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Emergence of West Nile Virus in Ivory Coast

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Abstract: West Nile fever is a neglected endemic pathology in West Africa. It is caused by a flavivirus. The objective of this work is to search from the national surveillance of yellow fever and Dengue viruses, the West Nile virus for the year 2019. Sera from samples diagnosed negative for yellow fever and dengue fever were used to detect specific anti-yellow fever virus IgM antibodies by immunocapture using the ELISA (Enzyme-Linked ImmunoSorbent Assay) technique. The real-time molecular method (RT-PCR) was also used to search for the viral genome through the envelope gene (E). Serological results revealed serum IgM antibodies against West Nile virus. We have 03 positive cases/132 cases studied with 3.17% positive in women and 1.67% of cases in men. It affected children aged 15 to 17 (0.76%) and adults over 40 (1.53%). Only the districts of Abidjan were exposed. Regarding the PCR test, all samples for the viral genome were negative. This study indicates the presence of antibodies against the circulating West Nile virus in Abidjan. These West Nile cases detected in the Abidjan health district have highlighted the reality of West Nile.

Keywords: West Nile Virus, ELISA (Enzyme-Linked ImmunoSorbent Assay), RT-PCR

1. Introduction

Vector-borne diseases account for over 17% of all infectious diseases, causing over 700,000 deaths per year. They can be caused by parasites, bacteria or viruses [1].

There is a potential threat of the spread of these diseases, both geographically and seasonally, due to climatic and ecological changes, human activities such as development projects, civil conflicts, urbanization and population movements.

This group of diseases includes West Nile fever or West Nile (WN) fever, the subject of this study. West Nile virus (VWN) belongs to the genus Flavivirus (family Flaviviridae) along with other pathogens, such as Dengue Fever, Zika, Yellow Fever, Japanese Encephalitis, Tick-borne Encephalitis, Usutu, St. Louis encephalitis and Powassan virus [2]. West Nile fever is endemic, neglected, first isolated in 1937 in Uganda [3, 4]. However, it can progress to severe encephalitis, meningitis or poliomyelitis-like acute flaccid paralysis [5, 6]. West Nile virus infection develops between 3 and 14 days after the bite of a WNV-infected mosquito and may persist for an additional 3 to 6 days, although severe cases may be biphasic and show symptoms for as long as at 60 days. When they do occur, symptoms include fever, rash, headache, muscle weakness/pain, and joint pain or hepatitis [7]. Reservoir hosts are wild birds, mainly passerines. Transmission to humans is possible through the Culex mosquito [8, 9]. Most people infected with the virus remain asymptomatic (80%) and go unreported [10]. Nevertheless, nearly 30% of infected people show symptoms ranging from a simple flu syndrome to an encephalitic syndrome with a case fatality rate of up to 17% [11, 12]. In horses, neurological disorders are observed in 10% of those infected [13, 14].

Since its first identification in East Africa, the virus has been identified on all continents. Today, it is endemic in the Mediterranean region, in Central Europe and in North America where it is responsible for fatal human cases as it

has been observed in mainland Greece, southern Italy and the United States [15]. Since then it has been isolated in western and central Asia, the Middle East, southern and eastern Europe, and the Western Hemisphere [16]. Like all viral illnesses, there is no effective specific treatment for West Nile virus. As a result, the control of the disease can only be achieved through targeted and effective means of control against the mosquito vector.

In human and animal health, no clinical case of the disease has so far been documented in Ivory coast. However, clinical signs resembling those of malaria in humans (flu syndrome) and diagnosed negative for malaria are extensively documented in the health structures of Ivory coast. And therefore, cases of fever in West Nile can go undetected either by a lack of diagnosis or by a misunderstanding of the disease. The objective of this work is to search from the national surveillance of Dengue yellow fever, the circulation of the West Nile virus for the year 2019.

2. Material

2.1. Type of Study

Figure 1. *Geographical positioning of the different health districts.*

This is a retrospective study that took place from January 2019 to December 2019, i. e. over 12 months, carried out at the Arbovirus and Hemorrhagic Fever Virus Unit, National Reference Center (CNR) for yellow fever and dengue at the Department of Epidemic Viruses of the Institut Pasteur in Ivory coast. Study areas and plans: This study was conducted in Ivory coast (4°5 and 10°5 Latitude North and between 2°5 and 8°5 West longitude) from January to December 2019. Ivory coast is an ecologically diverse territory (Sudanian Zone, Sub-Sudanian Zone, Lower Zone Ivory coast, Forest Zone, Middle Zone Ivory coast). The samples obtained come from the different districts of Ivory coast.

2.2. Selection Criteria

All blood samples from the health districts of Ivory coast for yellow fever surveillance during this study.

Inclusion criteria: All samples with a volume of 500 µL and an investigation form of suspected persons are sent to the National Reference Laboratory for research of dengue viral haemorrhagic fever or yellow fever.

Criteria for non-inclusion: Any sample with a positive result for dengue and/or yellow fever.

Any sample without identifier was excluded from our study.

Sampling

In each of the 7 groups defined by place of residence (interior of the country or health district of Abidjan), a sample for the study was randomly selected by systematic sampling. Empirically, we found that the number of samples in Abidjan was greater than those in the interior. Study sample sizes were set accordingly.

We targeted an approximate number of at least 500 samples in total, which would provide sufficient statistical power to answer the main study question. The selection of the sample and the analysis of the sera were carried out in December 2019. To obtain the number of our sample for the study, we multiplied 500 by the proportion of sera of the total serum by communes of Abidjan. All sera from the interior of the country have been retained to ensure national coverage. According to the kinetics of a viral infection, all the samples received at the level of the reference laboratory beyond 7 days from the date of onset of the disease on the date of reception, were used for the serological diagnosis and those less than 7 days for molecular biology. The samples were, in the majority of cases, transported under the conditions as recommended by the relevant WHO procedure manual and stored in the biobank at -80°C. The samples are then sent to Dakar for confirmation in the event of a positive serology or molecular test.

3. Methods

3.1. Serological Test

The sera obtained after 3500 revolutions for 3 min were tested at the Pasteur Institute of Ivory coast (IPCI) for the search for specific anti-yellow fever virus IgM type antibodies by immuno-capture, according to the ELISA technique (Enzyme -Linked ImmunoSorbent Assay) and developed by the Centers for Diseases Control (CDC) made available to national laboratories for yellow fever by the WHO [1]. The interpretation of the results is carried out by calculating the difference of the OD (ΔDO) obtained with the

antigens, negative and positive. A serum is considered positive if this ΔDO is greater than 0.300. A serum is negative if the ΔDO is less than 0.200.

3.2. PCR Test

Viral ribonucleic acid (viral RNA) was isolated from 140 µL aliquots of blood serum using the Qiagen RNeasy Mini Kit (Qiagen, Hilden, Germany). The RNA was eluted in 60 µL H_2O without RNase A direct search for the West Nile virus (VWN) was carried out on the blood samples, after the extraction of the viral RNA was carried out using a QIAGEN commercial kit and according to the supplier's instructions (RNeasy mini Kit, QIAGEN). The extracted nucleic acid was then stored at -80°C. RT-PCR amplification of viral RNA was performed using primers and probes for the envelope gene (E gene) [17].

WNproC-F (5'-CCTGTGTGAGCTGACAAACTTAGT-3')

WNproC-R (5'GCGTTTTAGCATATTGACAGCC-3')

WNproC S (6 FAM-CCTGGTTTCTTAGACATCGAGATCTXCGTGCp)

The reaction mix with a final volume of 25 µL consisted of 12.5 µL of the 2X Universal PCR Master Mix buffer (Applied Biosystems), 1.25 µL at 200 mM of each forward and reverse primers with 0.5 µL at 200 mM of the probe, 1 µL of AgPathIDTM One-Step RT-PCR enzyme and 5 µL of RNase-free water. An addition of 5 µL of extracted RNA was necessary to the reaction mixture for amplification. The fluorogenic probe for WNV detection was synthesized with a 5'-reporter dye 6-carboxyfluorescein (6-FAM) and a 3'-quencher dye 6-carboxytetramethylrhodamine (5'-TAMRA).

Amplification involves two major steps, cDNA synthesis and PCR.

Transcription from RNA to cDNA was performed for 10 min at 50°C. As for the PCR, it started with an initial denaturation and the activation of the polymerase for 10 min at 95°C. This denaturation is followed by 40 cycles comprising denaturation for 15 seconds at 95°C., and elongation of the polymerase after hybridization of the probe and the primers to their respective target sequences at a temperature of 60°C. for 1 min. One of the CNR thermal cyclers, Applied Biosystems™ 7500 Fast Real-Time PCR Systems, or Qantstudio 5 Applied Biosystems was used as an automaton.

Statistical analysis

Statistical analyzes were performed using Excel software Version 6. For the cartographic figure, the Geographic Information System (GIS), in this case QGIS version 2.16 was used.

Result

The results of our serological surveys are presented in Tables 1 and 2. Of all the districts from which the samples originated, only the districts in the Abidjan area were exposed. Exposure was observed for 03 positive cases in ELISA / 132 serological samples with in 3.17% of cases in women and 1.67% of cases in men. It affected children aged 15 to 17 (0.76%) and adults over 40 (1.53%). All samples less than seven days old were PCR negative.

Table 1. *Prevalence of patients infected with the West Nile virus according to age.*

AGE CLASS	ABIDJAN N (n%)	BIANKOUMA N (n%)	BOUAKE N (n%)	MAN N (n%)
0-4 years old	17 (0)	0	0	0
5-14 years old	21 (0)	1 (0)	0	5 (0)
15-17 years old	7 (0.76)	0	0	1 (0)
18-39 years old	50 (0)	0	1 (0)	3 (0)
40 years and over	37 (1.53)	0	0	2 (0)
TOTAL	132 (2.27)	1 (0)	1 (0)	11 (0)

N: Number of patients analyzed; (n%): Prevalence of infected patients

Table 2. *Prevalence of patients infected with the West Nile virus according to gender.*

GENRE	ABIDJAN N (n%)	BIANKOUMA N (n%)	BOUAKE N (n%)	MAN N (n%)
F	63 (3.17)	0	0	5 (0)
M	69 (1.45)	1 (0)	1 (0)	6 (0)
TOTAL	132 (2.27)	1 (0)	1 (0)	11 (0)

F: Female; M: Male; N: Number of patients analyzed; (n%): Prevalence of infected patients

4. Discussion

The results obtained made it possible to highlight the presence of IgM West Nile in human sera in the district of Abidjan. This presence of West Nile IgM shows that people have been infected with West Nile virus. The endemic nature has been demonstrated by numerous serological surveys in animals [18, 19, 20] and in man [21].

Our results would be consistent with the hypothesis of the existence of well-defined viral circulation foci within the same temporarily colonized geographical area [22, 23]. If West Nile virus infection is classically considered "endemic" in Africa, especially sub-Saharan Africa, the precise situation of the disease in Côte d'Ivoire, the subject of this study, has not been established until now. Indeed very few studies or reviews have taken into account the presence of the virus in sub-Saharan Africa, its burden on public health or the factors that lead to its spread to other regions of the world [24]. Human infections due to the West Nile virus have been

reported in Senegal, the Central African Republic and Nigeria [25, 26], but only an epidemic which occurred in 1998 in a military camp in Kisangani (Democratic Republic of Congo) is well documented [27]. Two factors would have favored the appearance of this outbreak. The heavy rains that preceded it produced floods favorable to the reproduction of the Culex mosquito vector.

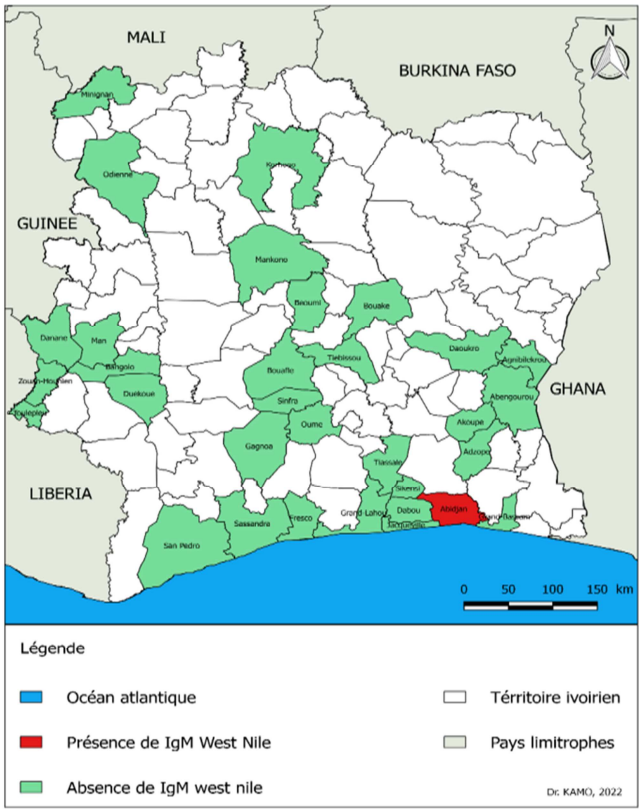

Figure 2. *Distribution of seroprevalence according to the health districts studied.*

Since the West Nile virus is known to show cross-reactions with many other flaviviruses belonging like it to the Japanese encephalitis serogroup [28] or to other serogroups (dengue fever), confirmation was HIV-positive people. PCR results did not yield conclusive results. This can be either due to poor storage of the samples, which has certainly degraded the viral RNA, or due to the low viral load rate or the packaging for transporting the samples, which is poorly understood by the staff of the health structures. The distribution, by sex, of seroprevalence in these different districts does not show any significant difference. However, a difference is observed according to age in Abidjan, with a higher frequency in subjects over 40 years old. One could suppose that this difference is due to an immune system in depression but which remains to be confirmed. West Nile virus is mainly spread when a mosquito is exposed to the virus by feeding on birds that develop high levels of the virus in their blood. Competent infected mosquitoes can transmit the virus if they feed on susceptible vertebrates, including humans [29]. The abundance of human biting mosquitoes in sub-Saharan Africa, where Côte d'Ivoire is located, presents a major risk. Indeed this abundance, the eating habits of infected mosquitoes, as well as the human behavior that influences their exposure to mosquitoes, influence the probability of WNV transmission [30]. This low incidence reported in our regions is probably due to the lack of surveillance of this virus in humans, animals and insect vectors of the virus.

5. Conclusion

The serological results of this study indicate that the West Nile virus is circulating in Abidjan. These cases of West Nile detected in the health district of Abidjan made it possible to highlight the reality of West Nile and to collect information relating to this arbovirosis which could be evoked but which becomes an obligatory differential diagnosis of yellow fever. We will have the prospect of conducting further studies at the level of the virus in mosquitoes in the district of Abidjan. This encourages us to carry out broader epidemiological studies on the whole country. It would also be important to institute active monitoring:

1. at the human level by systematic VWN serology in all cases of meningitis and meningoencephalitis or for other clinical pictures in which VWN may be incriminated;
2. at the animal level by setting up a sentinel system targeting the horse as an accidental host of the virus and the vectors (mosquitoes).

Competing Interests

The authors declare that they have no competing interests.

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