



Foraging range of arthropods with veterinary interest: New insights for Afrotropical *Culicoides* biting midges (Diptera: Ceratopogonidae) using the ring method



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ABSTRACT

The identification of blood meal source of arthropod vector species contributes to the understanding of host–vector–pathogen interactions. The aim of the current work was to identify blood meal source in *Culicoides* biting midge species, biological vectors of internationally important arboviruses of livestock and equids, using a new ecological approach. We examined the correlation between blood meal source identified in engorged *Culicoides* females collected in a suction light trap and the available vertebrate hosts along four rings (200, 500, 1000 and 2000 m) centered at the trap site and described the foraging range of the three main vector species of veterinary interest present in the study area, *Culicoides imicola*, *Culicoides kingi* and *Culicoides oxystoma*. The study was performed in four sites localized in the Niayes region of Senegal (West Africa) where recent outbreaks of African horse sickness occurred. Blood meal source identification was carried out by species-specific multiplex PCRs with genomic DNA extracted from the abdomen of engorged females collected during nine night collections for twenty-six collections. The four most abundant hosts present in the studied area (horse, cattle, goat and sheep) were surveyed in each ring zone. The blood meal source varied according to *Culicoides* species and host availability in each site. *C. oxystoma* and *C. imicola* females mainly fed on horses readily available at 200 m maximum from the trap location whereas females of *C. kingi* fed mainly on cattle, at variable distances from the traps (200 to 2000 m). *C. oxystoma* may also feed on other vertebrates. We discuss the results in relation with the transmission of *Culicoides*-borne arboviruses and the species dispersion capacities.

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1. Introduction

Finding a host is a crucial event in the life-history of haematophagous dipterans to obtain the mandatory blood meals for egg maturation. Feeding success on a given host relies on host preference, host availability and host defensive reactions. Host preference can be defined as the inherited tendency to select a

group of vertebrate hosts (mammals vs birds), or a given host species (horse vs cattle) to feed on (Balenghien et al., 2011; Takken and Verhulst, 2013). Host choice is the result of a trade-off between the advantages of finding the optimal host and the risk of dying before blood feeding. This determines opportunistic or specialized feeding behavior (Lyimo and Ferguson, 2009). The description of host preferences and foraging behavior of species involved in pathogen transmission is important to improve our understanding of epiological cycles (Gubbins et al., 2008). Host diversity and availability may impact pathogen transmission by allowing transmission between different host species or leading to a dilution effect (Balenghien et al., 2011; Schmidt and Ostfeld, 2001).

The genus *Culicoides* Latreille (Diptera: Ceratopogonidae) encompasses around 1358 species worldwide (Borkent, 2015). Certain *Culicoides* species are known vectors of bluetongue virus (BTV) in wild and domestic ruminants and African horse sickness virus

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(AHSV) in equids, both devastating animal diseases that lead to substantial economic losses (Carpenter et al., 2013; Mellor et al., 2000). African horse sickness affects equids in Sub-Saharan Africa and mortality rates frequently exceed 90% in horses (Mellor and Hamblin, 2004). In Senegal, epizootic outbreaks of AHS occurred in 2007 in two localities (Niague, Mbaou) in the Niayes area, before reaching other localities in the country (Diouf et al., 2012). These outbreaks affected 26 out of 34 departments in Senegal (Diouf et al., 2012) and caused important economic costs estimated at 1.37 million euros (Akakpo et al., 2011).

Feeding behavior of *Culicoides* species has received great attention recently in the Palearctic region (Ayllón et al., 2014; Blackwell et al., 1994; Calvo et al., 2012; Garros et al., 2011; Martínez-de la Puente et al., 2015; Ninio et al., 2010; Santiago-Alarcon et al., 2012). In the Afrotropical region, feeding behavior and host preferences of *Culicoides* species of veterinary interest are mainly described in South Africa (Meiswinkel et al., 2004; Nevill and Anderson, 1972). In Sudan, *Culicoides kingi* Austen is mainly attracted by cattle (El Sinnary et al., 1985). Previous studies in the Niayes region characterized the abundance of the five most abundant species in the vicinity of horses: *Culicoides imicola* Kieffer, *C. kingi*, *Culicoides oxystoma* Kieffer, *C. enderleini* Cornet and Brunhes and *C. nivosus* de Meillon (Diarra et al., 2014), and described a strong trophic preference for horses vs sheep for the first three species (Fall et al., 2015a, 2015c).

Different experimental designs have been developed in the field to analyze host feeding habits of *Culicoides* vector species and provided relevant information on host preferences of *Culicoides* species of veterinary interest in Europe (Viennet et al., 2013, 2011, 2012) and in Africa (Fall et al., 2015a,b,c). The most unbiased approach is to collect females directly on animal hosts but the small size of *Culicoides* makes it very difficult. Several authors also used animal-baited traps as for entomological investigations on West Nile fever virus (Balenghien et al., 2011; Fall et al., 2011). However, these direct methods are usually difficult to set up in the field and time consuming. Therefore, indirect approaches using serological or molecular identification assays have been developed. In most studies on trophic behavior, the host preferences are determined by examining the blood meal origin of field-collected specimens (using species-specific molecular assays or sequencing) (Garros et al., 2011; Lassen et al., 2012; Ninio et al., 2010) but feeding rates have rarely been linked quantitatively to host availability in the studied area. It is difficult to enumerate the numbers of available domestic and wild hosts in an area, and to estimate their spatial and seasonal variations (Balenghien et al., 2011). Furthermore, the collection of blood-fed *Culicoides* females is challenging especially because suction light traps do not allow the collection of large numbers of engorged females. Female *Culicoides* resting sites are largely unknown. Authors usually hypothesize that engorged females collected in a trap feed on the hosts present at the vicinity of the trap, except when no identified hosts are present at the trap location which allow inferring on dispersal and host preferences (Garros et al., 2011). Therefore, enumerating hosts in the close area of the trap site is required (Balenghien et al., 2011).

The ring method is frequently used in agricultural entomology and allows the investigation of dynamics and feeding behavior of polyphagous insect pests in relation to landscape composition and crop surfaces (Carrière et al., 2006; Sivakoff et al., 2013). In this study, we applied this ecological method to *C. imicola*, *C. kingi* and *C. oxystoma* in order to determine the foraging range of these three *Culicoides* species of veterinary interest abundant in the Niayes areas of Senegal. Indeed, *C. imicola* is regarded as the most important and proven vector species of Orbivirus of livestock diseases. This species has been associated with BTV (Venter et al., 2006, 1998) and AHSV (Paweska et al., 2003; Venter et al., 2000, 2009; Venter and Paweska, 2007). *C. oxystoma* is a well-known vector of bovine

arboviruses such as Akabane virus (Oem et al., 2013; Yanase et al., 2005) and is suspected of being vector of Epizootic Haemorrhagic Disease Virus in Israel (Morag et al., 2012). *C. kingi* is involved in the transmission of *Onchocerca gutturosa*, a widespread parasite of Sudanese cattle (Sinnary and Hussein, 1980). Breeding sites of these species are generally moist soil more or less enriched with organic matter for *C. imicola* (Meiswinkel et al., 2004), alkaline wet mud for *C. kingi* (Cornet, 1969) and aquatic or semiaquatic environment, such as paddy field, stream edge and pond margin for *C. oxystoma* (Yanase et al., 2013).

We investigated the correlation between blood meal source identified for the *Culicoides* females collected in a suction light trap, and the relative abundance of vertebrate hosts along various rings around the trap location with a peculiar interest for sheep, goat, cattle and especially horse because of the recent AHS outbreak in the area. Host survey was facilitated by the absence of wild ruminants and the absence of animal movements in the area.

2. Material and methods

2.1. *Culicoides* collection and survey of vertebrate hosts

Adult midges were collected using a suction light trap of the OVI type (Onderstepoort Veterinary Institute, South Africa) placed at four sites (2 traps per site) in the Niayes area, Senegal (Fig. 1) for nine night collections (Table 1). The Niayes area is a coastal band 25–30 km wide stretching over 180 km from Dakar to the southern tip of the Senegal River Delta. The climate is oceanic, typically warm and humid with strong, relatively constant winds. The total annual rainfall is 300–350 mm/year, with a maximum in September. Mean daily minimum and maximum temperatures range from 18 °C to 31 °C with an annual mean temperature of 27 °C (more details are provided in Faye et al. (1995)).

All collected specimens were preserved in 90% ethanol, identified and sexed under a stereomicroscope using the identification keys of Cornet and Brunhes (1994), Boorman (1989) and Meiswinkel (1989). Of 43,484 specimens collected in the light traps (Table 1), only 270 fully engorged females of *C. imicola*, *C. kingi* and *C. oxystoma* were considered and used in this study (Table 3). Information on vertebrate host position was collected around each trapping site on a radius of 2000 m (Table 1). Movements of domestic ruminants/horses are very much restricted in the Niayes area. Surveys showed that the spatial and seasonal movements of vertebrate hosts are negligible in this area during the studied period. Free wild fauna is completely absent from this area.

2.2. DNA extraction, primers design and PCR

Genomic DNA was extracted using commercial kits (Macherey-Nagel, Germany). Host primers were selected as described in the literature (Garros et al., 2011; Kocher et al., 1989) for identifying the origin of *Culicoides* blood meals (Table 2). Molecular identification was based on the amplification of the cytochrome b region of blood DNA as described in Garros et al. (2011). A multiplex PCR was performed to separate cattle, sheep and goat, and a simplex PCR was used to identify blood meal from horse (Garros et al., 2011).

2.3. Statistical analysis

Host positions and host densities (cattle, goat, horse, sheep and other vertebrates) were surveyed and calculated in the four concentric rings at distances of 200 m, 500 m, 1000 m and 2000 m around the trapping site (MapInfo® software, version 7.0). Selection of vertebrate hosts by *Culicoides* species was determined by correlation (Pearson's product-moment correlation). We examined the correlation between the percentage of the identified host as

Table 1
Diversity of vertebrate hosts and *Culicoides* specimen collection per site.

Trapping site	Latitude	Longitude	Vertebrate animals recorded in each ring (r) from the trapping site				Survey date	Culicoides collected	
			r = 200 m	r = 500 m	r = 1000 m	r = 2000 m		Total specimens of all species	Collection date
Parc de Hann ^a	14° 72' 83" N	17° 42' 98" W	80 horses, 28 sheep and 2 dogs	80 horses, 116 sheep and 4 dogs	80 horses, 2 cows, 8 goats, 253 sheep, 313 poultry, 11 lions, 1 tiger, 3 hyenas, 5 jackals, 6 antelopes, 21 crocodiles, 9 turtles, 7 pythons and 2 pumas	80 horses, 2 cows, 275 sheep, 8 goats, 313 poultry, 11 lions, 1 tiger, 3 hyenas, 5 jackals, 6 antelopes, 21 crocodiles, 9 turtles, 7 pythons and 2 pumas	26 and 30 April 2012	1272	17–18 May 2012 18–19 May 2012 17–18 June 2012 18–19 June 2012
Mbao	14° 74' 67" N	17° 33' 27" W	32 horses, 1 dog, 2 poultry and 3 goats	35 horses, 1 dog, 28 sheep, 3 goats and 127 poultry	38 horses, 1 dog, 3 goats, 181 sheep, 552 poultry and 8 rabbits	38 horses, 1 dog, 9 cows, 207 sheep, 7 goats, 566 poultry and 8 rabbits	17 and 18 May 2012	7847	17–18 May 2012 18–19 May 2012 17–18 June 2012 18–19 June 2012 18–19 July 2012
Niague	14° 82' 34" N	17° 24' 99" W	43 horses, 4 cows, 6 sheep, 1309 poultry and 1 dog	117 horses, 4 cows, 6 sheep, 7089 poultry and 1 dog	117 horses, 256 cows, 191 goats, 60 sheep, 12731 poultry, 10 dogs and 9 rabbits	117 horses, 426 cows, 86 sheep, 320 goats, 13059 poultry, 12 dogs and 9 rabbits	17, 18 and 19 July 2012	32,452	18–19 July 2012
Thiès	14° 79' 40" N	16° 95' 00" W	24 horses, 2 sheep, 50 poultry and 14 rabbits	24 horses, 27 sheep, 118 poultry, 14 rabbits and 2 dogs	24 horses, 41 cows, 75 goats, 123 sheep, 299 poultry, 7 dogs and 22 rabbits	24 horses, 41 cows, 142 sheep, 75 goats, 314 poultry, 7 dogs and 22 rabbits	20 and 21 May 2012	1913	20–21 May 2012 21–22 May 2012 20–21 June 2012 21–22 June 2012

^a Zoo is located about 800 m from the trap.

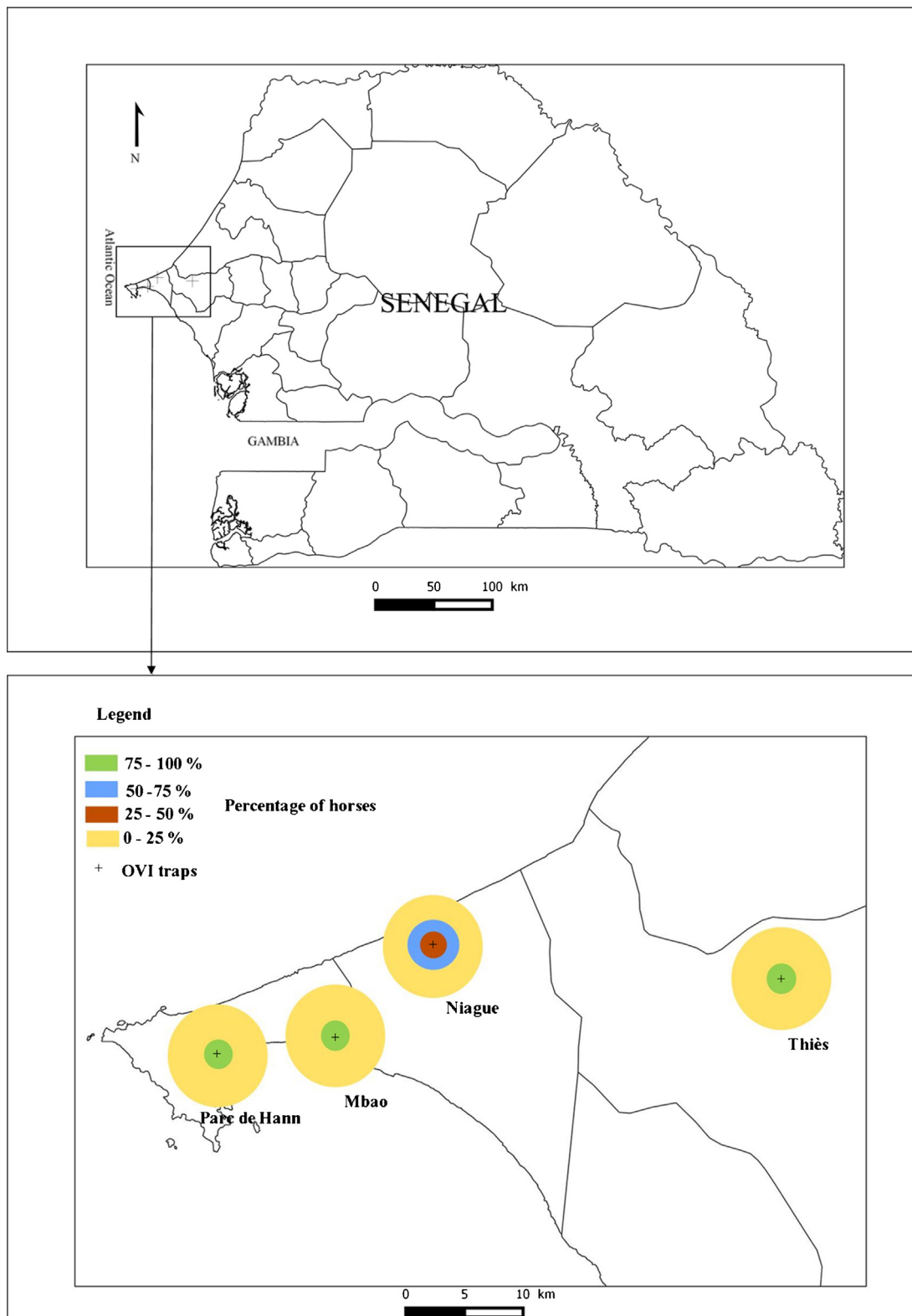


Fig. 1. Location of the four study sites in the Niayes area of Senegal. The circles present the relative abundance of horses around the trap site.

blood meal source and the percentage of availability of this host within each ring corrected by their weight. The average weight of hosts were estimated as follows: pigeon (0.5 kg), rabbit (4 kg), jackal (13 kg), dog (15 kg), turtle (20 kg), goat (30 kg), antelope (40 kg), puma (40 kg), sheep (40 kg), hyena (60 kg), ostrich (60 kg), crocodile (100 kg), python (100 kg), lion (160 kg), tiger (200 kg),

cattle (400 kg), horse (450 kg) (Data obtained at the veterinary services and livestock agents).

Table 2Primer set used for the identification of blood meal origin in *Culicoides* abdomens.

Primers	Primer sequence (5' → 3')	Length (pb)
VERT-F (Forward primer vertebrate)	CCATCCAACATCTCAGCATGATGAAA	357
VERT-R (Reverse primer vertebrate)	GCCCCTCAGAATGATATTTGTCCTCA	
Forward primer UNIV 3	TTTTTTTTTTTCGVTCCHATYCCHAAYAACTAGG	208
EQUUS-R (<i>Equus caballus</i>)	TACGTATGGGTGTTCCACTGGC	
Forward primer UNIV 2	TGAGGACAATATCATTYTGAGGRGC	287
BOS-R (<i>Bos taurus</i>)	TAAGATGTCCTAATGGTATAGTAG	
CAPRA-R (<i>Caprus hircus</i>)	TTAGAACAAGAATTAGTAGCATGGCC	313
OVIS-R (<i>Ovis aries</i>)	GGCGTGAATAGTACTAGTAGCATGAGATGA	336

Table 3Number of engorged females (N_{Eng}) relative to the total number of females collected (N_{T}).

	<i>C. kingi</i> ($N_{\text{Eng}}/N_{\text{T}}$)	<i>C. imicola</i> ($N_{\text{Eng}}/N_{\text{T}}$)	<i>C. oxystoma</i> ($N_{\text{Eng}}/N_{\text{T}}$)	Total
Parc de Hann	0/1	2/14	56/546	58/561
Mbao	10/3498	17/1177	42/1798	69/6473
Niague	53/30,292	4/548	2/355	59/31,195
Thiès	0/16	33/336	51/1328	84/1680
Total	63/33,807	56/2075	151/4027	270/39,909

3. Results

3.1. *Culicoides* collection

For nine night collections at 4 sites (twenty-six collections), 43,484 specimens of *Culicoides* species were collected (Table 1). Of these specimens, 39,909 were represented by *C. kingi*, *C. imicola* and *C. oxystoma*. A total of 270 engorged females belonging to these three species were obtained (Table 3).

3.2. Blood meal identifications

Identification of blood meal sources was successful for 155 out of 270 blood-fed females (57%), of which 110 were identified as horse, 24 as cattle and 21 on other vertebrates (Table 4). No blood meals were identified on sheep or goat.

3.3. Correlation between survey data of vertebrate hosts and blood meals identified

Fig. 2 shows the blood meal source identified at the 4 sites in relation to the composition of host species corrected by their weight along the transect. Different correlation coefficients were found according to *Culicoides* species (Fig. 3). For *C. imicola*, the highest correlation was observed in the lowest range (<200 m) ($r^2 = 0.998$, $t = 35.118$, $df = 3$, $p < 10^{-3}$). For *C. kingi*, the correlation was high from the 1000 to 2000 m range ($r^2 = 0.95$, $t = 5.299$, $df = 3$, $p = 0.013$). For *C. oxystoma*, similar correlation coefficients were found at all ranges in Parc de Hann ($r^2 = 0.543$, $t = 1.122$, $df = 3$, $p = 0.343$) and Mbao ($r^2 = 0.957$, $t = 88.77$, $df = 3$, $p = 0.014$). In Thiès, the highest correlation was observed for a range below 200 m ($r^2 = 0.999$, $t = 227.25$, $df = 3$, $p < 10^{-3}$), as also described for *C. imicola*.

4. Discussion

The characterization of host preferences of vector species is an important key to understand the transmission of vector-borne pathogens. In this study, we assessed the feeding preferences and foraging range of *Culicoides* species of veterinary interest in Senegal using the ring method and molecular blood meal identification.

The recent developments in molecular biology allow reliable and effective identification of blood meal source of field-collected insect specimens, as previously performed on mosquitoes (Fall et al., 2012; Gokool et al., 1993; Kent and Norris, 2005; Ngo and Kramer, 2003) and on *Culicoides*

(Ayllón et al., 2014; Elbers and Meiswinkel, 2014; Garros et al., 2011; Martínez-de la Puente et al., 2015). In this study, blood meals were identified by amplification of the cytochrome b region of genomic DNA extracted from abdomen of engorged females of *Culicoides* species using multiplex and simplex PCRs. However, blood meal source for 43% of engorged females failed to be identified. This unexpected loss of efficiency is probably due to the degradation of host DNA present in the blood meal. Indeed, the digestion processes quickly denature the genomic DNA of blood meal making identification difficult or impossible (Kent and Norris, 2005; Oshaghi et al., 2005). Moreover, the long-term conservation of specimens in alcohol (between 2 to 3 years) may also have contributed to degrade the genomic DNA of blood ingested by females. One limitation is the small number of engorged females collected. Therefore, sufficient numbers of identified blood meals to permit statistical analysis of host preference and foraging ratios were only obtained for certain sites and species.

In contrast to previous studies on feeding preferences of *Culicoides*, i.e. using indirect approaches (serological and molecular analyzes from the abdomens of females collected), our study has taken into account the diversity and availability of potential hosts around the sampling site. In this study, blood meal source varied according to *Culicoides* species and host availability. Blood meals were mainly taken around the trapping site (<200 m) for *C. imicola* and *C. oxystoma* in one site whereas *C. kingi* seems to present a different foraging strategy, with blood meal taken at an average of 2000 m. Potential breeding sites of these species are located in the vicinity of the trap sites, except those of *C. kingi*. Breeding sites of this species is the edge of the Lac Rose located at 2.5 km from the trap site of Niague (unpublished data).

Among vertebrate host studied, horse was the principal species attacked by *Culicoides* (71% of blood meal) and no blood meals were taken on sheep or goat. Although sheep and goat were present around the trapping sites, their relative abundance was very low in comparison to horse (horse = 73%, sheep = 9% and goat = 1% for all sites). Studies using direct approaches (i.e. to collect females directly on animal hosts) shown that *Culicoides* species are more attracted to horses than to sheep in the same area (Fall et al., 2015b, 2015c).

Local populations of *C. oxystoma* mainly fed on horses in the vicinity of the trapping site. In Parc de Hann, only 48% of *C. oxystoma* individuals fed on horses readily available at 200 m whereas 52% fed on other vertebrates (different from sheep, goats and cattle). This high rate of non-identified blood meal (52%) is probably related to

Table 4
Blood meal identification in *Culicoides* females collected from four sites in the Niayes area in Senegal.

Sites	<i>Culicoides</i> species	Cattle	Goat	Sheep	Horse	Other vertebrates	Total
Parc de Hann	<i>C. oxystoma</i>	–	–	–	11	12	23
	<i>C. imicola</i>	–	–	–	3	–	45
	<i>C. kingi</i>	–	–	–	5	–	–
	<i>C. oxystoma</i>	–	–	–	37	–	–
Niague	<i>C. kingi</i>	23	–	–	9	9	41
Thiès	<i>C. imicola</i>	1	–	–	22	–	46
	<i>C. oxystoma</i>	–	–	–	23	–	–
Total		24	–	–	110	21	155

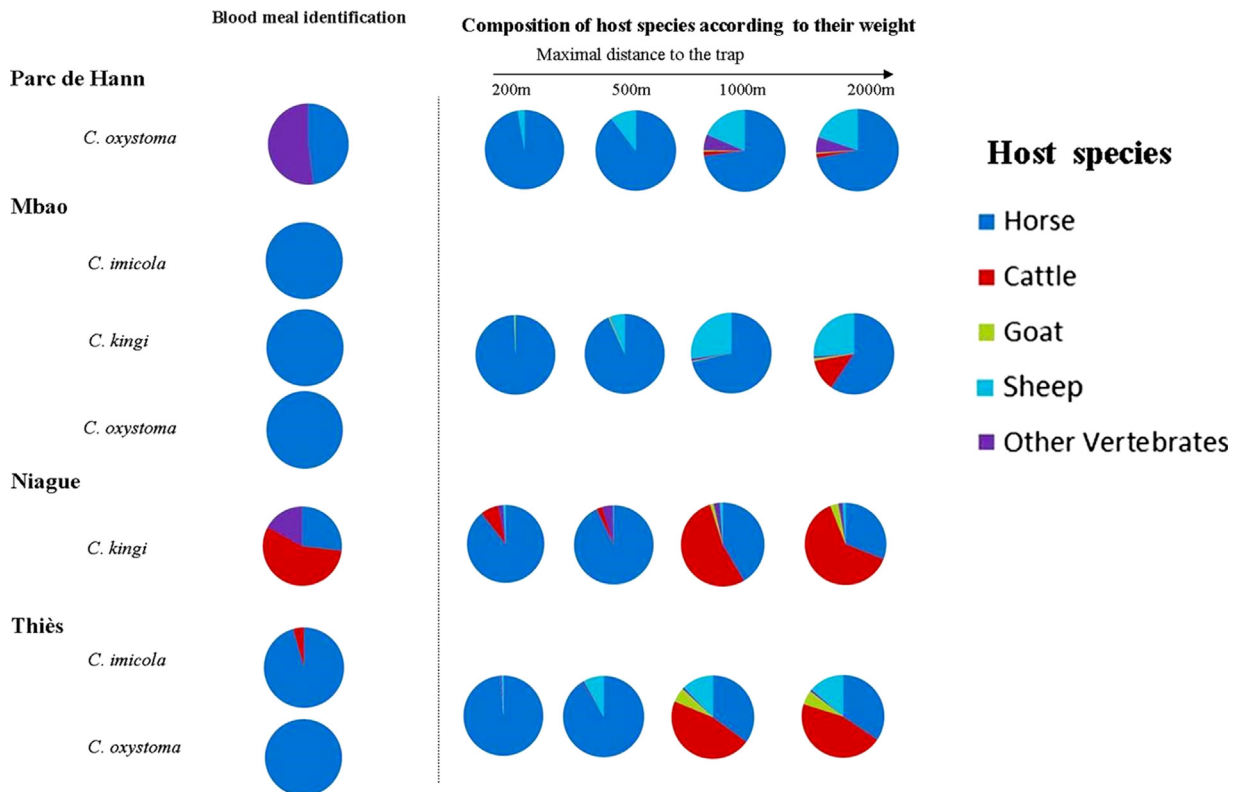


Fig. 2. Identified blood meals and composition of host species corrected by their weight at different distances from the trapping sites.

the presence of a zoo located at 800 m from the trap position. *C. oxystoma* showed a wide foraging range.

Considering *C. imicola*, horse was the favorite host in this study and this species mainly fed in the vicinity of the trapping sites in Thiès. Previous studies showed *C. imicola* is attracted by horses (Fall et al., 2015a, 2015c; Meiswinkel et al., 2004; Nevill and Anderson, 1972). Our results demonstrated a strong host preference for horses (96%) but *C. imicola* is also able to feed on cattle at nearly 1 km. This observation illustrates that an engorged female of *C. imicola* can be up to 1 km after a blood meal whether by active dispersal.

Our results show that *C. kingi* presents an opportunistic feeding behavior with a diverse range of vertebrate hosts and the best correlation with host availability was observed at 2000 m in the trapping site of Niague. This population of *C. kingi* seemed to be attracted by cattle in this site, as it was also the case in another study performed in Sudan using cow-baited traps (El Sinnary et al., 1985).

In Niague, cattle were more abundant after 1000 m than around the trapping site. The relative abundance of cattle after 1000 m was more correlated with the blood meals composition of *C. kingi* leading to suspect an active dispersal of *C. kingi* within a distance of 1000–2000 m. This foraging behavior leading to flight around 2000 m may imply important cost benefit trade-off in the bio-

ecology of this species. However, *C. kingi* could also have fed on cattle within the ring of 200 m from of the trapping site thanks to a strong specific selection of this species.

Generally, the active dispersion of *Culicoides* specimens is assumed to be short, average distance of more or less than 2 km from their breeding sites, as for *Culicoides obsoletus* (Kluiters et al., 2015) and *Culicoides pulicaris* (Kirkeby et al., 2013) in the Palearctic region. This has never been investigated for Afrotropical species. Beside the active dispersion, the movement of *Culicoides* can be aided by the wind. This passive dispersion could be several kilometers and plays an important role in the spatial dynamics of epidemics of *Culicoides*-borne diseases essentially the bluetongue (Braverman and Chechik, 1996; Eagles et al., 2014; Sellers et al., 1979).

The originality of our study relies on the use of a new method in medical and veterinary entomology applied to insect vectors allowing studying the foraging range of *Culicoides* species. The foraging behavior of the studied species varied with host availability and their foraging. It was found that *C. oxystoma* and *C. imicola* mainly fed on horse unlike *C. kingi* which appear to favor cattle but can also feed on horses in the absence of cattle. None of these species fed on goats and sheep although some of these hosts were available near trapping sites. These behavioral results reinforce the impor-

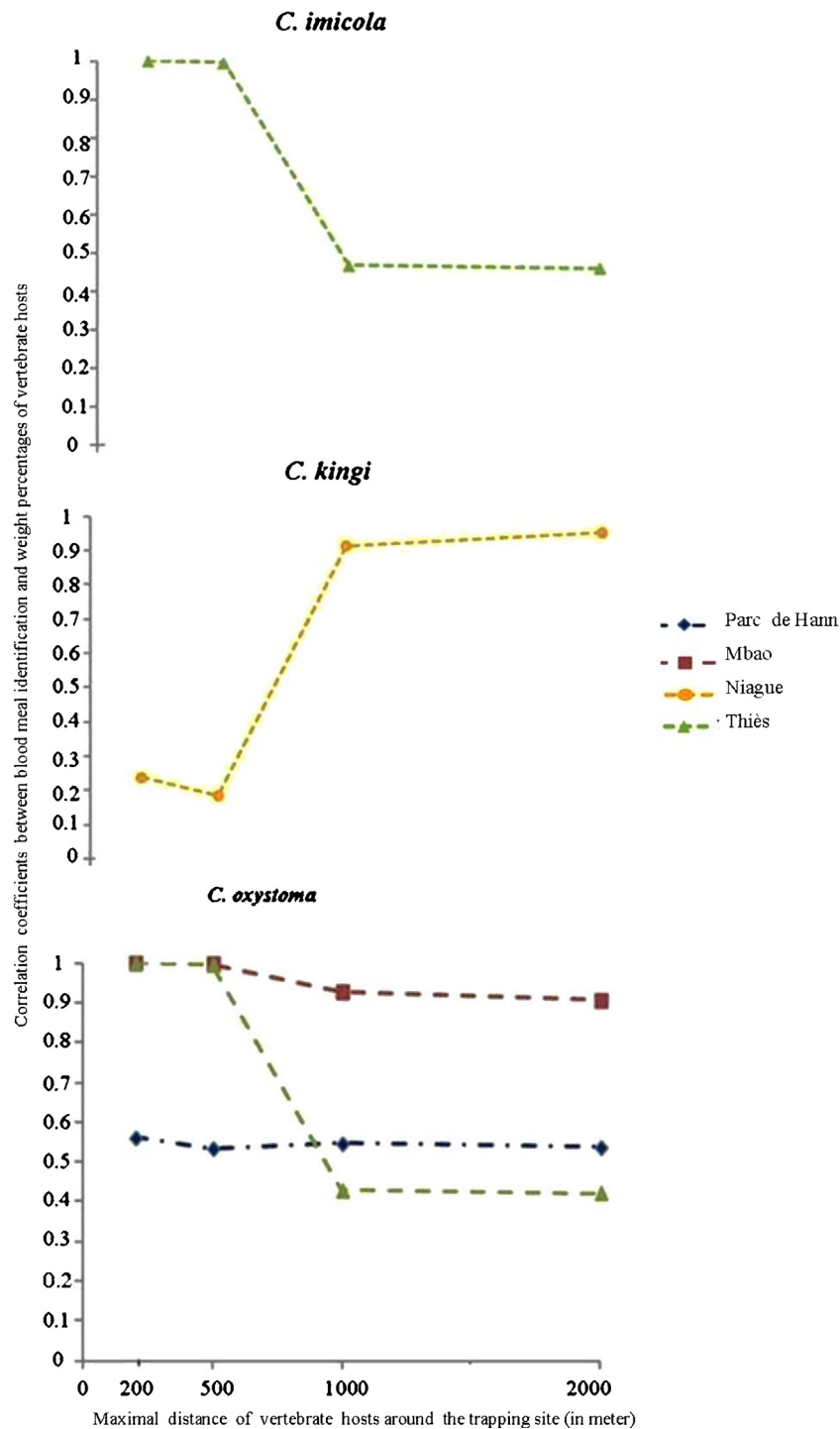


Fig. 3. Correlation between host selection by *Culicoides* species and vertebrate host availability.

tance of these species as putative vector species for AHSV in the Niayes region although their formal vector competence has still not been investigated. It would be interesting to replicate this study in controlled conditions in at different times to evaluate seasonal variations of the foraging range and in sites where horses are scarce compared to other potential hosts to evaluate the relative importance of innate preferences for these *Culicoides* species. This will improve our understanding and prevention of the transmission of *Culicoides*-borne viruses.

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Author contributions

Conceived and designed the experiments: MTB CG AGF MTS JB. Contributed the experiments: MTB CG MF LG IM MD. Analyzed the data: MTB CG TB JB. Wrote the first draft of the manuscript: MTB CG MF LG AGF TB MTS GG JB. All authors revised and approved the final version of the manuscript.

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