

## Short Communication

# Abundance of biting midge species (Diptera: Ceratopogonidae, *Culicoides* spp.) on cattle farms in Korea

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***Culicoides* biting midges were collected on three cattle farms weekly using light traps overnight from May to October between 2010 and 2011 in the southern part of Korea. The seasonal and geographical abundance of *Culicoides* spp. were measured. A total of 16,538 biting midges were collected from 2010 to 2011, including seven species of *Culicoides*, four of which represented 98.42% of the collected specimens. These four species were *Culicoides* (*C.*) *punctatus* (n = 14,413), *C. arakawae* (n = 1,120), *C. oxystoma* (n = 427), and *C. maculatus* (n = 318). *C. punctatus* was the predominant species (87.15%).**

**Keywords:** arboviruses, biting midges, cattle, *Culicoides*

Arthropod-borne viruses (arboviruses) are transmitted by several vectors, such as mosquitoes, *Culicoides* biting midges, and ticks [5]. *Culicoides* biting midges are among the smallest and most abundant hematophagous vectors. The genus *Culicoides* of the family *Ceratopogonidae* comprises >1,400 described species and is distributed throughout the world, with the exception of Antarctica and New Zealand [5]. *Culicoides* biting midges transmit several diseases caused by viruses, such as bluetongue virus, Akabane virus, and bovine ephemeral fever virus (BEFV) [5]. Recently, *Culicoides* (*C.*) *obsoletus* was identified as a vector for Schmallenberg virus (SBV) [8]. Virus prevalence largely depends on the vector and host distribution, which are governed by geographical and climatic factors.

In Korea, vaccination for Akabane virus and BEFV has been performed since the early 1990s. Only sporadic outbreaks of malformations and febrile illness have occurred after the use of attenuated and inactivated vaccines [1,4]. However, bovine encephalomyelitis caused by Akabane virus was reported in cattle in 2010 [7]. The reasons for these

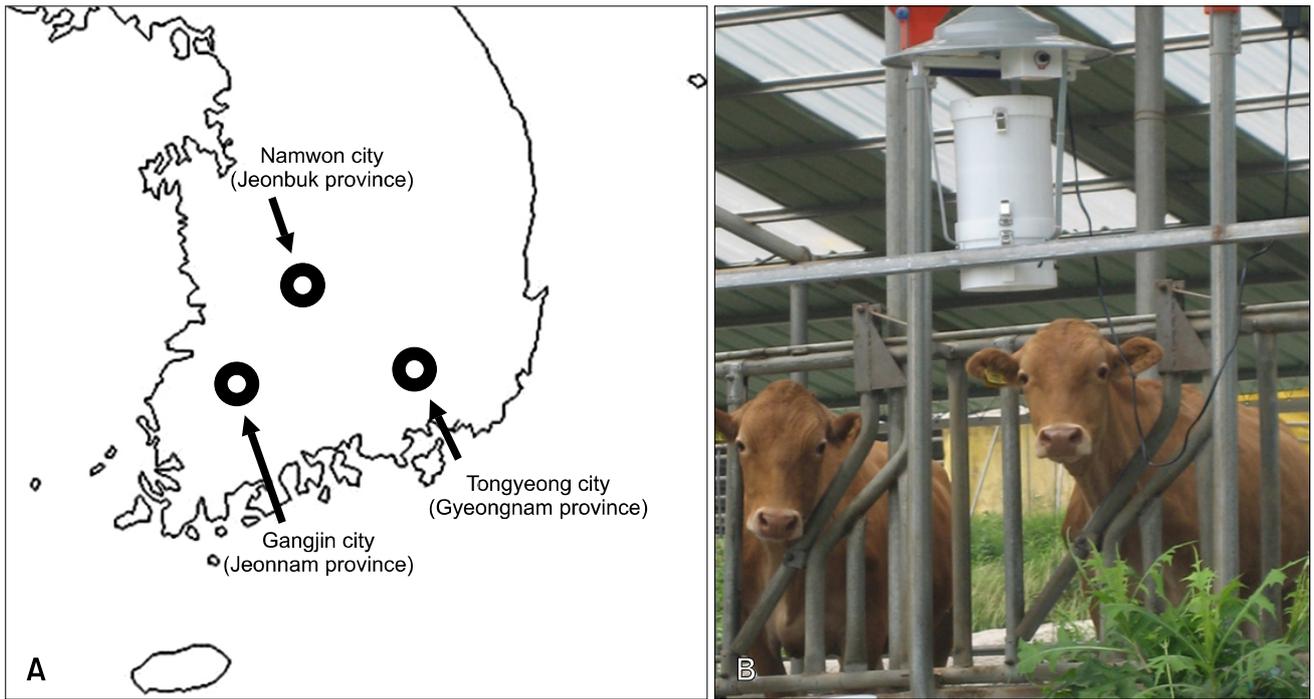
epizootics are not yet fully understood. In Japan, arboviruses were most frequently isolated from *C. oxystoma* between 1985 and 2002 [11]. It is important to identify the principal vectors in specific countries and regions for the control and prevention of arboviral diseases. However, a survey of *Culicoides* biting midges has not been performed in Korea since 1974 [2]. Therefore, we undertook this study to measure the current abundance of *Culicoides* biting midges on cattle farms in southern Korea.

*Culicoides* biting midges were collected on three cattle farms located in the suburbs of the cities of Gangjin and Tongyeong from May to October in 2010 and Namwon from May to October in 2011 (Fig. 1A). The three collection regions were chosen because they were located in the southern part of Korea, and Akabane viral encephalomyelitis in cattle was reported in these regions. Adult *Culicoides* biting midges were collected once a week using a light trap (SNC, Korea) that was equipped with three 8 W black lights and a downdraft suction motor (Fig. 1B). The traps were placed inside the barn with the cattle at a height of 1.4 m. The traps were set in the afternoon between 4 : 00 and 5 : 00 p.m. and were collected the next morning at 9 : 00 a.m. Collected samples were sent to the Animal, Plant, and Fisheries Quarantine and Inspection Agency (QIA), Korea and were sorted into species microscopically according to wing patterns [3].

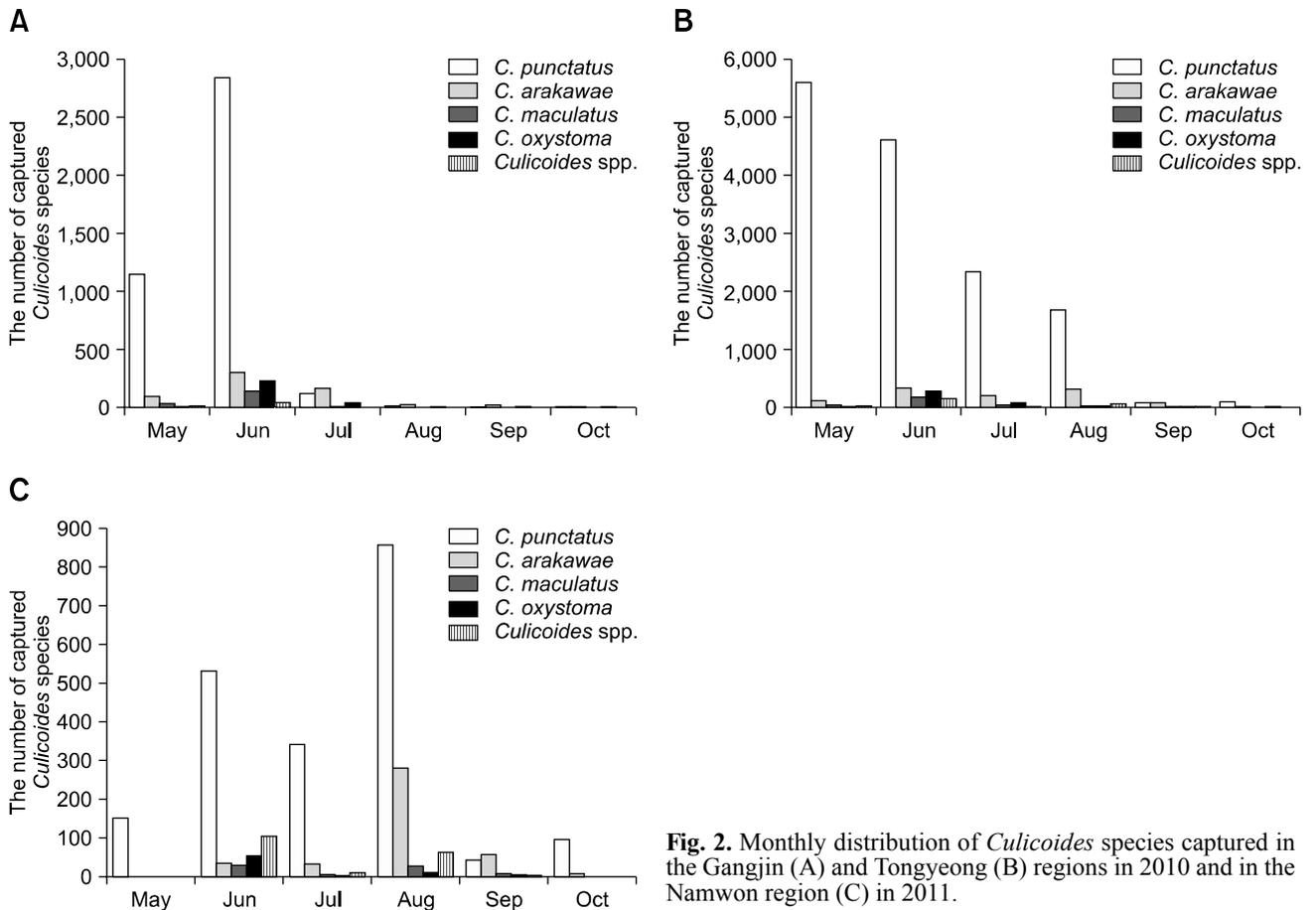
*Culicoides* species (30~50) were pooled into one sample and added to a 2 mL container with ceramic beads. Samples were ground for 30 sec with 1 mL of cold phosphate-buffered saline (PBS). Ground samples were centrifuged (5 min, 4°C), and supernatants were harvested for RNA extraction.

Akabane virus (93FMX), Aino virus (KSA9910), Chuzan virus (YongAm), Ibaraki virus (Imaizumai), and

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**Fig. 1.** (A) The location of the three sampling sites in the southern part of Korea. (B) The light trap, equipped with three 8 W lights and a downdraft suction motor that was placed on the cattle farm.



**Fig. 2.** Monthly distribution of *Culicoides* species captured in the Gangjin (A) and Tongyeong (B) regions in 2010 and in the Namwon region (C) in 2011.

BEFV (TongRae) were propagated at 37°C in Vero cells. RNA was extracted using an RNeasy mini-kit (Qiagen, USA) according to the manufacturer's instructions. Viral genes were amplified using the following conditions: initial denaturation at 94°C for 2 min, 35 cycles of reverse transcription at 50°C for 30 min, denaturation at 94°C for 30 sec, primer annealing at 58°C for 30 sec, and primer extension at 72°C for 1 min. Nested cycling conditions were the same as described for RT-PCR, except that 30 cycles were used. All procedures were conducted as described previously [9].

A total of 16,538 *Culicoides* biting midges were collected from 2010 to 2011. Seven species of *Culicoides* were collected, four of which represented 98.43% of the collected specimens. These four species were *C. punctatus* (n = 14,413), *C. arakawae* (n = 1,120), *C. oxystoma* (n = 427), and *C. maculatus* (n = 318). *C. punctatus* was the predominant species (87.15%) and was abundant during all collection periods (May through October). The three other species were *C. erairai*, *C. japonicas*, and *C. circumscriptus*.

The monthly abundance of *Culicoides* species captured at the Gangjin and Tongyeong regions in 2010 and the Namwon region in 2011 was investigated (Fig. 2). *C. punctatus* was the most abundant of all the *Culicoides* spp. collected monthly in the three provinces. In the Gangjin and Tongyeong regions, *C. punctatus* was abundant in early summer (May-June) in 2010, whereas *C. punctatus* was most abundant in August in the Namwon region in 2011. The different peak abundance of *C. punctatus* may be attributed to the fact that the Gangjin and Tongyeong regions are more southerly than Namwon. Also, the mean temperature and humidity of 2010 were higher than those of 2011. These differences may explain the different peak abundance of *C. punctatus*.

Akabane virus genes in four *Culicoides* spp. were detected by nested RT-PCR (Fig. 3). In four *Culicoides*

spp. samples (n = 276) collected in 2010, the numbers and percentages of Akabane virus gene-positive samples were 8 (7.0%), 4 (5.7%), 2 (4.5%), and 2 (4.2%) for *C. punctatus*, *C. arakawae*, *C. maculatus*, and *C. oxystoma*, respectively. Akabane virus genes were detected from May to September in 2010, and the frequency of detection was the highest in July. In four *Culicoides* spp. samples (n = 108) collected in 2011, the numbers and percentages of Akabane virus gene-positive samples were 1 (2.0%), 1 (4.2%), 0 (0%), and 1 (8.3%) for *C. punctatus*, *C. arakawae*, *C. maculatus*, and *C. oxystoma*, respectively. Akabane virus genes were detected in July and September in 2011, and the frequency of detection was the highest in September.

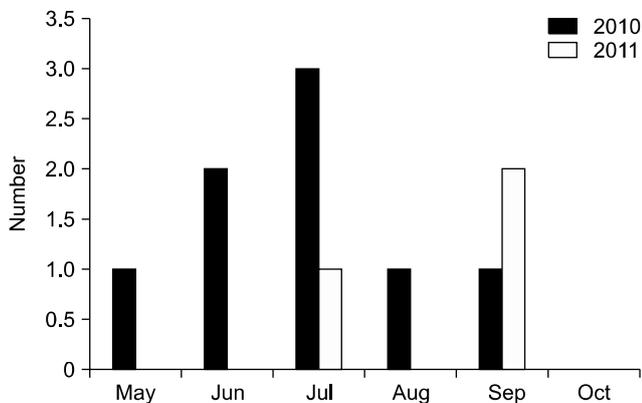
A large-scale epidemic of Akabane viral encephalomyelitis in cattle was reported in the southern part of Korea in 2010 [7]. Although no outbreak of bovine encephalomyelitis occurred on the sampling farms, the percentage of Akabane virus gene-positive samples in 2010 was twice that found in 2011. These data indicated that Akabane virus was circulated by *Culicoides* biting midges and might have caused bovine encephalomyelitis in Korean cattle in 2010. However, it was impossible to differentiate between Akabane virus genogroups by partial sequencing.

*Culicoides* biting midges are important vectors that transmit a number of infectious diseases, in both the veterinary and medical fields. Thus, it is important to investigate the abundance, population, and distribution of these vectors and associated pathogen infection rates. *C. oxystoma* was reported to be the most abundant species and a potential vector for bovine arboviruses in Japan [10]. In addition, *C. brevitarsis* is the principal vector of arboviruses, such as Akabane virus and bluetongue virus, in Australia [6]. The most abundant species collected from three Korean cattle farms was *C. punctatus* in this study. However, no potential vector species transmitting bovine arboviruses was identified because virus isolation from *Culicoides* biting midges was not attempted. Therefore, further studies are required to identify the exact vector that transmits bovine arboviruses through systemic monitoring and regular surveillance of *Culicoides* species.

This study is a starting point that will help elucidate the role of *Culicoides* biting midges in the transmission of bovine arboviruses. Recently, *C. brevitarsis* was recognized in Kyushu [10]. Global warming may have a considerable effect on vector distribution, such as *Culicoides* spp. Therefore, continuing investigation of the distribution and seasonal activity of *Culicoides* biting midges is needed to identify the main vector species. This is the first published report of *Culicoides* trapped on Korean cattle farms.

### Acknowledgments

This research was supported by a grant from the Animal, Plant and Fisheries Quarantine and Inspection Agency,



**Fig. 3.** Detection of Akabane virus genes in four *Culicoides* spp. by nested RT-PCR.

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