

Epidemiology and enzyme-linked immunosorbent assay of *Leptospira* spp. in humans in the Republic of Guinea

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World Journal of Advanced Research and Reviews, 2023, 19(03), 404–410

Publication history: Received on 23 July 2023; revised on 04 September 2023; accepted on 06 September 2023

Article DOI: <https://doi.org/10.30574/wjarr.2023.19.3.1789>

Abstract

Introduction: Leptospirosis is a zoonosis caused by spirochete bacteria called leptospire of the genus *Leptospira*.

Objective: To contribute to the knowledge and circulation of the *Leptospira* germ in humans in the Republic of Guinea.

Methods: This prospective and descriptive cross-sectional study lasted 18 months, from June 2019 to December 2020.

Results: Of 2346 human blood samples analysed in the laboratory using the enzyme-linked immunosorbent assay (ELISA) for IgG antibodies against leptospirosis, the largest number of samples was taken in Lower Guinea (31.58%), followed by Forest Guinea (27.66%), Middle Guinea (20.97%) and Upper Guinea (19.77%). More samples were taken from females (50.89%) than males (49.10%), for a sex ratio of 1.03 in favour of females. In terms of natural regions, Basse Guinée recorded the highest number of female samples (36.85%). On the other hand, the highest number of male samples was observed in Guinée Forestière with 28.55%. The results of anti-*leptospira* class G immunoglobulin tests identified the germ in 93 patients, representing a prevalence of 4%. The average age of the patients was 45, with extremes ranging from 10 to 90 years. Of the 93 positive cases, the prevalence of *Leptospira* infection was highest in subjects aged 31 to 40 (46.23%), followed by individuals in the 41 to 50 age group (18.27%). Prevalence was lower in the 11-20 age group (9.67%), the 51-60 age group (7.52%) and the 61-70 age group (3.22%). No cases of Leptospire infection were recorded in the 0-10, 71-80 and 81-90 age groups. Males accounted for 53.76% of Leptospire infections, compared with 46.23% for females, with a 95% confidence interval (CI) ranging from [13.08-20.82]. Lower Guinea recorded 25.80%, followed by Middle Guinea with 32.25%, Upper Guinea with 20.43% and Forest Guinea with a prevalence of 21.51%. The prevalence of *Leptospira* infection is higher among women in Middle Guinea (21.50%) than in the other natural regions. We also note that Lower Guinea recorded the highest rate of positivity among males at 18.28% compared with the other regions of Guinea. Farmers are the most affected by infections with 46.23%, followed by veterinarians with 25.80%, health workers with 18.28%, retired people with 4.30% and commercial workers with 3.22% (3/93). Students and workers were the least represented in this study, with 1 case each, representing a prevalence of 1.07%.

Conclusion: The results obtained prove that the leptospirosis pathogen does exist in the Republic of Guinea in the entire human population.

Key words: *Leptospira* spp; Human; ELISA; Natural regions; Republic of Guinea.

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1. Introduction

Leptospirosis is a cosmopolitan zoono-anthroposis caused by filamentous bacteria called Leptospire of the genus *Leptospira*. It attacks both humans and animals. It is a major public health problem. It affects almost a million people a year worldwide, including 60,000 deaths, but these figures are probably underestimated due to the lack of specificity of symptoms and the difficulty of using diagnostic techniques. Leptospirosis is endemic in countries with tropical climates, and climate change may increase its incidence [1]. It has been described all over the world, including in temperate countries. Conditions are very favorable for the circulation of leptospire in several tropical countries such as Asia, Latin America and Sub-Saharan Africa [1]. Worldwide incidence estimates put the number of cases at 1.03 million per year, giving an average incidence rate of 14 cases per 100,000 inhabitants and a mortality rate of 7%. These figures are underestimated due to a lack of information for certain areas of the world with no diagnostic facilities [2]. The areas most affected are those with tropical or subtropical climatic conditions, where the incidence varies from 13 to 150 cases per 100,000 inhabitants per year. The incidence appears to be higher in areas with a low standard of living, with 2 categories of group particularly at risk in developing countries: people working in agriculture, and people living in insalubrious conditions. Leptospirosis affects 80% of men, half of whom are in the 20-50 age bracket [3]. Leptospirosis is caused by spirochete bacteria called leptospire of the genus *Leptospira*. It attacks humans as well as domestic animals (cattle, pigs, dogs) and wild animals (rodents), which excrete the bacteria in their urine [4]. The pathogenic species, *Leptospira interrogans*, comprises more than 200 serovars grouped into twenty serogroups [5]. The usual symptoms vary from one individual to another, ranging from a simple fever to multiple organ failure, with mortality in 5 to 10% of cases. These bacteria are aerobic and are not resistant to dryness or hypertonicity, but can tolerate alkalisation down to a pH of 7.8 [6]. Global warming, heavy rainfall, increasing urbanisation, etc. are all factors in the spread of leptospire. People living in unhealthy urban or peri-urban habitats and subsistence farmers are particularly at risk. The bacteria are transmitted naturally from domestic or wild vertebrate animals (e.g. rodents) to humans, and vice versa, who excrete them in their urine [7]. In sub-Saharan Africa, many countries have climatic characteristics conducive to the development and transmission of leptospire, but the incidence and prevalence remain difficult to assess. However, Gabon has been reporting cases regularly since 1994 [8]. In Senegal, 3.1% of blood samples were found to be positive for leptospirosis in blood donors and a prevalence of 14.3% of canine leptospirosis in the Kaolack region, with serovar *L.icterohemorrhagiae* dominating over serovar Pomona, with 69% of positive cases compared with 23 [9]. In Nigeria, surveys of healthy populations showed leptospirosis seroprevalence in the Eastern and Plateau states, at 14% and 18% respectively [10].

2. Materials and working methods

2.1. Study environment and setting

Our study was carried out in the Republic of Guinea. The Guineo-Russian laboratory of Guinea's Applied Biology Research Institute (IRBAG) provided the setting for the study.

2.2. Working method

This was a prospective cross-sectional, descriptive and analytical study that lasted 18 months, from June 2019 to December 2020. It focused on three types of population, namely: Patients seen in consultation in a febrile state in various hospitals in Guinea; Bats captured in Guinea and Rodents captured in Guinea. Sampling was simple random and the sample size (n=2346) was obtained using the SCHWARTZ formula. All patients seen in consultation with a fever during the survey period were included in our study.

2.3. Bio-material

Our bio-material consisted of patient blood.

2.4. Study variables

2.4.1. Biological variables

- ELISA

2.4.2. Epidemiological variables

- Sampling regions
- Sex: Age

- Age; Occupation
- Occupation

2.5. Ethical considerations

Before beginning the data analysis phase, the study's research protocol was submitted to the Doctoral School management. The research team guaranteed the confidentiality of the information collected and the anonymity of the study participants.

2.6. Data collection and computer analysis

The data were compiled manually, entered into Word 2007 and processed and analysed using Excel and SPSS version 21.

2.7. Limitations and difficulties of the study

Small sample size for rodents and bats (due to COVID-19), storage of blood samples in health facilities and their transport to IRBAG.

3. Results and Discussion

The study of the seroprevalence of anti *leptospira* immunoglobulin G in humans, the detection of *Leptospira spp.* 16s RNA in humans. The results obtained are presented in the form of tables, interpreted, commented on and discussed according to the available literature data.

3.1. Results of blood sample collection in humans.

A total of 2346 human blood serum samples from the different natural regions of Guinea were tested for leptospiral immunoglobulin class G (IgG) using the ELISA test.

Table 1 Breakdown of different harvests by Natural Region

Natural Regions	Number of samples	Percentage
Lower Guinea	741	31.58
Middle Guinea	492	20.97
Upper Guinea	464	19.77
Forest Guinea	649	27.66
Total	2346	99.98

The table shows that the greatest number of samples were taken in Lower Guinea with 741 samples, i.e. 31.58%, followed by Forest Guinea with 649 samples, i.e. 27.66%, Middle Guinea with 492 samples, i.e. 20.97% and finally Upper Guinea with 464 samples, i.e. 19.77%. This situation is unpredictable, as it depends on how feverish patients are in the country's various hospitals.

Table 2 Distribution of samples by sex

Natural Regions	Number of samples by sex	
	Female	Male
Lower Guinea	440	301
Middle Guinea	300	322
Upper Guinea	134	200
Forest Guinea	320	329
Total/Gender	1194	1152
Total Général	2346	

We note that the number of samples taken is higher among women with 1194 cases, i.e. 50.89%, than among men with 1152 cases, i.e. 49.10%, for a sex ratio of 1.03 in favour of women. Lower Guinea recorded the highest number of female samples with 440 cases, or 36.85%. On the other hand, the highest number of male samples was observed in Guinée Forestière with 329 cases, i.e. 28.55%.

Table 3 Results of detection of class G antibodies against *Leptospira* agents in blood

Natural Regions	umber of samples	Positive cases	Percentage	95% CI
Lower Guinea	741	24	1.02	1.1-3.1
Middle Guinea	492	33	1.40	0.7-3.5
Upper Guinea	464	18	0.76	1.3-2.9
Forest Guinea	649	18	0.76	1.3-2.9
Total	2346	93	4	

In the analysis of human serum samples, class G anti-*leptospira* immunoglobulins were identified in 93 patients, representing a prevalence of 4% (table 3). This indicates contact between these patients and the leptospirosis pathogen. Immunoglobulins were detected in all natural regions, with a higher prevalence in Middle Guinea with 33 cases, i.e. a prevalence of 1.40%, followed by Lower Guinea with 24 cases, i.e. 1.02%, and the lowest prevalences were recorded in Forest Guinea and Upper Guinea with 18 cases each, i.e. 0.76%.

3.2. Results of blood sample analyses

Table 4 Results of detection of class G antibodies against *Leptospira* agents in the blood sera of the Guinean population by sex.

Regions Natural	Positive cases		Percentage		95% IC	
	Female	Male	Female	Male	Female	Male
	7	17	7.53	18.28	1.8-2.34	1.38-2.82
Lower Guinea	20	10	21.50	10.75	1.3-2.9	1.7-2.5
Middle Guinea	10	9	10.75	9.68	1.7-2.5	1.8-2.4
Upper Guinea	6	14	6.45	15.05	1.9-2.3	1.5-2.7
Forest Guinea	43	50	46.23	53.76	6.7-10.4	6.38-10.42
Total	93		100		13,08-20,82	

The results of this table show that the male sex with 50 positive cases, i.e. 53.76, is the most represented by *Leptospira* infection, compared with 43 cases, i.e. 46.23% for the female sex, with a Confidence Interval (95% CI) varying between [13.08-20.82]. Lower Guinea recorded 24 positive cases, i.e. 25.80%, followed by Middle Guinea with 30 cases, i.e. 32.25%, Upper Guinea with 19 cases, i.e. 20.43% and Forest Guinea with 20 cases, i.e. a prevalence of 21.51%.

The prevalence of *Leptospira* infection is higher among women in Middle Guinea (21.50%) than in the other natural regions. We also note that Lower Guinea recorded the highest rate of positivity among males at 18.28% compared with the other regions of Guinea.

The table 5 shows that the average age of patients was 45, with extremes ranging from 10 to 90 years. Of the 93 positive cases, the prevalence of *Leptospira* infection was highest in subjects aged 31 to 40, with 43 cases (46.23%), followed by individuals in the 41 to 50 age group, with 17 cases (18.27%). The prevalence was lower in the 11-20 age group, with 9 cases (9.67%), the 51-60 age group, with 7 cases (7.52%), and the 61-70 age group, with 3 cases (3.22%). No cases of *Leptospira* infection were recorded in the 0-10, 71-80 or 81-90 age groups.

Table 5 Seroprevalence of anti-*leptospira* IgG in humans, by age group

Age groups (years)	Positive cases	Percentage	95% IC
0-10	-	-	[0-0]
11-20	9	9.67	[1.8-2.4]
21-30	14	15.05	[1.6-2.6]
31-40	43	46.23	[0.3-0.039]
41-50	17	18.27	[1.4-2.8]
51-60	7	7.52	[1.81-2.39]
61-70	3	3.22	[2-2.2]
71-80	-	-	[0-0]
81-90	-	-	[0-0]
Total	93	4	[0-0]

Table 6 Prevalence of anti-*leptospira* IgG according to socio-occupational category.

N°	Socio-professional categories	Positive cases	Percentage
1	Farmers	43	46.23
2	Veterinarians	24	25.80
3	Health workers	17	18.28
4	Retired	4	4.30
5	Commercial agents	3	3.22
6	Student	1	1.07
7	Worker	1	1.07
Total		93	100

From this table, we see that farmers are the most affected by *Leptospire* infections with 43 cases, i.e. 46.23% (43/93), followed by veterinarians with 24 cases, i.e. 25.80% (24/93), health workers with 17 cases, i.e. 18.28% (17/93), retired people with 4 cases, i.e. 4.30% (4/93), and commercial workers with 3 cases, i.e. 3.22% (3/93). Students and workers were the least represented in this study, with 1 case each, i.e. a prevalence of 1.07% (1/93). This can be explained by the fact that farmers and veterinarians are in contact with animals.

4. Discussion

The discussion of patient results is based on the 2694 cases in which Antileptospire immunoglobulin class G (IgG) was detected by ELISA. The present study revealed a seroprevalence of 4% in the patients examined (93 cases/2694). This seroprevalence (4%) indicates that these patients had been in contact with the leptospirosis pathogen. Our results are lower than those found by Bertherat E et al (1999), who respectively found seroprevalences of 15% in north-eastern Gabon [11].

While people of all ages and sexes are likely to be infected, adult men, who are more often involved in high-risk activities, are more often affected. The age groups with the highest seroprevalence are 24-34 and 35-45. Thus, 54% of our patients (50/93) are male, indicating a clear predominance of this sex. This male predominance has been reported by many authors. In 2016, Dominique Blaise observed a more frequent contamination in the 26-48 age group, with a predominance of males, in his study, «Leptospirosis in the cirque of Cilaos (Ile de la Réunion)" [12].

In addition, two modes of contamination are often mentioned: direct (contact with animals) and indirect. Indirect contamination of humans occurs during work or leisure activities in an infected environment. In our study, 93/2346 cases had high-risk occupations, i.e. 4% of cases, including 43/93 (1.83% farmers), 24/93 (1.02% vets), 17/93 (0.72% healthcare workers) and 3/93 (0.12% shopkeepers). These findings have also been described in a series of studies, including:

- Swapna and al (2006) in their study "Seroprevalence of leptospirosis in high-risk groups in Calicut, North Kerala, India", the highest prevalence of the disease was observed among health professionals (56.2%), fishmongers (52.8%), bricklayers (40%), farmers (30%), sewage workers (28.2%), veterinary surgeons (13.3%) and laboratory workers (3.3%) [13].
- Yanagihara and al (2007), in their study "Current state of leptospirosis in Japan and the Philippines. Comparative immunology, microbiology and infectious diseases", 72.1% of cases were occupationally exposed, including refuse collectors, farmers and fishmongers. Nearly 80% of cases reported previous contact with suspect water (sewage, flooding) [14].

In our study, 1/93 and 4/93 were young students and retired people respectively, thus confirming the hypothesis of certain authors who have shown that leptospirosis can affect either a young population engaged in aquatic leisure activities, or a population of retired people engaged in leisure activities, for example. As demonstrated in the study by Merien F et al (2005) in New Caledonia [15].

5. Conclusion

This study on the epidemiology and enzyme-linked immunosorbent assay of *Leptospira spp.* in humans in the Republic of Guinea showed that this bacterium exists in all the natural regions of the country, with a prevalence of 4%. This study provided information on the circulation of *Leptospira* in the country's 4 geographical zones, age groups and socio-professional categories.

The existence of an immune stratum is one of the indicators of the circulation of *Leptospira* in the territory in particular, but the other concerns cases of Leptospirosis.

Compliance with ethical standards

Acknowledgments

The authors would like to thank the managers and technicians of the Russo-Guinean laboratory of IRBAG and those of the Gamal Abdel Nasser University of Conakry for their support in carrying out this work.

Disclosure of conflict of interest

No conflict of interest statement to be disclosed.

Statement of ethical approval

In accordance with international or academic standards, written ethical approval was obtained and retained by the authors.

Statement of informed consent

In accordance with international or academic standards, written parental consent was obtained and retained by the authors.

Authors' contributions

All authors contributed to this work. They have read and approved the final version of the manuscript.

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