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Research Article

Occurrence of *Culicoides* Latreille (Diptera: Ceratopogonidae) Collected from a Layer Farm in Yogyakarta

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Abstract

Background and Objective: *Culicoides* spp. compose the most studied vector genera and contain the species that is a vector of *Leucocytozoon*, the parasite that causes leucocytozoonosis. One hundred species of *Culicoides* spp. inhabit Indonesia and are scattered among 19 provinces, with great diversity in each region. The aim of this research was to study the species diversity and the dominance of *Culicoides* on layer farms. **Methodology:** Midges were obtained from layer farms in Sentolo, Kulon Progo (altitude ± 78 m); Pakem, Sleman (altitude ± 371 m) and Cangkringan, Sleman (altitude ± 745 m). Midges were collected using a light trap (New Jersey Standard #2858) for 12 h, from 6 pm-6 am. Identification was performed using dissecting microscope and classified using the identification key of Wirth and Hubert. **Results:** Eight species were found in this study: *Culicoides arakawae*, *C. huffi*, *C. oxystoma*, *C. guttifer*, *C. sumatrae*, *C. peregrinus*, *C. palpifer* and *C. fulvus*. There was diversity in the species collected from the three research areas. *Culicoides arakawae* was the dominant species found in Sentolo; in Pakem, the most collected species was *C. huffi* and in Cangkringan, it was *C. fulvus*. **Conclusion:** There was a difference in the number of midges collected from the areas with differing topography. The largest collection of *Culicoides* (270 midges) was obtained from Sentolo subdistrict, Kulon Progo Regency.

Key words: *Culicoides* spp., vector, *Leucocytozoon*, egg-layer farms, topography

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Culicoides spp. are vectors of some pathogenic diseases. While only a small portion of *Culicoides* spp. are vectors, *Culicoides* have a significant adverse impact through the transmission of arboviruses, bacteria, protozoa and parasitic worms between humans and animals. *Culicoides* is the group most studied, one species of which is a vector of *Leucocytozoon*, the organism that causes leucocytozoonosis^{1,2}. Leucocytozoonosis is a parasitic disease in poultry that can cause loss in productivity, such as a decline in egg production, loss of up to 30% of body weight and eventually, death^{3,4}.

Approximately 1400 species of this genus have been recorded throughout the world⁵. Only females of some *Culicoides* species, however, act as vectors of pathogens (viruses, protozoa and filarial nematodes) of medical and veterinary importance^{6,7}. Some *Culicoides* spp. are vectors or suspected vectors of leucocytozoonosis in Indonesia, including *C. arakawae*, *C. oxystoma*, *C. guttifer*, *C. huffi*, *C. hegneri*, *C. oxystoma*, *C. peregrinus* and *C. humeralis*. There are one hundred species of *Culicoides* spp. spread over 19 provinces in Indonesia⁸. There are different species in different areas. The diversity of species may be caused by differences in the climatic conditions in each region. Tropical areas are generally characterized by almost uniform climatic

conditions, however, topography can cause overall weather and climate differences, especially in temperature, humidity and rainfall. Weather and climate elements are heavily influenced by latitude, altitude, distance from sea, topography, soil type and vegetation. These elements affect the breeding sites of *Culicoides* spp. according to sites of each species⁹⁻¹¹.

The aim of this study was to provide information on the diversity of *Culicoides* spp., especially on egg-layer farms in different topographic areas. This information can be used as a reference to understand the risk of *Culicoides*-borne diseases in an area and aid in determining the development of effective control strategies specific to farm location.

MATERIALS AND METHODS

Sample sites: During this study, insect trapping was performed in locations where previous Leucocytozoonosis occurred. *Culicoides* were collected from 2 different sources in Sleman and Kulon Progo district in Daerah Istimewa Yogyakarta, Indonesia. The "Special Region" (province) of Daerah Istimewa Yogyakarta comprises 4 districts and 1 city: Kulon Progo district, Gunung kidul district, Bantul district, Sleman district and Yogyakarta city. *Culicoides* were collected from poultry farms in Sleman (107°15'03" 107°29'30" E, 7°47'51" and 7°47'30" S) and Kulon Progo (110°1'37"-110°16'26" E 7°38'42"-7°59'3" S) (Fig. 1).

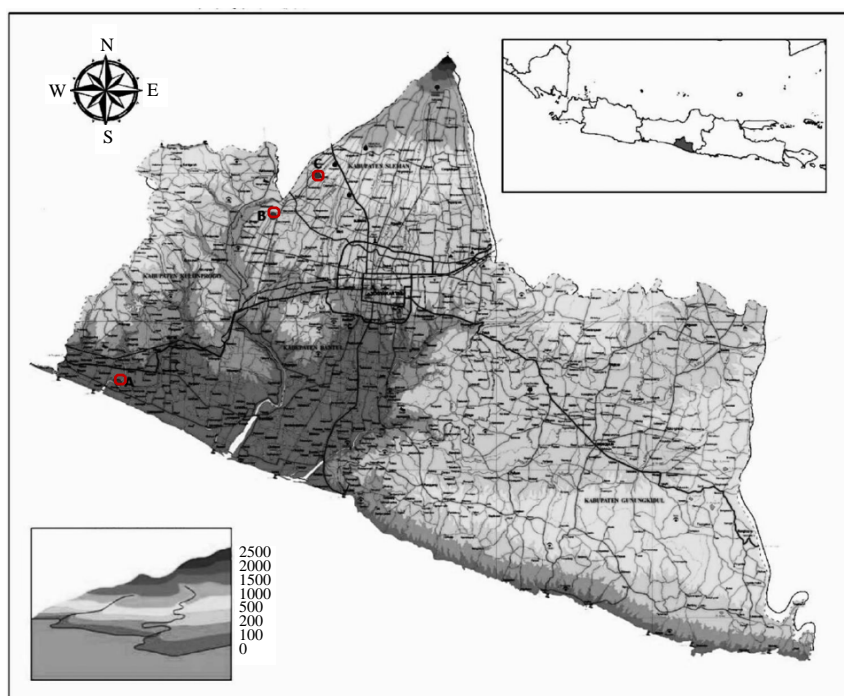


Fig. 1: Survey sites for *Culicoides* spp. collected in Yogyakarta, Indonesia

A: Sentolo subdistrict, Kulon Progo regency, B: Pakem subdistrict, Sleman regency and C: Cangkringan subdistrict, Sleman regency

Sampling and identification of *Culicoides*: Two traps were placed in each farm to collect specimens. The light traps were activated from 6 pm-8 am to coincide with dusk/dawn and night-time activity of most *Culicoides* species. Light traps were placed 1.5-2 m above ground and sheltered from wind and any source of artificial light. *Culicoides* specimens collected in the light traps were recovered in the morning and strained to separate the insects into appropriately labeled jars. All *Culicoides* specimens were subsequently separated and identified according to their wing pattern. Samples were preserved in 70% alcohol and stored at room temperature until identified.

Identification of *Culicoides* spp.: The collected samples were sorted to separate *Culicoides* spp. from other insects. Morphological identification of *Culicoides* species was conducted by examining the wing pigmentation pattern using a stereo microscope. Species identification was performed on the wing patterns of *Culicoides* spp. according to Wirth and Hubert¹² and Uslu and Dik¹³.

RESULTS

In this study, 405 *Culicoides* specimens were collected (270 from Sentolo, 54 from Pakem and 81 from Cangkringan) (Table 1). The captured specimens were classified into 8 species. The most abundant species was *C. huffi* (32.59%), which was collected in all of the locations. *C. huffi*, representing 32.59% of *Culicoides* collected, was the dominant species. *Culicoides arakawae*, *C. huffi* and *C. peregrinus* each contributed more than 10% to the species composition.

A total of 405 *Culicoides* midges comprising eight species collected from three areas of Yogyakarta. A total of 270 (66.67%) midges were recovered from Sentolo, 54 (13.33%) midges were recovered from Pakem, 81 (20%) midges were recovered from Cangkringan. Eight *Culicoides* species; *C. arakawae* (31.11%), *C. huffi* (32.59%), *C. oxystoma* (1.48%), *C. guttifer* (8.64%), *C. sumatrae* (0.74%), *C. peregrinus* (4.1%), *C. palpifer* (0.74%) and *C. fulvus* (15.31%) were identified (Table 2).

DISCUSSION

Midges were collected from commercial layer chicken farms in Sentolo sub-district, Kulon Progo regency; Pakem sub-district, Sleman regency and Cangkringan sub-district, Sleman regency. Climate data for each location can be seen in Table 1.

Table 1: Climate data

Location	Altitude (masl)	Temperature (°C)	Humidity (%)	Rainfall (mm)	Wind velocity (km h ⁻¹)
Sentolo	78	26.5	87	395	7.4
Pakem	371	26.7	87	343	25.9
Cangkringan	745	24.8	88	308	12.2

Source: BMKG Yogyakarta (2017)

Table 2: *Culicoides* spp. collected from three areas

Species	Sentolo	Pakem	Cangkringan	Total
<i>C. arakawae</i>	119	7	0	126
<i>C. huffi</i>	107	22	3	132
<i>C. oxystoma</i>	4	1	1	6
<i>C. guttifer</i>	32	5	1	38
<i>C. sumatrae</i>	1	2	0	3
<i>C. peregrinus</i>	6	2	27	35
<i>C. palpifer</i>	1	1	1	3
<i>C. fulvus</i>	0	14	48	62
Total	270	54	81	405

The farm located in Sentolo was at an altitude ± 78 masl with a temperature of 26.5°C, humidity of 87%, 395 mm of rainfall and wind velocity of 7.4 km h⁻¹. The farm located in Pakem is situated under the slopes of Mount Merapi, at an altitude ± 371 masl with a temperature of 26.7°C, almost the same temperature as Sentolo. Humidity in Pakem is similar to Sentolo (87%), but the rainfall is only 343 mm, which is less than Sentolo. The farm in Pakem has the highest wind velocity (25.9 km h⁻¹). Mellor *et al.*⁶ showed that wind speed most significantly affects populations of *Culicoides*. Wind speed affects flying activity and the incidence of insect bites. *Culicoides* that entered the trap were assumed to be adult midges. High wind speeds make it difficult for *Culicoides* to target hosts.

Cangkringan is located on the slopes of Mount Merapi and the farm in Cangkringan is located at an altitude of 745 masl. It has the lowest temperature of all the collection locations. The temperature is 24.8°C and the humidity is 88%. Rainfall in Cangkringan is the lowest (308 mm). The wind velocity in Cangkringan is 12.2 km h⁻¹, higher than the wind velocity in Sentolo and lower than the wind velocity in Pakem. The tropics are generally characterized by almost uniform climatic conditions. However, the difference in altitude above sea level (masl) can cause differences in weather and climate as a whole, but especially in temperature, humidity and rainfall. These weather and climate elements are heavily influenced by latitude, altitude, distance to the sea, topography, soil type and vegetation. Low altitudes are characterized by ambient temperature, high air and oxygen pressures and increased rainfall. The rate of temperature change is due to having different variations for each place⁹.

Puddles and bushy plants were found around the farms in each area. The research location in Sentolo was dominated



Fig. 2(a-c): Commercial egg-layer farms of survey sites

(a) Sentolo subdistrict, Kulon Progo regency, (b) Pakem subdistrict, Sleman regency and (c) Cangkringan subdistrict, Sleman regency

by teak plants with rice fields surrounding the farm. The Pakem site was dominated by shrubs with many puddles around the farm. The Cangkringan region was dominated by bamboo plants. The types of plants in each region varied and can be seen in Fig. 2. Many studies have shown that abiotic environmental factors, such as topographic parameters, can be important sources of variation of plant diversity¹⁴⁻¹⁶.

A total of 405 *Culicoides* midges comprising eight species were collected from the three areas of Yogyakarta. A total of 270 (66.67%) midges were recovered from Sentolo, 54 (13.33%) midges were recovered from Pakem and 81 (20%) midges were recovered from Cangkringan. Eight *Culicoides* species were identified: *C. arakawae* (31.11%), *C. huffi* (32.59%), *C. oxystoma* (1.48%), *C. guttifer* (8.64%), *C. sumatrae* (0.74%), *C. peregrinus* (4.1%), *C. palpifer* (0.74%) and *C. fulvus* (15.31%) (Table 2). Site differences caused by climate differences affect habitats and can result in different numbers of midges^{17,18}.

Culicoides arakawae was the dominant species, especially in Sentolo. Seven *C. arakawae* were found in Pakem. *Culicoides arakawae* was not found in Cangkringan.

In Sentolo, there were many trees and there were rice fields around the farms. These conditions support breeding sites of *C. arakawae*. *Culicoides huffi* was also commonly found in the Sentolo area and is the dominant species found in Pakem. Around the farm in Pakem were many shrubs and nearby were trenches with small amounts of flowing water. According to Wirth and Ratanaworabhan¹⁸, breeding sites of *C. arakawae* include muddy puddles, which are often found in rice fields. The breeding sites of *C. huffi* are usually on the sides of rivers or where there are many puddles. *Culicoides fulvus* was the dominant species found in Cangkringan. There are many cow and sheep farms in Cangkringan. Breeding sites of *C. fulvus* are not known. *Culicoides fulvus* are usually found sucking the blood of cows, buffalo, sheep and marsupials¹⁹. Generally, hematophagous species (such as mosquitoes and *Culicoides*) prefer a specific host on which to feed²⁰⁻²³, although some species are generalists. Some *Culicoides* species, however, exhibit opportunistic feeding behavior with respect to host distribution and density^{22,24-26}. Most species of the genus *Culicoides* are known to be either mammalophilic^{24,26,27} or ornithophilic^{21,27,28}.

Culicoides guttifer, *C. peregrinus*, *C. oxystoma* and *C. palpifer* were found in all areas. *Culicoides guttifer* are commonly found in Sentolo, where the area is dominated by poultry farms. Thirty-eight *C. guttifer* specimens were found: 32 from Sentolo, five from Pakem and one from Cangkringan. *Culicoides guttifer* has a preferred host of poultry⁸. *Culicoides peregrinus* is commonly found in Cangkringan. Twenty-seven *C. peregrinus* specimens were recovered from Cangkringan, six from Sentolo and two from Pakem. Mean temperature and relative humidity of the capture day, mean humidity between 21 and 19 days prior to capture event, density of ruminants, percentage cover of water bodies within a 2 km radius and interaction between temperature and humidity were predictors for presence of *C. oxystoma*. Mean rainfall, NDVI of the capture day and percentage cover of water bodies were indicators for *C. imicola* presence²⁹. Cangkringan had a mean temperature of 24.8°C and there are many tall bamboo trees blocking the sunlight. These conditions support breeding sites of *C. peregrinus*. *Culicoides oxystoma* and *C. palpifer* were found in every site in low numbers. Six *C. oxystoma* specimens were recovered; four specimens in Sentolo, one from Pakem and one from Cangkringan. Only one *C. palpifer* was recovered in each area. One *C. sumatrae* was found in Sentolo and two in Pakem. It has been previously reported that *Culicoides* species breed in various sites including mud rich organic matter, water reservoirs, along streams, rain puddles, mud around dams, reed sites and cow dung⁴.

The implication of this study is that the differing number of specimens between locations might be an indicator of the establishment and dynamics of settled *Culicoides* spp. The application of this study is to identify the various *Culicoides* species and use this information to prevent the transmission of diseases through *Culicoides*. The importance of this study is that the types of the *Culicoides* species can be one of the risk factors for the transmission of diseases transmitted by the vector *Culicoides*.

CONCLUSION AND FUTURE RECOMMENDATIONS

Based on the results, it can be concluded that there is a difference in the number of midges obtained from the three areas. Differences in climatic factors at sampling areas with different altitudes affect the habitat of *Culicoides*. The most *Culicoides* species (270 midges) were obtained from Sentolo subdistrict, Kulon Progo regency. This site had an altitude of 78 masl, a temperature at 26.5°C, humidity of 87%, 395 mm of

rainfall and a wind velocity at 7.4 km h⁻¹. *Culicoides arakawae* was the dominant species. The conditions at Sentolo support breeding sites of *Culicoides*.

This study can provide explanations of the risk factors of transmission of diseases transmitted by the variety of *Culicoides* vectors, especially in the Yogyakarta region. Further identification should be performed using PCR to more accurately reveal the *Culicoides* species.

SIGNIFICANCE STATEMENT

This study determined the diversity of *Culicoides* spp. at three locations in Yogyakarta. This article reports that *Culicoides arakawae* was the most common midge species found in Yogyakarta. *Culicoides arakawae* is a vector for leucocytozoonosis. This study is beneficial because it informs veterinarians about the risk of diseases caused by *Culicoides* sp. This study will help research efforts to understand the variety of *Culicoides* spp. in Yogyakarta that has not previously been described.

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