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1 Geographic distribution of anti-*Leptospira* antibodies in humans in Côte
2 d'Ivoire, West Africa.

3

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15 INTRODUCTION

16 Leptospirosis is a zoonotic bacterial disease with a worldwide distribution caused by pathogenic
17 bacteria of the genus *Leptospira* [1]. It is an endemic disease especially in subtropical and tropical
18 regions and outbreaks can occur after heavy rain or flooding [1]. Transmission occurs through direct or
19 indirect contact with urine of infected animals [1, 2].

20 Leptospirosis may be unrecognized or misdiagnosed because of the lack of specific clinical symptoms,
21 thus human incidence is commonly underreported.

22 The World Health Organization (WHO) Leptospirosis Epidemiology Reference Group (LERG)
23 estimated the leptospirosis incidence at 1.03 million cases and 58,900 deaths each year worldwide [3].
24 West African annual leptospirosis incidence was estimated to be 9.7 per 100,000 population [3].

25 In West Africa, few studies on *Leptospira* prevalence have been published. And prevalence of acute
26 human leptospirosis ranged from 0.0% to 7.8% in five studies on symptomatic patients [4, 5].

27 In Côte d'Ivoire, data are sparse, however an unpublished study carried out in 2013 by the "Centre
28 Suisse de Recherche Scientifique" in Yopougon (Abidjan) showed, 4% prevalence in both 124 febrile
29 patients and 99 rodents using MAT showing human and animal infection. Another study showed high
30 prevalence in 93 vaccinated dogs (58%) and 63 non-vaccinated dogs (47%) sampled in Abidjan and in
31 two neighbouring cities using MAT suggesting human exposure [6].

32 Thus, little is known about leptospirosis in the country and data are needed to inform burden estimates
33 and to inform intervention strategies. Mapping leptospirosis exposure could help find high-risk areas
34 and lead intervention choices against the disease. This study aims to determine the geographic
35 distribution of anti-*Leptospira* antibodies in humans in Côte d'Ivoire.

37 MATERIAL AND METHODS

38 This study was carried out from August to November 2015 in the 82 health districts of Côte d'Ivoire.

39 The study was carry out on stored human specimens previously collected for the national surveillance
40 system for communicable diseases in 2014. Only negative specimens were included in the study.

41 One to 13 serum samples were randomly selected per health district based on the number of notified
42 cases by health districts in the year to obtain a sample size of 384.

43 Blood samples were collected from each patient who matches the clinical WHO's case definition for
44 notifiable disease. Samples were sent, at +4°C, to the National Reference Centre at the Institut Pasteur
45 de Côte d'Ivoire for analysis. Anti-*Leptospira* antibody testing was carried out at Institut Pasteur de
46 Côte d'Ivoire (IPCI) and Institut Pasteur in Paris (IP). We used as screening methods the ELISA
47 against type IgG anti-*Leptospira* antibodies previously describe by Bourhy et al. (2013) in IPCI [7].
48 Confirmation was carried out by MAT in IP with a threshold of 1:50. As antigens, 16 serogroups were
49 used (table 1).

50 Socio-demographic and clinical data from the National Reference Centre, which manage the samples
51 and results for analysis performed on studied samples, were used to build a database using Epi Info
52 software (CDC, Atlanta, Georgia). Data analyses were done using R software 3.2.0 [8].

54 RESULTS

55 Age of patients ranged from 1 month to 78 years old with a mean age of 20.8 years. Patients less than
56 25 years were more represented with 60.9% of all patients. We noted a male predominance with a sex
57 ratio of 1.55.

58 Of the samples tested, ELISA positively screened 90. Of these, 36 were confirmed by MAT
59 representing 9.4 % prevalence. People with anti-*Leptospira* antibodies had a mean age of 34.5 years
60 and a Sex ratio of 2. Of the 82 health districts of the country, anti-*Leptospira* antibodies were found in
61 samples from 22. Health districts with anti-*Leptospira* antibodies were mostly located in the western
62 and the southern parts of the country (fig 1).

63 MAT anti-*Leptospira* antibodies titre ranged from 1:50 to 1:6400. Eight different serogroups of
64 *Leptospira* were found (table 2). Serogroup Panama was the most frequent (36.1 %) followed by
65 Louisiana (19.4 %) and Grippotyphosa and Sejroe (11.1% respectively).

67 **DISCUSSION**

68 This study focuses on anti-*Leptospira* antibodies in human specimens collected in the national
69 surveillance system for communicable diseases in Côte d'Ivoire. Few published data on *Leptospira* and
70 leptospirosis in Côte d'Ivoire are available [4, 5, 9]. Our study, which focus on type IgG antibodies
71 showed a prevalence of 9.4%. This result is in accordance with the LERG who estimated an annual
72 incidence for leptospirosis to 9.6 % in the West sub-Saharan Africa WHO region [3].

73 Samples from all the 82 health districts of the country were included in this study. So, the studied
74 population were representative of all the country. However, health districts with anti-*Leptospira*
75 antibodies found in this study were mostly located in the western and the southern parts of the country
76 (fig 1). These parts of the country are peculiar by their climate and forest cover. They are the most
77 humid parts of the country with higher forest cover and heavy raining season compare to the northern
78 part of the country, which is mostly arid. It's actually well known that leptospirosis is related to water
79 and wet soils were *Leptospira* find proper conditions for his maintenance in the environment [10, 11,
80 12]. In these parts of the country, some irrigated farming activities like rice, banana and vegetable
81 farming are associated with risk behaviours like walking barefooted in wet farm soil, sludge or working
82 without wearing appropriated personal protective equipment. The maintenance of *Leptospira* in the
83 environment is better when bacteria are protected from direct UV sunlight [13]. This situation could be
84 achieved in forests or in large farms with trees like cocoa, coffee or rubber plantations mostly seen in
85 the western and southern part of the country. These plantations also offer habitats to small mammals
86 that are considered a reservoir of bacteria and create a Human/ Animal/ Environment interface, which
87 could improve the transmission of the disease.

88 Regarding people with anti-*Leptospira* antibodies, they were young adults (mean age 34.5 years)
89 mostly men (sex ratio 2) living in rural area; corresponding to active low-income farmers working into
90 agricultural fields. These people could represent an at-risk population and it could be suitable to
91 implement prevention activities against leptospirosis related to them. Indeed, farming is a major
92 activity in Côte d'Ivoire representing 43.5 % of jobs.

93 This study highlights the circulation of leptospirosis among people in Côte d'Ivoire. Unfortunately, no
94 routine laboratory-based diagnostic strategies are available for leptospirosis or other diseases such as
95 rickettsia or viral hepatitis. Numerous patients suffering from infectious diseases are not correctly
96 diagnosed, and the burden of these diseases is underestimated. Early diagnosis and treatment reduce
97 occurrence of complications and improve recovery rate significantly [1]. There is a need to improve
98 public health by Ivorian authorities and their international partners.

99

100 **COMPLIANCE WITH ETHICAL STANDARDS**

101 **Funding:** No funding was received.

102 **Conflict of Interest:** The authors declare that they have no conflict of interest.

103 **Ethical approval:** The national ethic and research committee of Côte d'Ivoire approved this study
104 under the number 23/MSLS/CNER-dkn.

105 **Informed consent:** Not applicable.

106

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138 **FIGURE CAPTIONS**

139 **Fig 1** shows health districts where people with anti-*Leptospira* antibodies were found. Colors are related
140 to number of cases found