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Study of the prevalence of antibodies to some arboviruses in the population of the Republic of Guinea

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Introduction. Acute febrile diseases kill more than 250,000 people annually in West Africa. Malaria and typhoid fever traditionally occupy most of the total structure of registered fevers. However, these data do not fully reflect the true overall disease patterns in the West African region. This is due to the fact that diagnosis is mainly based on the clinical signs of the infectious process, suggesting that a certain number of diseases may be caused by arboviruses. The detection of specific antibodies (ABs) to infectious pathogens in the blood sera of residents of a particular area is a reliable indicator of the circulation of these pathogens in a particular territory.

The **aim** of this study was to determine the prevalence of antibodies to a number of arboviruses: Dengue (DENV), West Nile (WNV) (family *Flaviviridae*), Crimean-Congo hemorrhagic fever (orthonairo)virus (CCHFV), Batai (Batai virus), Bhanja (BHAV) (order *Bunyavirales*), Chikungunya (CHIKV), and Sindbis (SINV) (family *Togaviridae*) in the population of the Republic of Guinea.

Material and methods. In total, a panel of 2,620 blood serum samples from people living in all landscape and geographical areas of Guinea was collected for the study. Detection of IgG antibodies was performed using an enzyme-linked immunoassay (ELISA).

Results. In total, ABs to Batai virus were detected in 144 samples (5.5%), BHAV in 58 (2.2%), WNV in 892 (34.0 %), DENV in 659 (25.2 %), CCHFV in 58 (2.2 %), CHIKV in 339 (12.9 %), and SINV in 52 samples (2.0 %).

Discussion. The obtained results indicate serological evidence of the spectrum of arboviruses in the population of all landscape and geographical zones of the Republic of Guinea, confirming their active circulation in this territory.

Conclusion. Given the high epidemiological significance of arbovirus infectious diseases, it is an urgent task to continue studying its share in the structure of febrile diseases in the territory of the Republic of Guinea.

Key words: arboviruses, Republic of Guinea, antibody prevalence, IgG immunoglobulins, enzyme-linked immunosorbent assay

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Conflict of interest. The authors declare no conflict of interest.

Ethics approval. The study was conducted with the informed consent of the patients. The research protocol was approved by the National Ethics Committee Ministry of Health of the Republic of Guinea (Approval No. 129/CNERS/16 dated August 31, 2015).

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Определение уровня иммунной прослойки населения Гвинейской Республики к некоторым арбовирусам

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Введение. Ежегодно в странах Западной Африки от острых лихорадочных заболеваний погибают свыше 250 тыс. человек. Большую часть в общей структуре регистрируемых лихорадок традиционно занимают малярия и брюшной тиф. Однако эти сведения не в полной мере отражают реальные данные обо всей конъюнктуре заболеваемости в западноафриканском регионе. Это связано с тем, что в качестве критериев постановки диагноза используются только клинические признаки инфекционного процесса, и не исключена вероятность того, что некоторое количество заболеваний может быть вызвано арбовирусами. Выявление специфических антител (АТ) к возбудителям инфекционных болезней в сыворотках крови жителей той или иной местности является достоверным показателем циркуляции этих патогенов на определённой территории.

Цель работы – определение уровня иммунной прослойки населения Гвинейской Республики (Гвинеи) к ряду арбовирусов: денге (DENV), Западного Нила (WNV, ВЗН) (семейство *Flaviviridae*); Крымской-Конго геморрагической лихорадки (СCHFV, ККГЛ), Батаи (Batai virus), Бханджа (BHAV) (порядок *Bunyavirales*); чикунгунья (CHIKV) и Синдбис (SINV) (семейство *Togaviridae*).

Материал и методы. Для работы собрана панель из 2620 образцов сывороток крови людей, проживающих во всех ландшафтно-географических зонах Гвинеи. Выявление АТ класса IgG проводилось с помощью иммуноферментного анализа (ИФА).

Результаты. Всего за период исследования АТ к вирусу Батаи выявлены в 144 (5,5%) образцах; Бханджа – в 58 (2,2%); ВЗН – в 892 (34,0%); денге – в 659 (25,2%); ККГЛ – в 58 (2,2%); чикунгунья – в 339 (12,9%) и Синдбис – в 52 образцах (2,0%).

Обсуждение. Полученные результаты указывают на наличие иммунной прослойки населения всех ландшафтно-географических зон Гвинейской Республики к данному спектру арбовирусов, что является подтверждением их активной циркуляции на этой территории.

Заключение. С учётом эпидемиологической значимости арбовирусных инфекций сохраняется актуальность дальнейшего изучения вопроса о доле инфекционных агентов этой экологической группы в общей структуре лихорадочных заболеваний, зарегистрированных на территории Гвинеи.

Ключевые слова: арбовирусы, Гвинейская Республика, иммунная прослойка, иммуноглобулины класса IgG, иммуноферментный анализ

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Introduction

According to official data from the World Health Organization (WHO), more than 250,000 people die from acute febrile illnesses in West African countries each year [1]. Malaria and typhoid fever traditionally account for the highest number of reported fever cases. In the meantime, the above data do not give a complete picture of the disease activity in the West African region. Such incompleteness comes from the limited number of diagnostic criteria, which tend to rely only on clinical manifestations of an infectious process. Laboratory testing is either unavailable or underutilized [2, 3]. After the epidemic caused by the Ebola virus (*Filoviridae*; *Ebolavirus*: *Zaire ebolavirus*) disease (EVD), which hit the Republic of Guinea (Guinea), the Republic of Liberia, and the Republic of Sierra Leone in 2014–2016; the level of laboratory diagnosis of infectious diseases increased significantly in this region. At the same time, despite the proven indication techniques used for some pathogens, there are frequent cases caused by an unidentified etiological agent. It may be explained by doctors' poor knowledge about other infections as well as by the limitedness of diagnostic

products. There is also a possibility that some febrile illnesses can be caused by arboviruses, which are frequently neglected as potential infectious agents [4]. Therefore, there are currently no research data on the circulation of these viruses in West Africa and no reliable up-to-date information about arboviral infection rates among the population of this region.

Guinea is located in the western part of the African continent. As of August 2021, its population is around 14 million people and is very diverse in its ethnic composition [5]. The country is divided into four geographic regions (**Fig. 1**). Lower or Maritime Guinea located on the Atlantic Coast of West Africa is a coastal plain lying less than 150 meters above sea level. Another region known as Middle Guinea is principally defined by the large Fouta Djallon sandstone plateau with peaks reaching 1,400 meters. This massif traverses the country from north to south. This region is characterized by prevailing savannah landscapes and occasional mountain meadows. Upper Guinea is located east of Fouta Djallon, on the plains of the upper Niger River basin, being part of the African savanna

biome. The Forest Region is an area in the country's southeastern corner. Most of the landscapes are represented by savannas, though river valleys harbor tropical rainforests. Guinea has a tropical climate with distinct wet and dry seasons. The rainy season lasts from June through October (being slightly longer in the coastal part of the country than inland); the dry season lasts from November through May. Humidity depends on the location. For example, along the coast, near Conakry, the mean annual rainfall reaches 4,300 mm, while in inland areas, the mean annual rainfall is 1,300 mm. Average monthly temperatures in most areas of the country range from 18 to 28 °C [6].

In the 1970s–1980s, in the Republic of Guinea, the Soviet-Guinean Virological and Microbiological Laboratory started research in circulation of arboviruses, including dengue virus (DENV), yellow fever virus (YFV), West Nile virus (WNV), Zika virus (ZIKV) (the *Flaviviridae* family); Crimean-Congo hemorrhagic fever virus (CCHFV) (the *Nairoviridae* family); Rift Valley fever virus

(RVFV), Batai virus (the *Peribunyaviridae* family, order *Bunyavirales*); chikungunya virus (CHIKV) (the *Togaviridae* family), and other viral agents, which can cause febrile illnesses and death of people [7]. Markers of arboviruses are also detected in biomaterials from carriers and transmitters trapped in different geographical locations in Guinea [7, 8]. However, due to the adverse economic situation developing in the country for the in recent decades, the studies on circulation of pathogens of arboviral infections were discontinued for more than 30 years. At present, the studies in this field have been resumed.

Detection of virus-specific antibodies (Abs) in blood sera of people living in a certain locality is one of the main indicators pointing at the circulation of arboviruses in the studied region and at the presence of natural clusters of arboviral infectious diseases [9]. The previous studies focused on measuring the proportion immune to arboviruses among the population of the Kindia Region (the Republic of Guinea). The obtained data showed quite high prevalence of specific Abs to WNV, DENV, CCHFV, and

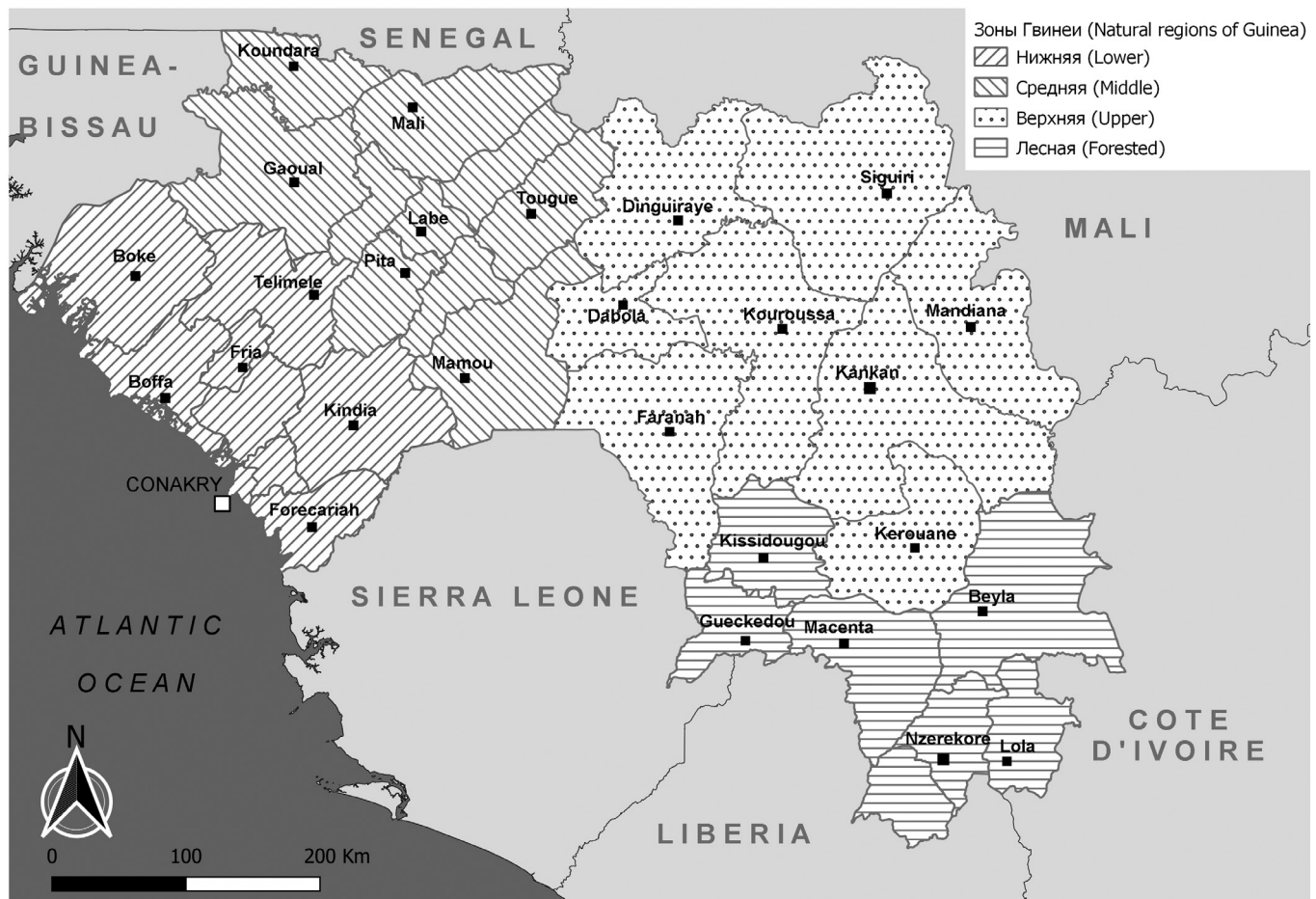


Fig. 1. Landscape and geographical zones of Guinea.
Рис. 1. Ландшафтно-географические зоны Гвинеи.

CHIKV [10, 11]. Subsequently, studies covered the entire territory of Guinea and a wider variety of arboviruses.

The study focused on a number of arboviruses, such as Dengue virus, WNV (*Flaviviridae*), CCHFV (*Nairoviridae*), Batai (*Peribunyaviridae*), Bhanja (*Phenuiviridae*), Chikungunya, and Sindbis (*Togaviridae*) viruses and used an enzyme-linked immunosorbent assay (ELISA).

Material and methods

Studies were performed by Russian and Guinean specialists at the Russian-Guinean Epidemiology and Infectious Disease Prevention Center, which was opened in 2017 and is located in the Research Institute of Applied Biology in Kindia, the Republic of Guinea [12].

Blood sera of relatively healthy people were collected by Guinean specialists at regional hospitals and delivered to the laboratory in compliance with the rules of biological safety and temperature conditions. The materials were collected by prior agreement, after the required documents had been signed.

Taking into consideration that in 2014–2016, Guinea was hit by EVD epidemic and to ensure biosafety during the research, the obtained blood samples were tested using reverse transcription-polymerase chain reaction (RT-PCR) and an AmpliSens EBOV Zaire-FL reagent kit (InterLabService Ltd., Russia). All the samples were Ebola virus RNA negative. The materials were examined using an immunochromatographic assay (ICA) to detect malaria plasmodium antigens (*Plasmodium malariae*, *Pl. vivax*, *Pl. falciparum*, *Pl. ovale*) using SD BIOLINE Malaria Ag P.f/Pan reagents (Standart Diagnostics, Inc., South Korea). The samples containing malaria antigens were not included in the subsequent studies.

Thus, the set of 2,620 blood sera from people representing all four geographic regions of Guinea was ready for tests. The study included representatives of all age groups ranging from 1 to 90 years: 1,224 (46.7%) women and 1,396 (53.3%) men.

The obtained sera were tested using ELISA to detect DENV, WNV, CCHFV, Batai, Bhanja, Sindbis, and CHIKV-specific IgG Abs. The samples were tested using reagent kits manufactured by the Bioservice Biotechnology Company, LLC (Russia) in accordance with the manufacturer's instructions. To exclude any acute phase of the disease and any risk of obtaining non-specific results, all the sera samples were also tested for presence of IgM immunoglobulins for all the above-listed viruses by using ELISA and reagent kits from the same manufacturer. The samples containing IgG Abs to 3 and more pathogens were not included in the further studies. Taking into consideration the large number of positive samples and possible cross-reactions, all the samples containing

WNV- and DENV-specific Abs were additionally tested using Anti-West Nile Virus ELISA (IgG) and Anti-Dengue Virus ELISA (IgG) reagent kits (Euroimmun, Germany). In all the cases, the results were comparable with those received earlier. Out of the above diagnostic products, the following reagent kits were registered for diagnostics: BioScreen-WNV (IgM), BioScreen-WNV (IgG) (FSR 2012/13840) (Bioservice, Russia), Anti-West Nile Virus ELISA (IgG), and Anti-Dengue Virus ELISA (IgG) (FSZ 2010/07294) (Euroimmun); the other reagents were certified for research use only.

When the results were processed, positive results in each sampling subset and 95% confidence intervals (CI) for immune proportions were calculated using the Wilson method [13]. The statistically significant differences between the immune proportions among residents of the Forest Region and the average proportions observed in the country were assessed by using Pearson's chi-squared (χ^2) test ($p = 0.05$). CI values were compared to check for presence of any statistically significant differences.

The study was performed with the informed consent of the patients. The study protocol was approved by the National Ethics Committee of the Ministry of Health of the Republic of Guinea (Approval No. 129/CNERS/16 of August 31, 2015).

Results

A total of 2,620 human sera samples were tested; arbovirus-specific IgG Abs were detected in 1,595 (60.9 % of the total number) cases, thus not excluding the possibility of infection and implying the exposure of examined people to pathogens. Positive samples were collected in all age groups, though no distinct association of immune proportions with the gender identity was found. The data on detected Abs to different arboviruses are presented in the **Table**.

Note that the tested sera most frequently contained Abs specific for 3 pathogens (WNV-specific 34.0%, dengue virus-specific 25.2%, and chikungunya virus-specific 12.9%) (**Fig. 2**).

Discussion

In Guinea, WNV immune people comprise one of the highest proportions (34.0% of all tested sera). This high rate of prevalence of Abs to this pathogen is statistically significant compared to the similar rate demonstrated by IgG immunoglobulins specific for other arboviruses, which is supported by the absent CI transgression (**Table**).

Interestingly, WNV-specific IgG Abs were detected much more frequently in residents of the Forest Region (39.5%) than throughout the country. The absent CI transgression also implies significance of the observed differences. At the same time, among residents of Upper Guin-

ea, Abs specific for this infection were detected much more rarely than generally in the country (25.1%).

Dengue viruses account for higher proportions in terms of immunity to arboviruses. As seen from the **Table**, the detected DENV-specific Abs account for 25.2% of the to-

tal number of sera samples. Most of the positive results were found in samples collected in the Forest Region (32.8% of cases). The lowest number of samples containing Abs to dengue viruses was recorded in Upper Guinea (16.5%). Similarly to WNV-related results, the statisti-

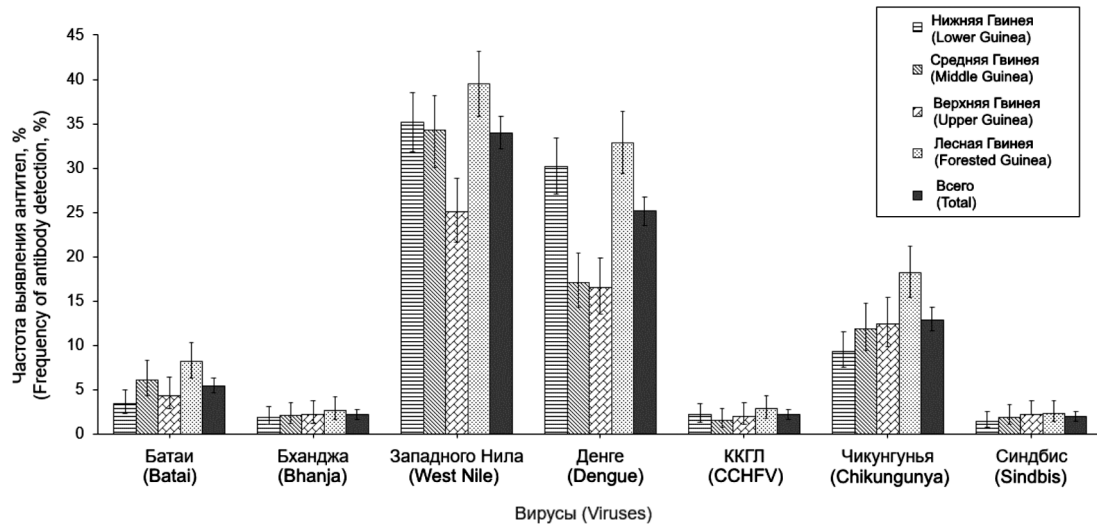


Fig. 2. Distribution of detection rates of specific antibodies to arboviruses in different landscape and geographical zones of the Republic of Guinea.

Рис. 2. Распределение специфических антител к арбовирусам в разных ландшафтно-географических зонах Гвинейской Республики.

Results of detection of antibodies to arboviruses in various landscape and geographical zones of the Republic of Guinea

Результаты выявления антител к арбовирусам в различных ландшафтно-географических зонах Гвинейской Республики

Landscape and geographical zones Ландшафтно-географические зоны	Total number of tested samples, <i>n</i> Общее количество исследованных образцов, <i>n</i>	Viruses Вирусы						
		Results of detection of IgG antibodies: number of positive samples, <i>n</i> ; proportion of positive samples, %; CI						
		Результаты выявления антител класса IgG: количество положительных проб, <i>n</i> ; доля положительных проб, %; ДИ						
		Batai Багаи	Bhanja Бханджа	West Nile Западного Нила	Dengue Денге	CCHFV ВККГЛ	Chikungunya Чикунгунья	Sindbis Синдбис
Lower Guinea Нижняя Гвинея	799	28 3,5 2,4–5,0	15 1,9 1,1–3,1	281 35,2 31,9–38,5	241 30,2 27,1–33,4	18 2,3 1,4–3,5	75 9,4 7,6–11,6	12 1,5 0,8–2,6
Middle Guinea Средняя Гвинея	578	35 6,1 4,4–8,3	12 2,1 1,2–3,6	198 34,3 30,1–38,2	99 17,1 14,3–20,4	9 1,6 0,8–2,9	69 11,9 9,5–14,8	11 1,9 1,1–3,4
Upper Guinea Верхняя Гвинея	545	24 4,4 2,9–6,5	12 2,2 1,3–3,8	137 25,1 21,7–28,9	90 16,5 13,6–19,9	11 2,0 1,1–3,6	68 12,5 9,9–15,5	12 2,2 1,3–3,8
Forest Guinea Лесная Гвинея	698	57 8,2 6,4–10,4	19 2,7 1,7–4,2	276 39,5 35,9–43,2	229 32,8 29,4–36,4	20 2,9 1,8–4,4	127 18,2 15,5–21,2	17 2,4 1,5–3,8
Total across the country Итого по всей стране	2620	144 5,5 4,7–6,4	58 2,2 1,7–2,8	892 34,0 32,2–35,9	659 25,2 23,5–26,8	58 2,2 1,7–2,8	339 12,9 11,7–14,3	52 2,0 1,5–2,6

Note. CCHFV, Crimean-Congo hemorrhagic fever (orthonairo)virus; CI is the confidence interval.

Примечание. ВККГЛ – вирус Крымской-Конго геморрагической лихорадки; ДИ – доверительный интервал.

cally significant differences between DENV-immune proportions of the population of the Forest Region (significantly higher) and Upper Guinea (significantly lower) were identified and compared with average proportions in the country.

The proportion of the population with immunity to CHIKV was quite high (12.9% of the total number of samples). Note that most of the positive results (18.2%) were detected in sera from people living in the Forest Region (significantly higher than overall average in the country), and the lowest number was detected in samples from Lower Guinea (9.4%).

The analysis of prevalence of antibodies to Batai virus (5.5% of the total number of samples) showed that their detection rate is much higher in the Forest Region than in other regions, accounting for 8.2%. Compared to other regions, the differences are statistically accurate, as no CI transgression is observed.

The assessment of levels of humoral immunity to the other arboviruses in the country demonstrated the following: CCHFV and Bhanja-specific IgG immunoglobulins were present in 2.2 % of the tested samples and Sindbis virus-specific IgG immunoglobulins were detected in 2.0% of the samples. Although the highest number of positive samples was detected in blood sera from residents of the Forest Region (suggesting the high probability of people's contact with pathogens), the CI transgression in the Ab detection rates challenges the statistical significance of differences.

Conclusion

Thus, the obtained results suggest that a wide variety of arboviruses circulates in all regions of Guinea. Note that the most active circulation of pathogens of arboviral infections is recorded in the Forest Region where the prevalence of WNV, DENV, Batai, and CHIKV-specific IgG Abs are significantly higher than average prevalence rates across the country; the lowest circulation is observed in Upper Guinea. This fact can be explained by differences in climatic conditions, population numbers and species composition of reservoirs and vectors in these regions.

Taking into consideration high epidemic proneness of arboviral infections, it is extremely important to identify their place among febrile illnesses recorded in Guinea. Besides, regular epizootological monitoring is required for detection and species identification among reservoirs /vectors of arboviruses in different geographic regions of Guinea.

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