



Analysis of Five Arboviruses and *Culicoides* Distribution on Cattle Farms in Jeollabuk-do, Korea

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Abstract: Arthropod-borne viruses (Arboviruses) are transmitted by arthropods such as *Culicoides* biting midges and cause abortion, stillbirth, and congenital malformation in ruminants, apparently leading to economic losses to farmers. To monitor the distribution of *Culicoides* and to determine their relationship with different environmental conditions (temperature, humidity, wind speed, and altitude of the farms) on 5 cattle farms, *Culicoides* were collected during summer season (May-September) in 2016 and 2017, and analyzed for identification of species and detection of arboviruses. About 35% of the *Culicoides* were collected in July and the collection rate increased with increase in temperature and humidity. The higher altitude where the farms were located, the more *Culicoides* were collected on inside than outside. In antigen test of *Culicoides* against 5 arboviruses, only Chuzan virus (CHUV) (2.63%) was detected in 2016. The Akabane virus (AKAV), CHUV, Ibaraki virus and Bovine ephemeral fever virus (BEFV) had a positive rate of less than 1.8% in 2017. In antigen test of bovine whole blood, AKAV (12.96%) and BEFV (0.96%) were positive in only one of the farms. As a result of serum neutralization test, antibodies against AKAV were generally measured in all the farms. These results suggest that vaccination before the season in which the *Culicoides* are active is probably best to prevent arbovirus infections.

Key words: *Culicoides* biting midges, arbovirus, akabane virus, cattle, Korea

INTRODUCTION

Culicoides biting midges (Diptera: Ceratopogonidae) are the smallest haematophagous insects, which transmit the fatal virus to ruminants [1]. More than 1,400 species of *Culicoides* have been described and present on all land with the exception of Antarctica and New Zealand [1,2]. A size of *Culicoides* is around 1-3 mm, and they have a complete metamorphosis life cycle going through the egg, larva, pupa, and adult stages [1,3]. Females preparing to spawn after mating with males suck up blood of human or animal to get animal protein at dawn or dusk [1,3]. Breeding sites of *Culicoides* are watery and nutritious streams that can help them grow and develop larvae [4]. Adult *Culicoides* have different wing patterns based on species, which helps in easy identification of different species [5].

Arthropod-borne viruses (arboviruses) are major pathogens

in the veterinary field and are transmitted by arthropods such as *Culicoides* biting midges [1]. Arboviruses infected with ruminants cause abortion, stillbirth, and congenital malformation [6,7]. Akabane virus (AKAV) and Aino virus (AINOV) belong to the family *Peribunyaviridae* [1,8]. Sporadic outbreaks of AKAV have been reported widely in Japan, Southeast Asia and the Middle East, including Korea [9-12]. In Japan, JaGAR39, the prototype of AKAV, was first isolated in 1959 [9]. In Korea, a case of AKAV was first reported in 1980 [12]. In 2010, meningitis associated with AKAV infection occurred in a large-scale in South Korea [13]. More than 500 cattle exhibited symptoms such as movement disorder, trembling, and in severe cases, the cattle exhibited an inability to stand. Chuzan virus (CHUV) and Ibaraki virus (IBAV) are members of the family *Reoviridae* [1,14]. CHUV was first emerged in Japan, 1985, and the clinical signs are neurological abnormalities and hydranencephaly [15]. IBAV infection is characterized by fever, salivation, anorexia, and a deglutitive disorder in cattle. Symptoms except deglutitive disorder with dysphagia are usually mild. Bovine ephemeral fever virus (BEFV) grouped in the family *Rhabdoviridae* is similar to the IBAV, which is characterized by acute fever [14]. High fever, nasal discharges, salivation, and inability to stand

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appear to the principal signs of cattle infected with BEFV.

The surface temperature of the earth is rising continuously due to global warming, which is expected to rise further [3]. Temperature is one of the environmental factors that affect the activity of poikilothermic *Culicoides* [16,17]. Viruses that spread faster due to increased vector activity in warmer atmospheric temperatures result in severe economic losses in the industrial animal field. The aim of this study was to investigate the *Culicoides* distribution in different cattle farms in the area where encephalomyelitis occurred on a large-scale in 2010, and environmental conditions which affect the *Culicoides* abundance.

MATERIALS AND METHODS

Collection and identification of *Culicoides* biting midges

Culicoides biting midges were collected once (2016) or twice (2017) per month using a light trap (SNC, Hanam, Korea) from 5 different cattle farms located in Gimje (35°50'34.4389" N; 126°51'21.3368" E), Gochang (35°24'42.0937" N; 126°39'05.0940" E), Wanju (36°03'20.7356" N; 127°11'12.1909" E), Jinan (35°51'17.6353" N; 127°20'19.2646" E) and Namwon (35°29'32.7782" N; 127°38'42.2509" E) areas, Jeollabuk Province from May to September in 2016 and 2017 (Supplementary Fig. S1). Each region differed in altitudes (about 50 m, 50 m, 300 m, 500 m and 600 m respectively).

Used light trap consisted of 8 W UV fluorescent light with a downdraft suction fan. The traps were located inside and outside (about 200 m from breeding farms) the farms to compare the differences in collection rates. The traps were set in the afternoon before sunset and were collected the next morning within 1 hr after sunrise. Collected *Culicoides* samples were sorted morphologically into species according to their wing patterns observed under the microscope [18,19]. After classification, *Culicoides* (-30) were pooled into one sample based on

collection date and species.

During June and July, whole blood of cattle (over six months) was drawn from the jugular vein using 10 ml syringes. Collected samples were into vacuum tubes treated anticoagulant, ethylenediaminetetraacetic acid (EDTA), and mixed.

Measurement of temperature, humidity, and wind speed

The temperature and humidity were measured using a digital thermo-hygrometer machine (LAVISEN, Namyangju, Korea) and the wind speed data were obtained through the Korea Meteorological Administration (<http://www.kma.go.kr>).

DNA/RNA extraction and Reverse transcription PCR analysis

Pooled *Culicoides* samples were homogenized using a Hand-held Pestle Cordless Motor (BIOFACT, Daejeon, Korea) with 500 µl of Dulbecco's Modified Eagle's Medium (DMEM). Homogenized samples were centrifuged at 13,000 rpm for 10 min at 4°C and the supernatant was used for DNA/RNA extraction using a Patho Gene-spin™ DNA/RNA Extraction Kit (iNtRON, Seongnam, Korea) according to the manufacturer's instructions. Bovine whole blood was centrifuged at 13,000 rpm for 5 min to get buffy coat and plasma. Collected supernatant was used for virus DNA/RNA extraction using a MinElute Virus Spin Kit (QIAamp, Hilden, Germany).

Extracted *Culicoides* and blood DNA/RNA samples were tested to detect 5 arboviruses (AKAV, AINOV, CHUV, IBAV and BEFV) using VDX® Single RT-PCR Kit (MEDIAN Diagnostics, Chuncheon, Korea). Table 1 showed the information including primer sequences used, target gene, and size. PCR program is as follows: cDNA synthesis at 50°C for 30 min and initial inactivation at 95°C for 15 min in the first cycle followed by 40 cycles of denaturation at 94°C for 20 sec, annealing at 55°C for 30 sec and extension at 72°C for 40 sec. Lastly, a final exten-

Table 1. Sequences of primer used and target information

No.	Target Virus	Primer	Gene	Size (bp)
1	AKAV	J424F: 5'-CAGAAGAAGGCCAAGATGGT-3' J425R: 5'-AATGCAGCCTTGACTGCGTCC-3'	S segments N gene	476
2	AINOV	J424F: 5'-CAGAAGAAGGCCAAGATGGT-3' J426R: 5'-GGGTGGGGTTTTACAGGAA-3'	S segments N gene	584
3	CHUV	J427F: 5'-CTGGCTTTCTGAGGCGTTTC-3' J428R: 5'-GGTTGCTCAATATGCCAAGCGA-3'	NS1 (S5)	306
4	IBAV	J431F: 5'-AGGATACGGAGGCGCCTTCTT-3' J432R: 5'-CCGGAGATACCTCCATTACC-3'	S segment3	411
5	BEFV	J429F: 5'-CGGTTGCACAGATGCGGTTAAG-3' J430R: 5'-GACTCTCACATCTGGTATCC-3'	G	262

sion was carried out at 72°C for 5 min in the last cycle [20].

Gel electrophoresis

Amplification samples (5 µl) were analyzed by electrophoresis in 1.5% agarose gels (GeneDireX®, Taiwan) containing 0.5 ml of RedSafe™ (iNtRON). Typically, 6 µl of 100 bp marker and samples were loaded in the gel and run at 100 V for 25 min. Subsequently, the gel images were captured using ImageQuant™ LAS 500 (GE Healthcare Life Science, Pittsburgh, Pennsylvania, USA).

Serology analysis in bovine blood against arboviruses

The AKAV strain 93FMX (KVCC-VR63), AINOV strain KSA 9910 (Korea Veterinary Culture Collection (KVCC) VR64), CHUV strain YongAm (KVCC-VR66), IBAV strain 08220 (KVC-CVR65) and BEFV strain TongRae (KVCC VR41) were used for serum neutralization tests (SNT) [8,20]. Vero cells (ATCC,

C-1586) were maintained in alpha- minimum essential medium (Gibco, Grand Island, New York, USA) supplemented with 5% fetal bovine serum and antimycoticantibiotics (Gibco). Bovine serum diluted to 2-fold serial was mixed with equal volumes of virus containing 200 TCID₅₀/0.1 ml and inoculated with Vero cells [8,20,21]. The plates were microscopically examined after 3 and 5 days to find virus-specific cytopathic effects (CPE). Antibody titer was defined as the reciprocal of the highest serum dilution at which CPE was inhibited. A titer of 4 folds or greater was considered to be positive.

RESULTS

Collected numbers of *Culicoides* species

In 2016, a total of 4,932 *Culicoides* were collected (Table 2; Fig. 1A). Collected *Culicoides* species were *C. arakawae* (n = 2,988, 60.6%), *C. punctatus* (n = 1,497, 30.4%), *C. nipponensis* (n = 246,

Table 2. Information about collected *Culicoides* species in 2016 and 2017

Year	Region	Total number	Species				
			<i>C. arakawae</i>	<i>C. punctatus</i>	<i>C. tainanus</i>	<i>C. nipponensis</i>	<i>Culicoides</i> spp.
2016	Wanju	605	272 (45.0)	264 (43.5)	36 (6.0)	0	33 (5.5)
	Gochang	1,237	868 (70.2)	93 (7.4)	2 (0.2)	246 (19.9)	28 (2.3)
	Gimje	304	295 (97.0)	6 (2.0)	2 (0.7)	0	1 (0.3)
	Jinan	1,328	996 (75.0)	294 (22.1)	9 (0.7)	0	29 (2.2)
	Namwon	1,458	557 (38.2)	840 (57.6)	12 (0.8)	0	49 (3.4)
	Total	4,932	2,988 (60.6)	1,497 (30.4)	61 (1.2)	246 (5.0)	140 (2.8)
2017	Wanju	664	348 (52.4)	309 (46.5)	4 (0.6)	0	3 (0.5)
	Gochang	2,904	2,636 (90.8)	165 (5.7)	2 (0.1)	87 (3.0)	14 (0.5)
	Gimje	220	215 (97.7)	5 (2.3)	0	0	0
	Jinan	2,981	2,129 (71.4)	813 (27.3)	23 (0.8)	0	16 (0.5)
	Namwon	3,954	2,011 (50.9)	1,927 (48.7)	6 (0.2)	0	10 (0.3)
	Total	10,723	7,339 (68.4)	3,219 (30.0)	35 (0.3)	87 (0.8)	43 (0.4)

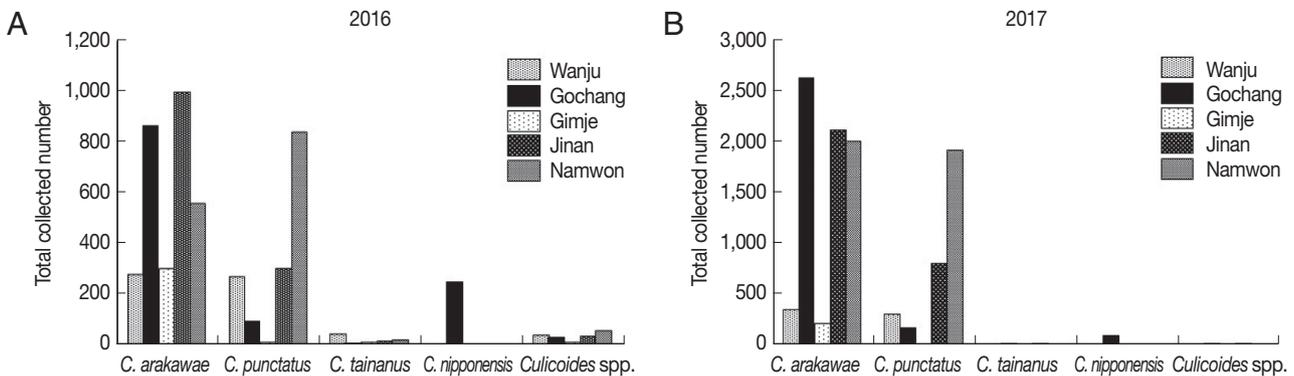


Fig. 1. Collected *Culicoides* species and numbers in 2016 (A) and 2017 (B). According to results of *Culicoides* collection in 2016 (once per month) (A) and 2017 (twice per month) (B), dominant species was *C. arakawae*, followed by *C. punctatus*. *C. nipponensis* was only collected in Gochang.

5.0%), *C. tainanus* (n=61, 1.2%) and *Culicoides* spp. (n=140, 2.8%). Dominant species were *C. arakawae* in 4 farms whereas *C. punctatus* was dominant in one farm (Namwon). *C. nipponensis* was collected only in one farm (Gochang).

A total of 10,723 *Culicoides* were collected in 2017 (Table 2; Fig. 1B). Species such as *C. arakawae* (n=7,339, 68.4%), *C. punctatus* (n=3,219, 30.0%), *C. nipponensis* (n=87, 0.8%), *C. tainanus* (n=35, 0.3%) and *Culicoides* spp. (n=43, 0.4%) were collected. *C. arakawae* was dominant species in all the regions and *C. nipponensis* was collected only in one farm (Gochang) similar to the collection in 2016.

Comparison of collected *Culicoides* numbers between inside and outside the cattle farms

Different collection rates of *Culicoides* inside and outside the cattle farms were observed depending on different altitudes.

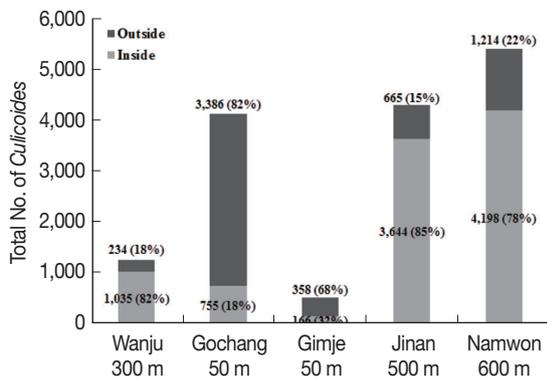


Fig. 2. Comparison of numbers of *Culicoides* collected between inside and outside the cattle farms. A majority of *Culicoides* were collected from low altitude area outside the farms compared to inside the farm. At farms located at relatively high altitude, more *Culicoides* were collected from inside the farm as compared to outside the farm.

The outside of the cattle farms located at low altitude (about 50 m) showed higher collection rates than inside the farm (Fig. 2). On the other hand, collection rates of *Culicoides* were higher inside the farm located at high altitude (over 300 m) compared to the outside of cattle farms.

Distribution of collected *Culicoides* species month-wise

Overall, the number of collected *Culicoides* was mainly concentrated in July 2016 (Fig. 3A) and 2017 (Fig. 3B). A total of 1,871 *Culicoides* were collected in July followed by June (n=1,084), August (n=967), September (n=740), and May (n=307) in 2016. In 2017, a total of 3,618 *Culicoides* were collected in July and followed by August (n=3,075), June (n=2,063), May (n=1,002), and September (n=965). Generally, *C. arakawae* was the most collected species during each month.

Correlation between collection rates of *Culicoides* and temperature, humidity, and wind speed

Three environmental factors (temperature, humidity, and wind speed) were recorded on the collection dates of *Culicoides*. The mean temperature was in the range of 16.4-26.9°C, and the mean humidity was in the range of 67.4-88.1%. The mean wind speed ranged from 1 to 2 m/sec. On the conditions of 25.3°C temperature, 87.3% humidity, and 1.6 m/sec of mean wind speed, the highest mean number of *Culicoides* collected was 364. On the other hand, the lowest mean number of *Culicoides* collected was 91 at 20.2°C temperature, 80% humidity, and 1.8 m/sec of mean wind speed. *Culicoides* tended to be more collected at a high mean temperature and humidity (Fig. 4A, B). There was no effect of mean wind speed on the rates of *Culicoides* collection (Fig. 4C).

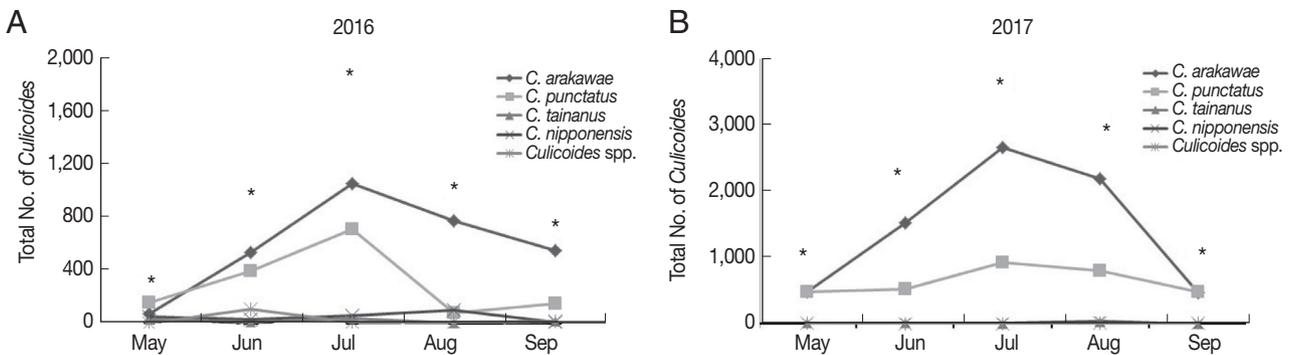


Fig. 3. Distribution of collected *Culicoides* species in the months of 2016 (A) and 2017 (B). *Total number of collected culicoides species.

Antigen detection of *Culicoides* and bovine whole blood against 5 arboviruses

Collected *Culicoides* were conducted to test the positive rates against 5 arboviruses. In 2016, only CHUV was detected twice in Jinan cattle farm with a positive rate of 2.63% in 2 of the 76 poolings (Table 3A). Each species of poolings showing positive were *C. arakawae* and *C. punctatus*. In 2017, 3 of the 166 poolings were positive for AKAV, 2 for CHUV, one for IBAV, and one for BEFV. AKAV were detected in one *C. arakawae* pooling at Gochang and 2 *C. arakawae* poolings at the farm

located in Jinan with a positive rate of 1.81%. Two CHUV were detected in one *C. arakawae* pooling and one *C. punctatus* pooling at the farm located in Namwon with a positive rate of 1.20%. IBAV and BEFV were positive in each *C. punctatus* and *C. arakawae* at the farm located in Wanju with a positive rate of 0.60%. In antigen test of bovine blood against 5 arboviruses, the antigen to AKAV (12.96%) and antigen to BEFV (0.93%) were detected in samples collected from the farm located in Gochang (Table 3B). To confirm the identification of arbovirus-specific detection, Basic Local Alignment Search

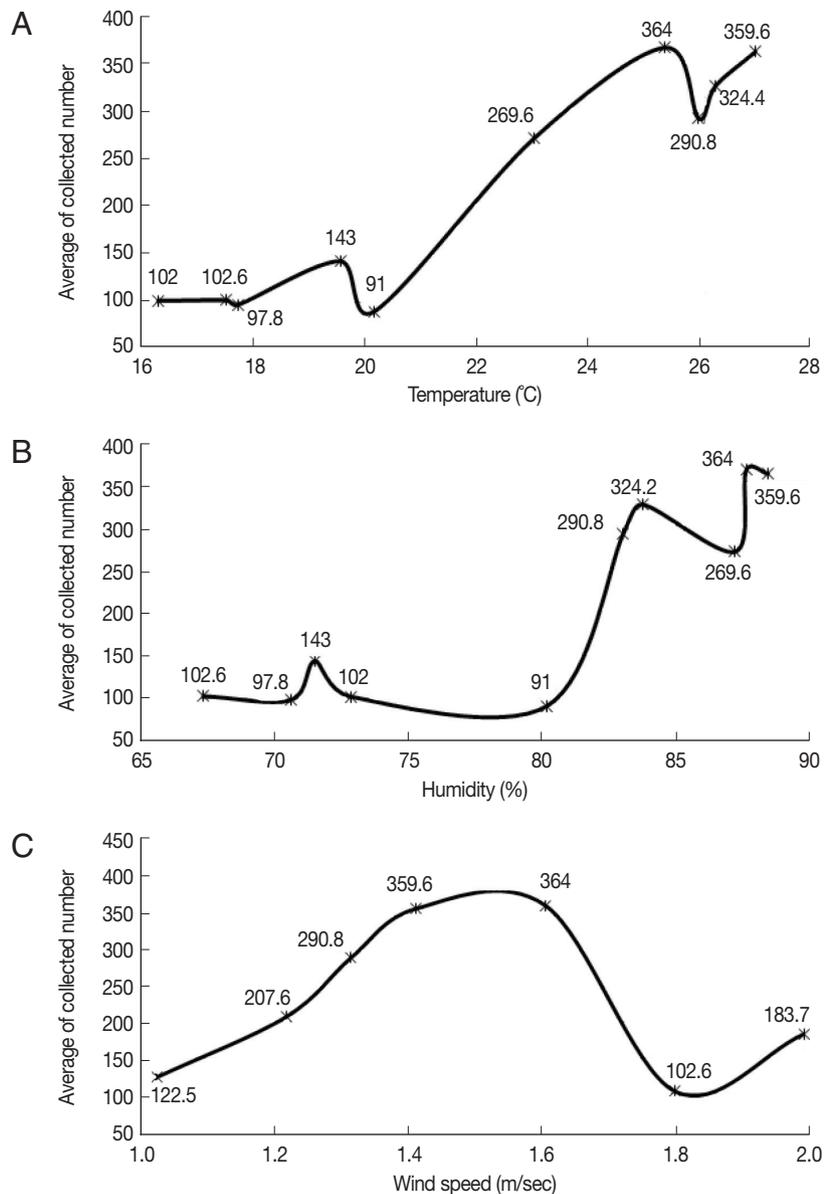


Fig. 4. Correlation between the number of collected *Culicoides* and temperature (A), humidity (B), and wind speed (C). More *Culicoides* were collected at higher temperature and humidity. Collected *Culicoides* was concentrated at 1.4-1.6 m/s.

Table 3. Antigen detection of *Culicoides* and bovine whole blood against 5 arboviruses

(A) In results of antigen detection rates against arboviruses in *Culicoides*, AINOV was not detected in any year, and CHUV was detected in 2016 (2.63%) and 2017 (1.20%). All of the detected viruses showed a detection rate within 3%

Year	Arboviruses				
	AKAV	AINOV	CHUV	IBAV	BEFV
2016	-	-	2.63% (2/76) In Jinan, 1 <i>C. arakawae</i> 1 <i>C. punctatus</i>	-	-
2017	1.81% (3/166) In Gochang, 1 <i>C. arakawae</i> In Jinan, 2 <i>C. arakawae</i>	-	1.20% (2/166) In Namwon, 1 <i>C. arakawae</i> 1 <i>C. punctatus</i>	0.60% (1/166) In Wanju, 1 <i>C. punctatus</i>	0.60% (1/166) In Wanju, 1 <i>C. arakawae</i>

(B) In bovine blood, only AKAV and BEFV were detected. The test was not carried out in Jinan

Region	Arboviruses				
	AKAV (%)	AINOV (%)	CHUV (%)	IBAV (%)	BEFV (%)
Wanju (n=20)	0	0	0	0	0
Gochang (n=108)	12.96 (14/108)	0	0	0	0.93 (1/108)
Gimje (n=20)	0	0	0	0	0
Namwon (n=42)	0	0	0	0	0
Jinan	The test was not carried out.				

Tool (BLAST) was conducted with bi-directional sequencing data of positive samples. The results showed that over 92% identity was observed in individual arboviruses (Supplementary Table S1).

Serum neutralization tests against 5 arboviruses

As a result of antibody test against arboviruses in bovine serum, antibody to AKAV (3.57%, 25.00%, 5.56%, 33.33%, and 11.36%) was positive in all the 5 farms (Table 4A). Antibody to CHUV (3.57% and 5.00%) was detected in blood samples collected from 2 farms (Wanju and Gochang). The antibody titer of BEFV detected was particularly high (64.29%) in blood samples collected from the farm located in Wanju (Table 4B). There were cases that 2 or more arboviruses were detected in one cattle in Wanju (21.4%) and Gochang (17.5%).

DISCUSSION

Transmission of arthropod-borne viral disease is difficult to prevent as they cannot be visually observed and spread quickly and widely. Surveillance has steadily been conducted in several countries to investigate the distribution of *Culicoides* that is a vector of arboviruses, and to test against target viruses for predicting the outbreak [2,22,23]. As these investigations have been carried out for a long time, haematophagous vector collection methods have been developed on a variety of occa-

sions, such as collection means using UV light with a wide range of wavelengths [24], and traps in which carbon dioxide (CO₂) is released at diverse concentrations to attract *Culicoides* [25]. They showed that the standard CDC UV light trap collected the *Culicoides* significantly more than the normal LED trap, and CO₂ trap releasing decanal or phenol collected highly compared to trap with CO₂ alone. These results will be a useful source to collect *Culicoides* more effectively. In addition, new species of *Culicoides* as a vector of arboviruses are continuously being discovered [25-27]. In Korea, new *Culicoides* species were discovered in 2013 [28]. Therefore, epidemiological studies on arthropod-borne are expected to experience continual growth.

In 2016 and 2017, a total number of 15,655 *Culicoides* were collected during the collection period. The species were classified as *C. arakawae*, *C. punctatus*, *C. nipponensis* and *C. tainanus*. In this study, *C. arakawae* was the extensively collected species while *C. arakawae* which is a transvector of leucocytozoon disease in Japan was generally collected from the chicken farm [29]. Previous studies showed that *C. arakawae* was rarely found in ruminant farms and is blood-sucking midges which parasitize chicken [29,30]. There were no chicken farms around the 5 cattle farms where *Culicoides* were collected in this study. The detection of antigens to AKAV, CHUV, and BEFV in *C. arakawae* suggests that *C. arakawae* can hold arboviruses antigen although it is not principal vector that transmit

Table 4. Serum neutralization tests of bovine blood against 5 arboviruses

(A) In data of arboviruses antibody positive based on regions, the AKAV antibody detected in all regions

Region	Arboviruses				
	AKAV (%)	AINOV (%)	CHUV (%)	IBAV (%)	BEFV
Wanju	3.57 (1/28)	0	3.57 (1/28)	0	64.29 (18/28)
Gochang	25.00 (10/40)	0	5.00 (2/40)	0	5.00 (2/40)
Gimje	5.56 (1/18)	0	0	0	5.56 (1/18)
Jinan	33.33 (2/6)	0	0	0	0
Namwon	11.36 (5/44)	0	0	0	0

(B) Antibody titer which was measured against arboviruses based on regions was considered to be positive if a titer of 4 folds or greater, and BEF showed high titer

Region	Arboviruses positive number of total poolings	Arboviruses					(2 ⁿ)
		AKAV	AINOV	CHUV	IBAV	BEFV	
Wanju	18 of 28	<2	<2	<2	<2	<2	32
		<2	<2	<2	<2	<2	16
		<2	<2	<2	<2	<2	32
		<2	<2	<2	<2	<2	16
		<2	<2	<2	<2	<2	64
		<2	<2	<2	<2	<2	64
		4	<2	<2	<2	<2	64
		<2	<2	<2	<2	<2	32
		2	<2	<2	<2	<2	8
		<2	<2	<2	<2	<2	64
		<2	<2	<2	<2	<2	32
		<2	<2	<2	<2	<2	32
		<2	<2	<2	<2	<2	128
		2	<2	16	<2	<2	16
		2	<2	<2	<2	<2	32
<2	<2	<2	<2	<2	64		
2	<2	<2	<2	<2	64		
2	<2	<2	<2	<2	16		
Gochang	12 of 40	4	<2	<2	<2	<2	
		4	<2	<2	<2	8	
		4	<2	2	<2	2	
		4	2	<2	<2	2	
		4	<2	<2	<2	<2	
		4	<2	<2	<2	<2	
		8	<2	<2	<2	<2	
		8	<2	4	<2	<2	
		2	<2	<2	<2	64	
		4	<2	<2	<2	<2	
		2	<2	4	<2	<2	
4	<2	2	<2	<2			
Gimje	2 of 18	8	<2	<2	<2	<2	
		<2	<2	<2	<2	16	
Jinan	2 of 6	8	<2	<2	<2	<2	
		8	<2	<2	<2	<2	
Namwon	5 of 44	4	<2	<2	<2	<2	
		4	<2	<2	<2	<2	
		4	<2	<2	<2	<2	
		4	<2	<2	<2	<2	
		4	<2	<2	<2	<2	

arboviruses to ruminants.

When compared with the antigen detection results against arboviruses in *Culicoides*, the antigen test in the bovine blood

did not show any correlation with the type of virus and region.

During the study period, there were no reported damages associated with arboviruses, and the appearance and epidemic

of 5 arboviruses seemed to be rare. On the other hand, antibody test in the bovine serum, antibody to BEFV was detected and the antibody titer was high in one farm (Wanju). As this farm was vaccinated only against AKAV, there is a possibility that the farm might get infected with BEFV. However, there were no virus-related clinical symptoms and the antibody titer can be considered as a maternal antibody. Serological tests using blood were not initially designed and there was a lack of sample number and collection period. In future studies, a large number of blood samples from various regions will be employed to perform the more specific serological examination. In this study, because blood samples were obtained from randomly selected cattle, it was difficult to determine the exact timing of the virus infection. In order to supplement these shortcomings, surveillance study using sentinel cattle that confirmed no-virus infection before investigated year should be conducted.

Previous studies have reported that *Culicoides* are highly influenced by various environmental factors like temperature, humidity, and wind speed [1,31]. Generally, infections caused by arboviruses and activity rates of *Culicoides* increase in proportion to rising temperature and are interrupted by wind speed. As a result of our study, we could not confirm the dependent effect of the wind speed. However, the measured values of all the environmental factors had a narrow range and it was difficult to confirm uniformity in results. Therefore, a more frequent sampling will be needed in future studies. Additionally, with respect to the collection of *Culicoides* from the farm, more *Culicoides* were collected from outside than inside at low altitude farms (50 m), while more *Culicoides* were collected from inside than outside at high altitude farms (300, 500, and 600 m). It appeared that *Culicoides* were attracted by the warmth of the cattle and gathered inside the farm due to lowering of temperatures at higher altitude. However, there was no significant difference in measured temperature inside and outside the farms during the collection period, so the close causal relationship between altitude and mobility of *Culicoides* was not clearly revealed in this study. In South Africa, more *Culicoides* were collected from old stable than new stable, and more *Culicoides* were collected from outside than inside the stable [31]. Published study reports about an experiment conducted to compare the sampling rates of females and males *Culicoides* from inside and outside the farm in Eastern Slovakia [32]. However, there exist not enough studies based on how altitude is related to the abundance of *Culicoides*. It is

not easy to conclude the relationship between the number of collected *Culicoides* and the environmental factors because altitude alone exactly affects environmental conditions. Therefore, it is necessary to study whether more various conditions affect behavior of *Culicoides*. Furthermore, through environmental studies on farms that can attract or avoid *Culicoides*, we may suggest the manuals that farmers can prevent the occurrence of *Culicoides* related diseases.

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

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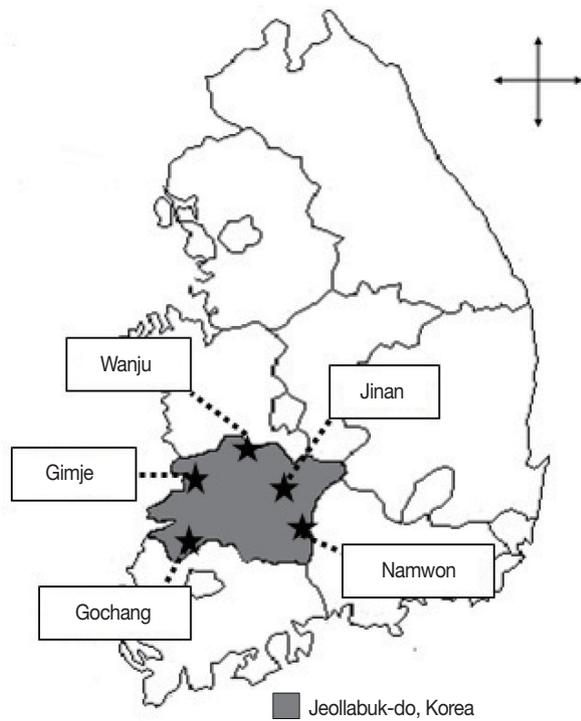
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Supplementary Table S1. The identity data of BLAST about 5 arboviruses sequence. BLAST results showed that over 92% identity was observed in 5 arboviruses

Virus	BLAST results		
		Detected sequence name	Identity (%)
AKAV	F*	Akabane virus strain 93 FMX S protein mRNA, partial cds	100
		Akabane virus isolate 93FMX nucleocapsid protein (N) gene, complete cds	99
	R*	Akabane virus strain 93 FMX S protein mRNA, partial cds	99
		Akabane virus isolate 93FMX nucleocapsid protein (N) gene, complete cds	98
AINOV	F	Aino virus N and NSs genes, segment S, genomic RNA, isolate 38K	99
	R	Aino virus N and NSs genes, segment S, genomic RNA, isolate 38K	99
CHUV	F	Chuzan virus genomic RNA for non structural protein NS1, complete cds	98
	R	Chuzan virus genomic RNA for non structural protein NS1, complete cds	95
IBAV	F	Epizootic hemorrhagic disease virus (serotype 2 / strain Ibaraki) segment 3	92
	R	Epizootic hemorrhagic disease virus (serotype 2 / strain Ibaraki) segment 3	99
BEFV	F	Bovine ephemeral fever virus isolate CS1935 surface glycoprotein (G) gene, partial cds	99
	R	Bovine ephemeral fever virus isolate CS1931 surface glycoprotein (G) gene, partial cds	99

F, Forward; R, Reverse.



Supplementary Fig S1. Five cattle farms where collected *Culicoides* biting midges in Jeollabuk-do, Korea. *Culicoides* biting midges were collected from 5 different cattle farms located in Gimje, Gochang, Wanju, Jinan, and Namwon areas, Jeollabuk-do. Two farms (Gimje and Gochang) were located in plain area with low altitude (about 50 m), and 3 farms (Wanju, Jinan, and Namwon) were mountainous areas with relatively high altitude (above 300 m).