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Short Communication

Seasonal abundance of *Culicoides imicola* and *Culicoides oxystoma* in Prachuap Khiri Khan province, Thailand

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Abstract

<u>Importance of the work</u>: *Culicoides* spp. (biting midges) are one of the most abundant hematophagous insects in livestock. However, limited information is available about their seasonal abundance in Thailand.

<u>**Objectives**</u>: To study the seasonal abundance of the most common *Culicoides* species and to assess their association with weather parameters.

<u>Materials & Methods</u>: The seasonality of *Culicoides* (Diptera: Ceratopogonidae) species was assessed monthly from November 2020 to October 2021 using four Center for Disease Control miniature light traps at a horse stable in Hua Hin district, Prachuap Khiri Khan province, Thailand.

Results: In total, 10,849 females of the genus *Culicoides* were identified, representing 23 species, consisting of *C. imicola* (43.4%), *C. oxystoma* (24.4%), *C. peregrinus* (16.7%), *C. tainanus* (3%), *C. actoni* (1.7%), *C. fulvus* (1.5%), *C. flavipunctatus* (1.3%), *C. huffi* (1%), *C. innoxius* (0.9%), *C. orientalis* (0.8%) and another 13 *Culicoides* species that accounted for 2%. Two predominant species—*C. imicola* and *C. oxystoma*—occurred throughout the entire study period. Both species showed no significant differences in seasonal abundance pattern. No significant correlations were identified between numbers of *C. imicola* and *C. oxystoma* with rainfall, temperature or relative humidity.

Main finding: This study was the first investigating the seasonal abundance of *C. imicola* and *C. oxystoma* in Thailand. More detailed studies are needed to better understand abiotic and biotic factors affecting *Culicoides* abundance and distribution.

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Introduction

Biting midges of the genus Culicoides Latreille are small nematocerous Diptera that belong to the family Ceratopogonidae and contain more than 1,300 species described worldwide (Borkent, 2014), except in Antarctica and New Zealand (Mellor et al., 2000). The Culicoides females are obligate blood suckers attacking mammals, including humans and birds. although a few species feed on turtles, frogs and insects, while males do not take blood, feeding on flower nectar or honeydew (Borkent, 2005). The biting habit of Culicoides spp. causes an allergic reaction in horses known as sweet itch, summer eczema or Queensland itch in many regions worldwide and also in humans, for whom their heavy bite causes painful itching and swelling that can lead to infection and dermatitis from scratching the bites in some regions (Borkent, 2005). In addition, many species of *Culicoides* are known to transmit a wide range of viruses, protozoa and filarial nematodes, especially the transmission of important diseases, such as African horse sickness (AHS) and bluetongue (BT) in horses and ruminants, respectively (Du Toit, 1944; Borkent, 2005). AHS was first reported in Thailand, when an outbreak of this disease in horses began in Pak Chong district, Nakhon Ratchasima province in March 2020 that subsequently spread to 17 provinces, including Prachuap Khiri Khan province (Bunpapong et al., 2021; Castillo-Olivares, 2021). Later, in September 2020, AHS was reported and confirmed in horses in Malaysia (Shere, 2021). These were the first outbreaks reported in Southeast Asia (King et al., 2020; Castillo-Olivares, 2021; Nelson et al., 2022). AHS is endemic in sub-Saharan Africa and has recently invaded Europe and Asia (Carpenter et al., 2017), affecting members of the family Equidae. Horses are the most susceptible to AHS, with a mortality rate of up to 95%, followed by mules, with mortality rates in the range 50-70%, while donkeys and zebras rarely show symptoms after infection (Wilson et al., 2009; Long and Guthrie, 2014). The mortality rate from outbreaks in Thailand has been reported as 93% of infected horses (Bunpapong et al., 2021). The African horse sickness virus (AHSV) is biologically transmitted by the bites of hematophagous biting midges of the genus Culicoides. Field and laboratory-based trials have demonstrated that Culicoides imicola Kieffer is a major vector of AHSV in South Africa (Paweska et al., 2003; Venter et al., 2000, 2006a, 2009). Notably, Culicoides oxystoma Kieffer is considered a potential vector for AHS in Senegal due to its abundance on horses and other widespread species (Fall et al., 2015a, b). These two

species have been found in Thailand (Wirth and Hubert, 1989; Thepparat et al., 2015) and Choocherd et al. (2022) observed that both species were the two most-abundant species in horse stables in Prachuap Khiri Khan province.

The seasonal incidence and abundance of adult female *Culicoides* populations are important parameters in determining the timing and intensity of *Culicoides*-borne arbovirus outbreaks (Sanders et al., 2011; Searle et al., 2014). The distribution and transmission of AHS is dependent on the temperature and season and the abundance and prevalence of vectors (Backer and Nodelijk, 2011; Zientara et al., 2015). The relationship between climatic variables and vector abundance can provide useful information about the epidemiological processes of vector-borne diseases.

Materials and Methods

This study was conducted at a horse stable, housing 20 horses (12°34'36.57"N, 99°53'09.00"E; 3 m above sea level, a.s.l) in Hua Hin district of Prachuap Khiri Khan province. This province is located in the lower central part of Thailand on the western coast of the Gulf of Thailand (10°57'–12°39'N and 99°8'–100°04'E; 0–10 m a.s.l) covering approximately 6,360 km² (Choowong et al., 2009). The climatic pattern within Prachuap Khiri Khan province is divided into three seasons: the hot season (mid-February to mid-May), the rainy season (mid-May to mid-October) and the dry season (mid-October to mid-February). In addition, rains can last through until November.

The insect collections were operated continuously between 1800 hours and 0600 hours using four Center for Disease Control miniature light traps with different light sources: an incandescent light, an ultraviolet light-emitting diode (UV-LED) light, a 4 W UV fluorescent light and a 4 W white fluorescent light. The experiment was conducted for 1 night/ mth from November 2020 to October 2021. Traps were hung on tripods, 1.5 m above the ground around the horse stable and 10 or 15 m apart from each other. To reduce any position-specific effect, all traps were rotated to the next trap position after each sampling (González et al., 2016). Captured Culicoides specimens were preserved using 95% ethanol until processing. Culicoides female specimens were identified to species by the wing pattern (Wirth and Hubert, 1989; Dyce et al., 2007; Bellis et al., 2015). Weather parameters were obtained from the Thai Meteorological Department Service at Hua Hin (Station 500202), as shown in Fig. 1.

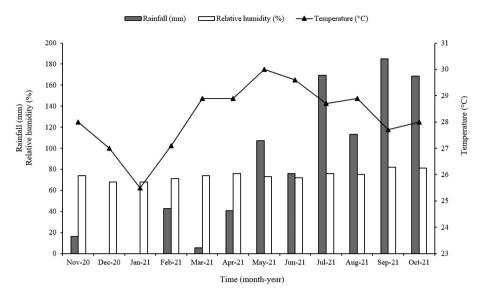


Fig. 1 Monthly total rainfall, mean temperature and mean relative humidity during survey in Hua Hin district, Prachuap Khiri Khan province, Thailand from November 2020 to October 2021

The data were tested for normality and data were log-transformed [log (n+1)] when needed. Then the data on monthly numbers of females of C. imicola and C. oxystoma were analyzed using a one-way analysis of variance and the least significant difference (LSD) post hoc test was used for mean comparisons. Pearson's correlation coefficient (r) was calculated to estimate relationships between abundances of females for C. imicola and C. oxystoma and weather parameters. All statistical tests were considered significant at p < 0.05. The data analyses were performed using the R statistical software version 4.1.2 (R Core Team, 2018).

Results

In total, 12,583 specimens of the *Culicoides* genus were collected, with 10,849 (86.2%) being females and 1,734 (13.8%) males, as shown in Table 1. The highest total number of *Culicoides* specimens was collected in the light traps baited with the 4 W UV fluorescent light (n = 7,667), followed by the 4 W white fluorescent light (n = 2,047) and the incandescent light (n = 234). The three most-abundant species were *C. imicola* 4,706 (43.4%), *C. oxystoma* 2,652 (24.4%) and *C. peregrinus* 1,810 (16.7%). Another 20 *Culicoides* species were identified: *C. tainanus*, *C. actoni*, *C. fulvus*, *C. flavipunctatus*, *C. huffi*, *C. innoxius*, *C. orientalis*. *C. brevitarsis*, *C. asiana*, *C. similis*, *C. clavipalpis*, *C. geminus*, *C. halonostictus*, *C. guttifer*, *C. arakawae*, *C. sumatrae*, *C. griffithi*, *C. brevipalpis*, *C. shortti* and *C. homotomus*.

During the entire study period, *C. imicola* individuals were present, indicating all-year-round occurrence, with three seasonal abundance peaks: the highest in November (dry season), another in March (hot season) and the third during June–September (rainy season). The lowest number was observed in April (Fig. 2). In addition, *C. oxystoma*, occurred throughout the year, with the first of the two seasonal peaks being a large peak during July–September (rainy season) and the second, smaller one in January (dry season), while low numbers occurred during February–April (Fig. 2).

Variation in the abundance levels of C. imicola and C. oxystoma was observed within three different seasons (hot, rainy, dry), as shown in Fig. 3. The highest numbers of C. imicola were collected in the dry season, whereas the highest numbers of C. oxystoma were collected in the rainy season. During the dry and hot seasons, C. imicola was highest in numbers, followed by C. oxystoma; however, the number of C. oxystoma collected was higher than for C. imicola during the rainy season. Both species were trapped in lower numbers during the hot season. However, there were no significant differences in the mean numbers of C. imicola (F-test value = 1.084; degrees of freedom, df = 2, 9; p = 0.379) and C. oxystoma (F-test value = 4.215, df = 2, 9; p = 0.051) collected among seasons.

Pearson's correlation tests showed non-significant correlation between the abundance of *C. imicola* and the following parameters: rainfall (r = -0.091, p = 0.778); temperature (r = -0.221, p = 0.49); and relative humidity (r = 0.007; p = 0.983). Rainfall and relative humidity were not significantly correlated with the number of *C. oxystoma* collected (r = 0.551, p = 0.063; r = 0.232, p = 0.468, respectively), and neither was temperature (r = -0.189, p = 0.556).

Table 1 Total numbers of *Culicoides* specimens collected using four light sources in Hua Hin district, Prachuap Khiri Khan province, Thailand from November 2020 to October 2021

Species	2020		2021										Total	%
	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct		
Culicoides imicola	1,753	690	115	159	515	16	63	303	275	62	510	245	4,706	43.4
Culicoides oxystoma	198	136	406	26	44	36	185	213	581	197	404	226	2,652	24.4
Culicoides peregrinus	625	268	9	18	13	21	14	41	362	67	211	161	1,810	16.7
Culicoides tainanus	0	1	0	0	0	9	3	47	57	3	115	88	323	3.0
Culicoides actoni	103	33	3	15	2	0	4	5	11	4	1	4	185	1.7
Culicoides fulvus	98	11	3	1	2	0	3	6	23	1	11	7	166	1.5
Culicoides flavipunctatus	28	5	0	4	0	0	1	5	11	6	25	54	139	1.3
Culicoides huffi	2	9	2	5	7	6	18	3	4	6	2	49	113	1.0
Culicoides innoxius	13	8	4	8	4	0	6	4	20	0	29	6	102	0.9
Culicoides orientalis	0	0	0	0	0	0	3	19	5	0	36	20	83	0.8
Culicoides brevitarsis	7	10	5	7	2	1	1	3	3	0	3	0	42	0.4
Culicoides asiana	3	1	0	0	19	0	1	7	3	1	5	1	41	0.4
Culicoides similis	1	7	2	1	4	3	2	1	1	4	0	3	29	0.3
Culicoides clavipalpis	1	0	1	0	1	1	2	0	10	2	2	0	20	0.2
Culicoides geminus	0	0	0	0	0	0	1	4	13	2	0	0	20	0.2
Culicoides halonostictus	0	0	3	0	0	2	2	1	4	6	0	0	18	0.2
Culicoides guttifer	2	1	0	1	0	0	0	0	0	3	1	4	12	0.1
Culicoides arakawae	2	1	1	0	0	0	0	0	0	1	0	6	11	0.1
Culicoides sumatrae	0	0	0	0	0	3	0	5	0	0	0	1	9	0.1
Culicoides griffithi	1	0	0	0	0	0	0	0	5	0	0	0	6	0.1
Culicoides brevipalpis	3	1	0	0	0	0	0	0	0	1	0	0	5	0.0
Culicoides shortti	0	1	0	1	0	0	0	0	0	0	0	0	2	0.0
Culicoides homotomus	0	0	0	1	0	0	0	0	0	0	0	0	1	0.0
Culicoides spp.	52	41	19	14	40	16	5	33	57	13	44	20	354	3.3
Female	2,892	1,224	573	261	653	114	314	700	1,445	379	1,399	895	10,849	86.2
Male	383	208	36	65	57	28	125	107	295	106	226	98	1,734	13.8
Total	3,275	1,432	609	326	710	142	439	807	1,740	485	1,625	993	12,583	

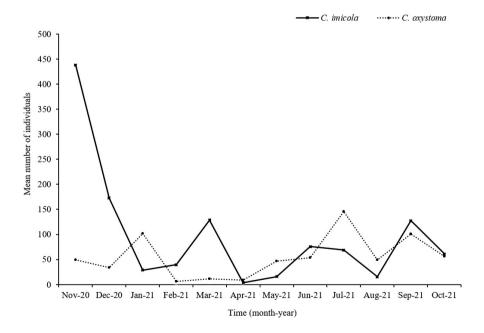


Fig. 2 Seasonal abundance of *Culicoides imicola* and *Culicoides oxystoma* collected in Hua Hin district, Prachuap Khiri Khan province, Thailand from November 2020 to October 2021

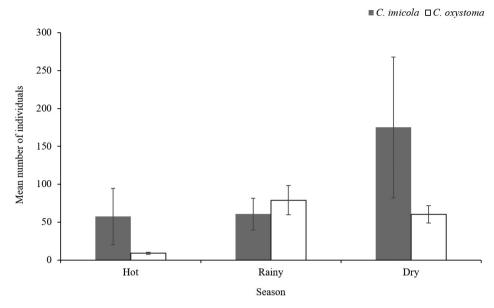


Fig. 3 Mean numbers \pm SE of *Culicoides imicola* and *Culicoides oxystoma* collected using four light sources during three seasons in Hua Hin district, Prachuap Khiri Khan province, Thailand from November 2020 to October 2021; no statistical difference (p > 0.05) was observed between seasons within a species

Discussion

During the survey, the two predominant species were C. imicola and C. oxystoma. Similar findings were reported in Senegal (Diarra et al., 2015) and India (Archana et al., 2016). Furthermore, C. imicola is the proven field vector of AHSV in South Africa (Rawlings et al., 1997; Venter et al., 2000, 2009) and is suggested to be the main vector of bluetongue virus (BTV), which infects domestic and wild ruminants in Africa and the Mediterranean (Venter et al., 1998; Mellor et al., 2000; Venter et al., 2006b; Nolan et al., 2008). C. oxystoma has been implicated in the transmission of several bovine arboviruses to livestock, such as Akabane in Japan (Kurogi et al., 1987; Yanase et al., 2005) and epizootic hemorrhagic disease virus in Israel (Morag et al., 2012). It is thought to be a potential vector of BTV in India (Dadawala et al., 2012) and China (Di et al., 2021). In Senegal, C. oxystoma has been suggested as a possible vector of the AHS and BT viruses (Fall et al., 2015b). These two species may be potential vectors involved in AHS transmission in Thailand. However, its vector competence is unknown under natural environmental conditions in Thailand.

In general, the seasonal abundance of vectors on livestock farms is likely to be linked to the efficiency of virus transmission and the occurrence of outbreaks (Miranda et al., 2014). In the current study, the seasonal abundance of the two predominant

species in Hua Hin district, Prachuap Khiri Khan province suggested that they were found every month of the year. The results were similar to those reported by Diarra et al. (2015) who found that C. imicola and C. oxystoma were present throughout the year in the Niaves area, Senegal. Furthermore, the former species was found in Nigeria (Herniman et al., 1983), Israel and Zimbabwe (Braverman et al., 1985), where the species was reported throughout the year. Although seasonal difference in abundance of C. imicola was not supported by the statistical tests, the abundance tended to be high during November and December (dry season), whereas C. oxystoma tended to have a period peak in abundance between July and September (rainy season), while these two species were present in relatively low numbers during the hot season. This was similar to the findings in a study conducted by Diarra et al. (2014) in the Niayes area, Senegal, where it was reported that the highest abundance of C. imicola was found in the cold dry season (February) and of C. oxystoma in the rainy season (August-October). In India, the seasonal prevalence revealed the highest numbers of Culicoides species (including C. imicola and C. oxystoma) in the rainy season and low numbers in summer, except for C. oxystoma whose low numbers coincided with the winter season (Archana et al., 2014; Sathiyamoorthy et al., 2021). The variations in the abundance peaks for the collected Culicoides species might have been due to variations in the sites and species (Diarra et al., 2014).

Temperature, humidity and rainfall are crucial factors influencing the activity, richness, abundance and survival of Culicoides populations (Silva and Carvalho, 2013; Gusmão et al., 2019). For example, temperature had a positive correlation with C. imicola abundance in Spain (Ortega et al., 1997) and on Réunion Island (Grimaud et al., 2019). The greatest numbers of C. imicola were recorded for daily minimum and maximum temperatures of 18°C and 38°C, respectively (Ortega et al., 1997). In Senegal, the abundance of *C. oxystoma* was positively correlated with temperature and relative humidity, while being negatively correlated with the interaction between temperature and rainfall; in contrast, the abundance of C. imicola was negatively correlated with relative humidity and rainfall (Diarra et al., 2015). Changes in climatic conditions, such as rainfall, which help to lower the temperature and increase the humidity and produce more suitable breeding sites, favor increasing Culicoides abundance (Gusmão et al., 2019). However, high rainfall may lead to a decrease in temperature and inhibit the activity of some Culicoides species (Diarra et al., 2015). In the current study, C. imicola and C. oxystoma were active in the study area throughout the year with no significant differences between seasons. Furthermore, temperature and relative humidity did not seem to influence the abundance levels for both C. imicola and C. oxystoma, while rainfall although seemed to be correlated with C. oxystoma abundance but without statistical support. There are probably other environmental factors that influence the abundance of arthropod vectors (including Culicoides species) comprising abiotic or biotic factors, such as climatic variables, animal host populations and vector larval habitats (Tabachnick, 2010). More research is necessary to better understand the abiotic and biotic factors affecting Culicoides abundance and distribution in AHS-affected areas in Thailand.

The current study has provided information on occurrence of *C. imicola* and *C. oxystoma*, which may be potential vectors for AHSV outbreak in Thailand. Thus, entomological surveillance should monitor the presence of these predominant species to prevent the emergence of new diseases (Gusmão et al., 2019).

Ethics statements

Animal care and all experimental procedures were approved by the Institutional Animal Care and Use Committee, Faculty of Veterinary Medicine, Kasetsart University, Bangkok, Thailand (Approval no. ACKU63-VET-043).

Conflict of Interest

The authors declare that there are no conflicts of interest.

Acknowledgments

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