Host Landing And Diel Activity of Potent Vectors of Bluetongue Disease, *Culicoides Oxystoma* and *Culicoides Peregrinus*

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Research

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Abstract

Background The spread of bluetongue virus depends on the vectorial ability of Culicoides affecting the susceptible host. Animal farms in West Bengal have reported prevalence of potent vectors of BTV (C. oxystoma, C. peregrinus and C. fulvus). Besides, high seroprevalence of BTV was also reported from this cattle dense region. Henceforth host-seeking activity of two important potent vectors, C. oxystoma and C. peregrinus on cattle were studied in two farm sites of West Bengal, India.

Methods The study was done in 2018-19 comprising of total 297 hours of collection over 27 nights. A comparison was made between the catches obtained by mouth aspirator and light trap. Hourly collections of Culicoides were done directly from cattle (oral aspirator) as well as light trap was operated in close vicinity of cattle at a different shed.

Results A total of 11,462 Culicoides belonging to C. oxystoma, C. peregrinus and C. fulvus were collected in light trap and aspirator. In aspirator 4764 midges were collected whereas 6698 individuals were collected in light trap. The following species were aspirated: C. peregrinus and C. oxystoma; however the light trap catches consisted of C. fulvus, C. oxystoma and C. peregrinus. Light trap collection exhibited crepuscular activity whereas aspirator collection was maximum between 4.00 am and 5.00 am. Likewise maximum landing of midges was observed in neck and hump region of cattle.

Conclusion It was observed that the preferred time of feeding of C. peregrinus and C. oxystoma on cattle were early morning hours though midges were ubiquitous from dusk to dawn. Surprisingly the preferential landing of the two vectors were mostly restricted to the neck and hump region of the cattle. The results obtained during the study warrants further insight into the factors influencing the landing site by the vectors which may be useful biological data in disease management and draw effective deterrent strategies.

Background

Among the hematophagous genera, Culicoides have assumed significance and notoriety worldwide due to its ability to transmit a wide range of pathogens of public and veterinary importance [1]. Presently India records 535.78 million as major farm animals i.e cattle, buffalo, poultry, sheep and goat including indigenous and exotic breeds, with a total bovine population of 302.79 million (buffalo, cattle, mithun, yak) [2]. Such presence of large numbers and variety of livestock and density of livestock is at risk in transmission of an orbivirus bluetongue (BT) by Culicoides midges and which may lead to outbreak of BTD. Besides, This virus causes bluetongue disease (BTD) in goats, sheep, cattle and other small and wild ruminants [3]. Although the disease was dominant in temperate zones, introduction of non-native breeds in virus endemic tropical and subtropical zones also caused BT outbreak [4]. The spread of range of BT in the temperate was also attributed to global warming [5]. Presently seroprevalence of bluetongue virus (BTV) has been reported across all the Indian states [6]. The BTD outbreak has been witnessed frequently owing to the significant density of livestock, high prevalence of the Culicoides along with conducive climatic conditions helping in propagation of vectors and the virus [7, 8]. BTV was first recorded from the state of Maharashtra [9] and also occurrences of BTV from Indian states covering western, northern and southern regions followed [10, 11]. Although eastern and north eastern India is yet to witness an outbreak, high seroprevalence in livestock has appeared in several scientific articles [11, 12]. Presently 22 of the 28 serotypes (worldwide) have been recorded from India [13]. Moreover BT seropositivity in different animals depicts 34% in buffalo, 16% in camel, 38% in cattle, 43% in goat, 39% in sheep and 66% in mithun [13]. According to [2] state of West Bengal shares 6.9% of total livestock population comprising 8.25% cattle, 0.8% of sheep and 10.93% goats’ population. The economy of West Bengal, especially in Burdwan district is agriculture based (mixed farming) in which livestock and animal husbandry plays a pivotal role. The villagers form a cluster of self help groups (SHG) which market livestock products to generate revenue [14]. The seroprevalence in goat (66.95%), sheep (57.66%) and cattle (52%) has been reported across 7 districts of West Bengal [15, 16]. Despite such high seroprevalence, BTD outbreak remains unreported from West Bengal [17]. BTD outbreak and its economic implications has been well documented from the southern Indian states, however, there remains critical knowledge gaps and understanding of biology of the potent vector species prevalent in this tropical region. Few studies on biology, ecology and taxonomy of the Culicoides spp were carried out [8, 17]. Besides, [18] has reported prevalence of 7 putative vectors C.oxystoma, C. fulvus, C. orientalis, C. dumrumi, C. imicola, C. peregrinus and C. brevitarsis from southern India. In the eastern state of West Bengal, C. fulvus, C. peregrinus and C. oxystoma were the prevalent species in mixed farms and cattle sheds [8, 17] thereby posing considerable risk in the spread of disease. Further a coloured LED light trap based extensive surveillance program was also conducted in the same site to ascertain the prevalence of the aforesaid species in the cattle sheds [8].

Information available on host seeking activity, ecology and biology of Culicoides are limited across the globe [19, 20]. The aforesaid information was imperative for implementation of control measures in disease epidemiology [20]. Although the disease causing pathogens vectored by Culicoides were a matter of concern, relatively scant information on its bionomics exists in India [18] and Europe [20, 21, and 22]. In the light of forecasting disease transmission, limited information on the host-vector association has led to assumptions that all the Culicoides species prevalent in the cattle sheds fed on host with equal facility on which various predictions were based [21]. Besides difficulty in aspirating Culicoides from the host body also limits studies on host seeking activity of the Culicoides [19].

In this study the catch data obtained by two different methods (light trap and aspirator) were evaluated and compared in order to separate two adult activity of Culicoides spp. in real time: flight activity and biting activity. The two study sites were selected at Burdwan district of West Bengal due to report of seroprevalence of BTV from this region [12] as well as high seasonal abundance of potent vector species of Culicoides associated with livestock from this district [17]. Moreover, the evaluation enables validation of the time and preferential landing of Culicoides on cattle body parts which is critical at farmer's level to reduce the host-vector contact.

Materials And Methods

Study site
This investigation was carried out in two rural villages of West Bengal 51 km apart i) Dharan (DH; 23°02'57.7"N, 87°51'47.5"E), ii) Sahibganj-Tantipara (ST; 23°44'29.14"N, 87°82'76.56"E). The biting midges were trapped throughout the seasons in DH during May to October, 2018 and in April to June, 2019, and in ST from May to October, 2018. In India, Indian Meteorological Department (IMD) recognizes the occurrences of four seasons: winter, December to February; summer or pre-monsoon season, lasting from March to May; monsoon or rainy season, lasting from June to September; Post-monsoon or autumn season, lasting from October to November. At ST village site, 12 night collections were made i.e., two catches/month, whereas in DH village site, monthly three collections were done (excepting for the months of October, April and June where single collections/every month). A total of 27 night catch data (297 hrs of collection) were considered for both the sites. In both the sites, the sheds housing the cattle were made of mud-brick adjoining to household. Geographically the villages are situated amidst agricultural fields in which extensive rice cultivation is practiced throughout the year, water logging even during dry seasons predominantly rice growing areas of West Bengal. Most households maintain livestock animals for their economic sustenance. It experiences tropical climate with annual rainfall of 1496 mm. The hygiene conditions are compromised within and outside the perimeter of the animal sheds as dung heaps and paddy straws were garbage. In ST, the cattle sheds were located on the embankment of a pond. Moreover, the drainage system of the village was found to empty into the pond. For this study one cattle shed at DH and two cattle sheds at ST were chosen.

Description of collection

The adults that landed on the host body surface was retrieved by aspirators (oral & mechanical) and flying adults were trapped by LED based light traps installed at close vicinity of cattle within the shed. Adults were aspirated covering various parts of the body surface of a white coloured adult cow. For our convenience the entire body surface of the cattle was subdivided [23] following the studies of [20] and [21]. Catches made were labeled as follows: head (H1), neck (H2), hump (H3), back (H4), leg (H5), belly (H6), hip (H7) (Figure 1). Each catch duration was restricted to 10 minutes/hour the entire process of aspiration initiated on 18.00hrs in the evening that continued up to 6.00hr in the morning (12 hours per diem). The catch period (designated as T1 hr to T11 hr) was followed: T1: 18.00-19.00, T2: 19.00-20.00, T3: 20.00-21.00, T4: 21.00-22.00, T5: 23.00-00.00, T6: 00.00-01.00, T7: 01.00-02.00, T8: 02.00-03.00, T9: 03.00-04.00, T10: 04.00-05.00, T11: 05.00-06.00. During the process of aspiration all other cattle stationed within the sheds were evacuated at least half an hour prior to collection. 4W dim white light was used during the process of collection of the Culicoides from cattle. The LED light trap was operated within the cattle shed (ST), 200 m apart from the shed where aspiration based collections were done. Although a mechanical aspirator was also used for the purpose; however, the mouth aspirator was tedious but proved handy in aspirating the adults tucked within the fur.

Statistical Analysis

A logistic regression was done on the proportion of engorged and non engorged females to justify the effects of time and site on host landing. The logistic regression was carried following the binomial GLM with logit link, using time and site as the explanatory variables on the assumptions of generalized linear model (GLM). The logistic regression equation form: (y) = 1/(1+exp(-(-0.99+0.11*time – 0.49*host landing + 1.75 – 02*time*host landing))); where the explanatory variables, x1, x2 and x3 represented the time of landing of Culicoides on host, site of landing on host and interaction between the two components respectively and y was the response variable. The regression analysis was performed on the assumptions that the landing of non-engorged and engorged Culicoides follow binomial distribution (n, p) with n replicates for each set of independent variables (time of landing, site of landing on host and interaction between host landing and time). Maximum likelihood method has been used as a measure to estimate the logit linked parameters through statistical software. Using the value of Wald's Chi square, the parameter of the models were tested for the significance at P= 0.05 level.

Results

In this investigation, the trapped number of C. oxystoma females caught were 1924 individuals further categorized as 1315 non-engorged, 609 engorged and C. peregrinus females consisted of 2070 individuals of which 1192 non-engorged and 878 engorged (Table 1a). In ST, a total catch of C. oxystoma were 2119 individuals (899 engaged, 1220 non-engorged) and C. peregrinus were 2645 individuals (1630 engaged and 1015 non-engorged) respectively (Table 1b). In ST collections, the following species were aspirated: C. peregrinus and C. oxystoma; however the light trap catches consisted of C. fulvis, C. oxystoma and C. peregrinus. The total Culicoides caught through aspiration were 4764 compared to light trap catch of 6698 individuals (Figure 2a; 2b). Significant numbers of Culicoides individuals landed on the upper portion of the cow, while very few individuals landed on the belly and legs. Landing of Culicoides was limited to hip due to continuous tail whipping, licking and kicking reflexes of the cattle. It appeared that a period of 30-40 min i.e. between 4 am and 5 am (dawn) was the actual feeding window of the C. oxystoma and C. peregrinus attacking the cattle (Table 2). During the feeding interval usually the Culicoides females swarm on to the host although both the species attacked the cattle with equal intensity, the proportion of engorged C. peregrinus aspirated were more (42.42%) compared to C. oxystoma (31.65%) (Figure 3). The results highlighted variation in abundance of the Culicoides analyzed through ANOVA with post hoc Tukey test considering the time of host-seeking activity and different body parts of cattle as the source of variations (Table 3a; Table 3b).

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GLM with logit link was done after aligning the data in binomial order to interpret the relation between specific age groups preferring particular landing sites on cattle bodies. In C. oxystoma, engaged (y) = 1/(1+exp(-(-1.19+0.13*time+0.59*host landing + 0.025*time*host landing))); The parameters significantly were at p<0.05 (intercept = -1.96±0.59; Wald χ² = 11.44; host landing = -0.599±0.2; Wld χ² = 9.3). For non-engorged (y) = 1/(1+exp(-0.099+0.11*time – 0.49*host landing + 1.75 – 02*time*host landing))); Significance at level of p<0.05 has been observed for the following parameters (intercept = -0.991±0.4; Wald χ² = 6.2; time = -0.110±0.05; Wald χ² = 4.2; host landing = -0.498±0.12; Wald χ² = 17.15). Similarly in C. peregrinus, the equation of the models for input variables, engaged (y) = 1/(1+exp((-1.78+0.17*time+0.37*host landing – 3.48 – 03*time*host landing))); The parameters significant at p<0.01 (intercept = -1.78±0.46; Wald χ² = 14.75; time = 0.17±0.06; Wald χ² = 7.3; host landing = -0.37±0.14; Wald χ² = 7.08) non-engorged (y) = 1/(1+exp((-2.07+0.18*time – 0.26*host landing – 1.32 – 02*time*host landing))); The model parameters considered significant at p<0.05 (intercept = -2.073±0.47; Wald χ² = 19.2; time = -0.184±0.06; Wald χ² = 8.6; host landing = -0.264±0.13; Wald χ² = 3.9).
For *Culicoides oxystoma*, the binomial GLM with logit link was done after conversion of the data to binary (presence of the *Culicoides* has been considered 1 or else 0). The equation obtained was engorged \( y = 1/(1+exp(-0.985+0.069*\text{time}+0.59*\text{host landing}+0.02*\text{time}*\text{host landing})) \). The following model parameters were significant at \( p<0.05 \) level (intercept = -0.985±0.5; Wald \( \chi^2 = 3.976; \text{host landing} = -0.585±0.2; \text{Wald} \chi^2 = 12.8 \)). It was observed that the pattern of engorged *Culicoides oxystoma* landing on cattle was a time-dependent variable. The following equation has been noted for non-engorged \( y = 1/(1+exp(-0.76+8.49*\text{time}+0.51*\text{host landing} + 1.76 - 0.02*\text{time}*\text{host landing})) \). The parameters significant at \( p<0.05 \) (host landing = -0.509±0.13; Wald \( \chi^2 = 15.31 \)). Likewise in *C. peregrinus*, engorged \( y = 1/(1+exp(-1.69+0.183*\text{time}+0.463*\text{host landing} + 9.387-0.3*\text{time}*\text{host landing})) \). The parameters observed were significant at \( p<0.05 \) (intercept = -1.69±0.5; Wald \( \chi^2 = 10.81; \text{host landing} = -0.463±0.16; \text{Wald} \chi^2 = 8.58 \)). For non-engorged \( y = 1/(1+exp(-1.87+0.18*\text{time}-0.23*\text{host landing} -0.02*\text{time}*\text{host landing})) \). The parameters observed significant were (intercept = -1.868±0.49; Wald \( \chi^2 = 14.61; \text{time} = 0.182±0.07; \text{Wald} \chi^2 = 7.59; \text{host landing} = -0.234±0.14; \text{Wald} \chi^2 = 2.96 \).

### Discussion

The *Culicoides* species investigated in this study was reported to be vectors of important diseases associated with farm animals’ worldwide [17]. Shielding of animals from attack of female *Culicoides* may be adopted as a measure to interrupt disease transmission hence information on peak activity of *Culicoides* from this region of world will be useful in disease management strategies [17]. The landing time and site of females belonging to *C. oxystoma* and *C. peregrinus* on the cattle in the early morning for the purpose of obtaining blood meal was observed. However, *C. fulvus* has been reported only in a light trap from one of the study sites thereby raising doubts on the host preference of the species. The aspirator based study validated the diel activity of the *C. oxystoma* and *C. peregrinus*. Significant proportion of engorged females in the study justified the usage of aspirator to intercept the host-seeking females. Moreover the present study ascertained that cattle not only attracted *Culicoides* but constituted one of the significant hosts. *C. oxystoma* and *C. peregrinus* were known to be one of the most prevalent species across India, from which the BTV serotypes have been isolated [24, 25] and also were enlisted as potent vectors of BTV from the subcontinent [18]. Moreover both the species were reported to be most abundant in cattle sheds of West Bengal [17] thereby contributing to the significance of the study. High proportion of engorged *C. peregrinus* and *C. oxystoma* feeding on the cattle suggests active blood-seeking behaviour of the species, which agrees with the opinion regarding fierceness of *C. peregrinus* [26]. *C. oxystoma* has been recognized as potential vectors of BTV from India [24] and Indonesia [27]. Japan has reported *C. oxystoma* as potent vector of Akbane virus [28, 29] and of epizootic hemorrhagic disease virus from Israel [30]. Moreover [31] has reported involvement of *C. oxystoma* in transmission of African horse sickness virus (AHSV) from Senegal.

The proportion of engorged to non-engorged females aspirated ascertains landing of *Culicoides* on host but does not warrant feeding. It was noted that 30-40% of the total midges landing actually fed on the cattle; the finding substantiated the conclusion drawn on activity of *Culicoides* midges from Ireland [32].

A bimodal distribution of *C. oxystoma*, *C. peregrinus* and *C. fulvus* was observed in light trap based collections within the animal shed. The plausible explanation was that the resting adults within the cattle shed after completion of blood meal were also attracted towards the light traps. However, *Culicoides* were observed to be prevalent in resting conditions in the cracks, crevices, walls, and roof within the shed housing cattle throughout the 24 hour period. Mere prevalence of *Culicoides* within cattle sheds does not warrant their blood feeding activity.

Amongst the different portions of cattle body, the desired landing site of the *C. oxystoma* and *C. peregrinus* were neck, hump followed by the head of the cattle (mostly in and around the ear). Although various studies suggested temperature to be a significant factor influencing the landing of *Culicoides* [33], our observation suggests that the thickness of epidermis and degree of vascularization might be two of the most important criteria influencing the landing of female *Culicoides*. Likewise [32] reported on the preference of *C. obsoletus*, *C. dewulfi*, *C. pulicaris*, *C. punctatus* and *C. nebeculosus* for mane and lower legs of horse. [33] remarked that 72% of the total collections were from the belly whereas 28% from the dorsal surface. *C. puncticollis* and *C. schultzei* preferred the belly region whereas *C. imicola* preferred the dorsal surface. A comparative preferential landing study of *Culicoides* on dairy cows, Shetland pony and sheep has been done [21]. It was observed that *C. chiopterus* favoured legs, *C. punctatus*, *C. achraysi* landed on the belly. *C. obsoletus*, *C. dewulfi* and *C. pulicaris* landed on head, back and flanks respectively. Moreover *C. chiopterus*, *C. punctatus*, *C. obsoletus/scoticus* favoured the belly region of horses [35].

Anatomically, vascularization has been complex in the neck and hump region [23], henceforth correlating to the preferred landing of the *Culicoides* at these sites. Likewise the thickness of epidermis was found to be less in the aforesaid body parts. The difference in preference could not be attributed to body surface temperature as there was not much difference in temperature except belly and hip, where the difference was 1°C. However in the present study *Culicoides* on belly was less compared to that of neck and hump.

This investigation recorded diel activity and host-seeking activity of the potent vector species associated with cattle in the state of West Bengal. Most of the cattle sheds in West Bengal were open type and cattle were either herded in this shed or in open yard at night. In such a setup there exists a high risk of cattle being exposed to *Culicoides* bite during the early morning. The present study argues that in order to minimize the contact between *Culicoides* and cattle, the cattle should be housed in a closed shed for at least 1 hour (i.e., between 4.00 am and 05.00 am). In closed sheds the activity of *Culicoides* has been observed to be reduced by 14 fold (unpublished). Owing to absence of proper closed sheds in most of the rural areas, an alternative approach could also be adopted by farmers. Blankets, badges or jute bags could be placed over the hump and neck region of the cattle during the peak activity time (between 4 am and 5 am) of midges thereby interfering with the preferred landing and subsequent feeding of the *Culicoides* midges.

### Declarations

**ETHICS STATEMENT**

No animals were harmed during the study.
AUTHORS CONTRIBUTION: First author: The entire work from data collection to data analysis and writing of manuscript. Corresponding author: Ensuring that entire process was accurate. Second and third author: Aided in insect sampling

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CONFLICTS OF INTEREST

The authors declare no conflict of interest

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Tables
| Time        | C. oxystoma | C. peregrinus |
|-------------|-------------|---------------|
| 0-1.0       | 0.0-0.0     | 0.0-0.0       |
| 0.1±0.07    | 0.0±0.0     | 0.0±0.0       |
| 0.0-1.0     | 0.0-0.0     | 0.0-0.0       |
| 0.0-1.0     | 0.0-1.0     | 0.0-0.0       |
| 0.0-1.0     | 0.0-1.0     | 0.0-0.0       |
| 0.0-1.0     | 0.0-1.0     | 0.0-0.0       |
| 0.0-1.0     | 0.0-1.0     | 0.0-0.0       |
| 0.0-1.0     | 0.0-1.0     | 0.0-0.0       |
| 0.0-1.0     | 0.0-1.0     | 0.0-0.0       |
| 0.0-1.0     | 0.0-1.0     | 0.0-0.0       |
| 0.0-1.0     | 0.0-1.0     | 0.0-0.0       |
| 0.0-1.0     | 0.0-1.0     | 0.0-0.0       |
| 0.0-1.0     | 0.0-1.0     | 0.0-0.0       |
| 0.0-1.0     | 0.0-1.0     | 0.0-0.0       |
| 0.0-1.0     | 0.0-1.0     | 0.0-0.0       |
| 0.0-1.0     | 0.0-1.0     | 0.0-0.0       |
| 0.0-1.0     | 0.0-1.0     | 0.0-0.0       |
| 0.0-1.0     | 0.0-1.0     | 0.0-0.0       |
| 0.0-1.0     | 0.0-1.0     | 0.0-0.0       |

Table 1

a. Mean and S.E. of female C. oxystoma and C. peregrinus aspired hourly from cattle (cow) body surface at Dharan (DH), West Bengal. Body part of cattle at H2, neck; H3, hump; H4, back; H5, leg; H6, belly; H7, hip.
|          | 18.00-19.00 | 19.00-20.00 | 20.00-21.00 | 21.00-22.00 | 22.00-23.00 | 00.00-01.00 | 01.00-2.00 | 2.00-3.00 | 3.00-4.00 | 4.00-5.0 |
|----------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|
| Engorged |             |             |             |             |             |             |             |             |             |             |
| H7       | 0.0±0.0     | 0.0±0.0     | 0.0±0.0     | 0.0±0.0     | 0.0±0.0     | 0.0±0.0     | 0.0±0.0     | 0.0±0.0     | 0.0±0.0     | 0.0±0.0     |
|          | 0.0±0.0     | 0.0±0.0     | 0.0±0.0     | 0.0±0.0     | 0.0±0.0     | 0.0±0.0     | 0.0±0.0     | 0.0±0.0     | 0.0±0.0     | 0.0±0.0     |
| H1       | 0.0±0.0     | 0.0±0.0     | 0.0±0.0     | 0.0±0.0     | 0.0±0.0     | 0.0±0.0     | 0.0±0.0     | 0.0±0.0     | 0.0±0.0     | 0.0±0.0     |
|          | 0.0±0.0     | 0.0±0.0     | 0.0±0.0     | 0.0±0.0     | 0.0±0.0     | 0.0±0.0     | 0.0±0.0     | 0.0±0.0     | 0.0±0.0     | 0.0±0.0     |
| H2       | 0.0±0.0     | 0.0±0.0     | 0.0±0.0     | 0.0±0.0     | 0.0±0.0     | 0.0±0.0     | 0.0±0.0     | 0.0±0.0     | 0.0±0.0     | 0.0±0.0     |
|          | 0.0±0.0     | 0.0±0.0     | 0.0±0.0     | 0.0±0.0     | 0.0±0.0     | 0.0±0.0     | 0.0±0.0     | 0.0±0.0     | 0.0±0.0     | 0.0±0.0     |
| H3       | 0.0±0.0     | 0.0±0.0     | 0.0±0.0     | 0.0±0.0     | 0.0±0.0     | 0.0±0.0     | 0.0±0.0     | 0.0±0.0     | 0.0±0.0     | 0.0±0.0     |
|          | 0.0±0.0     | 0.0±0.0     | 0.0±0.0     | 0.0±0.0     | 0.0±0.0     | 0.0±0.0     | 0.0±0.0     | 0.0±0.0     | 0.0±0.0     | 0.0±0.0     |
| H4       | 0.0±0.0     | 0.0±0.0     | 0.0±0.0     | 0.0±0.0     | 0.0±0.0     | 0.0±0.0     | 0.0±0.0     | 0.0±0.0     | 0.0±0.0     | 0.0±0.0     |
|          | 0.0±0.0     | 0.0±0.0     | 0.0±0.0     | 0.0±0.0     | 0.0±0.0     | 0.0±0.0     | 0.0±0.0     | 0.0±0.0     | 0.0±0.0     | 0.0±0.0     |
| H5       | 0.0±0.0     | 0.0±0.0     | 0.0±0.0     | 0.0±0.0     | 0.0±0.0     | 0.0±0.0     | 0.0±0.0     | 0.0±0.0     | 0.0±0.0     | 0.0±0.0     |
|          | 0.0±0.0     | 0.0±0.0     | 0.0±0.0     | 0.0±0.0     | 0.0±0.0     | 0.0±0.0     | 0.0±0.0     | 0.0±0.0     | 0.0±0.0     | 0.0±0.0     |
| H6       | 0.0±0.0     | 0.0±0.0     | 0.0±0.0     | 0.0±0.0     | 0.0±0.0     | 0.0±0.0     | 0.0±0.0     | 0.0±0.0     | 0.0±0.0     | 0.0±0.0     |
|          | 0.0±0.0     | 0.0±0.0     | 0.0±0.0     | 0.0±0.0     | 0.0±0.0     | 0.0±0.0     | 0.0±0.0     | 0.0±0.0     | 0.0±0.0     | 0.0±0.0     |
| H7       | 0.0±0.0     | 0.0±0.0     | 0.0±0.0     | 0.0±0.0     | 0.0±0.0     | 0.0±0.0     | 0.0±0.0     | 0.0±0.0     | 0.0±0.0     | 0.0±0.0     |
|          | 0.0±0.0     | 0.0±0.0     | 0.0±0.0     | 0.0±0.0     | 0.0±0.0     | 0.0±0.0     | 0.0±0.0     | 0.0±0.0     | 0.0±0.0     | 0.0±0.0     |
### Table 1

b. Mean and S.E. of female *C. oxystoma* and *C. peregrinus* aspirated hourly from cattle (cow) body surface at Sahebganj-Tantipara (ST), West Bengal. Body abbreviated: H1, head; H2, neck; H3, hump; H4, back; H5, leg; H6, belly; H7, hip.

| Time     | Non Engorged | Engorged |
|----------|--------------|----------|
|          | *C. oxystoma* | *C. peregrinus* |
| 18.00-19.00 | 0.0±0.1 | 0.0±0.2 |
| 19.00-20.00 | 0.0±0.0 | 0.0±0.0 |
| 20.00-21.00 | 0.0±0.0 | 0.0±0.0 |
| 21.00-22.00 | 0.0±0.0 | 0.0±0.0 |
| 22.00-00.00 | 0.0±0.0 | 0.0±0.0 |
| 00.00-01.00 | 0.0±0.0 | 0.0±0.0 |
| 01.00-02.00 | 0.0±0.0 | 0.0±0.0 |
| 02.00-03.00 | 0.0±0.0 | 0.0±0.0 |
| 03.00-04.00 | 0.0±0.0 | 0.0±0.0 |
| 04.00-05.00 | 0.0±0.0 | 0.0±0.0 |
|        | 18.00-19.00 | 19.00-20.00 | 20.00-21.00 | 21.00-22.00 | 22.00-00.00 | 00.00-01.00 | 01.00-2.00 | 2.00-3.00 | 3.00-4.00 | 4.00-5.00 |
|--------|-------------|-------------|-------------|-------------|-------------|-------------|------------|-----------|-----------|-----------|
| H7     | 0.0±0.0     | 0.0±0.0     | 0.0±0.0     | 0.0±0.0     | 0.0±0.0     | 0.0±0.0     | 0.0±0.0    | 0.0±0.0   | 0.0±0.0   | 0.0±0.0   |
| H1     | 0.0-5.0     | 0.0-0.0     | 0.0-0.0     | 0.0-0.0     | 0.0-0.0     | 0.0-0.0     | 0.0-0.0    | 0.0-1.0   | 0.0-5.0   | 0.0-9.0   |
|        | 0.5±0.42    | 0.0±0.00    | 0.0±0.00    | 0.0±0.00    | 0.0±0.00    | 0.0±0.00    | 0.0±0.00   | 0.1±0.08  | 0.7±0.46  | 1.0±0.73  |
| H2     | 0.0-3.0     | 0.0-3.0     | 0.0-16.0    | 0.0-9.0     | 0.0-0.0     | 0.0-3.0     | 0.0-1.0    | 0.0-8.0   | 0.0-11.0  | 0.0-167.0 |
|        | 0.8±0.32    | 0.7±0.33    | 1.6±1.31    | 0.8±0.75    | 0.0±0.00    | 0.4±0.26    | 0.1±0.08   | 1.8±0.89  | 19.2±11.22| 67.9±12.4 |
| H3     | 0.0-26.0    | 0.0-41.0    | 0.0-1.0     | 0.0-6.0     | 0.0-1.0     | 0.0-3.0     | 0.0-1.0    | 0.0-4.0   | 0.0-29.0  | 0.0-29.0  |
|        | 4.25±2.46   | 5.8±4.00    | 0.2±0.11    | 0.6±0.49    | 0.1±0.08    | 0.3±0.25    | 0.1±0.08   | 0.3±0.33  | 5.1±2.73  | 6.7±2.60  |
| H4     | 0.0-1.0     | 0.0-1.0     | 0.0-0.0     | 0.0-0.0     | 0.0-0.0     | 0.0-0.0     | 0.0-0.0    | 0.0±0.0   | 0.0±0.0   | 0.4±0.33  |
|        | 0.1±0.08    | 0.1±0.08    | 0.0±0.00    | 0.0±0.00    | 0.0±0.00    | 0.0±0.00    | 0.0±0.00   | 0.0±0.00  | 0.0±0.00  | 0.0±0.00  |
| H5     | 0.0-9.0     | 0.0-0.0     | 0.0-0.0     | 0.0-1.0     | 0.0-0.0     | 0.0-0.0     | 0.0-0.0    | 0.0±0.0   | 0.0±0.0   | 0.0-11.0  |
|        | 0.8±0.75    | 0.0±0.00    | 0.0±0.00    | 0.1±0.08    | 0.0±0.00    | 0.0±0.00    | 0.3±0.25   | 0.0±0.00  | 0.0±0.00  | 2.4±0.94  |
| H6     | 0.0-0.0     | 0.0-0.0     | 0.0-1.0     | 0.0-0.0     | 0.0-0.0     | 0.0-0.0     | 0.0-1.0    | 0.0±0.0   | 0.0±0.0   | 0.0-5.0   |
|        | 0.0±0.00    | 0.0±0.00    | 0.1±0.08    | 0.0±0.00    | 0.0±0.00    | 0.0±0.00    | 0.1±0.08   | 0.0±0.00  | 0.0±0.00  | 1.3±0.54  |
| H7     | 0.0-0.0     | 0.0-0.0     | 0.0-0.0     | 0.0-0.0     | 0.0-0.0     | 0.0-0.0     | 0.0-0.0    | 0.0±0.0   | 0.0±0.0   | 0.0±0.0   |
|        | 0.0±0.00    | 0.0±0.00    | 0.0±0.00    | 0.0±0.00    | 0.0±0.00    | 0.0±0.00    | 0.0±0.00   | 0.0±0.00  | 0.0±0.00  | 0.0±0.00  |

Due to technical limitations, table 2 is only available as a download in the Supplemental Files section.

**Table 3a.** Results of ANOVA using the time, host landing of *Culicoides* as the source of variations for the observed abundance in the cattle sheds in the study area (Dharan). The values in bold indicate significance at *P* < 0.0001 level. T = time, H = Host body parts.

(a) *Culicoides oxystoma*
| Source       | SS    | MS    | DF | F    |
|--------------|-------|-------|----|------|
| TIME         | 12813.434 | 1281.343 | 10 | 17.546 |
| HOST LANDING | 10408.242 | 1734.707 | 6  | 23.754 |
| TIME*HOST LANDING | 49505.948 | 825.099 | 60 | 11.298 |
| Error        | 78725.333 | 73.029  | 1078 |
| Total        | 151452.958 | 1154  |

| Contrast    | Difference | Contrast | Difference | Contrast | Difference |
|-------------|------------|----------|------------|----------|------------|
| T10 vs T7   | 11.629     | T11 vs T1| 2.771      | T2 vs T6 | 0.219      |
| T10 vs T5   | 11.610     | T1 vs T7 | 1.000      | T2 vs T3 | 0.181      |
| T10 vs T6   | 11.562     | T1 vs T5 | 0.981      | T2 vs T4 | 0.171      |
| T10 vs T3   | 11.524     | T1 vs T6 | 0.933      | T2 vs T8 | 0.038      |
| T10 vs T4   | 11.514     | T1 vs T3 | 0.895      | T8 vs T7 | 0.248      |
| T10 vs T8   | 11.381     | T1 vs T4 | 0.886      | T8 vs T5 | 0.229      |
| T10 vs T2   | 11.343     | T1 vs T8 | 0.752      | T8 vs T6 | 0.181      |
| T10 vs T9   | 11.019     | T1 vs T2 | 0.714      | T8 vs T3 | 0.143      |
| T10 vs T1   | 10.629     | T1 vs T9 | 0.390      | T8 vs T4 | 0.133      |
| T10 vs T11  | 7.857      | T9 vs T7 | 0.610      | T4 vs T7 | 0.114      |
| T11 vs T7   | 3.771      | T9 vs T5 | 0.590      | T4 vs T5 | 0.095      |
| T11 vs T5   | 3.752      | T9 vs T6 | 0.543      | T4 vs T6 | 0.048      |
| T11 vs T6   | 3.705      | T9 vs T3 | 0.505      | T4 vs T3 | 0.010      |
| T11 vs T3   | 3.667      | T9 vs T4 | 0.495      | T3 vs T7 | 0.105      |
| T11 vs T4   | 3.657      | T9 vs T8 | 0.362      | T3 vs T5 | 0.086      |
| T11 vs T8   | 3.524      | T9 vs T2 | 0.324      | T3 vs T6 | 0.038      |
| T11 vs T2   | 3.486      | T2 vs T7 | 0.286      | T6 vs T7 | 0.067      |
| T11 vs T9   | 3.162      | T2 vs T5 | 0.267      | T6 vs T5 | 0.048      |

| Contrast    | Difference | Contrast | Difference | Contrast | Difference |
|-------------|------------|----------|------------|----------|------------|
| H3 vs H7    | 8.903      | H1 vs H5 | 1.485      | H2 vs H6 | 0.006      |
| H3 vs H5    | 8.818      | H1 vs H4 | 1.418      | H6 vs H7 | 0.412      |
| H3 vs H4    | 8.752      | H1 vs H6 | 1.158      | H6 vs H5 | 0.327      |
| H3 vs H6    | 8.491      | H1 vs H2 | 1.152      | H6 vs H4 | 0.261      |
| H3 vs H2    | 8.485      | H2 vs H7 | 0.418      | H4 vs H7 | 0.152      |
| H3 vs H1    | 7.333      | H2 vs H5 | 0.333      | H4 vs H5 | 0.067      |
| H1 vs H7    | 1.570      | H2 vs H4 | 0.267      | H5 vs H7 | 0.085      |

(b) *Culicoides peregrinus*
Table 3b. Results of ANOVA using the time, host landing of *Culicoides* as the source of variations for the observed abundance in the cattle sheds in the study area (Sahebganj-Tantipra). The values in bold indicate significance at P < 0.0001 level. T- time, H – Host body parts.

(a) *Culicoides oxystoma*
| Source                        | SS       | MS       | DF  | F     |
|-------------------------------|----------|----------|-----|-------|
| Time                          | 17941.411| 1794.141 | 10  | 33.205|
| HOST LANDING                  | 12727.071| 2121.179 | 6   | 39.258|
| Time*HOST LANDING             | 56263.952| 937.733  | 60  | 17.355|
| Error                         | 45765.083| 54.032   | 847 |       |
| Total                         | 132697.518| 923     |     |       |

### Contrast Differences

| Contrast       | Difference | Contrast       | Difference | Contrast       | Difference |
|----------------|------------|----------------|------------|----------------|------------|
| T10 vs T5      | 15.821     | T11 vs T1      | 1.345      | T2 vs T6       | 0.810      |
| T10 vs T4      | 15.786     | T1 vs T5       | 1.952      | T2 vs T8       | 0.774      |
| T10 vs T6      | 15.702     | T1 vs T4       | 1.917      | T2 vs T7       | 0.738      |
| T10 vs T8      | 15.667     | T1 vs T6       | 1.833      | T2 vs T3       | 0.333      |
| T10 vs T7      | 15.631     | T1 vs T8       | 1.798      | T3 vs T5       | 0.595      |
| T10 vs T3      | 15.226     | T1 vs T7       | 1.762      | T3 vs T4       | 0.560      |
| T10 vs T2      | 14.893     | T1 vs T3       | 1.357      | T3 vs T6       | 0.476      |
| T10 vs T9      | 14.345     | T1 vs T2       | 1.024      | T3 vs T8       | 0.440      |
| T10 vs T1      | 13.869     | T1 vs T9       | 0.476      | T3 vs T7       | 0.405      |
| T10 vs T11     | 12.524     | T9 vs T5       | 1.476      | T7 vs T5       | 0.190      |
| T11 vs T5      | 3.298      | T9 vs T4       | 1.440      | T7 vs T4       | 0.155      |
| T11 vs T4      | 3.262      | T9 vs T6       | 1.357      | T7 vs T6       | 0.071      |
| T11 vs T6      | 3.179      | T9 vs T8       | 1.321      | T7 vs T8       | 0.036      |
| T11 vs T8      | 3.143      | T9 vs T7       | 1.286      | T8 vs T5       | 0.155      |
| T11 vs T7      | 3.107      | T9 vs T3       | 0.881      | T8 vs T4       | 0.119      |
| T11 vs T3      | 2.702      | T9 vs T2       | 0.548      | T8 vs T6       | 0.036      |
| T11 vs T2      | 2.369      | T2 vs T5       | 0.929      | T6 vs T5       | 0.119      |
| T11 vs T9      | 1.821      | T2 vs T4       | 0.893      | T6 vs T4       | 0.083      |

### Contrast Differences (b)

| Contrast       | Difference | Contrast       | Difference | Contrast       | Difference |
|----------------|------------|----------------|------------|----------------|------------|
| H2 vs H7       | 11.008     | H3 vs H4       | 3.136      | H5 vs H1       | 0.098      |
| H2 vs H4       | 10.970     | H3 vs H6       | 2.939      | H1 vs H7       | 0.644      |
| H2 vs H6       | 10.773     | H3 vs H1       | 2.530      | H1 vs H4       | 0.606      |
| H2 vs H1       | 10.364     | H3 vs H5       | 2.432      | H1 vs H6       | 0.409      |
| H2 vs H5       | 10.265     | H5 vs H7       | 0.742      | H6 vs H7       | 0.235      |
| H2 vs H3       | 7.833      | H5 vs H4       | 0.705      | H6 vs H4       | 0.197      |
| H3 vs H7       | 3.174      | H5 vs H6       | 0.508      | H4 vs H7       | 0.038      |

(b) Culicoides peregrinus
| Source                      | SS      | MS      | DF  | F    |
|-----------------------------|---------|---------|-----|------|
| Time                        | 29269.818 | 2926.982 | 10  | 29.522 |
| HOST LANDING                | 23410.734 | 3901.789 | 6   | 39.355 |
| Time*HOST LANDING           | 118813.742 | 1980.229 | 60  | 19.973 |
| Error                       | 83975.250 | 99.144  | 847 |      |
| Total                       | 255469.544 | 923  |      |      |

| Contrast                      | Difference | Contrast                      | Difference | Contrast                      | Difference |
|-------------------------------|------------|-------------------------------|------------|-------------------------------|------------|
| T10 vs T7                     | 20.226     | T9 vs T11                     | 1.738      | T2 vs T6                      | 0.929      |
| T10 vs T5                     | 20.226     | T11 vs T7                    | 2.274      | T2 vs T4                      | 0.821      |
| T10 vs T6                     | 20.119     | T11 vs T5                    | 2.274      | T2 vs T3                      | 0.619      |
| T10 vs T4                     | 20.012     | T11 vs T6                    | 2.167      | T2 vs T8                      | 0.119      |
| T10 vs T3                     | 19.810     | T11 vs T4                    | 2.060      | T8 vs T7                      | 0.917      |
| T10 vs T8                     | 19.310     | T11 vs T3                    | 1.857      | T8 vs T5                      | 0.917      |
| T10 vs T2                     | 19.190     | T11 vs T8                    | 1.357      | T8 vs T6                      | 0.810      |
| T10 vs T1                     | 18.464     | T11 vs T2                    | 1.238      | T8 vs T4                      | 0.702      |
| T10 vs T11                    | 17.952     | T11 vs T1                    | 0.512      | T8 vs T3                      | 0.500      |
| T10 vs T9                     | 16.214     | T1 vs T7                     | 1.762      | T3 vs T7                      | 0.417      |
| T9 vs T7                      | 4.012      | T1 vs T5                     | 1.762      | T3 vs T5                      | 0.417      |
| T9 vs T5                      | 4.012      | T1 vs T6                     | 1.655      | T3 vs T6                      | 0.310      |
| T9 vs T6                      | 3.905      | T1 vs T4                     | 1.548      | T3 vs T4                      | 0.202      |
| T9 vs T4                      | 3.798      | T1 vs T3                     | 1.345      | T4 vs T7                      | 0.214      |
| T9 vs T3                      | 3.595      | T1 vs T8                     | 0.845      | T4 vs T5                      | 0.214      |
| T9 vs T8                      | 3.095      | T1 vs T2                     | 0.726      | T4 vs T6                      | 0.107      |
| T9 vs T2                      | 2.976      | T2 vs T7                     | 1.036      | T6 vs T7                      | 0.107      |
| T9 vs T1                      | 2.250      | T2 vs T5                     | 1.036      | T6 vs T5                      | 0.107      |

| Contrast                      | Difference | Contrast                      | Difference | Contrast                      | Difference |
|-------------------------------|------------|-------------------------------|------------|-------------------------------|------------|
| H2 vs H7                      | 14.886     | H3 vs H4                      | 3.386      | H5 vs H1                      | 0.318      |
| H2 vs H4                      | 14.803     | H3 vs H6                      | 3.265      | H1 vs H7                      | 0.538      |
| H2 vs H6                      | 14.682     | H3 vs H1                      | 2.932      | H1 vs H4                      | 0.455      |
| H2 vs H1                      | 14.348     | H3 vs H5                      | 2.614      | H1 vs H6                      | 0.333      |
| H2 vs H5                      | 14.030     | H5 vs H7                      | 0.856      | H6 vs H7                      | 0.205      |
| H2 vs H3                      | 11.417     | H5 vs H4                      | 0.773      | H6 vs H4                      | 0.121      |
| H3 vs H7                      | 3.470      | H5 vs H6                      | 0.652      | H4 vs H7                      | 0.083      |

Figures
The body of cow was divided into head (H1), neck (H2), hump (H3), back (H4), leg (H5), belly (H6), hip (H7)

(a) Nulliparous

Figure 2
Comparative graphical representation on sampling of Culicoides oxystoma by two trapping methods (aspirator, light trap) during May-October, 2018 from Sahebganj-Tantipara

Comparative graphical representation on sampling of Culicoides peregrinus by two trapping methods (aspirator, light trap) during May-October, 2018 from Sahebganj-Tantipara

**Figure 3**

Graph represents the proportion of engorged females, Culicoides oxystoma (COX) and Culicoides peregrinus (CPE) trapped on different body surfaces of cow at two collection sites: a) Dharan, b) Sahebganj-Tantipara respectively. Region of body surface abbreviated as H1, head; H2, neck; H3, hump; H4, back; H5, leg; H6, belly; H7, hip. Duration of collection (T1-T11) hours, T1: 18.00-19.00; T2: 19.00-20.00; T3: 20.00-21.00; T4: 21.00-22.00; T5: 23.00-00.00; T6: 00.00-01.00; T7: 01.00-02.00; T8: 02.00-03.00; T9: 03.00-04.00; T10: 04.00-05.00; T11: 05.00-06.00.

**Supplementary Files**

This is a list of supplementary files associated with this preprint. Click to download.

- Table2.docx
- Graphicalabstract.jpg