019, and 3 more species were found compared to the 12 species in 2019. By environment, 9,116 birds (TI. 506.4) were collected in the Wanju barn, followed by the bird migratory site and Jeonju city center. In Gunsan, the largest number of birds were collected in the women's toilet, and in Jeonju, the largest number of birds were collected in Deokjin Park (**Tables 4 and 5**). In 2014, collections in the Jeonbuk region were in the order of urban areas, rural areas, and bird migratory sites. However, in 2015 and 2019, the highest number of collections were consistently made at Wanju barn, and in the forested area of Deokjin Park in the city center of Jeonju, aligning with the findings of this study. The number of collections in urban areas like Jeonbuk University and Deokjin-gu Office was lower. This may be attributed to the surrounding maintenance projects and intensive disinfection activities due to the increasing interest in public health. In addition, the barn had the highest TI of 506.4, which means that cattle breeding contributes to mosquito proliferation. Therefore, understanding the blood-feeding and resting habits of mosquitoes in such areas is crucial for environmental improvement and proactive preventive measures against the outbreak of infectious diseases.

In terms of timing, in 2019, the highest mosquito collection months were July, June, and August, whereas in 2021, in the Jeonbuk region, the sequence was July, August, and June (Table 6). This can be attributed to climate change factors, including the monsoon season. The unusually high temperatures and reduced rainfall in July and August are considered factors contributing to the decrease in mosquito populations.

In 2019, the results of the same regional survey in Jeonbuk province were similar to those of this study, with *Anopheles spp.* being the dominant species at 2,538 (30.9%), followed by *Aedes vexans* at 1,709 (20.7%) and *Culex pipiens pallens* at 1,579 (19.2%), showing some changes in the collection rate of existing individuals. This is believed to be due to differences in the environment of collection and climate change. *Anopheles spp.*, the dominant species, started to be collected in March and was collected the most in July, August, and June, and there was a sharp decrease from September onwards. *Culex pipiens pallens*, the next most abundant species, was collected from March to November, with a peak in September and a gradual decrease in the following months. In July, 8,127 *Anopheles spp.* were collected (TI, 580.5), followed by 479 *Culex pipiens pallens* (TI, 34.2) and 455 *Aedes vexans* (TI, 32.5). *Anopheles spp.* were most abundant in January with 735 individuals (TI, 52.5), followed by 397 *Culex pipiens pallens* mosquitoes (TI, 28.4), 440 *Aedes vexans* (TI, 31.4), and 317 (TI, 22.6) *Mansonia uniformis* (**Table 6**).

Anopheles spp. mosquitoes, which transmit malaria, were the dominant species with 10,364 (65%) of the total collected mosquitoes, and as malaria continues to occur in the northern part of Gyeonggi Province, Korea, efforts should be made to control and prevent malaria.

According to a study by Jegal et al.^[16] based on the collection results in Hwaseong of Gyeonggi-do and urban areas in Incheon from 2016 to 2018, the most commonly isolated mosquito species was *Aedes vexans*, accounting for 49.13% (17,333/35,280). This was followed by *Culex pipiens pallens* (31.78%), *Ochlerotatus dorsalis* (13.62%), *Ochlerotatus koreicus* (1.68%), and *Culex tritaeniorhynchus* (1.50%). These findings contrast with the results of this current study.

Besides, in a study by Kim et al.^[17] in 2015, *Aedes vexans* (50.7%) was the most commonly isolated mosquito, followed by *Culex tritaeniorhynchus* (36.6%), *Culex pipiens* (7.5%), *Ochlerotatus dorsalis* (2.1%), and *Culex bitaeniorhynchus* (1.3%), and *Culex tritaeniorhynchus* (36.6%), which was in contrast to the study by Jegal et al.^[16] and this study.

Culex tritaeniorhynchus is a major vector worldwide, including in Asia. *Culex vishnui* and *Culex bitaeniorhynchus* have recently been implicated as vectors of Japanese encephalitis in Korea, India, and other parts of the world^[18,19].

In 2019, Seo and Chung^[20] reported the density and distribution of mosquito populations in Jeju Island in 2018, and the total number of mosquitoes collected was 1,847 mosquitoes belonging to 6 genera and 12 species, while the total number of mosquitoes collected in this study was 15,975 mosquitoes belonging to 7 genera and 15 species. In terms of the types of mosquitoes collected in Jeju, *Culex pipiens pallens* was the dominant species with 1,421 individuals (76.9%), and *Aedes albopictus* with 164 individuals (8.9%), which was in contrast to the results of this study. This may be due to differences in the collection environment, regional climate, and temperature. However, the ranking by collection area was consistent with our results, in the order of livestock farms, migratory sites, and Seogwipo.

Culex pipiens pallens, compared to other mosquito species, is believed to exhibit some variation in its habitat and breeding locations due to the effects of global warming, depending on the region.

The virus infection status of the mosquitoes collected this time yielded negative results, confirming the absence of detected viruses. However, several researchers have reported negative results as well. Nevertheless, this study's results report the presence of species that can transmit Japanese encephalitis, such as *Culex tritaeniorhynchus*, dengue fever by *Aedes albopictus*, malaria by *Anopheles spp.*, and West Nile virus by *Culex pipiens*. Considering the expanding distribution of disease vectors and the increased possibility of vector introduction due to global warming, this study aimed to provide fundamental data for epidemic prediction and management strategies based on mosquito distribution in the Jeollabuk-do region.

5. Summary

This survey investigated mosquitoes as vectors for Japanese encephalitis, malaria, West Nile virus, Zika virus, and yellow fever in the Jeollabuk-do region. From March to December 2021, mosquitoes were collected twice a month to monitor the occurrence frequency of disease vectors and pathogens. The total collection consisted of 15,975 individuals from 15 species within seven genera. The majority, 9,116 individuals (TI, 506.4), were collected in Wanju barn, followed by bird migratory sites and downtown Jeonju. In Gunsan's bird migratory site, 3,217 individuals were collected from the reed forest in the bird protection area (TI, 178.7), 356 individuals in the men's toilet (TI, 19.7), and 1,948 individuals in the women's toilet (TI, 108.2). In downtown Jeonju, 677 individuals were collected in Deokjin Park (TI, 37.6), 358 individuals near Deokjin-gu Office (TI, 19.8), and 303 individuals near Jeonbuk University (TI, 16.8). The women's restroom in Gunsan's migratory bird habitat and Deokjin Park in downtown Jeonju yielded the highest number of individuals. All pathogen test results were negative. This research provides essential data for disease prevention and control of mosquito-borne diseases in the Jeollabuk-do region.

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Disclosure statement

The authors declare no conflict of interest.

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