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SHORT COMMUNICATION

The mosquito *Aedes (Aedimorphus) vexans arabiensis* as a probable vector bridging the West Nile virus between birds and horses in Barkedji (Ferlo, Senegal)

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Abstract. Active catches of adult females of *Aedes vexans arabiensis* Patton, (Diptera: Culicidae) Patton by nets or aspirator, were conducted in 2003 and 2004 in the vegetation at the edge of temporary ponds in Barkedji, Senegalese Ferlo area. Two hundred and forty-one engorged females were captured, dissected and the gut content adsorbed on a Whatman filter paper and analysed using the enzyme-linked immunosorbent assay (ELISA) technique to determinate the bloodmeal origin. Results indicated that *Ae v. arabiensis* fed primarily on mammals, including horses (35.7% of the bloodmeals), but also on birds (10%). Moreover, associations between horses and birds accounted for 42% of the mixed bloodmeals. These results show an opportunistic feeding behaviour and suggest that *Ae v. arabiensis* is a probable vector bridging the West Nile virus between horses and birds hosts in the Ferlo area.

Key words. *Aedes vexans arabiensis*, Barkedji, birds, blood meal, mammals, vector, West Nile, Ferlo, Senegal.

Introduction

West Nile fever is a wide-spread viral zoonosis that affects humans, birds, domestic and wild mammals. It is mainly transmitted by mosquitoes from the *Culex* genus (Komar, 2003). Birds are the main biological amplifying hosts and are strongly incriminated in the dissemination of the virus through their migrations, for example in Romania (Savage *et al.*, 1999). Horses and humans can be infected but are considered as epidemiological impasses (Hubalek & Halouzka, 1999) because they do not often develop a viremia sufficient to perpetuate the virus transmission cycle. However, fatal febrile infections have been observed in humans (Petersen & Marfin, 2002).

In sub-Saharan Africa, notably in Senegal, West Nile fever is endemic in horses on which it has been found during serological investigation, with prevalence rates of 78.3 and 92%, respectively, by Chevalier *et al.* (2006) and Cabre *et al.* (2005). In addition, a serological West Nile investigation conducted in the Ferlo area showed a global prevalence rate of 5.5% on migratory and resident birds (Chevalier *et al.*, 2009).

Aedes vexans, represented here by the *arabiensis* Patton Afrotropical subspecies, is very closely related to the Palaearctic *Aedes vexans* Meigan commonly found in Europe, Asia, America and the Pacific (Edwards, 1941; White, 1975).

In spite of the fact that mosquitoes of the *Culex* genus are generally considered as the main vectors of West Nile (Hubalek & Halouzka, 1999; Komar, 2003), *Aedes v. arabiensis* could

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play the role of a secondary vector in the epidemiological cycles of the West Nile fever because of the following observations: (a) wild *Aedes v. arabiensis* were often found infected by the West Nile virus in Senegal (Digoutte, 1995; Fontenille *et al*., 1998), and in the U.S.A. (for *Aedes vexans*) (Molaei & Andreadis, 2006); (b) *Aedes v. arabiensis*, which is one of the most abundant mosquitoes in the Ferlo area (Fontenille *et al.*, 1998; Mondet *et al.*, 2005), is well adapted to local conditions with a high survival rate and a high longevity in the field (Ba *et al*., 2005) and showed a feeding tropism for both horses and birds (Fontenille *et al.*, 1995, 1998; Ba *et al.*, 2006); (c) although it has not been assessed for the *arabiensis* subspecies, the vector competence of *Aedes vexans* for the West Nile virus was experimentally demonstrated (Turell *et al*., 2005; Tiawsirisup *et al.*, 2008).

Furthermore, a recent study of the host-feeding pattern of *Aedes v. arabiensis* in the same area clearly indicated its epidemiological importance in the transmission of Rift valley fever (Ba *et al*., 2006) without making it clear for the West Nile fever. The knowledge of the host-feeding patterns of vector species constitutes a key element in the assessment of their epidemiological importance in a given environment. The present study on the host-feeding pattern of *Aedes v. arabiensis* is therefore a contribution to the assessment of this importance in the transmission of West Nile around temporary pounds in the Ferlo area of Senegal.

Materials and methods

Study area

Barkedji (15°16′78″N, 14°52′07″W) is located in the Ferlo area, department of Linguere, region of Louga. The climate is Sahelian, marked by two seasons: the rainy season from June–July to October and the dry season from November to May–June, according to the year. The mean annual rainfalls vary between 300 and 400 mm (Fig. 1) and permanent rivers are absent. Only fossil valleys are found, relics of old rivers that flowed through the region in the past. The Barkedji area is strewed with clay depressions forming temporary ponds which duration depends on the annual rainfalls, evapotranspiration and infiltration (Diop, 2004). Ngao (15°14′27″N, 14°51′08″W) and Kangaledji (15°16′17″N, 14°50′35″W) are two temporary ponds located in the Barkedji rural community near settlements with the same name, and served as sampling sites (Fig. 2).

Bloodmeal analysis

Resting mosquitoes were captured in the vegetation bordering ponds by aspiration or by using an insect net in 2003 (July, August and October) and 2004 (July and August 2004).

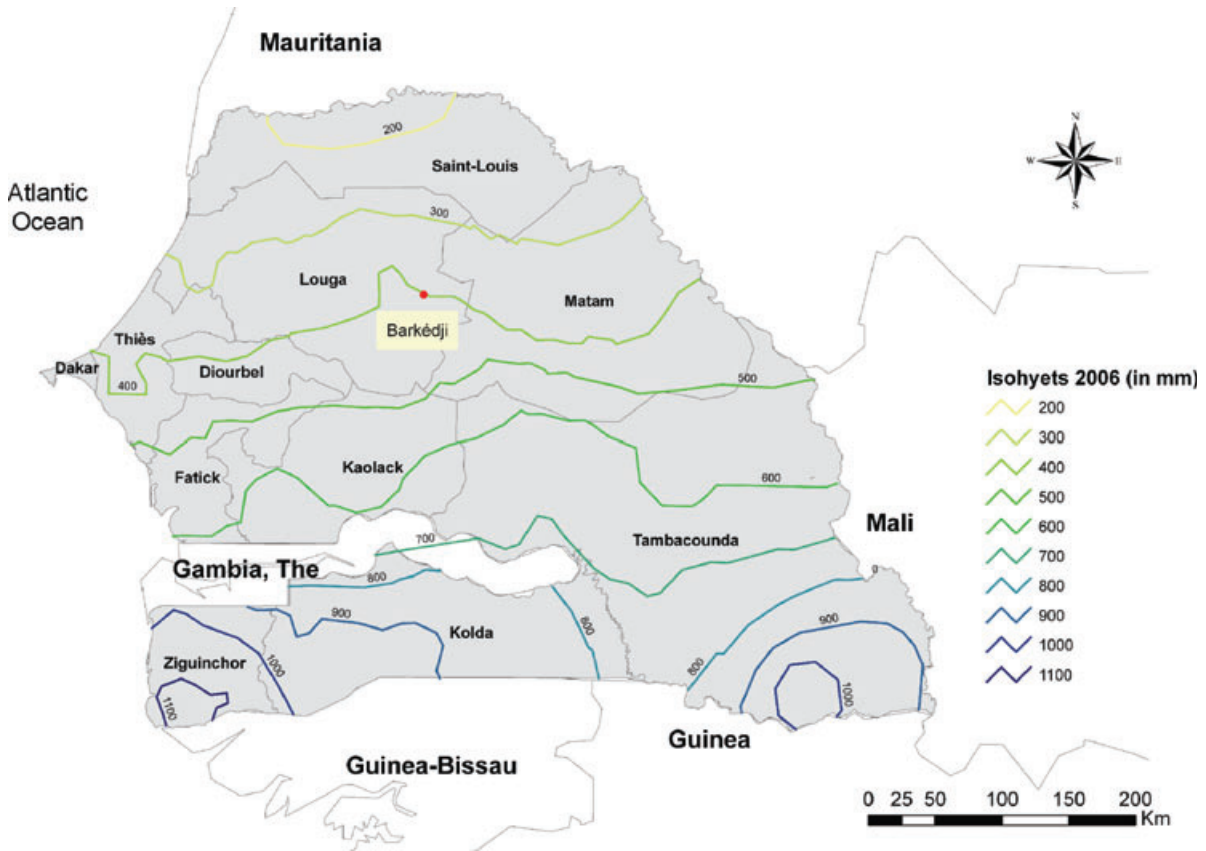

Fig. 1. Map of Senegal with the isohyets for the year 2006 and the location of the Barkedji area.

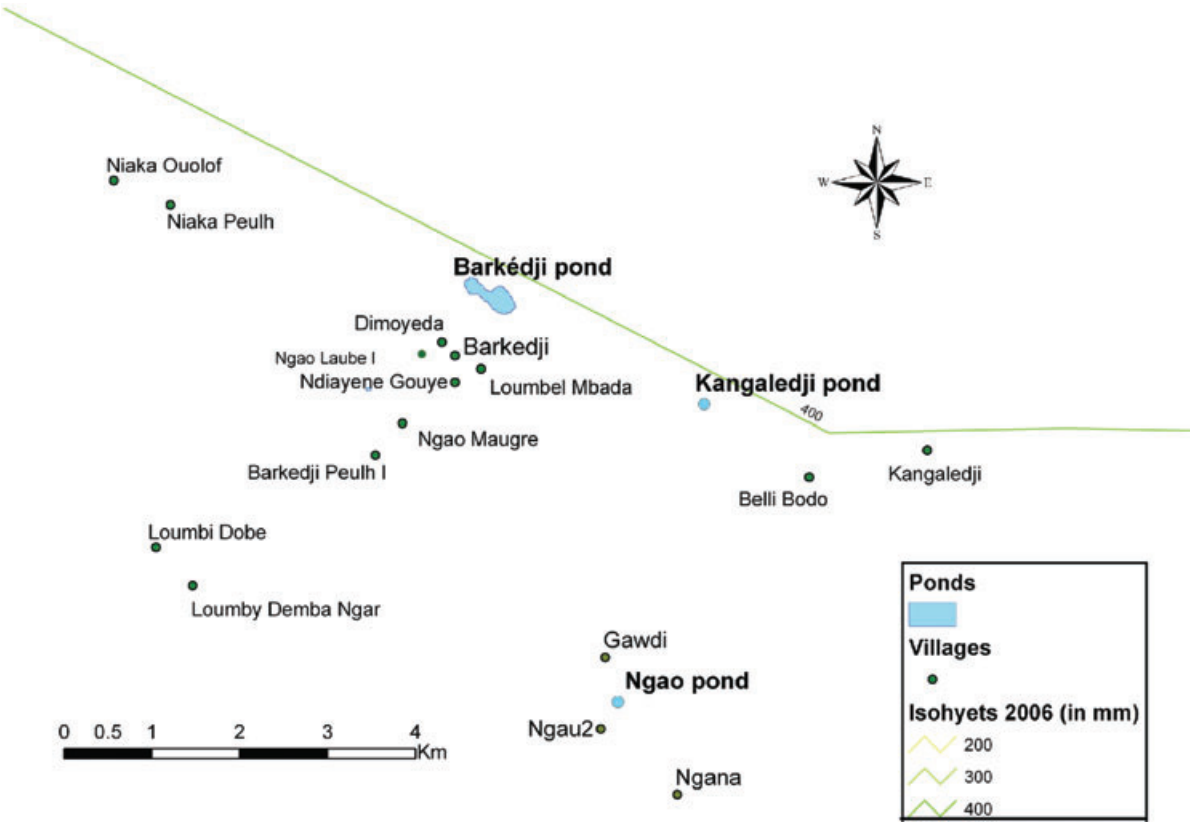

Fig. 2. Map of Barkedji with the location of the sampling sites.

Engorged *Ae. v. arabiensis* females were dissected, the gut content stained on filter paper and stored at −20 °C. The samples were analysed with the enzyme-linked immunosorbent assay (ELISA) protocol developed by Beier *et al*. (1988) and modified by Fontenille (unpublished data). The choice of antibodies was done taking into account the potential hosts of *Aedes v. arabiensis* which frequent the ponds and the neighbouring settlements, such as humans and domestic animals. The following peroxidase antibodies obtained from the Sigma-Aldrich laboratories (Steinheim, Germany) were thus used: anti-human IgG, anti-sheep IgG, anti-goat IgG, anti-bovine IgG, anti-chicken IgG, anti-dog IgG and anti-horse IgG. Positive controls (blood spots of 5 μL placed on filter paper) were collected from animals in a slaughterhouse in Dakar, in a veterinary clinic and in the Laboratoire National de l'Elevage et de Recherches Vétérinaires. Chicken serum was used as a negative control for mammals and bovine serum as a negative control for the chicken. Cross dilutions were performed in preliminary tests to determinate the optimal dilutions for bloodmeals and antibodies. The spots of collected blood were cut out as small discs (≈5 mm in diameter). Each disc was put in a 1.4-mL U-bottom tube range (Micronic Systems, Lelystad, the Netherlands) and blood from the filter paper was eluted with 1 mL of phosphate-buffered saline (PBS), pH 7.4, and left for 1 h at room temperature (25 °C). Each tube was then homogenized and the disc of filter paper was stirred inside and removed. Except the first column (Blanks), 50 μL of the positive control, the negative control and bloodmeals solutions were distributed in two contiguous well of the 96F MaxiSorp nunc immuno plates (ThermoFisher Scientific, Roskilde, Denmark) which were covered and incubated at 4 °C overnight. The next morning each well was washed twice with 300 μL of PBS, pH 7.4, containing 0.05% Tween 20. Then, 50 μL of a mixed solution of peroxidase host-specific conjugate (at indicated dilution), dilutions of heterologous sera (1/1000) (except between sheep and goat) and bloodmeal buffer solution were added to each well except in the blank column. For 0.5 L of blood meal buffer, 2.5 g of casein was added in 50 mL of 0.1N NaOH (boiled until dissolution) + 450 mL of PBS 0.05% Tween 20 + 0.05 g of Thiomerasal + 0.01 g of red Phenol. After 1-h incubation at room temperature, wells were emptied and washed four times with PBS 0.05% Tween 20. Equal volumes of TMB (3,3',5,5'-tetramethylbenzidine) peroxidase substrate (Kirkegaard & Perry Laboratories, Maryland) and peroxidase solution B (H_2O_2) (Kirkegaard & Perry Laboratories, Maryland) were mixed and added to each well (50 μL) of all the plates. Then the plate was covered and incubated in the dark for 30 mn at room temperature. Positive wells will be coloured in blue. The addition of 50 μL 4N H_2SO_4 showed a yellow colour in positive wells. Absorbance was read at 450 nm with the ELISA reader Multiskan Ascent (Thermo Labsystems, Helsinki, Finland). Samples with absorbance values upon the mean of negative controls + 2.5 times the standard deviation (SD) were considered positive. However, in such tests, sheep

and goat antibodies are known to cross-react. As a precaution the results of bloodmeals taken from goat and sheep were pooled as bloodmeals taken from small ruminant.

The percentage of unmixed bloodmeals on each host in each site was calculated as the ratio of the number of bloodmeals taken on that host on the total number of samples, including mixed meals. The percentage of mixed bloodmeals in each site was calculated as the ratio of the number of mixed meals on the total number of samples.

Results

The origin of the bloodmeal was successfully determined for 97% of the samples (241 blood meals). The 3% remaining could not be determined with the antibodies listed upon. From the 234 identified bloodmeals, 196 (83.8%) were simple meals. Only four (2%) were taken on humans, giving a high zoophilic rate of 98% (192 bloodmeals). Overall, the determined animal hosts were horses (35.7% of the bloodmeals), cattle (16.6%), small ruminants (17.0%), birds (10.0%) and dogs (0.4%) (Table 1).

In total, 38 bloodmeals (15.7% of the identified samples) were from a mixed origin and came from two, three and four different hosts in 32 (84.2%), 5 (13.2%) and 1 (2.6%) cases, respectively. Mixed meals involving birds and horses were the most frequent ones, with 16 (42.1%) multiple meals, whereas only 6 (15.8%) bloodmeals associating birds with other mammals (small ruminant, bovine and humans) were observed (Table 2).

Discussion

In the present study, approximately 1 bloodmeal over 10 identified was taken from birds, 1 over 3 from horses, 2 mixed bloodmeals over 5 associated horses and birds and 1 over 7 associated birds and other mammals including humans.

These results are similar to those of Fontenille *et al*. (1998) who found 6.2 and 33.6% of *Ae v. arabiensis* females fed on chicken and horse, respectively, in Barkedji. By contrast to these observations, Ba *et al.* (2006) found that only 0.3% of *Aedes v. arabiensis* fed on chicken and almost the same percentage as the previous studies on horses (33.7%) in the Niakha temporary pound, which is close to the study area. This shows that the feeding behaviour of *Ae. v. arabiensis*, and thus its importance as a bridging vector between horses and birds, may be heterogeneous in space and time, according to the hosts' relative availability and given the opportunistic feeding behaviour of this species. Indeed, in the ecosystem of Barkedji, the arrival of migratory birds is closely related to the filling of temporary ponds at the beginning of the rainy season. Moreover, regular rainfalls would limit the vectorial role of *Ae v. arabiensis* to the first half of the rainy season, corresponding to its peak of abundance; *Culex* mosquitoes would then take over during the remaining part of the rainy season. On the other hand, a season with irregular rainfalls would promote several peaks of abundance of *Ae v. arabiensis* (Mondet *et al.*, 2005), extending its involvement in the transmission of WNV, together with *Culex* mosquitoes. Hence, the weak prevalence rates obtained by Chevalier *et al.* (2009) could result from the seasonality of transmission which occurs in this area.

The high frequency and heterogeneity of mixed bloodmeals, especially in early winter (from late July to early August 2004), could be related to the scarcity of hosts. Indeed, the beginning of the rainy season in Barkedji is characterized by a low population of domestic hosts, whose return from transhumance is closely related to the establishment of the rainy season. Thus, the few animals available are overexposed to the bites of a high population density of *Ae. v. arabiensis*, fastly growing thanks to stocks of eggs from the previous rainy season. The self-defence reflexes developed by such animals may even

Table 2. Bloodmeals from mixed origins: hosts associated with *Ae. vexans arabiensis* females caught at the edges of the Ngao and Kangaledji temporary water ponds.

	Collection site		
Mixed bloodmeals	Ngao Nb (%)	Kangaledji Nb (%)	Total Nb (%)
Small ruminant–bovine	5 (50.0)	2 (7.1)	7 (18.4)
Small ruminant–horse	0 (0.0)	1 (3.6)	1 (2.6)
Human–horse	1 (10)	4 (14.3)	5 (13.2)
Birds–bovine	3 (30)	1 (3.6)	4 (10.5)
Birds–horse	1 (10)	14 (50.0)	15 (39.5)
Dog–human–horse	0 (0.0)	2 (7.1)	2 (5.3)
Small ruminant–bovine–horse	0 (0.0)	2 (7.1)	2 (5.3)
Small ruminant–birds–bovine	0 (0.0)	1 (3.6)	1 (2.6)
Human–birds–horse–small ruminant	0 (0.0)	1 (3.6)	1 (2.6)
Total	10	28	38

Table 1. Origin of bloodmeals collected from resting *Aedes vexans arabiensis* females caught on the edges of the Kangaledji and Ngao temporary water ponds.

		Vertebrate hosts (%)							
Location	Total number of samples	Horse	Bovine	Small ruminant	Birds	Human	Dog	Mixed bloodmeals (%)	Not identified blood meals (%)
Ngao	140	44 (31.4)	30 (21.4)	30 (21.4)	14 (10.0)	4 (3.0)	1 (0.7)	10 (7.1)	7 (5.0)
Kangaledji	101	42 (41.6)	10 (9.9)	11 (10.9)	10 (9.9)	0 (0.0)	0 (0.0)	28 (27.7)	0 (0)
Total	241	86 (35.7)	40 (16.6)	41 (17.0)	24 (10.0)	4 (1.7)	1 (0.4)	38 (15.7)	7 (2.9)

increase the rate of interrupted bloodmeals, favouring shifts between host species. This further illustrates the opportunism of *Aedes v. arabiensis.* The high rate of mixed meals also suggest that host-fidelity is not likely to occur in this species, unlike what is observed in other vectors (Bouyer *et al.*, 2007), or that its impact is lowered by starvation.

The endemicity of the West Nile virus in a given location is perpetuated through a cycle between wild birds and mosquitoes mainly from the *Culex* genus. Serological studies conducted in the Ferlo area of Senegal and the Senegal River valley identified 13 bird species, including 5 migratory species, as potential reservoirs of the West Nile virus (Chevalier *et al.*, 2009). In such a cycle, mosquitoes that predominantly ornithophile will, therefore, be the most efficient in maintaining the virus. This is the case of *Culex (Culex) pipiens* Linnaeus in the U.S.A. (Hamer *et al*., 2009). By contrast, opportunistic mosquitoes such as *Aedes v. arabiensis*, which feed on a wide range of hosts (especially mammals and birds), may be less effective in maintaining the West Nile virus between birds but could be good bridging vectors by feeding on viremic birds and subsequently on susceptible hosts such as humans and horses (Molaei & Andreadis, 2006; Hamer *et al.*, 2009).

In the context of the Ferlo, if *Aedes vexans arabiensis* is as competent as *Ae vexans* in the transmission of the WN virus (Turell *et al*., 2005; Tiawsirisup *et al*., 2008), our observation (*Ae v. arabiensis* feed both on birds and horses) combined with numerous West Nile virus isolations (Digoutte, 1995; Fontenille *et al*., 1998), the high population densities of this mosquito in the area (Mondet *et al*., 2005) and its good adaptation to local conditions (Ba *et al*., 2005), strongly support the hypothesis that *Aedes v. arabiensis* is a bridge vector for the West Nile virus in Barkedji.

Finally, without excluding possible cross reactions the specific antibodies used showed a good specificity. However, the use of more specific tests such as cytochrome B heteroduplex analysis (Boakye *et al*., 1999) would be interesting to confirm our results. Further studies are also needed to assess the vector competence of *Aedes v. arabiensis* for WNV to confirm its role in the epidemiology of West Nile fever in the Ferlo area. Actually, WNV isolation from this species does not necessarily imply that it is a competent WNV vector but sometimes simply that it fed on viremic hosts.

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