LEPTOSPIROSIS IN THE REPUBLIC OF GUINEA

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ЛЕПТОСПИРОЗ В ГВИНЕЙСКОЙ РЕСПУБЛИКЕ

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Abstract

Leptospirosis is an acute zoonotic infection caused by pathogenic bacteria from the genus *Leptospira*. Leptospirosis creates significant though underdiagnosed public health problems in Africa. The Republic of Guinea (Guinea) is a country in West Africa, its capital is Conakry. The study objective was to assess the prevalence of the leptospirosis in febrile patients in the Republic of Guinea. We analyzed 827 blood serum samples obtained from febrile patients who applied to municipal health services, in the period from 2020 to 2024. RT-PCR was used to test 627 blood serum samples for the presence of genetic material of pathogenic *Leptospira spp.* Genotyping of positive samples was performed with the help of primers selected for a fragment of the *secY* gene. As a result of our study, IgG antibodies to *Leptospira spp*. were detected in 202 blood serum samples, which amounted to 24.4%.154 blood serum samples (18.6%) were positive for IgM to *Leptospira spp*. In 80 of the 276 positive samples both antibodies (IgG and IgM) were detected, 122 samples contained only IgG, while 74 only IgM. *Leptospira spp*. DNA was detected in 5 (0.8%) of 657 serum samples tested for the presence genetic markers of pathogenic leptospira. Sanger sequencing was applied to all 5 samples, and for three samples it resulted in successful leptospira genotyping, but it failed for two other samples may be due to insufficient amount of DNA or its degradation. When analyzed according to the BLAST algorithm, the obtained sequences revealed 100% similarity to those of *L. kirschneri* that was further confirmed by clustering when constructing the phylogenetic tree. Our study revealed high level of antibodies to leptospirosis in febrile patients, which bear witness to the wide spread of this pathogen in the Republic of Guinea. Joint research with international organizations can provide valuable information on the epidemiology of leptospirosis and improve control and prevention of the disease.

Keywords: Leptospirosis; Leptospira; Guinea; Africa; Zoonotic diseases; Seroprevalence.

Резюме

Лептоспироз – острое зооантропонозное заболевание, вызываемое патогенными видами бактерий рода *Leptospira*, и являющееся значимой, но недостаточно диагностируемой проблемой общественного здравоохранения в Африке. Гвинейская Республика (Гвинея) - страна Западной Африки, со столицей г. Конакри. Целью данной работы являлась оценка распространения лептоспироза среди лихорадящих больных, проживающих на территории Гвинейской Республики. Нами было проанализировано 827 образцов сыворотки крови, полученных от лихорадящих пациентов, обратившихся в муниципальные службы здравоохранения, в период с 2020 по 2024 год. 627 образцов сывороток крови были также исследованы методом ПЦР-РВ на наличие генетического материала патогенных видов *Leptospira spp*. Генотипирование положительных образцов проводили с использованием праймеров, подобранных к фрагменту гена *secY*. В результате проведенного исследования IgG-антитела к *Leptospira spp*. были обнаружены в 202 образцах сывороток крови, что составило 24,4%.154 образца сывороток крови были положительными на наличие IgM к *Leptospira spp*., что составило 18,6% от общего числа исследованных образцов. Из 276 положительных образцов, в 80 случаях выявлялись антитела обоих классов (как IgG, так и IgM), в 122 случаях были выявлены только IgG, а в 74 - только IgM. Из 657 образцов сывороток крови, исследованных на наличие генетических маркеров патогенных лептоспир, ДНК *Leptospira spp*. была обнаружена в 5 пробах (0,8%). Методом секвенирования по Сэнгеру успешно были генотипированы образцы лептоспир, полученные лишь из трех проб, что может быть связано с недостаточным количеством ДНК или ее деградацией в двух пробах. При анализе с использованием алгоритма Blast, полученные последовательности имели 100% сходство с последовательностями вида *L. kirschneri*, что подтвердилось кластеризацией при построении филогенетического дерева. Проведенное исследование выявило высокий уровень антител к лептоспирозу среди лихорадящих больных, что подчеркивает распространенность данного возбудителя на территории Гвинейской Республики. Совместные исследования, проводимые с международными организациями, могут предоставить ценную информацию об эпидемиологии лептоспироза, а также повысить контроль и уровень профилактических мероприятий против данного заболевания.

Ключевые слова: Лептоспироз; лептоспира; Гвинея; Африка; зоонозные заболевания; серопревалентность.

1 **Introduction**

Leptospirosis is an acute zoonotic infection caused by pathogenic bacteria from the genus *Leptospira*. Leptospirosis creates significant though underdiagnosed public health problems in Africa. The spread of leptospirosis is facilitated by the tropical climate, floods, poor sanitary and hygienic conditions, as well as contacts of humans with animals [8].

The Republic of Guinea (Guinea) is a country in West Africa, its capital is Conakry. According to the World Health Organization, 14,405,468 people lived in Guinea in 2023. In 2021 malaria was one of the main causes of death in Guinea, its mortality rate being 78.6 and 75.2 per 100,000, in men and in women respectively [18]. Many cases of leptospirosis are misdiagnosed as malaria or yellow fever due to diagnostic limitations, lack of laboratory infrastructure, ignorance of medical personnel and an unclear disease pattern of this infection [5].

The regional climate with its rainy season that brings flooding and swamping provides ideal conditions for the growth of bacteria, including *leptospira*. Limited access to clean water, poor housing conditions, unsanitary conditions and frequent contacts with farm animals and rodents also increase the risk of acquiring leptospirosis pathogen by humans [2, 19].

Pathogenic leptospires are shed in the urine of infected animals. Humans become infected through contact with urine or urine-contaminated environs through damaged skin or mucous membranes [16].

Further to the global burden of leptospirosis in African countries, there is also the risk of this infection introduction to other countries through migration, tourism or import of animals [3, 4, 15].

The study objective was to assess the prevalence of the leptospirosis in febrile patients in the Republic of Guinea.

2 **Materials and Methods**

We analyzed 827 blood serum samples obtained from febrile patients who applied to municipal health services, in the period from 2020 to 2024. Blood was sampled from inhabitants of all four landscape-climatic zones: Lower Guinea - Boké (102), Conakry (59), Kindia (45); Middle Guinea - Labé (110), Mamou (122); Upper Guinea - Faranah (170), Kankan (61); Forest Guinea - Nzérékoré (158). Blood was sampled into vacuum tubes according to the standard protocol followed by centrifugation and storage of sera samples at –70°C.

All 827 samples were examined by the enzyme immunoassay method using commercial reagent kits “Leptospirosis-IFA-IgG” and “Leptospirosis-IFA-IgM” manufactured by OMNIKS LLC, Russia, in accordance with the manufacturer’s instructions.

RT-PCR was used to test 627 blood serum samples for the presence of genetic material of pathogenic *Leptospira spp*. The reagent kit “Riboprep” was used for nucleic acids isolation, and the reagent kit “LPS Detection of pathogenic leptospires” manufactured by the Central Research Institute of Epidemiology was used to detect DNA of the pathogenic leptospires.

Genotyping of positive samples was performed with the help of primers selected for a fragment of the *secY* gene (5’- ATGCCGATCATTTTTGCTTC - 3’; 5’- GAGTTAGAGCTCAAATCTAAG - 3’); both the amplification mixture composition and the amplification program are described by us elsewhere [1]. Visualization of the amplification products was performed in 1.5% agarose gel with the addition of ethidium bromide. The corresponding electrophoresis conditions were as follows: 150 V for 20 min.

Sequencing was performed with reagents ABI PRISM BigDye Terminator v3.1 (Applied Biosystems, USA) using ABI 3500 genetic analyzer (Applied Biosystems. USA).

Identification and confirmation of genomic species was performed using the BLAST algorithm, NCBI GenBank and MEGA 11. For constructing the phylogenetic tree we used reference sequences taken from the international GenBank NCBI database (Fig. 2); the technique applied for the tree constructing was Maximum Likelihood, with a bootstrap of 1000.

The resulting sequences of the *secY* gene fragment were deposited in the international GenBank database under accession numbers PV392372–PV392374.

3 **Results**

As a result of our study, IgG antibodies to *Leptospira spp*. were detected in 202 blood serum samples, which amounted to 24.4%. The maximal percent of seropositive samples 27.5% was found in children (under 18 years), and the minimal 14.8% was detected in the most old age group (over 70 years). From the territorial distribution of patients whose serum samples contained IgG to leptospirosis pathogens one may conclude that residents of the Kindia region were most frequently infected (46.7%), but this may be due to the small size of the evaluable set. The minimal number of seropositive patients (15.6%) was detected in the Mamou area No statistically significant difference was found between males and females (24.4%).

154 blood serum samples (18.6%) were positive for IgM to *Leptospira spp*. The distribution of seropositive individuals by age and by area of residence was similar to that of IgG: the maximal percent was detected in children (24.6%) and in the residents of Kindia (31.1%), and the minimal percent was detected in people over 70 years (7.4%) and in Mamou region (9.8%). IgM were detected more often in women (21.0%) than in men (16.8%).

In 80 of the 276 positive samples both antibodies (IgG and IgM) were detected, 122 samples contained only IgG, while 74 only IgM.

Leptospira spp. DNA was detected in 5 (0.8%) of 657 serum samples tested for the presence genetic markers of pathogenic *leptospira*. All positive samples were gathered in the Nzérékoré region from residents of various age groups. Two samples contained both DNA of pathogenic *leptospira* and IgM antibodies to *leptospira*, while in three samples neither IgM nor IgG antibodies were detected. Sanger sequencing was applied to all 5 samples, and for three samples it resulted in successful *leptospira* genotyping, but it failed for two other samples may be due to insufficient amount of DNA or its degradation.

When analyzed according to the BLAST algorithm, the obtained sequences revealed 100% similarity to those of *L. kirschneri* that was further confirmed by clustering when constructing the phylogenetic tree (Fig. 2).

The length of the obtained sequences ranged from 377 to 388 nucleotide base pairs. When intercomparing the obtained sequences a single substitution of adenine for guanine was detected in sample 64, however, the international GenBank database involves some sequences with absolute similarity, which possibly testify to the variability of this nucleotide.

4 **Discussion**

A systematic review published in 2023 analysed all available studies on leptospirosis conducted in sub-Saharan Africa [8]. Total prevalence of the leptospirosis pathogen was 12.7% according to ELISA method, while 4.5% according to PCR. The review did not concern studies conducted in the Republic of Guinea, however, it involved some studies conducted in countries bordering Guinea: in Senegal the *Leptospira spp*. seroprevalence was 8% in apparently healthy population, while in Côte d'Ivoire it was 23.4% in patients with possible infectious diseases [8, 16, 10]. In Sierra Leone, *leptospira* DNA was PCR- detected in 4.2% of sera sampled from jaundice patients [20]. In an earlier review published in 2015, in febrile patients the overall leptospirosis prevalence was 19.8%, i.e. less than in this our study (24.4% for IgG) [2].

Variation in *Leptospira spp*. seroprevalence in different regions of the Republic of Guinea results possibly from the variation in climatic and geographical features and socio-economic factors. Thus, in Lower Guinea, unlike the mountainous regions, the climate is more humid and rice cultivation is widespread. In the Middle Guinea cattle breeding is developed, while Forest Guinea is characterized by hunting and eating wild animals. The prefectures of Upper Guinea are characterized by agriculture and livestock farming. In addition, trade between different towns and villages favors transmission of zoonotic diseases as farmers transport their livestock to markets in large cities [9, 11]. In our study the maximal values of IgG and IgM prevalences were detected in the Lower and Upper Guinea regions.

The difference in *Leptospira spp*. seroprevalence between men and women results may be from the regional socio-economic features. Women are often involved in rice cultivating, doing laundry at natural conditions, cleaning houses, and use protective equipment (gloves, rubber boots, etc.) less often then men. Men are more often engaged in agricultural activities, farming, hunting and fishing [13, 14]. In our study, the level of class G antibodies was the same for men and women, while class M antibodies were more often detected in women.

Low seroprevalence in the elderly patients may result both from their lower involvement in activities that involves the risk of leptospirosis infection and from their age-related impairment of immunological reactivity [7]. Quite to the contrary, high seroprevalence in children is mostly associated with their pronounced immunological response and such behavioral patterns as low personal hygiene and more frequent contact with water bodies.

In our study, genetic material of *L.kirschneri*, one of the most common representatives of pathogenic *leptospira*, was detected in three sera samples. According to published studies this type of *leptospira* is most often isolated from rodents or farm animals [6, 12, 17].

Our study revealed high level of antibodies to leptospirosis in febrile patients, which bear witness to the wide spread of this pathogen in the Republic of Guinea. Leptospirosis may be responsible for febrile illnesses often overlooked and misdiagnosed as malaria or other infectious diseases. The lack of laboratory infrastructure in many West African countries hampers diagnostics of leptospirosis. Routine laboratory techniques are largely unavailable outside of research institutions. Further research is needed to investigate risk factors and transmission dynamics. Joint research with international organizations can provide valuable information on the epidemiology of leptospirosis and improve control and prevention of the disease.

ТАБЛИЦЫ

Table 1. Results of serological testing of samples.

	Number of examined persons	*% of positive samples , IgG*	+/-	*% of positive samples , IgM*	+/-
Distribution by provinces					
Boké	102	34,3	4,7	25,5	4,3
Conakry	59	25,4	5,7	22	5,4
Faranah	170	22,9	3,2	15,3	2,8
Kankan	61	32,8	6,0	24,6	5,5
Kindia	45	46,7	7,4	31,1	6,9
Labé	110	16,4	3,5	18,2	3,7
Mamou	122	15,6	3,3	9,8	2,7
N'zérékoré	158	22,2	3,3	17,7	3,0
Distribution by gender					
Men	475	24,4	2,0	16,8	1,7
Women	352	24,4	2,3	21	2,2
Distribution by age					
1-18	349	27,5	2,4	24,6	2,3
19-29	216	21,8	2,8	15,3	2,4
30-39	97	23,8	4,3	14,4	3,6
40-49	62	25,8	5,6	16,1	4,7
50-59	40	20	6,3	12,5	5,2
60-69	36	22,2	6,9	11,1	5,2
70+	27	14,8	6,8	7,4	5,0

РИСУНКИ

Figure 1. Map of the Republic of Guinea. The names of regions where blood was sampled are given.

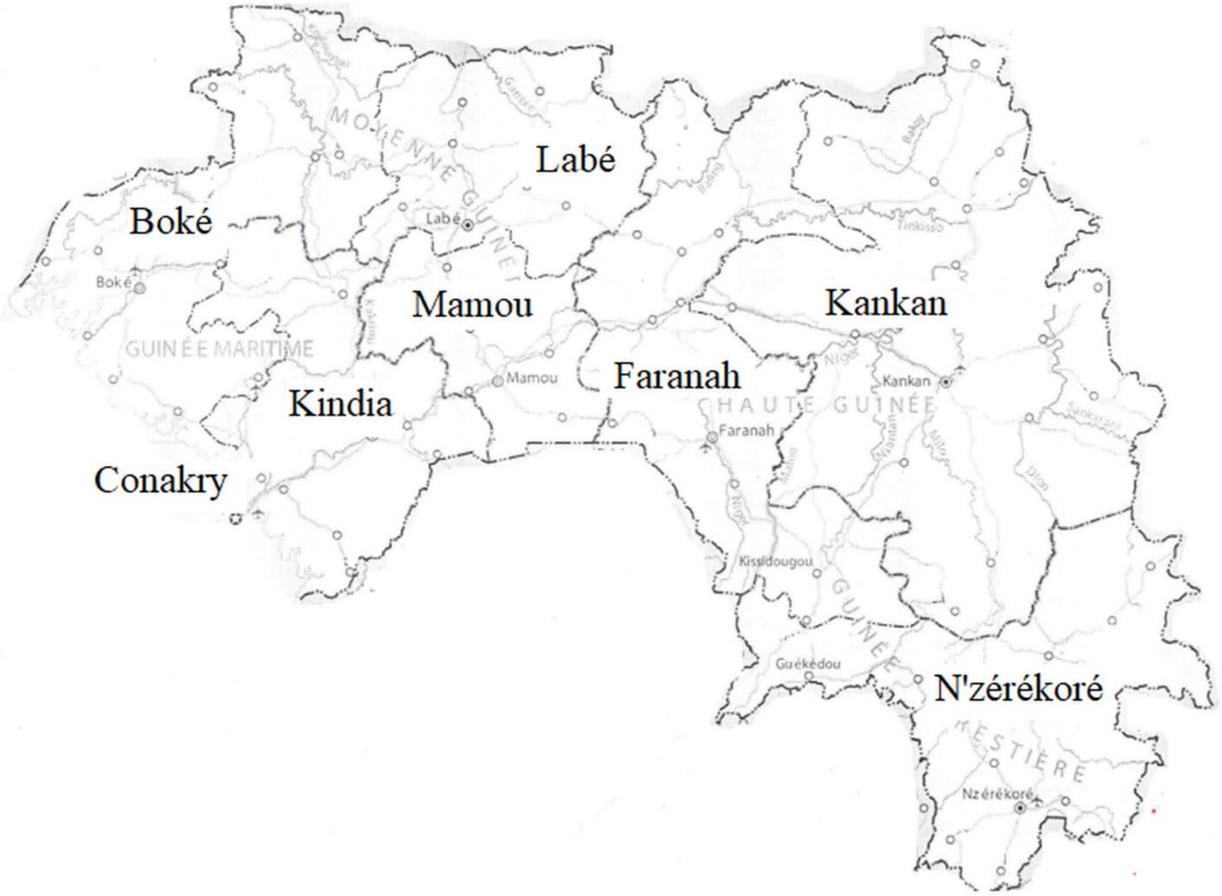
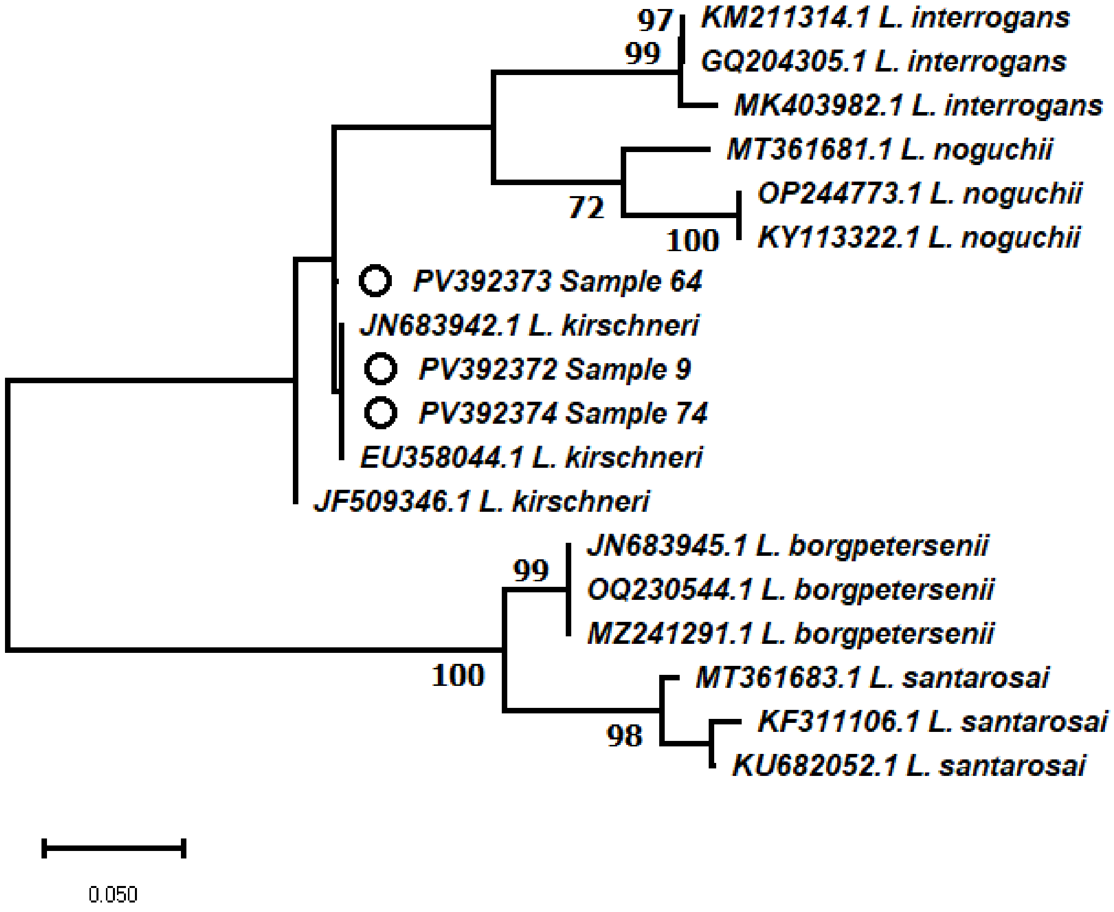


Figure 2. Phylogenetic tree constructed on the basis of the obtained sequences of the *secY* gene fragment, in comparison with the sequences available in the international GenBank database. The sequences obtained in this study are marked out.



ТИТУЛЬНЫЙ ЛИСТ_МЕТАДАННЫЕ

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Блок 3. Метаданные статьи

LEPTOSPIROSIS IN THE REPUBLIC OF GUINEA

ЛЕПТОСПИРОЗ В ГВИНЕЙСКОЙ РЕСПУБЛИКЕ

Сокращенное название статьи для верхнего колонтитула:

LEPTOSPIROSIS IN THE GUINEA

ЛЕПТОСПИРОЗ В ГВИНЕЕ

Keywords: Leptospirosis; Leptospira; Guinea; Africa; Zoonotic diseases; Seroprevalence.

Ключевые слова: Лептоспироз; лептоспира; Гвинея; Африка; зоонозные заболевания; серопревалентность.

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Номер ссылки	**Авторы, название публикации и источника, где она опубликована, выходные данные**	**ФИО, название публикации и источника на английском языке**	**Полный интернет-адрес (URL) цитируемой статьи и/или DOI**
1.	Баимова Р.Р., Рябико Е.Г., Останкова Ю.В., Токаревич Н.К. Оптимизация способа детекции и генотипирования патогенных лептоспир в биологических образцах. Клиническая лабораторная диагностика. 2025; 70 (3): 210-217	Baimova R.R., Ryabiko E.G., Ostankova Yu.V., Tokarevich N.K. (2025). Optimization of the method for detection and genotyping of pathogenic leptospires in biological samples. Clinical laboratory diagnostics (Russian journal) vol.70(3), pp.210-217	https://doi.org/10.51620/0869-2084-2025-70-3-210-217
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