Host landing and diel activity of potent vectors of bluetongue disease, *Culicoides oxystoma* and *Culicoides peregrinus*

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Abstract

Background

The spread of bluetongue virus (BTV) 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*). Additionally, high seroprevalence of BTV was also reported from this cattle dense region. To minimize the risk of disease outbreak a strategy to reduce the exposure of cattle to midges bite is necessary. Therefore 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 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 taken 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 15,456 *Culicoides* belonging to *C. oxystoma*, *C. peregrinus* and *C. fulvus* were collected in light trap and aspirator during the study. 8,758 midges were collected by aspirator, whereas 6,698 individuals were collected in light trap. The following species were aspirated: *C. peregrinus* and *C. oxystoma*; while 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 hr and 5:00 hr. Maximum landing of midges was observed in the neck and hump region of the 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 535.8 million as major farm animals i.e cattle, buffalo, poultry, sheep and goat including indigenous and exotic breeds, with a total bovine population of 302.8 million (buffalo, cattle, mithun, yak) [2]. The presence of large and diverse populations of livestock increases transmission risk of bluetongue virus (BTV) by *Culicoides* which may lead to outbreak of bluetongue (BT). This virus causes BT 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 BTV in the temperate was also attributed to global warming [5]. Presently seroprevalence of BTV has been reported across all the Indian states [6]. The BT 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 BTV seropositivity in different animals depicts 34.0 % in buffalo, 16.0 % in camel, 38.0 % in cattle, 43.0 % in goat, 39.0 % in sheep and 66.0 % in mithun [13]. The state of West Bengal shares 6.9% of total livestock population comprising of 8.3% cattle, 0.8% sheep and 10.9% goats' population. The economy of West Bengal, especially in Burdwan district was 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 (67.0 %), sheep (57.7 %) and cattle (52.0 %) has been reported across 7 districts of West Bengal [15, 16]. Despite such high seroprevalence, BT outbreak remains unreported from West Bengal [17]. BT 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]. It has been reported prevalence of 7 putative vectors *C. oxystoma*, *C. fulvus*, *C. orientalis*, *C. dumdumi*, *C. imicola*, *C. peregrinus* and *C. brevitarsis* from southern India [18]. 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, 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]. Difficulty in aspirating *Culicoides* from the host body also limits studies on host seeking activity of the *Culicoides* [19].

In the present 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 midges may undertake flight for foraging, mating, seeking of resting place or to oviposit. Biting activity points to the association of vectors to its host, probable route of transmission of disease depicted. 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) and 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 West Bengal high abundance of midges has been observed during April to October after which the population declines [17]. At ST village site, 12 night collections were made i.e., two catches/month, whereas in DH village site, three monthly 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 1,496 mm. The hygiene conditions were compromised within and outside the perimeter of the animal sheds as dung heaps and paddy straws were dumped. 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. The two farms at ST consisted of single white cattle each and at DH two white cattle were present. The farms were chosen in areas (district) where seroprevalence of BTV has been reported. Moreover high cattle density with prevalence of putative vectors of bluetongue (*C. oxystoma*, *C. peregrinus* and *C. fulvus*) led to selection of farms.

Description of collection

The adults that landed on the host body surface was retrieved by aspirators (oral & mechanical) and flying adults were trapped by UV LED light traps [23, 8] 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 [24] 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) (Fig. 1). Each catch duration was restricted to 10 minutes/hour. Entire process of aspiration was initiated at 18.00 hr in the evening that continued up to 6.00 hr in the morning (12 hours per diem). The catch period (designated T1 hr to T11 hr) as 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. Prior to initiation of the study, a pilot trial was conducted using a mechanical and mouth aspirator for collection of midges from cattle. It was observed that mouth aspirator was more effective in collection compared to the mechanical aspirator for *Culicoides*. During the study entire sampling was done using mouth aspirator at both the sites (DH & ST). In each collection, midges from single white cattle were aspirated by a person. Half an hour before the sampling, one cattle was kept in the shed and others were removed. The midges were visually located on the body of the white cattle and collected with mouth aspirator. For easy visualization of midges on the cattle a 4W dim light was used during the collection. At ST, UV LED light trap was operated within the cattle shed 200 m apart from the shed where aspiration based collections were done. *Culicoides* species were mounted on glass slides and identified on the basis of salient morphological characters [25]. The females were age graded on the basis of abdominal pigmentation [26]. However in the present study the unfed nulliparous and unfed parous collections were clustered as non-engorged and rest as engorged. Gravid females were mentioned.

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: $(y) = 1/(1 + \exp(-(a + b_1x_1 + b_2x_2 + b_3x_3)))$; where the explanatory variables, x_1 , x_2 and x_3 , 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

A total of 15,456 *Culicoides* spp. were collected during the study at both the sites. In DH, of the total 3994 individuals collected, *C. oxystoma* females were 1,924 individuals which were further categorized into 1,315 non-engorged and 609 engorged individuals. *Culicoides peregrinus* females consisted of 2,070 individuals of which 1,192 non-engorged and 878 engorged individuals (Table 1a). In ST, a total catch of *C. oxystoma* were 2,119 individuals comprised of 1,220 non-engorged, 899 engorged individuals. *Culicoides peregrinus* comprised of 2,645 individuals, of which 1015 were non-engorged and 1,630 engorged females (Table 1b). In ST collections, the following species were aspirated: *C. peregrinus* and *C. oxystoma*; however the light trap catches consisted of *C. fulvus*, *C. oxystoma* and *C. peregrinus*. The total *Culicoides* caught through aspiration were 4,764 compared to light trap catch of 6,698 individuals (both female and male) (Fig. 2a; 2b). Significant numbers of *Culicoides* individuals landed on the upper portion of the cattle, 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 4:00 hr and 5:00 hr (dawn) was the actual feeding window of the *C. oxystoma* and *C. peregrinus* attacking the cattle (Fig. 3). During the feeding intervals *Culicoides* females were observed to swarm on the host and they attacked the cattle with equal intensity. The proportion of engorged *C. peregrinus* aspirated were more (42.42%) compared to *C. oxystoma* (31.65%) (Fig. 4a and 4b).

Table 1

a. Number of *C. oxystoma* and *C. peregrinus* collected from body parts of cattle at various time (hr) in Dharan, West Bengal. H1, head; H2, neck; H3, hump; H4, back; H5, leg; H6, belly; H7, hip of the cattle.

		hump, H4, back, H5, leg, H6, belly, H7, hip of the cattle.											
		18.00– 19.00	19.00– 20.00	20.00– 21.00	21.00– 22.00	23.00– 00.00	00.00– 01.00	01.00– 02.00	02.00– 03.00	03.00– 04.00	04.00– 05.00	05.00– 06.00	
		n = 18	n = 18	n = 18	n = 18	n = 18	n = 18	n = 18	n = 18	n = 18	n = 18	n = 18	
Culicoides oxystoma	Non- engorged	H1	28	7	3	1	1	3	0	3	14	108	43
		H2	2	3	0	5	2	1	0	1	3	20	20
		H3	30	15	4	5	3	5	2	8	25	668	193
		H4	0	0	0	3	0	0	0	1	0	8	6
		H5	0	0	0	1	0	0	0	0	2	5	4
		H6	8	1	2	1	0	1	2	1	8	22	7
		H7	0	0	0	0	0	0	0	0	0	1	1
	Engorged	H1	13	0	2	0	0	0	0	2	0	25	8
		H2	0	1	1	0	0	0	0	0	2	10	0
		H3	26	6	2	0	0	1	0	12	14	339	113
		H4	0	1	0	0	0	0	0	1	0	6	1
		H5	2	0	1	0	0	0	0	0	0	1	0
		H6	0	0	0	0	0	0	0	1	0	12	4
		H7	0	0	0	0	0	0	0	0	0	0	0
	Female	H1	41	7	5	1	1	3	0	5	14	133	51
		H2	2	4	1	5	2	1	0	1	5	30	20
		H3	56	21	6	5	3	6	2	20	39	1007	306
		H4	0	1	0	3	0	0	0	2	0	14	7
		H5	2	0	1	1	0	0	0	0	2	6	4
		H6	8	1	2	1	0	1	2	2	8	34	11
		H7	0	0	0	0	0	0	0	0	0	1	1
Culicoides peregrinus	Non- engorged	H1	17	3	1	0	0	3	1	3	11	50	35
		H2	2	0	0	2	0	1	0	9	2	4	5
		H3	16	5	3	0	2	1	0	5	30	653	242
		H4	0	0	3	1	0	0	0	9	4	18	0
		H5	0	1	2	0	1	0	0	0	0	0	4
		H6	8	0	0	4	0	1	0	8	1	11	3
		H7	0	0	0	0	0	0	0	0	0	1	0
	Engorged	H1	6	5	2	2	1	2	0	4	17	32	27
		H2	5	3	0	1	0	0	0	30	2	1	2
		H3	8	16	5	1	3	1	2	9	31	384	221
		H4	2	0	0	1	0	0	0	2	0	6	0
		H5	1	1	0	0	0	0	0	0	0	3	7
		H6	2	0	0	2	0	0	1	1	7	8	1
		H7	0	0	0	0	0	0	0	0	0	0	0
	Female	H1	23	8	3	2	1	5	1	7	28	82	62
		H2	7	3	0	3	0	1	0	39	4	5	7

		18.00– 19.00	19.00– 20.00	20.00– 21.00	21.00– 22.00	23.00– 00.00	00.00– 01.00	01.00– 02.00	02.00– 03.00	03.00– 04.00	04.00– 05.00	05.00– 06.00
		n = 18	n = 18	n = 18	n = 18	n = 18	n = 18	n = 18	n = 18	n = 18	n = 18	n = 18
	H3	24	21	8	1	5	2	2	14	61	1037	463
	H4	2	0	3	2	0	0	0	11	4	24	0
	H5	1	2	2	0	1	0	0	0	0	3	11
	H6	10	0	0	6	0	1	1	9	8	19	4
	H7	0	0	0	0	0	0	0	0	0	1	0

Table 1

b. Number of *C. oxystoma* and *C. peregrinus* collected from body parts of cattle at various time (hr) in Dharan, West Bengal. H1, head; H2, neck; H3, hump; H4, back; H5, leg; H6, belly; H7, hip of the cattle.

			18.00– 19.00 n = 12	19.00– 20.00 n = 12	20.00– 21.00 n = 12	21.00– 22.00 n = 12	23.00– 00.00 n = 12	00.00– 01.00 n = 12	01.00– 02.00 n = 12	02.00– 03.00 n = 12	03.00– 04.00 n = 12	04.00– 05.00 n = 12	05.00– 06.00 n = 12
Culicoides oxystoma	Non- engorged	H1	16	2	0	3	0	0	1	2	2	20	15
		H2	48	12	7	1	3	3	15	7	39	639	89
		H3	13	5	1	0	0	2	2	1	3	117	40
		H4	2	0	1	0	0	0	0	0	1	4	0
		H5	4	1	0	1	1	1	1	1	1	69	5
		H6	1	0	2	0	0	0	0	0	1	12	2
		H7	1	0	0	0	0	1	0	0	0	0	0
	Engorged	H1	7	0	0	0	0	0	0	0	1	11	9
		H2	38	28	29	2	0	7	2	4	67	359	59
		H3	38	35	12	1	1	1	0	3	14	78	56
		H4	0	0	0	0	0	0	0	0	0	1	0
		H5	1	0	0	0	0	0	0	0	0	16	0
		H6	0	0	3	0	0	0	0	0	0	8	6
		H7	0	0	0	0		0	0	0	0	0	2
	Female	H1	23	2	0	3	0	0	1	2	3	31	24
		H2	86	40	36	3	3	10	17	11	106	998	147
		H3	51	40	13	1	1	3	2	4	17	195	96
		H4	2	0	1	0	0	0	0	0	1	5	0
		H5	5	1	0	1	1	1	1	1	1	85	5
		H6	1	0	5	0	0	0	0	0	1	20	8
		H7	1	0	0	0	0	1	0	0	0	0	2
Culicoides peregrinus	Non- engorged	H1	6	0	0	1	0	0	0	1	0	5	11
		H2	56	4	15	3	0	4	1	31	28	603	27
		H3	4	4	0	1	2	1	1	16	13	82	17
		H4	0	0	0	0	0	0	0	2	0	0	2
		H5	8	2	0	0	0	0	0	0	0	53	3
		H6	1	2	2	0	0	0	0	0	1	2	0
		H7	0	0	0	0	0	0	0	0	0	0	0
	Engorged	H1	6	0	0	0	0	0	0	1	8	12	20
		H2	10	8	19	10	0	5	1	22	230	815	73
		H3	51	70	2	7	1	3	1	4	61	81	36
		H4	1	1	0	0	0	0	0	0	0	5	0
		H5	9	0	0	0	1	0	0	3	0	29	5
		H6	0	0	1	0	0	0	0	1	0	16	1
		H7	0	0	0	0	0	0	0	0	0	0	0
	Female	H1	12	0	0	1	0	0	0	2	8	17	31
		H2	66	12	34	13	0	9	2	53	258	1418	100
		H3	55	74	2	8	3	4	2	20	74	163	53

		18.00– 19.00 n = 12	19.00– 20.00 n = 12	20.00– 21.00 n = 12	21.00– 22.00 n = 12	23.00– 00.00 n = 12	00.00– 01.00 n = 12	01.00– 02.00 n = 12	02.00– 03.00 n = 12	03.00– 04.00 n = 12	04.00– 05.00 n = 12	05.00– 06.00 n = 12
	H4	1	1	0	0	0	0	0	2	0	5	2
	H5	17	2	0	0	1	0	0	3	0	82	8
	H6	1	2	3	0	0	0	0	1	1	18	1
	H7	0	0	0	0	0	0	0	0	0	0	0

Table 1c. Collection of *Culicoides* spp. using sampling methods (Aspirator & UV light trap) at Sahibganj-Tantipara, West Bengal

		Aspirator n=12	UV LED light trap n=12
<i>C. oxystoma</i>	Male	0	83
	Female	2119	1146
	Non-engorged	1220	652
	Engorged	899	435
	Gravid	0	59
<i>C. peregrinus</i>	Male	0	46
	Female	2645	5197
	Non-engorged	1015	3718
	Engorged	1630	1418
	Gravid	0	61
<i>C. fulvus</i>	Male	0	14
	Female	0	212
	Non-engorged	0	67
	Engorged	0	143
	Gravid	0	2

Dharan

GLM with logit link depicted that the following parameters were significant at $p < 0.05$ for all the observations: engorged *C. oxystoma* exhibited significance for preferential landing on host ($\chi^2 = 9.3$) with time ($\chi^2 = 11.44$); non-engorged females preferred hump and neck for landing at specific time interval ($\chi^2 = 6.2$). Similarly, engorged *C. peregrinus* exhibited preference for specific body part of the cattle ($\chi^2 = 14.75$) with time ($\chi^2 = 7.3$) and for non-engorged similar observation was noted with respect to time ($\chi^2 = 8.6$) and choice of the individuals to descend at specific body part ($\chi^2 = 3.9$).

Sahebganj-Tantipara (ST)

For *C. oxystoma*, the binomial GLM with logit link exhibited that the following parameters were significant at $p < 0.05$ level for all the variables in each observation: for engorged individuals notable variance in response has been observed with respect to preferential host landing against time ($\chi^2 = 3.976$). Similar pattern for non engorged individuals has been observed: variation in preference for landing on different body parts of host ($\chi^2 = 15.31$) has been exhibited. For engorged *C. peregrinus* females the factors were observed significant for interaction between choice of landing with time ($\chi^2 = 10.81$) and similarly for non-engorged females also ($\chi^2 = 14.61$).

Discussion

The *Culicoides* species investigated in this study were 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 the 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. *Culicoides oxystoma* and *C. peregrinus* were known to be one of the most prevalent species across India, from which the BTV serotypes have been isolated [27, 28] 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* [29]. *Culicoides oxystoma* has been recognized as potential vectors of BTV from India [27] and Indonesia [30]. Japan has reported *C. oxystoma* as potent vector of Akabane virus [31, 32] and of epizootic hemorrhagic disease virus from Israel [33]. Moreover [34] 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 45% of the total midges landing actually fed on the cattle; the finding substantiated the conclusion drawn on activity of *Culicoides* midges from Ireland [35]. The work in Ireland was carried out as an extension of the project work on aetiology of sweet itch in horse. During the study, predominantly *C. obsoletus* and *C. dewulfi* were collected using aspirator from host body. Other species were *C. pulicaris* and *C. nebulosus*. The study reported 40.3% of midges actually fed of the total landing (i.e., 4097 *Culicoides* fed of the total landing of 10,178). However preferential landing site of *Culicoides* was different in both the studies due to different host.

The pattern of distribution observed from the light trap catch data to be non-linear random with two or more peaks observed between dusk and dawn. The prevalence of the *Culicoides* in light trap within cattle shed validates more of their crepuscular nature. Nonetheless, *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. However contrasting their nocturnal peaks between dusk and dawn, biting activity of *Culicoides* was limited only to early morning (4:00–5:00 hr). The present study emphasizes that 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* [36], our observation suggests that the thickness of skin and closeness of vascularization to body surface might be two of the most important criteria influencing the landing of female *Culicoides*. Likewise [35] reported on the preference of *C. obsoletus*, *C. dewulfi*, *C. pulicaris*, *C. punctatus* and *C. nebulosus* for mane and lower legs of horse. [36] remarked that 72% of the total collections were from the belly whereas 28% from the dorsal surface. *Culicoides 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. achrayi* landed on the belly. *Culicoides 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 [37].

The difference in preferential landing could not be attributed solely 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. Feeding by ectoparasitic midges on host depends on varying degree of skin thickness regionally differentiated at different body parts of host [38]. The thickness of skin in neck region of cattle was relatively less (~ 3.3 mm) compared to an average 4.2 mm of rest of the body [39]. Minute hematophagous midges like *Culicoides* possess small mouthparts hence they need to feed in region with vascularization close to the body surface [38] facilitating a easy reach to blood vessels through skin

Conclusion

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 hr and 05.00 hr). 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 4:00 hr and 5:00 hr) 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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COMPETING INTEREST

The author(s) declare(s) that they have no competing interests.

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Figures

Figure 1

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

(a) Nulliparous

(a) Nulliparous

(b) Parous

(b) Parous

(c) Engorged

(c) Engorged

A

B

Figure 2

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

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

(a) *C. oxystoma* at Dharan

(b) *C. peregrinus* in Dharan

(c) *C. oxystoma* in Sahebganj-Tantipara

(d) *C. peregrinus* in Sahebganj-Tantipara

Figure 3

The graphical presentation of the landing sites at different time intervals observed against *C. oxystoma* and *C. peregrinus* as observed in Dharan and Sahebganj-Tantipara, West Bengal, India. A total of 27 nights (297 hours) were included for the observations in each of the sites. H1, head; H2, neck; H3, hump; H4, back; H5, leg; H6, belly; H7, hip. x-axis: Time (in hour) 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: 01.00-2.00; T8: 02.00-03.00; T9: 03.00-04.00; T10: 04.00-05.00; T11: 05.00-06.00. y-axis: mean value of log (n+1) abundance of Culicoides

Figure 4

a. Graphical representation of proportion of engorged females of *Culicoides* collected from different body parts of cow at different time interval at Dharan. COX, *C. oxystoma*; CPE, *C. peregrinus*. Time (in hr) 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. x-axis: H1, head; H2, neck; H3, hump; H4, back; H5, leg; H6, belly; H7, hip. y-axis: mean value of log (n+1) abundance of *Culicoides*

b. Graphical representation of the proportion of engorged females of *Culicoides* collected from different body parts of cow at different time interval at Sahebganj-Tantipara. COX, *C. oxystoma*; CPE, *C. peregrinus*. Time (in hr) 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. x-axis: H1, head; H2, neck; H3, hump; H4, back; H5, leg; H6, belly; H7, hip. y-axis: mean value of log (n+1) abundance of *Culicoides*

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