

Seroprevalence of West Nile virus antibodies in equids in the North-East of Algeria and detection of virus circulation in 2014

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ABSTRACT

West Nile fever (WNF) is a viral disease of wild birds transmitted by mosquitoes. Humans and equids can also be affected and suffer from meningoencephalitis. In Algeria, since the 1994 epidemic, no data on WNV circulation was available until 2012. In September 2012, a fatal human case of WNV neuro-invasive infection occurred in Jijel province. This study describes the first seroprevalence study of West Nile virus (WNV) antibodies conducted in the equine population in Algeria. During 2014, serum samples were collected from 293 equids (222 donkeys and 71 horses) asymptomatic and unvaccinated for WNV in three localities in Northeastern wetlands of Algeria. Antibodies against WNV were found in 51 samples (seroprevalence 17.4%) of sampled equids, distributed as follows: 19 (seroprevalence 26.8%) horses and 32 (seroprevalence 14.4%) donkeys. Moreover 7 horses coming from Blida, in the center of Algeria, were tested before and after an 8-months stay in North-East Algeria. We observe a seroconversion in 2 horses, showing WNV circulation in 2014 in this specific region of Algeria.

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1. Introduction

West Nile virus fever (WNVF) is a mosquito-borne virus and belonging to the genus *Flavivirus*, *Flaviviridae* family. WNF is a mosquito-borne viral infection transmitted in natural cycles between birds and mosquitoes, particularly *Culex* spp. Consequently, WNV transmission is sensitive to environmental conditions: it is strongly associated with the presence of wetlands and the occurrence of rainfall and flooding, as well as the abundance of avifauna and mosquitoes [1]. Many bird species have been found infected by WNV and migrating birds are involved in its long-distance transmission [2]. In humans, WNV infection is usually an asymptomatic or mild febrile illness. However, in the United States of America, there were more fatalities, and not only in older and

immunocompromized patients [3]. WNV is also a cause of animal disease, especially in equids (horses and donkeys) in which possibly fatal meningoencephalitis cases are observed. Both humans and horses are dead-end hosts for WNV: viremia is low and does not allow the infection of mosquitoes feeding on these hosts [4]. As for humans, most horses seroconvert without clinical disease after exposure to WNV. Nonetheless, severe WNV disease with neurological symptoms develops in approximately 8% of exposed naive horses [4]. WNV transmission has been confirmed during the last years in Europe and in the Mediterranean Basin, due to the virus' repeated introduction by infected migratory birds and/or to an endemic circulation within sedentary bird populations [5]. Sporadic cases and outbreaks of WNF in humans and equids have been reported after WNV discovery in 1937 in the West Nile Province of Uganda. In the late 1990s, outbreaks were increasingly reported in Europe: Romania (1996), Russia (1999) and the Mediterranean basin (1994, 1997, 1998–2000), with hundreds of human cases [6]. In North Africa (Maghreb), WNV circulation has been described in Morocco during equine encephalitis outbreaks in 1996 [7], 2003

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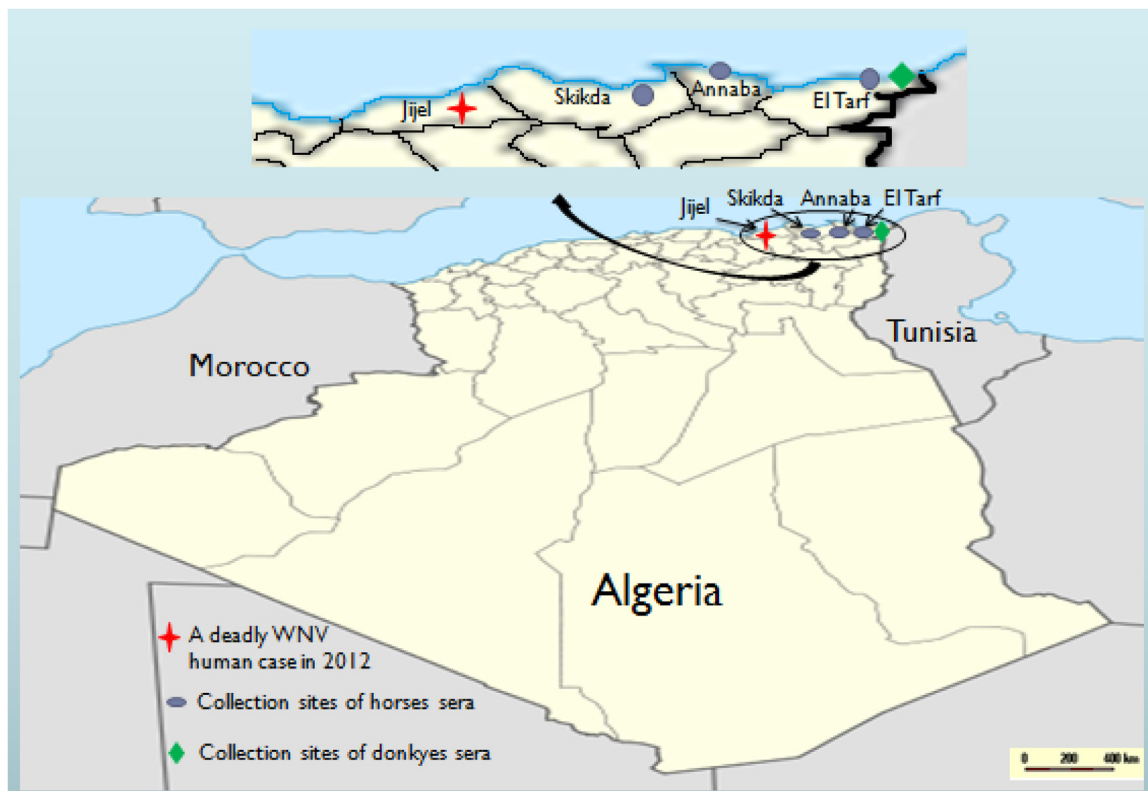


Fig. 1. Location of a deadly WNV human case and collection sites of equids sera in North-East of Algeria.

[8] and 2010 [9]; and in Tunisia with several human WN cases and seroprevalence studies in equid [10]. In Algeria, since the 1994 epidemic [11], no data on WNV circulation was available until 2012. In September 2012, a fatal human case of WNV neuro-invasive infection occurred in Jijel province (Episouth weekly Epi Bull, 2012, Leparc-Goffart personal communication). Our study was conducted to determine the prevalence rate of WNV antibodies in equids from the Northeastern wetlands of Algeria because of the proximity to the town where the human case was observed to determine seroprevalence of equids in these areas.

2. Materials and methods

2.1. Ethic statement

Risk assessment was submitted to and approved by the ethics committee and decision board of the riding clubs of Annaba, Skikda and El Kala's National Reserve. These institutions are affiliated with the Algerian Ministry of Agriculture and Rural Development (Directions des Services Veterinaires).

To facilitate fieldwork, collaborations were established with veterinary doctors and their assistants working in these establishments.

2.2. Sample collection and preparation

The study was performed in 2014, a non-epidemic year on equids that were neither symptomatic nor vaccinated against WNV. During 2014 (from 29 January 2014 through 6 November 2014), blood samples were collected on 71 adult horses in three localities, El Kala (El Tarf locality) (36.9000°N/8.4500°E), Annaba (36.9000°N/7.7667°E) and Skikda (38.8667°N/6.9000°E), and on 222 donkeys all in El Kala's National Reserve (Fig. 1). Sera were centrifuged within the next 24 h after collection, separated, frozen

at -20°C , and sent to the French National Reference Center for Arboviruses (Marseille, France). Each sample was systematically tested for IgG against WNV by using an in-house ELISA with precipitated and inactivated WNV as antigen [12]. The negative antigen was prepared from a supernatant of noninfected cells. Specific binding was demonstrated by using a peroxidase-labeled goat anti-horse IgG conjugate (Jackson) at a dilution of 1:10,000 for horse samples, and peroxidase-labeled goat antidonkey IgG conjugate (ThermoFisher) at a dilution of 1:10,000 for donkey samples. Serum samples were considered as positive if the ratio of the optical density at 450 nm of the sample on WNV antigen and on the negative antigen was over 3. Owing to antigenic cross-reactivity among *Flaviviruses*, all positive samples were confirmed as true positive both by Western Blot test and by Seroneutralization of West Nile virus as described previously [12,13]. The p values and 95% confidence interval were calculated separately for each variable using the Epi Info Software (v5.01; CDC Atlanta). The chi-square test was used to evaluate associations ($\alpha = 5\%$). The differences were considered statistically significant when $p \leq 0.05$. The Fisher exact test was used to test for significance when the chi-square test was not appropriate.

3. Results

Seropositive animals were identified in the three surveyed locations of the Algerian Northeastern wetlands. Table 1 shows the characteristics of the populations of the study and summarizes the results of the serology. The prevalence of WNV infection among equid were detected in the sera of 51 individual of 293 analyzed, giving an overall equids seroprevalence rate of 17.4% with an confidence interval of [CI: 13.4–21.4]. The prevalence of WNV infection among horses were detected in the sera of 19 horses out of the 71 tested, giving an overall horse seroprevalence rate of 26.8% with an confidence interval of [CI: 16.8–36.8], and anti-WNV antibodies were detected in the sera of 32 donkeys out of the 222 tested,

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