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Highlights

- We described host-seeking activity of *C.oxystoma* and *C.imicola* in Senegal
- We compared host preferences of these *Culicoides* for horse and sheep
- We ed the nuisance associated with *Lasiohelae*sp for animals
- Midges preferred horse compared to sheep and are mainly crepuscular

Abstract

African horse sickness- and bluetongue virus are orbiviruses transmitted by *Culicoides* biting midges (Diptera: Ceratopogonidae) to horses and to ruminants respectively. Since the last epizootic outbreak of African horse sickness in 2007 in Senegal, extensive investigations have been undertaken to improve our knowledge on *Culicoides* species involved locally in the transmission of the virus. The purpose of this study was to compare and quantify the host preferences of potential vectors of these orbiviruses on horse and sheep and to study their circadian rhythm. We found that *Culicoides oxystoma* and species of the sub-genus *Avaritia* (*C.imicola*, *C. bolitinos* and *C. pseudopallidipennis*) had a preference for horse when compared to sheep (the predicted ratio between horse and sheep was 80 for *C. oxystoma* and 26 for *C. imicola*), and were mostly crepuscular: *C. oxystoma* had continuous activity throughout the diel with peaks in numbers collected after sunrise and sunset, while *C. imicola* was mostly nocturnal with peak after sunset. Unexpectedly, species of the subgenus *Lasiohelea* was also collected during this study. This diurnal biting species was a nuisance pest for both animal species used as bait.

Key words: *Culicoides*, Orbivirus transmission, host-feeding pattern, diel host-seeking activity, Thiès (Senegal)

Introduction

African horse sickness (AHS) and bluetongue (BT) are endemic diseases in West Africa; caused by Orbiviruses (*Reoviridae*) transmitted by small hematophagous insects of the genus *Culicoides* (Diptera: Ceratopogonidae). In southern Africa, *Culicoides imicola* Kieffer and *Culicoides bolitinos* Meiswinkel are proven vectors of African horse sickness virus (AHSV) (Venter *et al.*, 1999; Venter *et al.*, 2000; Meiswinkel & Paweska, 2003) and of bluetongue virus (BTV) (Rawlings *et al.*, 1997; Venter *et al.*, 2000; Mellor & Hamblin, 2004). Little is known about the biology and ecology of *Culicoides* species in West Africa, in particular those involved in the transmission of AHS and BT, which impedes the development of efficient vector control methods.

Culicoides oxystoma Kieffer was detected for the first time in Senegal in 2011 (Bakhoum *et al.*, 2013). This species is abundant in light-traps in the Niayes area(Diarra *et al.*, 2014), a region particularly affected by the previous epizootic outbreak of AHS in 2007; it is also aggressive towards horses (Fall *et al.*, 2015). Furthermore, these studies collected up to 41

Culicoides species (this increased the number of described *Culicoides* species found in Senegal from 34 to 53) and highlighted that *Culicoides kingi* Austen reaches abundances on horse equivalent to those of *C. imicola*. It may be that *C. oxystoma* and *C. kingi* also contribute to the transmission of the AHSV in Senegal, and more broadly in West Africa (Fall *et al.*, 2015). *Culicoides oxystoma* has been reported as a putative vector for BTV in northern India (Prasad *et al.*, 1999), as involved in the transmission of bovine arboviruses such as Akabane virus in Japan (Kurogi *et al.*, 1987; Yanase *et al.*, 2005) and is suspected of transmitting the epizootic haemorrhagic disease virus (EHDV) in Israel (Morag *et al.*, 2012). *Culicoides kingi* has been found in the field to be infected by EHDV in Sudan (Mellor *et al.*, 1984).

Outside of South Africa, little research has been conducted on the biology and ecology of the African species of the genus *Culicoides*. The only in-depth investigations were conducted in Cameroon (Nicholas, 1953), Gabon (Auriault, 1977) and Congo (Vattier-Bernard *et al.*, 1986; Itoua *et al.*, 1987) and primarily related to *Culicoides grahamii* Austen. This species is anthropophilic and its peak feeding activity occurs at sunrise and sunset, causing a nuisance to humans in some areas and during abundance seasons. However, most *Culicoides* species studied in Central Africa are zoo-anthropophilic in their feeding behaviour, e.g. *Culicoides kumbaensis* Callot, Kremer, Mouchet and Bach, *Culicoides fulvithorax* Austen, *Culicoides dubitatus* Kremer, Rebholtz-Hirtzel and Delécolle and *Culicoides trifasciellus* Goetghebuer (Itoua *et al.*, 1987). Outside southern Africa, the only African species of veterinary significance studied so far are *C. kingi* in Sudan which displays two biting peaks: one after sunrise and the other close to sunset (El Sinnary *et al.*, 1985).

This illustrates how fragmentary current data about the life history and ecology of *Culicoides* is in Africa. The present study describes host preferences, as key elements in the transmission of arboviruses, of *Culicoides* that are potential vectors of the AHSV and BTV in Senegal by comparing their attraction to horse and sheep and determining their circadian rhythm.

Materials and methods

1) Study Area

The study was conducted at the Thiès national stud farm between 29 August and 28 September, 2013 in the middle of the rain season. This stud farm was located in a forested area. This season was selected as optimal for *Culicoides* abundance, and this site was selected because *C. imicola* and *C. oxystoma* were both abundant (Diarra *et al.*, 2014).The city of Thiès is in the Niayes area; it is characterised by depressions between dunes that flooded during the rainy season. These depressions are located from Dakar in the south to the Senegal River Delta in the north. This is an area where commercial garden crops and fruit are intensively grown. Dairy and poultry farms are also found there together with a number of riding centres that accommodate purebred horses. Rainfall rarely exceeds 500 mm/year with a rainy season ranging from July to October. Ocean vicinity is conducive to a high relative moisture rate ranging from 90% to 15%, depending on the distance from the sea and time of year.

2) Host-baited trap collections

We compared two hosts on two sites using a Latin-square design. One horse and two sheep were used. No insecticide was applied on animals the year before the start of the experiment. The horse was across-bred English yearling weighing between 110 and 120 kg. The sheep were two ewes of a local breed weighing between 25 and 30 kg each. The baited traps used were identical to that used by Fall *et al*. (2015).The animal-baited trap consists of a net box (3.5 m × 2.5 m × 2.5 m, with mesh of 1.5 mm × 0.3 mm) with an open space of 15 cm fromthe ground allowing *Culicoides* to enter, to engorge or not on animal, and avoiding the escape of trapped midges. The traps were placed at two locations separated by a distance of 150 m (without visual contact between them) to minimise between-trap interactions. Other horses were present in vicinity of the stud farm for pasturage and a cattle herd was grazing at a distance of more than 1,000 m.

Collections were conducted in three sessions. During each session, midges were collected every three hours over two periods of 24 hours – midges trapped inside the tents were collected for 10-15 min using an electric vacuum cleaner.Each 24-h period was spread over two consecutive days, stretching from midday (day 1) to midday (day 2). The two 24-h periods were separated by a 3-h interval to allow the switch of animals between the two locations. Thus, horse and sheep were exposed during 24 x 2 x 3 = 144 hours each.

3) *Culicoides* identification

The insects collected were killed by low temperature and identified. Morphological identification of *Culicoides* species relied on an examination of wing patterns under a stereomicroscope(Zeiss, Stemi DV4) using the morphological keys for the Afrotropical region (Boorman & Dipeolu, 1979; Boorman, 1989; Meiswinkel, 1989; Glick, 1990; Meiswinkel, 1991; Cornet & Brunhes, 1994). When needed, specimens were dissected and slide-mounted in accordance with the Wirth and Marston (1968) technique. *Culicoides* were counted by species and sex, and females were categorized as nulliparous or parous (Dyce, 1969), engorged or unengorged, and gravid or not. The specimens were placed in Eppendorf tubes and stored in 90% alcohol.

4) Statistical analysis

Abundances on respective hosts were compared by species using the raw data and data adjusted for host weight. Engorgement rates were computed by dividing the number of engorged females by the total number of females collected.

Unadjusted abundance of *Culicoides* species was modelled using a Poisson mixed-effect model fitted with a method providing an adaptive Gauss-Hermite approximation to the maximized log-likelihood. We used the trapping day (1 to 6) as a random effect, and the location and the host (horse, sheep) as fixed effects. Selection of effects in the abundance model was based on a likelihood ratio test, the confidence intervals for parameters in the fitted model, and the Akaike information criterion (AIC). The procedure proposed by Nakagawa and Schielzeth (2013) was used as an overall test for goodness of fit.

All data analyses were performed using R statistical packages(R_Core_Team, 2014).

Results

Collections were carried out during the rainfall season. Weather was cloudy during the two first collection sessions and sunny during the last session. Temperatures varied from 23.2 to 32.8°C the first collection session, from 26.4 to 34.8°C the second session, and from 25.2 to 33.6°C the third session and humidity varied from 55.1 to 90.6%, from 49.3 to 84.6% and from 47.0 to 85.6% for the three sessions. Intra-session amplitudes of both temperature and humidity were higher (about 9°C and 37%) than inter-session amplitudes (1-3°C and 1-9%).

Host preference

A total of 1,259 *Culicoides* specimens were collected, of which 54 were males. In total, 1,215 *Culicoides* were collected in the horse-baited trap, i.e. 96.5% of overall collections, vs. 44 in the sheep-baited trap, i.e. 3.5% (Table 1). We identified 11 different species, all of which were found in the horse-baited trap, while only 8 were found in the sheep-baited trap. *Culicoides oxystoma*, the only representative species of the Schultzei group, was predominant in the collections, with 928 specimens (73.7%). The medium-abundance species belonged to the Imicola group, namely *C. imicola* with 214 specimens (16.9%), *C. bolitinos* with 55 specimens (4.3%) and *Culicoides pseudopallidipennis* Clastrier with 34 specimens (2.7%). Taken together, these 4 species accounted for 97.8% of the *Culicoides* collected (Table 1). The engorgement rate for these species ranged from 40 to 50% on the horse and was 20% on sheep for *C. oxystoma* (Table 1). The other species – *C. trifasciellus*, *Culicoides translucens* Khamala and Kettle, *Culicoides nivosus* De Meillon, *Culicoides nigripennis* Carter, Ingram and Macfie and *Culicoides moreli* Clastrier were uncommon. Finally, *Culicoides accraensis* Carter, Ingram and Macfieand *Culicoides leucostictus* Kieffer were not attracted by the horse and sheep baits and their collection was incidental since only gravid females or males were identified. The *Culicoides* population was relatively young with a nulliparous rate of 70%.

Overall, *Culicoides* numbers were fairly balanced between sites (593 in one site and 600 in the other). The number of females caught varied significantly between trapping sessions. Both the raw and the host weight-adjusted figures were always much greater for the horse than for the sheep-baited trap for *C. oxystoma, C. imicola, C. bolitinos* and *C. pseudopallidipennis* (Figure 1); from 96 to 98% of females were collected on this host (Table 1).

To predict the abundance of *C. oxystoma* females, we selected the full model with the site and the host as the fixed effects, and the trapping day as the random effect. The conditional R^2 (percentage of variance explained by both fixed and random effects) was very high (99.6%), whereas the marginal R^2 (percentage of variance explained by fixed effects) was high (marginal R^2 = 64.6%), and mainly (96.0%) explained by the host effect. For the fixed effects, the model predicted a ratio of 80 between females collected on the horse and those collected on the sheep (the horse weighing twice as much as the ewes) and a ratio of 2.5 between the two sites. To predict the abundance of *C. imicola* females, we selected the full model. The conditional R^2 was very high (97.0), whereas the marginal R^2 was high (63.8), and mainly (94.2%) explained by the host effect. For the fixed effect, this model predicted a ratio of 26 between females collected on the horse and those collected on the sheep and a ratio of 2.2 between the two sites.

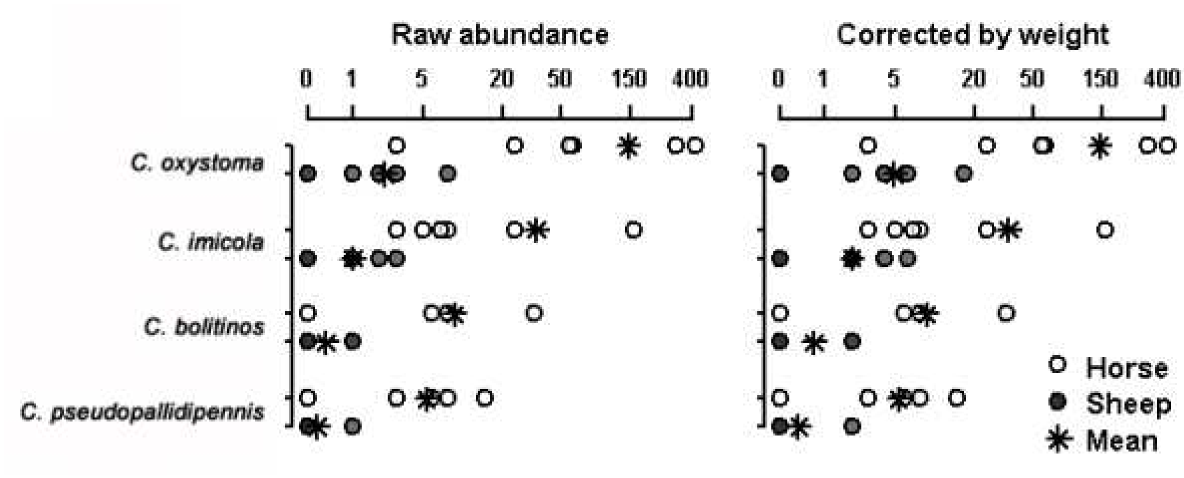

Figure 1: Raw abundance and host weight-adjusted abundance of *Culicoides oxystoma, Culicoides imicola*, *Culicoides bolitinos*, and *Culicoides pseudopallidipennis* females collected on each host. Both plots look quite similar as weight-correction (which is limited as two sheep were used against one horse) was applied on the numbers of *Culicoides* collected on sheep, which were always low.

Table 1. Number of *Culicoides* collected during the 6 trapping days on horse and sheep baits

***Culicoides* species**	**Total**						**Horse-baited trap**							**Sheep-bai**	
	Rank	**(C%)**	**F**	**(P)**	**M**		**F**	**bf F**	**(bf R)**	**N**	**P**	**G**	**M**	**F**	**bf**
C. oxystoma	1	73.7	881	35.8	47		867	381	43.9	308	176	2	36	14	[illegible]
C. imicola	2	90.7	212	12.8	2		206	99	48.0	91	14	2	1	6	[illegible]
C. bolitinos	3	95.1	55	16.1	0		53	22	41.5	26	4	1	0	2	[illegible]
C. pseudopallidipennis	4	97.8	33	5.6	1		32	14	43.8	17	1	0	0	1	[illegible]
C. trifasciellus	5	98.7	11	-	0		11	3	-	6	2	1	0	0	
C. translucens	6	99.1	4	-	1		1	0	-	0	0	1	1	3	[illegible]
C. nivosus	7	99.4	3	-	1		0	0	-	0	0	0	1	3	[illegible]
C. nigripennis	8	99.7	3	-	1		2	0	-	0	2	0	1	1	[illegible]
C. accraensis	9	99.9	2	-	0		1	0	-	0	0	1	0	1	[illegible]
C. moreli	10	100.0	0	-	1		1	0	-	0	1	0	0	0	
C. leucostictus	11	100.0	1	-	0		0	0	-	0	0	0	1	0	
TOTAL			**1,205**	**30.4**	**54**		**1,174**	**519**	**44.2**	**448**	**200**	**7**	**41**	**31**	[illegible]

(C%): cumulative percentages of abundance; F: number of females; (P): parous rate; M: number of males; bfF: number of engorged females; (bfR): engorgement percentage; N: number of nulliparous females; P: number of parous females; G: number of gravid females.

Circadian rhythms

Figure 2 shows the overall abundance of *C. oxystoma*, *C. imicola*, *C. bolitinos*, and *C. pseudopallidipennis* females over the diel regardless of host. For each species, observed abundances in each trapping session were correlated for nulliparous, parous, gravid and engorged females, thus suggesting that there was no activity difference related to time of day associated with differences in the physiological status of females. *Culicoides oxystoma* was collected at all times, with activity peaks immediately after sunrise, and immediately after sunset. *Culicoides imicola* was nocturnal and its activity peaked just after sunset. The activity of *C. bolitinos* and *C. pseudopallidipennis* peaked just after sunset and sunrise but the low numbers collected renders it difficult to validate this result.

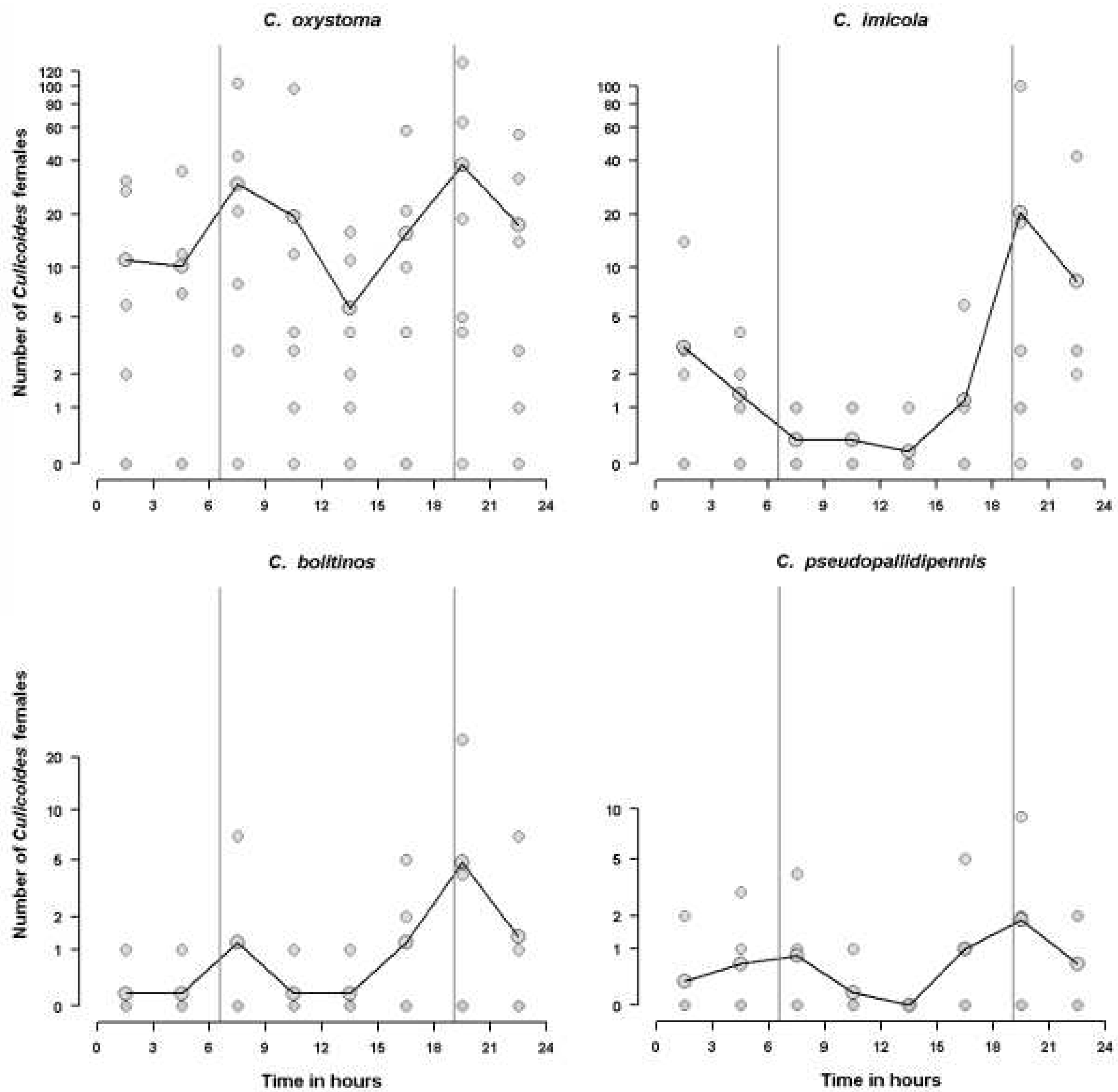

Figure 2: Circadian activity of female *Culicoides oxystoma*, *C. imicola*, *C. bolitinos*, and *C. pseudopallidipennis*, collected in host-baited traps regardless of the host. The black, broken line represents the average of observations. The vertical grey lines illustrate the time of sunrise and sunset.

Additionally, we identified another Ceratopogonidae species of the *Forcipomyia* genus and of the *Lasiohelae* sub-genus that was remarkably aggressive towards the hosts, particularly the horse. No male was collected and we were unable to identify the specimens to the species level. A total of 735 specimens were collected, 644 of which were found on the horse (87.6%), with an engorgement rate of 80.2%, as compared with 91 specimens collected on the sheep (12.4%), with an engorgement rate of 70.3%. This species of the *Lasiohelea* subgenus was markedly diurnal and its peak of activity is the pre-sunset period (Figure 3).

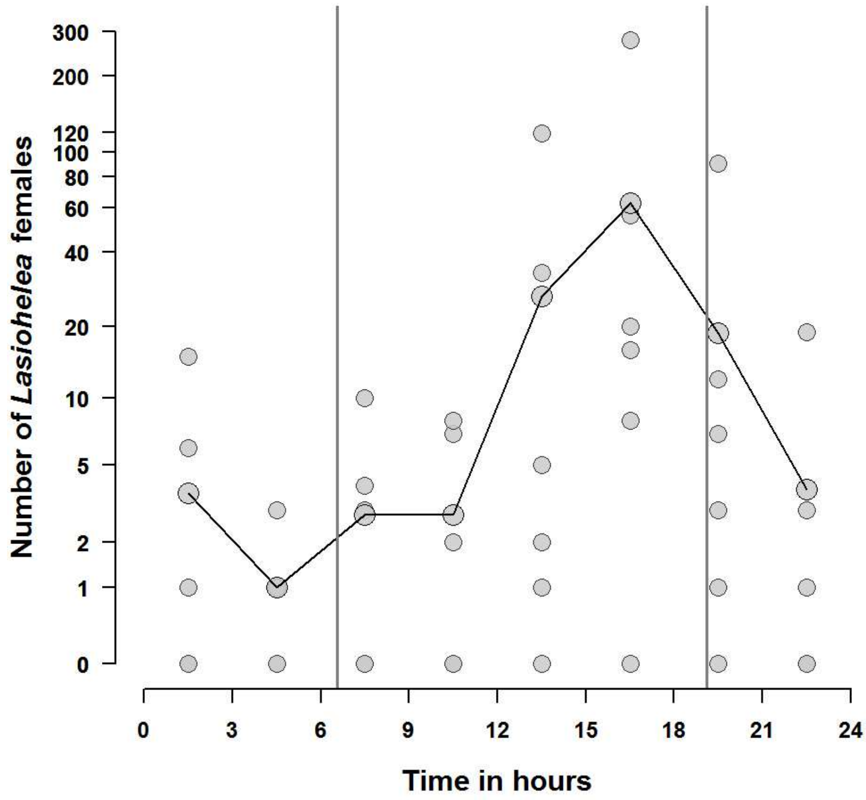

Figure 3: Circadian activity of females belonging to the unknown of the *Lasiohelea* subgenus, i.e. overall number of females collected in each catch regardless of host. The black, broken line represents the mean of the observations. The vertical grey lines illustrate time of sunrise and sunset.

Discussion

The predominance of *C. oxystoma* and *C. imicola* in UV-light traps and horse-baited traps has previously been reported in the region (Diarra *et al.*, 2014; Fall *et al.*, 2015).This study showed a host preference of *C. oxystoma* and *Culicoides* species from the *Avaritia* sub-genus for horse as compared to sheep. Similar host preference for horse was found by Viennet *et* *al.*(2012) for *Culicoides* species of veterinary significance using host-baited traps in the Palearctic region. The authors did not however reach the conclusion that horse were more attractive than other hosts, or that there was a non-linear relationship between weight and attractiveness (the horse being by far the largest host). In our study, the limited difference in weight between the horse and the sheep suggested that this difference in abundance was connected with greater attractiveness of horse for the 4 most abundant *Culicoides* species collected.

The number of midges collected varied between days. The numbers collected in baited traps depended not only on factors inherent to the host but also on weather conditions. Indeed, Cornet (1969) and Walker (1977) reported that as soon as night temperatures were somewhat higher in the Afro-tropical region, the number of specimens collected by light trap increased. Moreover, it is classically reported that catches in light trap or on human bait were generally higher on moonless nights in Sub-Saharan Africa (Cornet, 1969; Auriault, 1979; Itoua *et al.*, 1987) and in other regions (Mellor *et al.*, 2000). Host attractiveness to haematophagous insects can be dependent on the size of the host, its body temperature, the carbon dioxide (CO2) its other specific odours or its colour, as it has been highlighted by many studies conducted on mosquitoes (Dow *et al.*, 1957; Edman *et al.*, 1974; Lardeux *et al.*, 2007; Takken & Verhulst, 2013). Wind can also disrupt the odour flows emanating from the host or interfere with insect activity: Almost all activity was suppressed at wind speeds greater than three m/s for *C. imicola* in Kenya (Walker, 1977). Host colour does not seem to have any influence on engorgement rate of *Culicoides sanguisuga* Coquillett and *Culicoides guttipennis* Coquillett (Humphreys & Turner, 1973). Carbon dioxide attracted specimens of the Variipennis complex in northern America (Nelson, 1965; Mullens, 1995) and *Culicoides impunctatus* Goetghebuer in Scotland (Bhasin *et al.*, 2001). Also, greater host size, corresponding to higher quantity of carbon dioxide released by the host, resulted in greater host attractiveness for *Culicoides* to hosts (Humphreys & Turner, 1973; Tanner & Turner, 1974; Raich *et al.*, 1997).

The predominance of *C. oxystoma* in the horse-baited trap in the Niayes region was in line with observations made by Fall *et al.*(2015) using the same trap. Interestingly, *Culicoides subschultzei* Cornet and Brunhes was consistently collected in very small numbers in studies on *Culicoides* associated with livestock collected by light trap in southern Africa (Venter *et al.*, 1996; Mushi *et al.*, 1998) and in western Africa (our study) whereas this species was previously considered using light traps as highly abundant in the south and eastern Africa region (Cornet & Brunhes, 1994). This reputation was however based on taxonomic investigations in forested areas, which might explain this apparent mismatch. Scheffer *et al.*(2012) showed there were no *C. subschultzei* among the insects collected on horses by direct suction in South Africa. *Culicoides oxystoma* is regarded as abundant in light trap collections in the vicinity of livestock in areas where it is present (Oem *et al.*, 2013; Satheesha *et al.*, 2014). Following the detection of *C. oxystoma* in the Afro-tropical region (Bakhoum *et al.*, 2013) and due to the taxonomic imbroglio pertaining to the Schultzei group (Cornet & Brunhes, 1994), the status of the species in this group needs to be clarified. Further research is needed on host preferences in different ecosystems and different seasons, including in other African countries, to supplement the observations made in this study.

The night-time host seeking activity of *C. imicola* recorded in our study matched previous findings (Barnard, 1997; Meiswinkel *et al.*, 2000). As this species is exophilic, enter horses into closed holdings during the night might protect them from *C. imicola* bites and then against AHSV transmission. *Culicoides bolitinos* and *C. pseudopallidipennis* are also nocturnal.Although *C. bolitinos* does not seem to use horse manure as a larval developing site, it is considered as a vector of AHSV. The presence of cattle near horses may increase the numbers of *C. bolitinos*, and the risk of AHSV being transmitted, in a given area.

Culicoides trifasciellus was only collected in the horse-baited trap. Due to the low numbers collected, no firm conclusions can be drawn as to its feeding patterns. However, this species is undoubtedly zoo-anthropophilic (Itoua *et al.*, 1987). Its taxonomic status is uncertain as for other species such as *C. brosseti* and *C. dubitatus*, the male specimens of *C. dubitatus* being highly characteristic (Kremer *et al.*, 1975). Added to this, no taxonomic keys based on morphological characters are available for these species. It should also be noted that only one *C. nivosus* specimen – a male – was found on the horse whereas 3 females, 2 of which were engorged, were found on sheep. During a previous study performed with a horse-baited trap (Fall *et al.*, 2015), no engorged female of this species was found despite a high abundance in light-trap collections in the area (Diarra *et al.*, 2014).

We observed females belonging to the *Lasiohelea* subgenus to cause major nuisance to the horse and sheep, in particular during the day with pre-sunset peak of activity. No information is available regarding the role of species of this subgenus in virus transmission. In Australia, an undescribed species of day-feeding midge belonging to this subgenus was recently identified as possible vector of *Leishmania enrietti* complex to the red kangaroo (*Macropus* *rufus*) (Dougall *et al.*, 2011). Further research is needed to identify this species and its potential role in Orbivirus transmission. However, due to the pattern of AHSV and BTV outbreaks observed worldwide, it was always accepted that night active insects will play a more important role in Orbivirus transmission.

Conclusion

The eco-epidemiological features of *Culicoides* midges populations in the Niayes area of Senegal differs from southern Africa, with the predominance of *C. oxystoma* (vs. *C. imicola*), the attractiveness of horses to this species, its continuous activity (mostly crepuscular), and the lack of information regarding its vector competence for Orbiviruses, notably for the AHSV. Knowledge is too scarce to allow the development of operational methods for controlling populations of these midges in the field (Carpenter *et al.*, 2008). In particular, further research on the larval ecology and resting behaviour of this species would provide relevant information for developing more efficient control methods. Moreover, similar work should be done in other ecosystems in Senegal where other *Culicoides* species - particularly those of the *Avaritia* sub-genus, are more abundant.

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