Journal of Animal and Veterinary Advances 16 (8-12): 113-118, 2017
ISSN: 1680-5593

Contribution to a Seroprevalence Survey of West Nile Fever on Horses in Northern Algiers "2013/2014"

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Abstract: West Nile Fever (WNF) is a vector zoonosis caused by a flavivirus transmitted by the bite of Culex mosquitoes from birds, resulting on flu-like symptoms and may develop into nervous symptoms like meningitis type and meningoencephalitis, even death of horses as well as human beings. To get an appreciation of health status and in view of the lack of data on prevalence in Algeria of equine and birds WNV (West Nile Virus) infection since 1994, a serological survey was conducted on 209 sera horses tested in ELISA "IgG competititon" resulting from a prevalence of 12% with equestrian clubs, stables and private owners in various areas of Algiers, Boumerdes and Blida. Seropositive horses are present in nearby areas of migration of birds, wetlands and less humid ones in boxes, stall-paddock and even in the presence of means of vector control. However, the parameters sex, age, breed, color are to be reconsidered for future study by a more expanding population with homogeneous proportions. These horses-revealing epidemiological-give evidence of WNV but not to conclude an emerging nature of WNF. Epidemiological measures should be introduced to protect human and animal health; hence other studies should be carried out on domestic and wild birds to assess the prevalence later on.

Key words: West Nile fever, horse, migratory birds, Algiers, ELISA IgG competition, seroprevalence

INTRODUCTION

An ancient disease, presently making headlines, the West Nile fever (WNF) is a re-emerging zoonosis vector. Occupying the front of the scientific and media scene, it is caused by an arthropod-borne virus (arbovirus) of the genus *Flavivirus* of the family Flaviviridae, pathogen of humans, horses and some birds (Colpitts *et al*., 2012).

It was isolated in 1937 at Omogo in the West Nile District in Northern Uganda, at West Nile Albert in a blood of woman suffering from mild febrile illness (Anderson *et al*., 1999; Smithburn *et al*., 1940).

So, this virus has been expanded so much during the last years that it is now considered to be one the most widespread arboviruses in the world (Kramer *et al*., 2008; Brault, 2009).

The epidemiology and ecology of WNV was characterized during the occurrence of many outbreaks of this one in the Mediterranean basin in the early 1950's and 1960's (Murgue *et al*., 2001).

The virus is transmitted to a variety of vertebrate species through the bite of infected mosquitoes, mainly of the *Culex* genus. Birds play a key role in viral amplification while mammals, human beings as well as the horse are accidental hosts-dead-ends to the cycle because of the low level WNV viremia that cannot support infection of mosquito vectors (Kramer *et al*., 2008). *Culex pipiens* pipiens is a major vector and appears to be responsible for the vast majority of virus transmission (Bernard *et al*., 2001).

But these latters can develop the infection in an asymptomatic form, this one can be associated with an influenza-like illness (moderate to high fever, weakness and myalgia). Only infrequently in <1% (Porter *et al*., 2011; Sambri *et al*., 2013) infections in human beings and 10% of infections in horses, to severe West Nile Neuroinvasive Disease (WNND) (Sambri *et al*., 2013; Pradier *et al*., 2012) do acute meningitis, encephalitis or flaccid paralysis develop (reported only in humans). Horses can also develop paralysis of the limbs, facial tremors which is lethal in approximately one third of the cases in horses (Pradier *et al*., 2012).

For few years the WNV has caused several epizooties of meningoencephalitis on human beings and horses in the Mediterranean, Europe, Asia and North America (Rossi *et al*., 2010; Schuffenecker *et al*., 2005) with increasing impact on animal and human health with a geographical distribution of WNV at present in parts of the world, except Antarctica (Beck *et al*., 2013).

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Within that scope, human cases of meningo-encephalitis with fatalities occurred in Algeria in 1994 for 20 cases including 8 deaths (Guenno *et al*., 1996) and in Tunisia in 1997 making 173 cases including 8 deaths, (Triki *et al*., 2001), Russia in 1999 (Platonov *et al*., 2001), in Romania in 1997 and 1998 (Cernescu *et al*., 2000), the Czech Republic in 1997 (Hubalek and Halouzka, 1999) and France in 2003 (Giudice *et al*., 2004). Whereas epizootics in horses werereported in Morocco in 1996 (Harrack *et al*., 1997). In the following years, cases were reported again: in Tunisia in 2003 (Garbouj *et al*., 1997) and 2008 (Hassine *et al*., 2011) and in Morocco in 2003 and 2010 (Schuffenecker *et al*., 2005; WOAH., 2010) indicating that WNV is still circulating in the region in Italy in 1998 (Autorino *et al*., 2002) and France in 2004 (Durand *et al*., 2005) in 2004 Costa Rica (Hobson-Peters *et al*., 2011).

Following an epidemiological survey in autumn 2004 of horses which had not been vaccinated against WNV or traveled outside of Cordoba and Sucre in the Caribbean region of Colombia, the first detection of the WNV in the South America was registered (Berrocal *et al*., 2006; Mattar *et al*., 2011). From 1999-2014, a total of 38 259 human infections with 1654 deaths and more than 20000 equine deaths of WNV disease have been reported in the USA.

An outbreak of WNV occurred in Turkey in 2010-11, causing 47 cases including 40 WNND cases and 10 fatalities (Kalaycioglu *et al*., 2012) and from 2010-2013 in Austria, Bosnia and Herzegovina, Croatia, Greece, Hungary, Italy, Kosovo, the Former Yugoslav Republic of Macedonia, Montenegro, the Russian Federation, Serbia, Spain and Ukraine (ECDPC., 2013; Papa *et al*., 2013; Pem-Novosel *et al*., 2014) Greece and Russia recorded high WNV activity each year from 2010-2013 and 302 cases were reported from Serbia in 2013 (ECDPC., 2013ab). In Europe, the WNV is considered as one of the most threatening flaviviruses (Colpitts *et al*., 2012).

The lack of data as regards west nile infection prevalence on horses and birds in Algeria goes back to 1994 since, it is considered as notifiable disease as well as that of an epidemiological surveillance whilst this infection is spreading to regain activity in the Mediterranean, North Africa and in other parts of the world where it arouses great interest, to learn more about this question. What is the current health status of the infection in Algeria?

The objectives of this study are to: measure the health status by establishing a seroprevalence survey in horses population. Have an overview into the situation regarding this arbovirus infection in Algeria by a search for possible below circulation of the virus in the study regions in order to locate risk focuses, pointing predisposing and contributory parameters.

To shed light, an experimental study is presented in operating horse sera samples from horses (a seroprevalence survey was conducted on horses population) resulting to rural and urban regions to Algiers, Boumerdes and Blida in cooperation with veterinaries, breeders and horse owners.

MATERIALS AND METHODS

The study was conducted from July 2013 until April 2014. The cross-sectional study of descriptive nature is performed on regions that are selected on the following criteria: (horses population the flyways of birds migration and a climate favorable to the emergence) (Fig. 1).

These areas fall into 3 North of Algeria departments (Algiers, Blida, Boumerdes) (Fig. 2), however, the distribution of the horses was divided into 4 zones (Central-East-West and South) to the geographic location of each one to facilitate the statistical analysis.

- Department of Algiers, where is found the lake Reghaia which is a nature reserve for migratory birds
- Department of Blida: Mitidja wetlands at 50 km of Algiers
- Department of Boumerdes: coastal city at 45 km from Algiers with high rainfall

An indirect ELISA test was conducted "ELISA ID-Screen® West Nile Competition" recommended by the OIE (WOAH., 2010) for the detection of IgG Envelope Protein (Pr-E) on a sera which sample of 209 horses obtained to a venous blood centrifuged than collected and the results are obtained after spectrophotometer reading by measuring optical density at a wavelength of 450 nm in the Bacteriology Department of the National Institute of Veterinary Medicine of El Harrach (Algiers).

The data were inserted in an Excel file to determine seropositive horses WNV to different parameter according Excel application, χ^2-test of independence with risk 0.05 to calculate a varied dual variable and

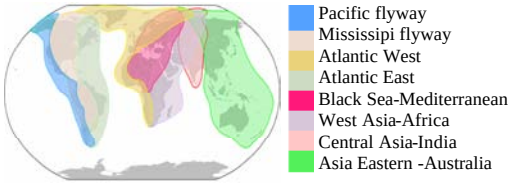

Fig. 1: The flyways of migratory brids in the world

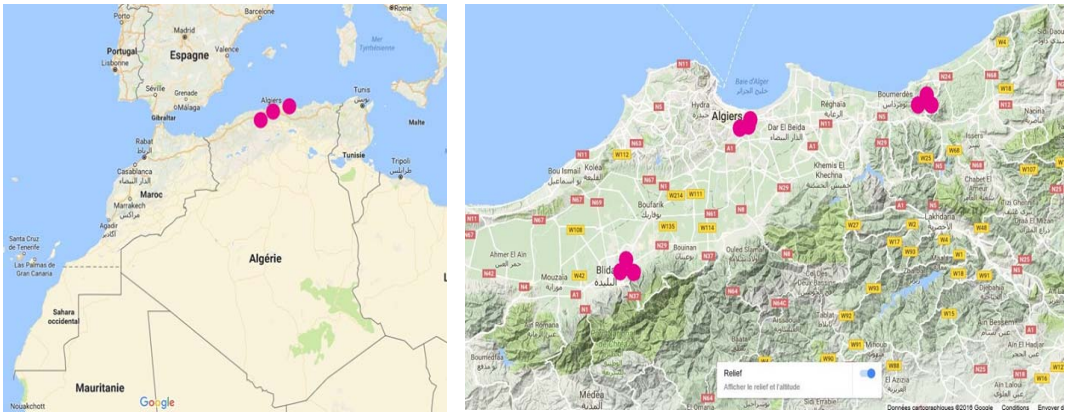

Fig. 2: Survey circulation of West Nile Virus (Algeria-demarcated areas)

calculating odds ratio formula is performed if the condition of the probability rate is less than 0.05 (significant test) and the odds ratio is calculated with a Confidence Interval (CI) of 95% and the result is:

- Lower than 1: the studied parameter is considered a protective factor
- Higher than 1: the studied parameter is a risk factor

RESULTS AND DISCUSSION

Amongst a population composed of 209 horses, 26 of them are seropositive West Nile Virus by ELISA test competition, representing hence, a prevalence rate of about 12%.

This bivariate analysis showed no statistical significance between the seroprevalence and factors (sex, age, color coat, race, season, humidity rate reading on the various stations according to the national office of meteorology, the setting up of anti-vector control means), therefore they have no influence on the occurrence of the infection.

However, there is a statistical significance between the prevalence and factors (Table 1) as follows where the odds ratio calculation allowed to make out the various risk factors to have positive serology to WNF.

The aim of this survey is qualitative, not quantitative, has led to a probable West Nile Virus circulation in the Northern Algiers in which a prevalence rate of 12% was recorded. To be noted that a prevalence rate of 27.1% was recorded in 2008 in the north west of Tunisia (Hassine *et al*., 2011) and another study was carried out between 2007 and 2011 in Northern Serbia with a prevalence rate of 28.6% of the 252 equine serum samples (Medic *et al*., 2014).

Table 1: The parameters influencing the occurrence of WNF

Parameters	Rate probability (p) CI (95%)/α (0.05)	Odds ratio	Confidence interval (95%)/α (0.05)
East region	0.008	2.27	(1.1-6.62)
Presence of migratory and sedentary birds	0.02	0.37	(0.16-0.86)
Prsence of migratory birds	0.0035	3.77	(1.54-9.21)
Box habitat	0.0044	0.24	(0.09-0.64)
Paddock stalls habitat	0.01	2.87	(1.23-6.69)

There is not sex, age, race, coat color real influence parameter to predisposing to infection, the extension and proportional dispatching to horses sample in a future study is to be recommended to reconsider their influence on the appearing of infection.

To explain the statistical results, it seems that WN infection depends on the geographical location. In this study in comparison with other areas it appears that the area "East" is significantly affected and constitute a risk factor owing to the presence of an "ideal" biotope for the development of a cycle of West Nile fever. All actors are present but the existence of particularity in comparison with other areas would be the vicinity of flyways of bird's migration: reghaia lake which is wetland and nature reserve for migratory birds, they are reservoir hosts and amplifiers (Lecollinet *et al*., 2010) and even represent an ecosystem of a nesting site (Fouque *et al*., 2004).

The resident birds are amplifying hosts of the virus (Zeller and Murgue, 2001) while that migratory birds would ensure the release, the introduction of new viral strains from one country to another (Bardos, 1969) hence, an establishment of the concept of lineage and the establishment of a phylogenetic tree due to genetic variability (Castillo-Olivares and Wood, 2004).

The presence of sedentary in association with migratory birds is a protective factor, this could be explained by the fact that the mosquito would be limited to an enzootic cycle with a limited spread of the virus towards mammals.

This also could be explained by the dissolution of the virulence of the strains introduced. That while the presence of migratory birds is a risk factor to causing of the disease, this could be explained by the acquisition of a higher pathogenicity and the introduction of more virulent strains.

The horses in individual boxes are less exposed to mosquito bites than those group living in stall paddock, so, this habitation would constitute a protector factor to contact West Nile fever infection, The horses in their stalls are immune to the bites compared with those who spend part of their day in a paddock or meadow where they could be subject to a contact with the vector, so, a horse in its stall is not necessarily protected from the vector this is explained by the fact that the vector has a greater chance of carrying the virus because it benefits from the gregarious behavior of these horses.

When the horse is seropositive, it does not mean that the mosquito has a seasonal preference for biting the host. It could be that the horses have been contaminated over the hot season or cold one during the previous year(s) and the presence of IgG would explain the WNF seropositivity as the IgG can persist over several years.

It would be advisable to do IgG kinetics on horses at precise intervals or other serological test "seroneutralization PRNT" to determine if the season is in fact a contributing factor or not to the development of the infection taking into account the vector and its evolution depending on temperature and humidity rate parameters.

Culex is found in mediterranean regions (Toral, 2005) when high temperatures associated with high humidity rate. It seems that horses can be infected in less as in humid zones which have shown the opposite to popular belief, where the mosquito was supposed to be found only in humid zone, so, the humidity factor does not exert a great influence. This could be explained by the fact that the mosquito circulates in these areas and has been adapted to humidity changes either for development or for the transmission of the virus.

According to the survey, there were many more infection cases in wetland than in less wetland this later has not to be isolated any more, further studies would be interesting to check the culex circulating type and to follow the evolution and evaluation of EIP Extrinsic Incubation Period or move to a different type vector.

It is to be noted that global warming will result necessarily on an impact on the biotope and consequently on the changing parameters of the Culex or of other vectors.

It would seem that these setting up of anti vector control means (fly-killer lamps, slaked lime and insecticides) prove to be insufficient or inefficient, this would be explained by:

- The lack of knowledge of the circulating Culex type and the choice of the insecticide
- The non respect of dosage of the insecticide (usually under dosage), irregular frequency of its use, "Fly-killer lamps" not replaced and the bad scalding in the stalls
- A possible resistance of Culex in front of anti vector control means

You should know that whatever the ELISA kit used (as with all the currently available commercial kits), its first quality is sensitivity.

In this ELISA test WNF, the IgG are revealed, because these antibodies may persist up to several years, however neither the exact moment nor the amount of Ac present in a sample of serum can be known, for this reason, ELISA IgG test competition could be associated to seroneutralization (a Plaque Reduction Neutralization assay (PRNT); even though, this one requires the handling of the virus which requires biosafety laboratory level 3 which is not yet available in Algeria.

The seroneutralization allows in this respect the establishing of the quantitative value the neutralizing antibodies titre in a serum sample that is not found with ELISA test.

CONCLUSION

According to the results of the serological survey, the presence of West Nile fever is confirmed by ELISA "competition" IgG with a prevalence rate of about 12% on a sample of 209 horses inside the riding clubs and with the horses owners in Northern Algiers.

Although, the prevalence seems low, the risk of emerging/re-emerging arbovirus endemic even epidemic is not impossible due to climate change.

The horses are epidemiological indicative of WNV circulation and of the vector in the regions studied. It seems that seropositive horses would be present near areas of migration with the presence of migratory birds in humid and less humid zones in boxes and paddock stalls and even within presence anti vector control means.

However, sex, age, breed, coat color parameters have to be reconsidered for further study to widen population with homogeneous proportions.

Now, this disease spreads around the Mediterranean and other regions of the world. A phenomenon is being observed that would be evolving with low noise in Northern Algiers with these epidemiological indicatives but this does not conclude to an emerging nature of this vector zoonosis.

On the veterinary side, it seems therefore essential to establish diagnostics routine means for the identification of the WNF. Knowing that WNV causes meningitis and meningo-encephalomyelitis with horses and human beings, the registration of the serological diagnosis within the scope of obligatory research in services for infectious diseases hospitals of our country is expected to protect human and animal health.

REFERENCES

Anderson, J.F., T.G. Andreadis, C.R. Vossbrinck, S. Tirrell and E.M. Wakem *et al*., 1999. Isolation of West Nile virus from mosquitoes, crows and a Cooper's hawk in Connecticut. Science, 286: 2331-2333.

Autorino, G.L., A. Battisti, V. Deubel, G. Ferrari and R. Forletta, 2002. West Nile virus epidemic in horses Tuscany region Italy. Emerg. Infect. Dis., 8: 1372-1378.

Bardos, V., 1969. Epidemiology of West Nile Infections in the South of France. In: Arboviruses of the California Complex and the Bunyamwera Group, Hannoun, C., R. Panthier and B. Corniou (Eds.). SAS Publishers Bratislava, Slovakia, pp: 379-387.

Beck, C., M.A. Jimenez-Clavero, A. Leblond, B. Durand and N. Nowotny *et al*., 2013. Flaviviruses in Europe: Complex circulation patterns and their consequences for the diagnosis and control of West Nile disease. Int. J. Environ. Res. Public Health, 10: 6049-6083.

Bernard, K.A., J.G. Maffei, S.A. Jones, E.B. Kauffman and G. Ebel *et al*., 2001. West Nile virus infection in birds and mosquitoes, New York State, 2000. Emerging Infect. Dis., 7: 679-685.

Berrocal, L., J. Pena, M. Gonzalez and S. Mattar, 2006. West Nile virus: Ecology and epidemiology of an emerging pathogen in Colombia. J. Public Health, 8: 218-228.

Brault, A.C., 2009. Changing patterns of West Nile virus transmission: Altered vector competence and host susceptibility. Vet. Res., 40: 1-19.

Castillo-Olivares, J. and J. Wood, 2004. West Nile virus infection of horses. Vet. Res., 35: 467-483.

Cernescu, C., N.I. Nedelcu, G. Tardei, S. Ruta and T.F. Tsai, 2000. Continued transmission of West Nile virus to humans in southeastern Romania, 1997-1998. J. Infect. Dis., 181: 710-712.

Colpitts, T.M., M.J. Conway, R.R. Montgomery and E. Fikrig, 2012. West Nile Virus: Biology, transmission and human infection. Clin. Microbiol. Rev., 25: 635-648.

Durand, B., G. Dauphin, J. Labie, H. Zeller and S. Zientara, 2005. Results of a serological survey on West Nile virus infection in Equids in the Var in 2003. Environ. Risks Health, 4: 114-118.

ECDPC., 2013a. Disease data from ECDC surveillance atlas West Nile fever. European Centre for Disease Prevention and Control, Solna Municipality, Sweden. http://ecdc.europa.eu/en/healthtopics/west_nile_fever/West-Nile-fever-maps/Pages/index.aspx.

ECDPC., 2013b. Historical data by year. European Centre for Disease Prevention and Control, Solna Municipality, Sweden. http://ecdc.europa.eu/en/healthtopics/west_nile_fever/West-Nile-fever-maps/Pages/historical-data.aspx.

Fouque, C., E. Corda, J.L. Tesson, J.Y. Mondain-Monval and C. Barthe *et al*., 2004. Breeding chronology of anatids (Anatidae) and Coots (Fulica atra) in France. Game Wildl. Sci., 21: 73-105.

Garbouj, M., M. Bejaoui, H. Aloui and M.B. Ghorbal, 2003. La maladie du Nil occidental. Bull. Epidemiologique, 3: 4-6.

Giudice, P.D., I. Schuffenecker, F. Vandenbos, E. Counillon and H. Zeller, 2004. Human West Nile virus, France. Emerging Infect. Dis., 10: 1885-1886.

Guenno, B.L., A. Bougermouh, T. Azzam and R. Bouakaz, 1996. West Nile: A deadly virus?. Lancet, 348: 1315-1315.

Harrack, M.E., B.L. Guenno and P. Gounon, 1997. Isolation of West Nile virus in Morocco. Virologie, 1: 248-249.

Hassine, T.B., S. Hammami, H. Elghoul and A. Ghram, 2011. Detection of circulation of West Nile virus in equine in the North-West of Tunisia. Bull Soc. Pathol. Exot., 104: 266-271.

Hobson-Peters, J., C. Arevalo, W.Y. Cheah, B.J. Blitvich and C. Tan *et al*., 2011. Detection of antibodies to West Nile virus in horses, Costa Rica, 2004. Vector Borne Zoonotic Dis., 11: 1081-1084.

Hubalek, Z. and J. Halouzka, 1999. West Nile fever--a reemerging mosquito-borne viral disease in Europe. Emerg. Infect. Dis., 5: 643-650.

Kalaycioglu, H., G. Korukluoglu, A. Ozkul, O. Oncul and S. Tosun *et al*., 2012. Emergence of West Nile virus infections in humans in Turkey 2010 to 2011. Euro Surveill, 17: 1-6.

Kramer, L.D., L.M. Styer and G.D. Ebel, 2008. A global perspective on the epidemiology of West Nile virus. Annu. Rev. Entomol., 53: 61-81.

Lecollinet, S., N. Poncon, A. Leblond, P. Hendrikx and Z.S. Breve, 2010. Le virus West Nile gagne du terrain en Europe. Bull. Epidemiologique Sante Anim. Alimentation, 41: 1-19.

Mattar, S., N. Komar, G. Young, J. Alvarez and M. Gonzalez, 2011. Seroconversion for West Nile and St. Louis encephalitis viruses among sentinel horses in Colombia. Memoirs Oswaldo Cruz Inst., 106: 976-979.

Medic, S., R.V.D. Hoven, T. Petrovic, D. Lupulovic and N. Nowotny, 2014. Serological evidence of West Nile virus infection in the horse population of Northern Serbia. J. Infect. Developing Countries, 8: 914-918.

Murgue, B., S. Murri, H. Triki, V. Deubel and H.G. Zeller, 2001. West Nile in the Mediterranean Basin: 1950-2000. Ann. N. Y. Acad. Sci., 951: 117-126.

Papa, A., E. Papadopoulou, E. Gavana, S. Kalaitzopoulou and S. Mourelatos, 2013. Detection of West Nile virus lineage 2 in Culex mosquitoes, Greece, 2012. Vector Borne Zoonotic Dis., 13: 682-684.

Pem-Novosel, I., T. Vilibic-Cavlek, I. Gjenero-Margan, N. Pandak and L. Peric *et al*., 2014. First outbreak of West Nile virus neuroinvasive disease in humans, Croatia, 2012. Vector Borne Zoonotic Dis., 14: 82-84.

Platonov, A.E., G.A. Shipulin, O.Y. Shipulina, E.N. Tyutyunnik and T.I. Frolochkina *et al*., 2001. Outbreak of West Nile virus infection, Volgograd Region, Russia, 1999. Emerg. Infect. Dis., 7: 128-132.

Porter, R.S., A. Leblond, S. Lecollinet, P. Tritz and C. Cantile *et al*., 2011. Clinical diagnosis of West Nile Fever in Equids by Classification and Regression Tree (CART) analysis and comparative study of clinical appearance in three European countries. Transboundary Emerging Dis., 58: 197-205.

Pradier, S., S. Lecollinet and A. Leblond, 2012. West Nile virus epidemiology and factors triggering change in its distribution in Europe. Rev. Sci. Tech, 31: 829-844.

Rossi, S.L., T.M. Ross and J.D. Evans, 2010. West Nile virus. Clin. Lab. Med., 30: 47-65.

Sambri, V., M. Capobianchi, R. Charrel, M. Fyodorova and P. Gaibani *et al*., 2013. West Nile virus in Europe: Emergence, epidemiology, diagnosis, treatment and prevention. Clin. Microbiol. Infect., 19: 699-704.

Schuffenecker, I., C.N. Peyrefitte, M.E. Harrak, S. Murri and A. Leblond *et al*., 2005. West Nile virus in Morocco, 2003. Emerging Infect. Dis., 11: 306-309.

Smithburn, K.C., T.P. Hughes, A.W. Burke and J.H. Paul, 1940. A neurotropic virus isolated from the blood of a native of Uganda1. Am. J. Trop. Med. Hyg., 1: 471-492.

Toral, G., 2005. Evaluation In vitro the effectiveness of Fipronil on Culex pipiens pipiens. Med. Vet. Toulouse, 99: 53-55.

Triki, H., S. Murri, B. Le Guenno, O. Bahri, K. Hili, M. Sidhom and K. Dellagi, 2001. West Nile viral meningo-encephalitis in Tunisia. Med. Trop., 61: 487-490.

WOAH., 2010. West Nile Virus in Morocco: Immediate notification report. World Organisation for Animal Health, Paris, France.

Zeller, H.G. and B. Murgue, 2001. [The role of migrating birds in the West Nile virus epidemiology (In French)]. Med. Infect. Dis., 31: 168-174.