



# Surveillance of *Culicoides* biting midges in northern Honshu, Japan, during the period of Akabane virus spread

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**ABSTRACT.** A surveillance of *Culicoides* biting midges with light suction traps was conducted in the northern region of Honshu, main island of Japan, during the summers and autumns of 2009 and 2010. A total of 106 trap collections across 37 cattle farms were investigated for the structure and distribution of *Culicoides* species. Forty-thousand and one hundred forty-nine specimens of *Culicoides* biting midges were identified at the species level, and  $\geq 19$  species were included in the specimens. *Culicoides oxystoma*, which is a known major vector of Akabane virus (AKAV), appeared not to have expanded in northern Honshu during the surveillance. Of the potential AKAV vectors suggested by a previous laboratory experiment, *C. tainanus* and *C. punctatus* widely infested cowsheds across northern Honshu. The AKAV circulation was confirmed by serological surveillance of sentinel cattle in northern Honshu during the summer and autumn of 2010 and, consequently, >200 calves affected by the virus were identified as of spring 2011. Our surveillance demonstrated that *C. tainanus* and *C. punctatus* were widely spread and often dominated at cattle farms in/around the seroconverted regions, and our results thus suggest that these species played a critical role in the AKAV transmission in 2010. Because the distribution ranges of *C. tainanus* and *C. punctatus* cover almost all of mainland Japan, a potential risk of AKAV transmission might be expected even in areas outside the range of *C. oxystoma*.

**KEY WORDS:** Akabane virus, arbovirus, congenital malformation, entomological surveillance, vector competence

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*Culicoides* biting midges (Ceratopogonidae; Diptera) are hematophagous insects and are known as primary vectors of ruminant arthropod-borne viruses (arboviruses) [7]. Over 1,250 species of the genus have been recorded [5], and at least 82 of them are distributed in Japan [49]. The species structure of *Culicoides* biting midges varies widely within individual geographical regions and seems to be affected by environmental factors such as the climate, vegetation, landscape, and land use [29]. Investigations of the species structure and abundance in the areas with arbovirus spread are therefore essential to determine the actual vector species and assess the risks of arboviral diseases depending on their distribution and active periods.

Akabane disease is an arboviral disease of livestock ruminants that is characterized by fetal damage resulting in abortions, premature birth, stillbirth, and congenital malformations [20–23, 40]. Akabane disease is caused by Akabane virus (AKAV), which is a member of the species *Akabane orthobunyavirus* of the genus *Orthobunyavirus* within the family *Peribunyaviridae* [1]. Several AKAV strains which are generally grouped within a single genogroup have been found to be associated with epizootic encephalomyelitis in postnatally infected cattle [18, 30, 35, 52]. The prevalence of AKAV ranges through tropical, sub-tropical, and temperate zones of Asia, Africa, the Middle East, and Oceania [15]. Akabane disease has continuously impacted the livestock industries of several countries, including Japan, Korea, and Australia, and the strategic vaccination of breeding cows has been conducted to prevent this disease [13, 15].

Although in Japan AKAV was initially isolated in 1959 from mosquitoes, i.e., *Aedes vexans* and *Culex tritaeniorhynchus* [38], *Culicoides* biting midges are known to be primary vectors for the virus at present [15]. The virus has been preferentially isolated/

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detected from limited *Culicoides* species including *C. brevitarsis* in Australia [9, 10] and *C. imicola* in the Middle East [3, 43]. Because AKAV has been most frequently isolated from *C. oxystoma*, this species is considered the most incriminated vector in Japan [14, 19, 54].

A laboratory experimental infection also indicated that *C. oxystoma* has capacity as a vector of AKAV [53]. In the past, the typical epidemic regions of Akabane disease have been limited to the southern half of Japan, including Kyushu, Shikoku, and the western part of the main island of Honshu, and these regions overlapped with the distribution range of *C. oxystoma*. However, several epidemics of Akabane disease were irregularly expanded over the known range of this species [13, 52]. More than 7,000 affected calves and lambs were reported between autumn 1985 and spring 1986 in six prefectures of northern Honshu [32, 36]. In a 1999 epidemic, Akabane disease spread across the same region and into a further northern area located in the southern part of the island of Hokkaido [26, 51].

These outbreaks reminded us of two possibilities regarding the AKAV transmission in northern Honshu. The first possibility is that *C. oxystoma* transiently or stably expanded its distribution range to the affected regions. The second is that other *Culicoides* species which are indigenous to northern Honshu had a primary role in the AKAV transmission. The northward expansion of *Aedes albopictus*, a mosquito vector of dengue virus, has been observed in northern Honshu [17, 41]. Those investigations revealed that the average temperature has gradually risen in northern Honshu, and the rise in the temperature probably enhanced the establishment of *A. albopictus*. This phenomenon seems to support the validity of the first possibility.

In 2006, bluetongue virus (BTV), a *Culicoides*-borne pathogen of ruminants, had newly emerged in northern Europe in areas where the original vector species, *C. imicola*, was absent. Virus detection from *Culicoides* biting midges collected in the affected area showed that several northern Palaearctic *Culicoides* species (rather than *C. imicola*) served as vectors [8]. More recently, Schmallenberg virus (SBV), which is also *Culicoides*-borne and belongs to the genus *Orthobunyavirus*, emerged and caused a large outbreak of congenital malformations in ruminants throughout Europe [2]. Entomological surveillance clearly showed that indigenous *Culicoides* species in northern Europe also contributed to the SBV transmission [4].

These outbreaks suggest the necessity of searching for other potential AKAV vector species rather than *C. oxystoma* in northern Honshu to test the second possibility described above. Unfortunately, the fauna of *Culicoides* species in northern Honshu remains uncertain, except for a surveillance of *Culicoides* associated with cowsheds in Aomori Prefecture, which is located at the northernmost end of Honshu [45].

The present study describes the results of our 2009 and 2010 entomological surveillances conducted to improve the knowledge of *Culicoides* species in northern Honshu. Coincidentally, seroconversion to AKAV was observed in sentinel cattle in northern Honshu during the summer and autumn of 2010, and subsequently, AKAV-infected calves showing arthrogryposis and hydranencephaly were reported until the following spring [31, 44]. A total of 218 affected calves were reported during the epidemic [27]. Therefore, to identify potential vector species in northern Honshu, we clarified the distribution and diversity of *Culicoides* species and examined the collected data along with the sero-surveillance data of AKAV occurrence in 2010.

## MATERIALS AND METHODS

### *Insect collection*

During the summers and autumns of 2009 and 2010, we selected a total of 37 cattle farms across nine prefectures (Aomori, Iwate, Akita, Miyagi, Yamagata, Fukushima, Ibaraki, Niigata and Tochigi) for the investigation (Table 1, Fig. 1). These sites were selected for thorough coverage across the northern Honshu region. For the surveillance in 2010, Ibaraki and Niigata Prefectures were added in place of Fukushima Prefecture. Collections were carried out at 28 sites in 2009 and 22 sites in 2010. Of them, 13 sites were surveyed in both years. Most of the midge trappings took place from July to September, when arbovirus transmissions have frequently occurred in Japan [14, 52, 54].

Because *C. oxystoma* prefers to use damp ground for immature breeding [55], we set most of the trapping sites (the exceptions were sites 4 and 35) in the vicinity of a paddy field (Table 1). The midge collections were conducted using light-suction traps equipped with a 6W black-light tube. The traps were deployed approximately 1.5 m aboveground inside cowsheds and operated overnight (for approximately 18 hr from 4:00 p.m.). The collected midges were killed by freezing at  $-20^{\circ}\text{C}$  and preserved in 70% ethanol. The species identification was performed under a stereoscopic microscope according to the morphological keys provided by Wada [49]. If it was difficult to distinguish the species based on wing patterns, the head and wings were separated with needles from the other parts of the body, and the dissected parts were slide-mounted in NEO-SIGARAL (Shiga Konchu Fukyusha, Tokyo) for detailed observation. When abundant midges were caught, subsampled specimens (approximately 500–1,000) were sorted to estimate the ratio of each *Culicoides* species. The presence/absence of *C. oxystoma* was checked in all catches.

### *Serological surveillance data*

In Japan, nationwide sero-surveillance for AKAV using sentinel cattle has been conducted every year since 1985 [33, 48]. Approximately 50 sentinel calves were selected in each of Japan's 47 prefectures every year. Sera of naïve calves with no previous exposure to AKAV were collected four or five times between June and November, and virus neutralization tests for AKAV were performed using the established method as described previously [46]. Antibody titers that were increased more than fourfold were defined as positive for neutralization antibodies to AKAV in the sentinel calves. We obtained the sero-surveillance data of AKAV in 2010 from Japan's Ministry of Agriculture, Forestry and Fisheries, and examined the locations and timing of seroconversion. Arc GIS software (ver. 10.6; Esri, Redlands, CA, U.S.A.) was used to generate a map.

**Table 1.** Location of cattle farms where *Culicoides* trapping was performed in northern Honshu in 2009 and 2010

Site ID	Location	Prefecture	No. of collection	Collection period		No. of counted midges <sup>a)</sup>		Ecological factors in the immediate vicinity <sup>b)</sup>		
				2009	2010	2009	2010	Paddy field	Forest	River
1	Imabetsu	Aomori	4	Jun., Aug.	Jul., Aug.	<i>1,122</i>	1,038	+	+	+
2	Higashidoori		4	Jun., Aug.	Jul., Aug.	<i>1,075</i>	<i>2,078</i>	+	+	+
3	Hirosaki		2	Aug.	-	1	-	+	-	-
4	Tsugaru		2	Jun., Aug.	-	10	-	-	+	-
5	Towada		2	Jun., Aug.	-	62	-	+	+	+
6	Gonohe		4	Jun., Aug.	Jul., Aug.	947	<i>2,043</i>	+	+	-
7	Hashikami		2	Jun., Aug.	-	400	-	+	+	-
8	Kitaakita	Akita	4	Aug.	Jul., Aug.	179	440	+	+	+
9	Akita		4	Aug.	Jul., Aug.	102	21	+	-	+
10	Yuzawa		2	Aug.	-	<i>1,348</i>	-	+	+	+
11	Yokote		4	Aug.	Jul., Aug.	104	79	+	-	-
12	Sakata A	Yamagata	2	Aug.	-	46	-	+	-	-
13	Sakata B		4	Aug.	Jul., Aug.	157	<i>1,745</i>	+	-	+
14	Funagata		4	Aug.	Aug., Sep.	93	152	+	-	+
15	Nakayama		4	Jul., Aug.	Jul., Aug.	31	12	+	-	+
16	Yamagata		2	Jul., Aug.	-	4	-	+	-	+
17	Esashi	Iwate	4	Aug.	Jul., Aug.	<i>1,000</i>	<i>2,092</i>	+	+	+
18	Tanzawa A		2	Aug.	-	932	-	+	+	+
19	Shizukuishi A		2	Aug.	-	14	-	+	+	+
20	Shiwa		4	Aug.	Aug.	90	417	+	-	-
21	Shizukuishi B		1	-	Oct.	-	353	+	+	+
22	Tanzawa B		1	-	Oct.	-	83	+	+	+
23	Tome	Miyagi	4	Jul., Aug.	Jul.	769	1,482	+	-	-
24	Iwanuma		4	Aug., Sep.	Aug.	393	1,198	+	+	+
25	Koriyama A	Fukushima	2	Jul., Aug.	-	<i>1,000</i>	-	+	+	-
26	Koriyama B		2	Jul., Aug.	-	<i>1,000</i>	-	+	+	+
27	Aizubange		2	Aug.	-	80	-	+	+	+
28	Yugawa		2	Aug.	-	83	-	+	+	+
29	Nishigo A		5	Jul. to Sep.	-	3,105	-	+	+	+
30	Nishigo B		3	Aug. to Oct.	-	3,408	-	+	+	+
31	Niigata	Niigata	4	-	Aug., Sep.	-	594	+	-	+
32	Tainai		4	-	Aug., Sep.	-	3,608	+	+	+
33	Tochigi	Tochigi	2	-	Jul., Aug.	-	15	+	-	+
34	Utsunomiya		2	-	Jul., Aug.	-	15	+	-	+
35	Nasushiobara		2	-	Jul., Aug.	-	556	-	+	-
36	Daigo	Ibaraki	2	-	Jul., Aug.	-	<i>2,520</i>	+	+	+
37	Hitachiomiya		2	-	Aug., Sep.	-	<i>2,053</i>	+	+	+
Total			106			17,555	22,594			

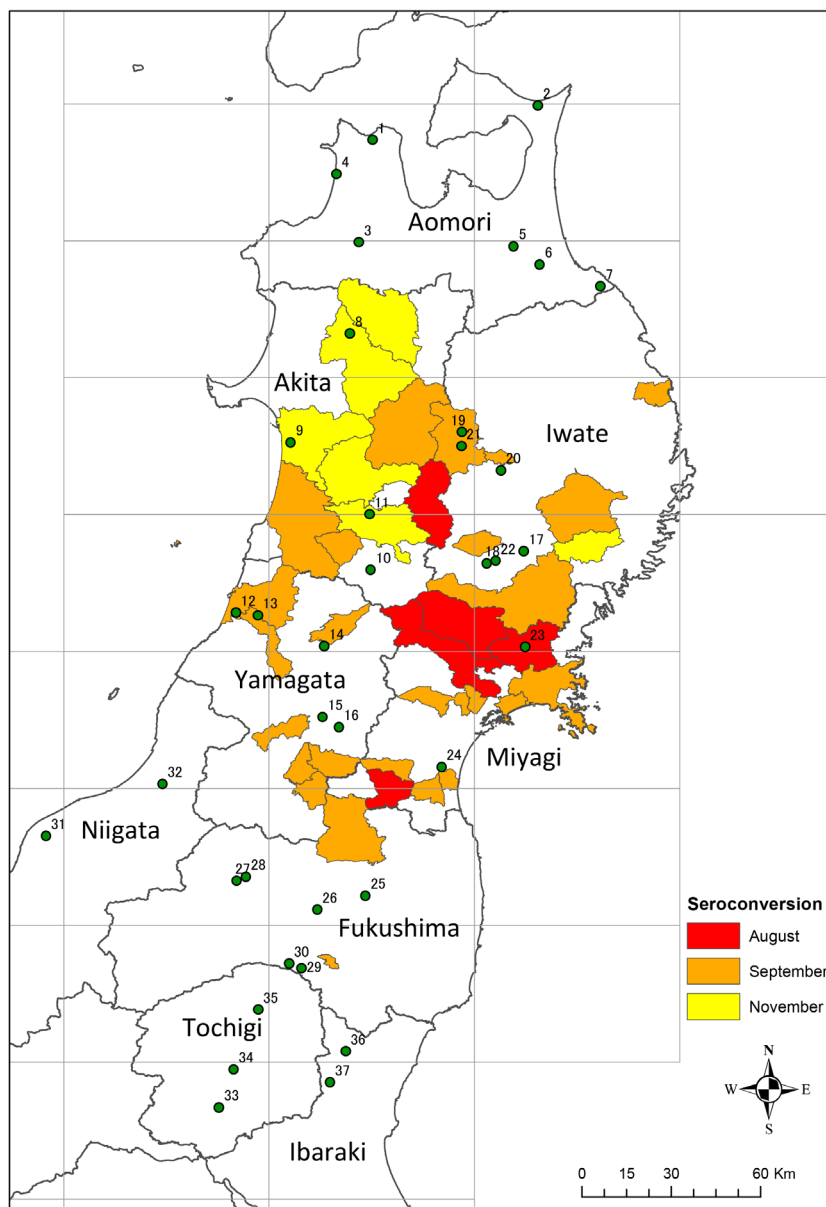
a) Numbers for which a portion of collected midges was counted are written in Italic. b) + and - means presence and absence of each habitat, respectively.

## RESULTS

A total of 106 separate light trap collections (60 and 46 collections in 2009 and 2010, respectively) were conducted in northern Honshu during the study period (Table 1). Of them, 104 collections included one or more *Culicoides* biting midges (Fig. 2). Of the collected midges, 40,149 specimens (17,555 and 22,594 collected in 2009 and 2010, respectively) were identified to the species level. A total of 19 *Culicoides* species were found during the surveillance. In the samples collected in 2010, *C. sanguisuga* was not found, but *C. arnaudi*, *C. jacobsoni* and *C. nipponensis* were newly identified. Spatial and temporal heterogeneity of the abundance and structure of *Culicoides* species was observed in both the 2009 and 2010 collections.

The most widespread species during the surveillance was *C. tainanus*, which was recorded from 28 sites in 2009 and 20 sites in 2010. *Culicoides tainanus* was also dominant in 25 collections at 16 sites in 2009 and in 8 collections at 5 sites in 2010. The highest proportion of *C. punctatus* was recorded in 13 collections at 8 sites in 2009 and in 11 collections at 10 sites in 2010.

The collected midges from 9 collections at 6 sites in 2009 and 12 collections at 6 sites were dominated by *C. arakawae*. The species *C. erairai*, *C. lungchiensis*, *C. matsuzawai* and *C. ohmorii* were occasionally dominant in 1–5 collections in each year. No *C. oxystoma* was included in any of the samples. Male midges of 11 species (with the exceptions of *C. arnaudi*, *C. dubius*, *C. humeralis*, *C. jacobsoni*, *C. kibunensis*, *C. matsuzawai*, *C. sanguisuga* and *C. saninensis*) were collected through the surveillance



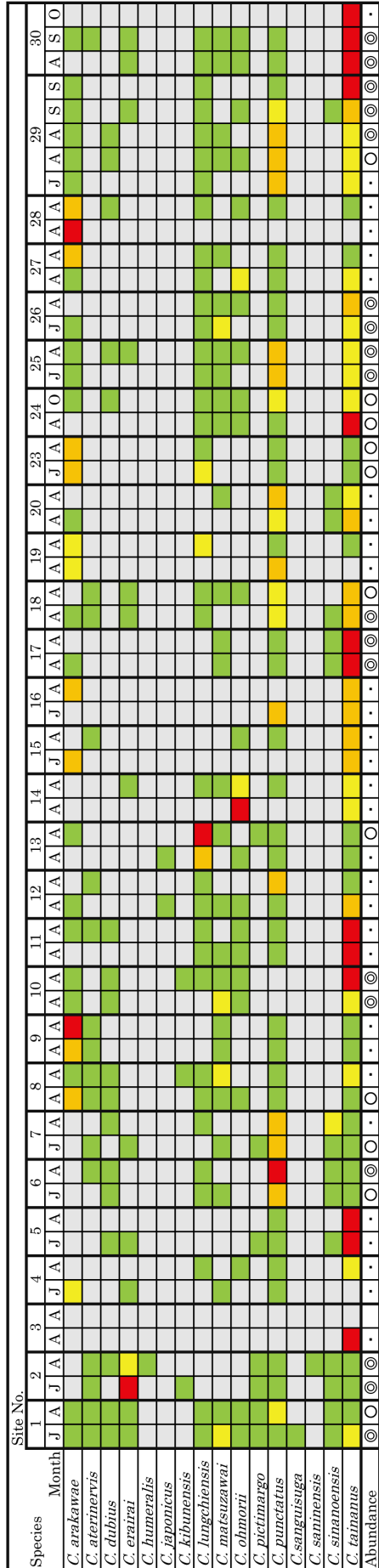
**Fig. 1.** The location of trapping sites in northern Honshu in 2009 and 2010. The location names are provided in Table 1. Akabane virus-seroconversion positive municipalities in 2010 are indicated by red (August), orange (September) and yellow (November).

and accounted for 1.1 and 4.5% of the counted midges in 2009 and 2010, respectively. Males of *C. lungchiensis* became subdominant in the total collections of midges at 2 farms in 2010, i.e., 29.0% at site 6 and 41.1% at site 13. Overall, female midges generally predominated in the counted midges at most of the collection sites. The proportions of blood-fed *Culicoides* females were highly variable depending on the species and collection sites (0–100%).

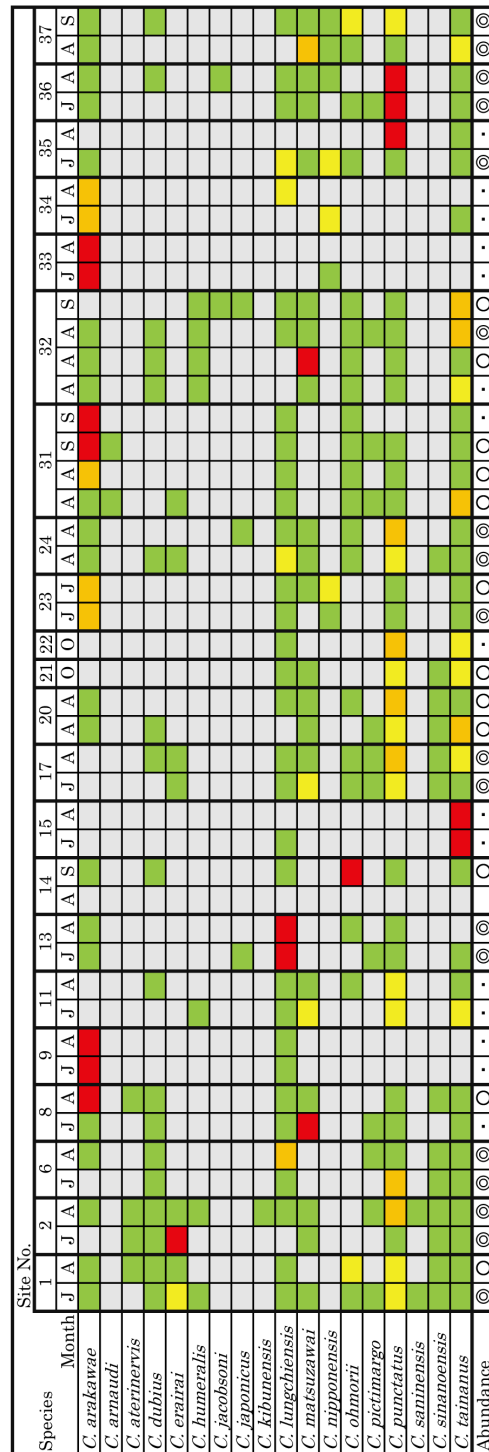
Among the 104 collections, the average feeding proportion of the counted female midges was 34.9%. Of the 19 species, 14 species were found to be engorged. No engorged female of *C. arnaudi*, *C. japonicus*, *C. kibunensis*, *C. pictimargo* or *C. sanguisuga* was included in the counted midges. Although *C. arakawae* were collected in 68 of the 104 collections, the blood-feeding ratio ranged from zero to very low (average blood feeding ratio: 3.0%). Five species (*C. dubius*, *C. erairai*, *C. ohmorii*, *C. punctatus* and *C. tainanus*) which were included in over 20 collections showed high average feeding proportions (46.9–66.8%).

In 2010, seroconversion of AKAV in sentinel cattle was observed in 37 municipalities in northern Honshu (Fig. 1). Among them, seroconversion occurred in August in 6 municipalities, in September in 25 municipalities, and in November in 6 municipalities. During that period, 12 trapping sites (sites 8, 9, 11, 13–15, 17, and 20–24) were located at or nearby municipalities where seroconversion to AKAV in sentinel cattle was observed (Fig. 1). At 5 trapping sites, *C. punctatus* was most dominant. *Culicoides arakawae* and *C. tainanus* were predominant at 2 sites. *Culicoides lungchiensis* and *C. ohmorii* dominated at 1 site. *Culicoides*

2009



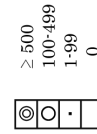
2010



Proportion of each species



Abundance of midges



J: July  
A: August  
S: September  
O: October

Fig. 2. The structures of collected *Culicoides* species at cowsheds in northern Honshu in 2009 and 2010.



*lungchiensis* was collected at all 12 sites. *Culicoides tainanus* and *C. punctatus* were recorded from 11 and 10 sites, respectively. In contrast, *C. arakawae* and *C. ohmorii* were sporadically trapped at the farms (6 or 7 sites).

## DISCUSSION

Historically, *C. oxystoma*, which is a major vector of AKAV in Japan, has been unevenly distributed in lower latitude regions in Japan [49], probably due to the mild winter in those regions, which appears to be favorable for the overwintering and continuous population of *C. oxystoma*. Recent climate changes have potentially enhanced the northward expansion of its range. However, our present investigation did not reveal any evidence of the existence of *C. oxystoma* in northern Honshu in 2009 and 2010. Our results suggest that, at the very least, *C. oxystoma* was not established in northern Honshu and did not contribute much to the AKAV transmission in the summer and autumn of 2010. Although it cannot be denied that *C. oxystoma* initially brought AKAV from its epizootic region to northern Honshu, our results strongly support the concept that indigenous *Culicoides* species transmitted AKAV through the 2010 epizootic.

Of the species collected during the surveillance, *C. tainanus*, *C. punctatus* and *C. jacobsoni* were confirmed to be infected with AKAV by laboratory experimental infection [53]. *Culicoides tainanus* and *C. punctatus* were widely distributed in northern Honshu, and their range overlapped with the seroconverted regions during the 2010 AKAV spread. In addition, these species were predominant at many trapping sites and their blood-feeding ratio was generally high. Although the blood meal origins of the collected midges were not determined in this study, engorged midges trapped inside cattle sheds have likely fed on cattle, as shown in previous studies [24, 34]. It was also reported that both the species prefer to feed on cattle [37]. *Culicoides tainanus* and *C. punctatus* may thus have played crucial roles in the AKAV transmission in 2010. It should be noted that *C. punctatus* in East Asia is uncertain to be conspecific with that in Europe, because of the high genetic diversity between them [28, 39]. Further research into these suspected cryptic species will be essential for accurate revision of the distribution of *Culicoides*.

*Culicoides jacobsoni* were only identified at sites located in Niigata and Ibaraki Prefectures, where no seroconversion to AKAV was observed in sentinel cattle in 2010. This species has often been recorded in tropical and subtropical zones of Asia and Oceania, and its previous northernmost record in Japan was from Tokyo [49, 50]. The collected sites were probably close to the northern limit of the expansion of *C. jacobsoni*. During the study, *C. arakawae* was caught at many trapping sites, probably because the sites were close to paddy fields, which are a major breeding site of this species [55]. However, this species prefers to feed on birds [16], and its feeding proportion was much lower than those of other engorged species.

*Culicoides lungchiensis* was also predominant/subdominant at several collection sites in/around the seroconverted regions in 2010. However, AKAV has not been detected in field-collected specimens of this species in the epizootic areas in southern Japan, and no laboratory experimental infection has suggested its vector capacity for the virus [14, 53, 54]. Our current knowledge may not be sufficient to elucidate the role of *C. lungchiensis* as a vector.

Unfortunately, the detection of AKAV RNA from 2,060 midges collected in/around the seroconverted regions in the summer and autumn of 2010 failed (data not shown). Thus, at present, there is no conclusive evidence of a potential role of *Culicoides* species in northern Honshu. Because the circulation of AKAV seems to be ephemeral in the environments around individual infected farms [14, 19, 54], the opportunities for AKAV RNA detection from midges may be very few. In fact, the proportion of infected midges collected from fields has been reported to be quite low during the epizootic of SBV in Europe [4]. Further large-scale and frequent insect collections will be needed to identify infected *Culicoides* species during the AKAV circulation.

The distribution ranges of *C. tainanus* and *C. punctatus* almost cover mainland Japan [49]. Immature habitats of these species are normally present around cowsheds in Japan: *C. tainanus* and *C. punctatus* emerged from soil mixed with cattle dung around a compost barn and in a paddock, and from mud on the margin of a stream and a spring, respectively [37]. These habitats are normally present around cowsheds in Japan, and these species might easily become abundant in such environments. In addition, *C. tainanus* maintained its flight activity under lower temperature compared to *C. oxystoma*, indicating that it has a longer active period than *C. oxystoma* [47]. Hence, there is a potential risk of AKAV transmission throughout Japan, even in areas out of the range of *C. oxystoma* and during the periods when it is not active.

After the AKAV epizootic in northern Honshu in 2010, seroconversion to the virus was not observed in that region in 2011. This indicates that the virus could not overwinter. Low temperatures in northern Honshu during the winter might not allow the activity and survival of adult midges in the field, and therefore the circulation of AKAV probably ceased. In contrast, the resurgence of BTV and SBV was observed over several consecutive years in northern Europe [2, 42]. A previous study indicated that adult midges, probably containing the infected individuals, were often active and survived inside warm stables during the cold winter months [25]. Differences in the vector species and the styles of stables between northern Honshu and northern Europe might affect the capacity of midge survival in winter.

As described above, our entomological survey indicated that *C. tainanus* and *C. punctatus* could be potential vectors of AKAV in northern Honshu, Japan. However, to understand the AKAV epizootics and assess the risk of arbovirus transmission, further research is required to improve our knowledge of the ecology of *Culicoides* biting midges. For this, ecological analyses using environmental data such as climate, vegetation, and land use data would be useful to understand factors associated with the distribution and abundance of *Culicoides* biting midges. Although the sudden appearance of AKAV in northern Honshu indicated that the infected midges were directly transported on winds from overseas, the mechanism of introduction remains unclear. Previous studies in other regions indicated that long-distance dispersals of *Culicoides* biting midges from overseas contribute the range expansion of arboviruses [6, 11]. Further investigations of the long-distance incursion of *Culicoides* biting midges using

meteorological models would be helpful to identify the potential source of infected midges and forecast the risk of incursion [12]. In addition, global warming may lead to expansions in vector distributions and an increase in the length of transmission seasons for *Culicoides*-borne arboviruses in higher-latitude regions. Continuous monitoring of the distribution, species structure, and active period of *Culicoides* biting midges in northern Honshu is essential. The insights arising from such efforts would promote the development of surveillance programs and preventive strategies.

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