

# Ethological Risk Factors of West Nile Infection of Wild Birds in Senegal

Chevalier, V.<sup>1</sup>, Reynaud, P.<sup>2</sup>, Lefrançois, T.<sup>1</sup>, Lancelot, R.<sup>1</sup>, Baillon, F.<sup>2</sup>, Gaidet, N.<sup>1</sup>  
Balança, G.<sup>1</sup> and Sylla, S.<sup>3</sup>

<sup>1</sup> Centre International de Recherche Agronomique pour le Développement (CIRAD), Montpellier, France ; <sup>2</sup> Institut de Recherche pour le Développement (IRD), Dakar, Sénégal ; <sup>3</sup> Direction des Parcs Nationaux Dakar, Sénégal

## Abstract

Serological studies performed in 2003, as well as viral isolations demonstrated that West Nile fever (WNF) was endemic in Senegal (West Africa). Large populations of potentially infected birds fly each year from West Africa to Europe. The goal of this study was to identify potential reservoirs of West Nile virus among Senegalese birds and ethological features related to WN infection.

In October 2003, wild birds were trapped and sampled in two different locations: the Ornithological Djoudj National Park, located in the Senegal River Basin and in a village located in the Ferlo area (northern Senegal). Sera were analysed using an inhibition immunocapture enzyme-linked immunosorbent assay. Serological data were analysed using a generalized linear Poisson model. Seven ecological features that may influence the exposure of birds to mosquito bites were included as explanatory variables: the trapping location, the migrating status, the feeding site, the resting site, the nesting type, the herd instinct level, and affinity with urban areas. A total of 422 birds representing 49 species were trapped and sampled. The overall prevalence rate was 5.5%. Resident birds building platform or cup nests, as well as birds feeding on soil or having low urban areas affinity were more at risk than others ( $p=0.01$ ,  $p=0.02$  and  $p=0.02$  respectively). Potential consequences of these results on virus dissemination and WN reservoir birds are discussed.

## Introduction

West Nile Fever (WNF) is an emerging arthropod borne zoonosis caused by the WNF virus (WNFV) (*Flavivirus*, *Flaviviridae*). The basic transmission cycle involves wild and domestic birds as main hosts and mosquitoes mainly from the *Culex* genus as vectors. This basic cycle may be amplified when ecological conditions are adequate. At this occasion, humans or horses may be infected. Human and equine disease is often asymptomatic or limited to a non specific mild febrile signs. However, severe clinical forms, such as meningitis or encephalitis may occur and lead to death.

During the last decades, several outbreaks were reported in Europe, USA and Northern Africa. As it was probably the case in Camargue (France, 2000) (Durand, Chevalier et al. 2002), the dissemination of the virus and its introduction in previously free areas have often been attributed to the migration of infected birds (Rappole, Derrickson et al. 2000). Identifying reservoir migrating birds could help foreseeing the locations and periods of future outbreaks. In Africa, the epidemiological situation has not been well documented. However, serological studies performed in 2003 demonstrated that West Nile fever was endemic in the Ferlo area (northern Senegal, West Africa) (Chevalier et al, in press). In addition, several WNV isolations suggested that the disease may be endemic in the Senegal river basin (Traore-Lamizana, Zeller et al. 1994). The goal of this study was to identify potential reservoirs of West Nile virus among Senegalese birds and ethological features related to WN infection.

## Methods

Two study sites were selected based on previous serological studies and ornithological knowledge. The first site was located in the close vicinity of the Barkedji village (14° 52' W, 15° 16' N), in the Ferlo area (northern Senegal). This typically sahelian area is characterized by (i) a succession of dry and rainy season; (ii) a temporary pond system. During the rainy season, temporary ponds are flooded and

constitute a favourable biotope for *Aedes vexans* and *Culex poicilipes* which both may transmit the disease. In addition, many species of endemic and migratory birds are attracted by the water and abundant food, and nest around these ponds. In 2003, more than 350 sedentary or migrating bird species were identified in Barkedji (Reynaud, unpublished data).

The second site was in the Djoud'j National Park (PNOD). The Djoud'j National Park is a 16,000-ha wetland located at the border between Mauritania and Senegal, in the Senegal-river delta. It is made of a large lake surrounded by streams, ponds and backwaters. Land cover is mainly composed of acacia swamps, muddy areas, reed bed, and rice fields. This ecosystem is one of the main pathways of migratory birds from Europe to Africa.

Birds were trapped from 28th September to 7th October 2003 (Barkedji) and from 12th to 21st November 2003 (Djoud'j). Ground-level mist nets were used. Nets were operated from sunrise to 11 a.m. and from 17 p.m. to sunset. Birds were sampled from the wing or jugular vein. Blood samples were centrifuged and sera were stored at 4°C until they were transported to Dakar. Sera were analysed using an inhibition immunocapture enzyme-linked immunosorbent assay (Blitvich, Marlenee et al. 2003).

Serological data were analysed using a generalized linear Poisson model. The prevalence (positive or negative) was the response, aggregated at the species level. The logarithm of the sample size of each species was used as an offset in the model.

Seven ecological features that may influence the exposure of birds to mosquito bites were included as explanatory variables: the trapping location (Barkedji, Djoud'j), the migrating status (resident, migratory), the feeding site (soil, half-height, flying), the resting site (soil, bush, canopy), the nesting type (ground nest, platform, cup or cavity nest –so called medium-, bulky nest), the herd instinct level (high, moderate, small), affinity with urban areas (high, moderate, small). The biological signification of the nesting type was different with regards to resident or migrating birds. Thus, the nesting variable was nested in the status variable. The most plausible models were set up according to the opinion of ornithologists. There were fitted and compared with the Akaike information criterion (Burnham and Anderson 2002). Because the number of parameters in the models was high compared

$$AIC_c = AIC + \frac{2k(k+1)}{n-k-1}$$

with the number of observations, a small-sample correction of AIC was used:

where n was the number of observations and k was the number of parameters in the model.

## Results

A total of 422 sera were analysed, representing 49 species. The most highly represented families were *Muscicapidae* (15.8%) and *Colombidae* (12%). A total of 170 birds were trapped in Barkedji, 252 in the PNOD. Most birds trapped in Barkedji were resident (89.4%). Conversely, the majority of birds trapped in the PNOD were migrating (79.8%). Anti-WNFV antibodies were detected in 13 species, among which five were migrating: *Lanius senator*, *Anthus trivialis*, *Hippolais pallida*, *Jynx torquilla* and *Cercotrichas galactotes*. The overall observed prevalence rate was 5.5% (n = 422). The best-selected model was additive with nesting type, feeding site, migrating status and urban area affinity as the explanatory variables. There was no difference between the prevalence rate of birds trapped in the Djoud'j (4.8%) and in Barkedji (6.5%). Resident birds building platform or cup nests, as well as birds feeding on soil or having low urban areas affinity were more at risk than others (p= 0.01, p=0.02 and p =0.02 respectively).

## Discussion

The overall prevalence rate in resident birds (8.3%) confirmed that WN disease was transmitted to wild birds in Senegal either in the Ferlo or in the Senegal River basin. This rate was smaller than that were found in other infected countries. In 1950, Taylor et al sampled 420 resident birds in an endemic area of the Nile Delta (Egypt). The seroprevalence rate was 40%, ranging from 13% in domestic chickens (n=15) to 65% in hooded crows (n=163) (Taylor, Work et al. 1956). The seroprevalence rate was 20.6% on resident birds in Israel in 1996-1997, with 29% in White wagtail (*Motacilla alba*) (n=234) (Malkinson and Banet 2002). Nevertheless, comparison to other serological studies may not be relevant since all species were not equally represented. Birds such as shorebirds,

which were probably highly exposed to mosquito bites given their habitat, could not be trapped. The prevalence rates may have been underestimated.

The age of birds could not be estimated during the sampling. The average survival time of each bird species was provided by expert opinion. However, data were conflicting. In addition, given the sampling period, a high proportion of migrating birds were probably very young. We assumed that the variable age would have been irrelevant. It was discarded from the analyses.

Anti-WN antibodies were found from 5 migrating species. These species may act as reservoir of WNFV virus. However positive serological status of migrating birds only provides assumption about their potential involvement in the virus dissemination. There is little information concerning the immunological response of wild birds to WNFV infection. Further ecological and virological knowledge, such as viremia titre, viremia duration or migration duration, are needed to assess the risk of introduction of the virus in Europe by these birds. Moreover, WNFV circulation depends on the spatial and temporal conjunction of at least two components: high density of competent and ornithophilic vectors, and high density of susceptible birds. Habitat is determinant either for birds or mosquitoes. In order to build a risky map of WN outbreaks, it would be relevant to identify ecological factors linked to bird infection and habitats favourable both to potential reservoir birds and vectors of WN.

Resident birds nesting in medium type nest were more at risk than birds nesting in bulky nests. The structure of bulky nests is much more complex and compact than the ground nests or medium ones. Access to outside is rather reduced. Thus, the exposition to mosquito bite of birds building this kind of nest is probably weak. Nesting type as a risk factor of WN infection will have to be considered in future researches as fledglings may be important reservoirs: their limited mobility and their exposed, unfeathered skin presumably make them easy prey for mosquito vectors [11]. Statistical analyses did not provide evidence that sociability was a risk factor. This may be explained by small sampling size. This factor will have to be taken into account as bird density is probably very attractive for mosquitoes, and social birds more exposed than others (Marra, Griffing et al. 2004).

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