Scientific Research Publishing

Journal of Biosciences and Medicines, 2025, 13(4), 99-114
https://www.scirp.org/journal/jbm
ISSN Online: 2327-509X
ISSN Print: 2327-5081

SARS-CoV-2 Detection on One Laboratory of COVID-19 Monitoring in Douala Cameroon and Associated Risk Factors of Viremia

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How to cite this paper: Ngo-Malabo, E.T., Nda Mefo'O, J.-P., Mbele Onana, C.L., Mengue, E.R., Wangso Wanlao, D., Olemba, C., Akono, C., Imandy, G., Fanga, B., Temb, A., Ngounou, C., Mouchilli, L., Ngo Ngue, M., Dikum, S., Tchatchueng Mbougua, J.B., Eyangoh, S.I., Wanji, S., Okalla Ebongue, C. and Namme Luma, H. (2025) SARS-CoV-2 Detection on One Laboratory of COVID-19 Monitoring in Douala Cameroon and Associated Risk Factors of Viremia. *Journal of Biosciences and Medicines*, **13**, 99-114.
https://doi.org/10.4236/jbm.2025.134010

Received: February 5, 2025
Accepted: April 11, 2025
Published: April 14, 2025

Abstract

Background: As soon as the COVID-19 epidemic outbreak in Cameroon, the Douala General Hospital (DGH) was performed to monitor the evolution of this infection with RT-PCR analysis. The aim of this study was to identify risk factors associated with viremia of samples analyzed by molecular biology at the Douala General Hospital laboratory, in the context of a major SARS-CoV-2 epidemic. **Methodology:** A cross-sectional study was carried out in the Molecular Biology Unit of the Clinical Biology Laboratory at the Douala General Hospital. All nasopharyngeal samples received for diagnosis or screening by PCR were analyzed. Samples were obtained from approved sampling centers in Douala (11 Health Districts) and from many departments at the DGH. Real-time RT-PCR targeting the N and Orf genes using the "Daan Gene Co., Ltd., Sun Yat-sen University" kit was performed according to the manufacturer's recommendations. The results were interpreted according to the appearance of the gene signal, with a detectability threshold of 37 cycles. A sample was declared positive if both genes had a Ct < 37. Statistical analysis was performed

using SPSS 22.0 software and quantitative results (Ct values for positive results) were collected. **Results:** From 28 December 2020 to 31 December 2022, 45,243 non-repeated samples were received and analyzed. The sex ratio was 1.52. The average age was 40 years (0 - 104), and the most represented age group was 30 - 45 years (39.4%). Of these samples, 47.2% (21,351/45,243) and 46.6% (21,065/45,243) came from travelers and contact cases, respectively. Only 2.9% (1304/45,243) were symptomatic. Molecular analysis revealed an overall positivity rate of 8.4% (3808/45,243). A proportion of 31.1% (1185/3,808) was classified as highly positive with CT values < 30. The year 2021 recorded the highest number of tests performed, 30,144 (66.6%), and the highest positivity rate, 11.2% (3376/30,144), as compared to 2022, having a positivity rate of 2.9% (432/15,099). **Conclusion:** RT-PCR was used to establish an adequate biological diagnosis of SARS-CoV-2 infection in a highly turbulent health context. In the context of a major epidemic, this technique could be necessary for the follow-up of this infection, with a clear diagnosis of low viral load (CT > 30) with seasonal peaks in positivity.

Keywords

SARS-CoV-2, RT-PCR, Orf and N Genes, Risk Factors, Douala General Hospital

1. Introduction

The Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), responsible for the COVID-19 infection, was first identified in 2019 [1]. In January 2020, this outbreak was declared a public health emergency of international concern, and by March 2020, it was classified as a pandemic [2]. Since its emergence, the virus has spread rapidly worldwide. Epidemiological data on the infection was widely across continents and countries [3]. North America was the most affected region, with almost 50% of cases and 47% of deaths, while Africa had recorded 216,999 cases for 4874 deaths [4]. In Cameroon, the first case was reported on March 6, 2020, and within days, it declared a state of emergency that included travel bans, lockdowns, widespread testing, and quarantine [5]. As of May 30, 2020, the Ministry of Public Health, which regularly communicated on figures at a national level, reported 5659 persons tested positive, 185 deceased and 3441 recovered cases [6]. The perceived risk of contracting the disease prompted several governments to implement various control measures. By the end of June 2022, the pandemic had affected 224 countries and territories, with 518,801,767 cases recorded globally; in Africa, 11,859,004 cases were confirmed, and in Cameroon, 119,947 cases were reported [3]. SARS-CoV-2 is an enveloped Ribonucleic Acid (RNA) virus belonging to the Coronaviridae family [7].

As part of the fight against this disease, several diagnostic methods have been developed, ranging from clinical diagnosis, including assessment of the patient's condition, chest X-rays, and CT scans, to biological diagnosis. Biological diagnosis in-

volves detecting antibodies through serological tests (e.g. rapid tests, ELISA, LFA, and CLIA) and molecular tests (e.g. PCR) to identify viral antigens [8]. Efforts to control the spread of COVID-19, such as the implementation of quarantine and isolation measures and clinically appropriate patient management, all require effective screening and diagnostic tools. The WHO published guidelines for the follow-up of COVID-19 cases on January 31, 2020 [4]. Studies have shown that, in the context of a major epidemic, RT-PCR plays a crucial role in patient management, particularly in decisions to isolate patients and in ruling out differential diagnoses [8] [9].

Cameroon has actively participated in the fight against this virus. Several diagnostic techniques, both serological and molecular, have been used in this country to facilitate diagnosis and patient management. Taking into account the cost of some of these reagents, the ease of use they offer and the time it takes to carry out tests from analysis to delivery of results, these reagents have been standardized. A national response program was established, including a diagnostic algorithm and a selection of serological and molecular tests for diagnosis in line with WHO recommendations [10]. A health coverage pyramid was set up, comprising accredited analysis laboratories, patient isolation follow-up centers, and containment facilities. In this framework, RT-PCR techniques targeting the ORF1ab and N genes, widely used in laboratories worldwide, were adopted for COVID-19 diagnosis in Cameroon. These techniques enable the determination of relative viral load, which is generally associated with transmission risk, disease severity, and the morbidity and mortality linked to this virus [11] [12].

To monitor and control the COVID-19 epidemic in Cameroon, laboratory confirmation was done initially by the Centre Pasteur du Cameroun, a reference biological laboratory. Then, several laboratories were chosen and designated as reference centers for antigen and PCR testing by regions in Cameroon. It is in this setting that the Molecular Biology Unit of the Clinical Biology Laboratory at the Douala General Hospital (DGH) was established for the littoral region in the city of Douala. Douala is the economic capital of Cameroon, which has the busiest international airport in the country. It is a cosmopolitan city, recording the largest number of confirmed cases in the country. It is a town of about 3.7 million inhabitants as of 2015. Several studies have been carried out on COVID-19 and its associated factors in the city of Douala, most of them on hospitalized patients [5] [13]. However, given the national and international human interactions particularly in this city and general in the littoral region, with the presence of an international airport and even a deep-water port, it would be interesting to know the impact of this context on the epidemiology of SARS-CoV-2 in this region. This is why the aim of this study was to identify risk factors associated with viremia of samples analyzed by Molecular Biology Unit at the Douala General Hospital Laboratory after two years of monitoring, in the context of a major SARS-CoV-2 epidemic.

2. Methodology

2.1. Type, Location, and Study Period

A retrospective cross-sectional study was conducted in the Molecular Biology Unit

of the Clinical Biology Laboratory at Douala General Hospital from January 2021 marking the beginning of SARS-CoV-2 RT-PCR activity at this hospital, to December 31, 2022. DGH was the designated focal point for reception, treatment, and isolation of COVID-19 cases in the Littoral region. It is a first-category multidisciplinary specialist hospital equipped with a level-2 laboratory featuring state-of-the-art equipment. The laboratory comprises seven units: hematology, parasitology, bacteriology, serology, biochemistry, molecular biology, and a blood bank.

2.2. Study Population, Inclusion Criteria, and Sampling

All samples received during the two-year study period were included. These samples originated from the aggregated health districts of Douala (Bangue, Cité des Palmiers, Logbaba, Japoma, Deido, Bonassama, Nylon, Boko, Congo II, Douala delegation and New Bell), hospital departments (outpatient units, emergencies, pediatrics, hemodialysis, gynecology, isolation Internal and external medicine, and other inpatient units), or the DGH aggregated sampling site. The samples consisted of nasopharyngeal swabs collected for the diagnosis or screening of SARS-CoV-2 infection. Samples were collected every weekday between 6 a.m. and 3 p.m. at the above-mentioned sampling sites. Once the daily sampling was complete, the samples were transported on the same day to DGH laboratory molecular biology unit.

For each sample, forms were completed to capture information on socio-demographic data (name, age, sex, place of sampling, etc.), epidemiological context (contact case, traveler, sporting event), clinical context (symptoms suggestive of infection or follow-up), the date, and the reason for sampling. Samples were collected using single-use plastic swabs with nylon or cotton tips. After collection, the swab was placed into 1 ml of viral transport medium contained in a 1.5 ml screw-top tube. The samples were packed in triplicate with ice packs. Once the accompanying information was verified and the samples were registered, they were promptly sent to the Molecular Biology Unit of the laboratory, adhering to proper biological substance transport conditions (swabs in viral transport medium maintained at 2˚C - 8˚C).

In the laboratory, samples stored at +4˚C were either analyzed immediately or within 48 hours. Samples were excluded from the study if they were submitted in unlabeled tubes, with non-compliant transport media, or without adhering to proper transport conditions.

2.3. Technical Analysis

2.3.1. Viral RNA Extraction

We used a manual extraction as follows. Nucleic acids were extracted using the RNA/DNA purification kit (Spin Column) (Daan Gene Co., Ltd, Schiffgraben, Germany) according to the manufacturer's instructions [1]. The extraction process involved separating negatively charged RNA from other components of the biological sample using a positively charged silica column through ion-exchange chromatography, following the analysis of cell membranes and precipitation of the ex-

tracted materials. After a washing step, the extracted product was eluted in 50 µl of pre-warmed elution solution. The elevators were stored under the following conditions based on the intended amplification timeline: at 2˚C - 8˚C for immediate amplification, at −20˚C for amplification within 24 hours, or at −70˚C for amplification beyond 24 hours.

2.3.2. Amplification

The "Detection Kit for 2019 Novel Coronavirus (2019-nCoV) RNA™ (PCR-Fluorescence Probing)" (DAAN GENE CO., Ltd., Schiffgraben, Germany) was used for real-time RT-PCR amplification following the manufacturer's recommendations [14]. The viral genes targeted were ORF1ab (Open Reading Frame1) and N (Nucleocapsid) as well as an endogenous human fragment (as an internal control for the analysis). The viral genes targeted were ORF1ab (Open Reading Frame 1) and N (Nucleocapsid), as well as an endogenous human fragment (as an internal control for the analysis). These three fragments were detected by probes specific to each target: FAM (maximum emission at 518 nm) for the N gene, VIC (maximum emission at 552 nm) for the ORF1ab gene, and CY5 (maximum emission at 667 nm) for the endogenous internal control. The internal control is used to guarantee the presence of human cells, validating RNA extraction, absence of inhibitors, and PCR amplification, thereby reducing the risk of false negative results. The volume of the amplification reaction mix was 25 µl. This included 20 µl of PCR mix and 0.5 µl of RNA extract. The AMPLIlab™ thermal cycler (Adaltis) was used for the different amplifications. The thermal profile was as follows: 45˚C for 10 min, 95˚C for 2 min, and 45 cycles (95˚C for 15 s, 60˚C for 30 s). The total analysis time was approximately two hours.

2.3.3. Results Interpretation and Turnaround Time

Results were interpreted according to the manufacturer's recommendations, based on the appearance of 3 genes at a detectability threshold of 37 cycles (37 Ct). Any sample showing an obvious amplification curve in the FAM and VIC wells for a Ct value < 37 was considered positive for coronavirus 2019 (SARS-CoV-2) infection. Any sample marked by the absence of an amplification curve in wells FAM and VIC or by the presence of an amplification curve with a Ct value > 37 in wells FAM and VIC and with an amplification curve in well Cy5 was considered negative for coronavirus 2019 infection (SARS-CoV-2). Where the sample tested had a Ct value < 37 in a single FAM or VIC well, with an amplification curve in the Cy5 well, the sample was classified as doubtful and retested. If the results were consistent, it was concluded that the sample was positive for coronavirus 2019 (SARS-CoV-2) infection. If, on the other hand, the results of the retest were negative, the sample was negative for this infection. In the event of amplification inhibition (absence of amplification curves on the three genes), the series was retested by including a 1/10 dilution of the RNA extract. Following the Ct threshold values defined by the control and surveillance strategy for SARS-CoV-2 infection in Cameroon, samples found positive for this infection were classified into two main groups: strong posi-

tives (Ct < 30) and weak positives (30 < Ct < 37).

2.4. Statistical Analyses

Socio-demographic and clinical data, qualitative results (positive, negative), and quantitative results (Ct values for positive results) were collected using an Excel spreadsheet. The various proportions were calculated with a 95% confidence interval. Univariate and multivariate logistic regression tests were performed to assess the risk factors associated with positivity. Only tests returning values of $P < 0.05$ were considered to show significant differences. All analyses were performed using IBM SPSS Version 22.0 software.

3. Results

During the two years of activity in the diagnosis of COVID-19 at the Douala General Hospital, 45,517 nasopharyngeal samples were analyzed by PCR at the Molecular Biology Unit of the Clinical Biology Laboratory of this hospital.

3.1. Samples Origin

Overall, most of these samples, 31,753 (75.5%), came from the health districts of the city of Douala, while 2207 (5.0%) had been collected during the various major sporting events held in Cameroon. The remaining 7625 samples (19.5%) came from the various medical departments of the Douala General Hospital and from the aggregate sampling site available within the same hospital (Table 1).

Table 1. Samples origin (N= 45,517).

Sample origin	Number of samples	Percentage (%)
Sports meeting	2207	5.0
Health districts	31,753	75.5
Douala general hospital	7625	19.5

Global distribution of samples received for analysis by sampling site. The percentage of the different groups is the ratio between the number of people in each group (number of samples) and the total number of samples received in the study (N = 45,517).

3.2. Socio-Demographic Data and PCR Results

In this study, sex ratio was 1.52 with predominance of men at 60.3% (27,441/45,517) to 39.7% of women (18,076/45,517) (Table 2). The people tested ranged in age from 0 to 95 years, with a median of 40 years. The age group most represented was [30 - 44] years (39.4%) (Table 2).

The main reasons for testing were travel and contact cases: 46.9% (21,351/45,517) and 45.1% (20,517/45,517), respectively. Symptomatic people accounted for 3.0% of all people tested. Majority of the tests were carried out in the year 2021 (30,143; 66.2%) and the year equally had the most positive results (3380; 11.2%). Overall, most of the samples analyzed, 41,622 (91.4%), were PCR negative, and 3895 (8.6%)

were PCR positive.

Table 2. PCR results and general characteristics of the study population.

Categories		PCR results (n_1)		Total$_1$	
		Negative (%)	Positive (%)	n_2	%
Gender	Male	25,230 (91.9)	2211 (8.1)	27,441	60.3
	Female	16,392 (90.7)	1684 (9.3)	18,076	39.7
Age ranges	0 - 14	2224 (93.7)	149 (6.3)	2373	5.2
	15 - 29	8092 (92.1)	696 (7.9)	8788	19.3
	30 - 44	16,520 (92.0)	1432 (8.0)	17,952	39.4
	45 - 64	11,824 (91.1)	1150 (8.9)	12,974	28.5
	65+	2292 (85.9)	377 (12.0)	2669	5.9
	No information	670 (88.0)	91 (12.0)	761	1.7
Samples collection year	2021	26,763 (88.8)	3380 (11.2)	30,143	66.2
	2022	14,859 (96.7)	515 (3.3)	15,374	33.8
Testing reason	Symptomatic	962 (70.0)	413 (30.0)	1375	3
	Contact cases	19,071 (93.0)	1446 (7.0)	20,517	45.1
	Travel	19,382 (90.8)	1969 (9.2)	21,351	46.9
	Sports meeting	2207 (97.1)	67 (2.9)	2274	5
Total$_2$ (%)	Each total result	41,622 (91.4)	3895 (8.6)	45,517	100

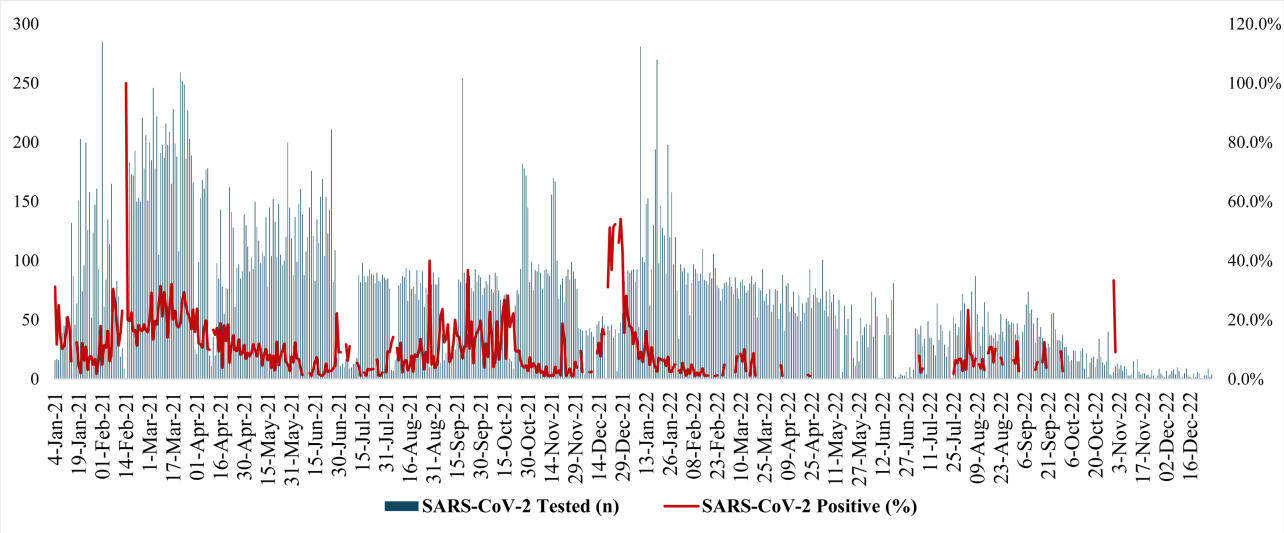
Overall view of positives results. The percentage of the different groups is the ratio between the number (n_1) of each group and the number (n_2) of the corresponding line. The percentage of **total$_1$** is the ratio between the total number of each line of category and the total number of samples received in the study (N = 45,517). The percentage of **total$_2$** is the ratio between the total number of each Colom of PCR results and the total number of samples received in the study (N = 45,517).

3.3. Positives Results Analysis

Analysis of the results for the two years shows an overall positivity rate of 8.6% (3895/45,517) (Table 2). For 2021, the highest number of tests was carried out in March, but the highest positivity rate was February over the year. For 2022, the highest number of tests was carried out in January, and the highest positivity rate was also recorded in the same month. December was the month with the lowest activity in both years (Figure 1). Four peaks in COVID-19 epidemics were observed over the two years, in March and September 2021 and January and august 2022, with rates of 33.1%, 6.4%, 53.4%, and 12.4%, respectively per months.

3.4. Positivity Variation According to Ct Values

In this study, positives were grouped into 3 categories. The positive cases with Ct values below 30 (strong positives), the positives cases with Ct values between 30 and 37 (weak positives), and the last category concerns samples judged positive



In blue: The number of samples received per day. In brown: The percentage of positives obtained per day during the study period. The percentage of positives obtained per day is the ratio between the positives obtained per day and the total number of samples received this day.

Figure 1. Evolution of the positive rate during the two years.

by the expression of only one of the two genes tested (potential positives). Analysis including these three divisions showed that more than half of the positive samples obtained in this study (50.6%) were weak positives and that only 8.7% of these samples could be classified as potential positives (Table 3). Observation of the variation in Ct according to the reason for testing the samples revealed that most of the samples with high positivity thresholds came from symptomatic cases (56.7%). In contrast, for the other reasons for testing, contact cases, travel, and sporting events, most Ct values were between 30 and 37 (50.1%, 53.6%, and 47.8%, respectively). All this information is presented in Table 3.

3.5. Associated Factors for Positivity

Univariate and multivariate analysis showed that among the 3895 positive samples, high viremia was associated with the test pattern parameter, with a risk of 1.7 of having high viremia in symptomatic patients compared with contact cases, travelers, and sport encounters (p = 0.001 [1.3 - 2.3]). According to gender, men had a higher risk of high viremia than women OR = 0.811 (0.708 - 0.929), p = 0.003. December was the month with the highest incidence of high viremia, unlike the other months of the year (Table 4).

4. Discussion

Declared a pandemic in March 2020, the SARS-CoV-2 infection responsible for COVID-19 has become a public health problem with many cases reported and many deaths [1]. The aim of our study was to make an assessment of the molecular diagnosis of COVID-19 two years after the introduction of this technique in the Molecular Biology Unit of the Clinical Biology Laboratory at Douala General

Table 3. Variation of Ct according to test purpose.

Categories		Ct values [n_1 (%)]			Total$_1$	
		Ct < 30	30 < Ct < 37	Ct < 37 single gene	n_2	%
Reason of testing	Symptomatic	234 (56.7)	160 (38.7)	19 (4.6)	413	10.6
	Contact cases	595 (4.1)	724 (50.1)	127 (8.8)	1446	37.1
	Travel	740 (37.6)	1055(53.6)	174 (8.8)	1969	50.6
	Sportive Meeting	18 (26.9)	32 (47.8)	17 (25.4)	67	1.7
Age ranges	0 - 14	61 (40.9)	79 (53.0)	9 (6.0)	149	3.8
	15 - 29	291 (41.8)	353 (50.7)	52 (7.5)	696	17.9
	30 - 44	584 (40.8)	709 (49.5)	139 (9.7)	1432	36.8
	45 - 64	445 (38.7)	611 (53.1)	94 (8.2)	1150	29.5
	65+	162 (43.0)	182 (48.3)	33 (8.8)	377	9.7
	No information	44 (48.4)	37 (40.7)	10 (11.0)	91	2.3
Samples origin	Health Districts	982 (37.9)	1392 (58.9)	18 (8.4)	2591	66.5
	DGH	587 (47.5)	547 (49.6)	103 (8.3)	1237	31.8
	Sports meeting	18 (37.0)	32 (46.2)	17 (25.4)	67	1.7
Samples collection year	2021	1367 (40.4)	1687 (49.9)	326 (9.6)	3380	86.8
	2022	220 (42.7)	284 (55.1)	11 (2.1)	515	13.2
Total$_2$ (%)	**Ct category/value**	**1587 (40.7)**	**1971 (50.6)**	**337 (8.7)**	**3895**	**100**

Overall view of the variation in positivity according to the Ct and the reason for testing. The percentage of the different groups is the ratio between the number (n_1) of each group and the number (n_2) of the corresponding line. The percentage of the different groups for total$_{1\&2}$ is the ratio between the total number of samples corresponding to the Ct value **(for total$_1$)** or corresponding to the reason for testing **(for total$_2$)** and the total number of samples found positive in the study (N = 3895).

Table 4. Multivariate analysis associated factors with high viremia (positive samples: **N = 3895**).

Categories		Ct Values [n_1 (%)]				95% CIS2		
		Total (N = 3895)	High viremia (n = 1587)	Low viremia (n = 2308)	Adjusted Odds ratio	Lower	Upper	p-value
Reason of testing	Travel	1969 (50.6)	740 (37.6)	1 229 (62.4)	Reference			
	Contact cases	1446 (37.1)	595 (41.1)	851 (58.9)	1.040	0.820	1.319	0.748
	Sportive meeting	67 (1.7)	18 (26.9)	49 (73.1)	/	/	/	0.142
	Symptomatic	413 (10.6)	234 (56.7)	179 (43.3)	1.745	1.303	2.336	0.001
Gender	Male	2211 (56.8)	926 (41.9)	1 285 (58.1)	Reference			
	Female	1684 (43.2)	661 (39.3)	1023 (60.7)	0.811	0.708	0.929	0.003
Samples origin	Sports meeting	67 (1.7)	18 (26.9)	49 (73.1)	Reference			
	DGH	1237 (31.8)	587(47.5)	650 (52.5)	1.806	0.957	3.409	0.068
	Health districts	2591 (66.5)	982 (37.9)	1609 (62.1)	1.538	0.860	2.749	0.146

Continued

Months of samples collection	December	229 (5.9)	167 (72.9)	62 (27.1)	Reference			
	November	96 (2.5)	13 (13.5)	83 (86.5)	0.061	0.032	0.118	<0.001
	October	211(5.4)	55 (26.1)	156 (73.9)	0.137	0.089	0.209	<0.001
	September	247 (6.3)	114 (46.2)	133 (53.8)	0.304	0.205	0.451	<0.001
	August	191 (4.9)	115 (60.2)	76 (39.8)	0.521	0.337	0.805	<0.001
	July	65 (1.7)	33 (50.8)	32 (49.2)	0.367	0.203	0.661	<0.001
	Jun	121 (3.1)	40 (33.1)	81 (66.9)	0.187	0.113	0.309	<0.001
	May	239 (6.1)	60 (25.1)	179 (74.9)	0.130	0.084	0.201	<0.001
	April	350 (9.0)	127 (36.3)	223 (63.7)	0.217	0.147	0.318	<0.001
	March	1168 (30.0)	484 (41.4)	684 (58.6)	0.289	0.205	0.406	<0.001
	February	537 (13.8)	220 (41.0)	317 (59.0)	0.277	0.193	0.398	<0.001
	January	441 (11.3)	159 (36.1)	282 (63.9)	0.226	0.158	0.324	<0.001

Hospital. Although Africa has been qualified as having an unsatisfactory level of diagnosis in the face of this pandemic, our laboratory has nevertheless been able to carry out a large number of screening tests for COVID-19 by RT-PCR. During the first two years of activity in this unit, 45,517 samples of nasopharyngeal swabs were analyzed by RT-PCR from January 2021 to December 2022. After analysis, the results showed an overall positivity rate of 8.6% over the two years (**Table 2**). This positivity rate is similar to another epidemiological study conducted in Douala [15]. Douala is one of big town of Cameroon and is therefore fairly heterogeneous and representative of the general population.

The distribution of the samples analyzed according to social demographic data showed that most of these samples came from the health districts of the city of Douala; 19.5% came from the various departments of the Douala General Hospital, and only 5.0% had been collected during the various major sporting events held in Cameroon during which Douala was a focal point. More men than women were tested. The ages ranged from 0 to 95 years, with a median of 40 years (30 - 49 years), and the most represented age group was [30 - 44] years. Our results are similar to those obtained in an epidemiological study in Cameroon, where the predominant age group was 30 - 49 years [16]. These data also confirm the results published by the WHO in 2022, which showed that the 30 - 49 years age group is the most commonly tested and that males are the most affected in Africa [17]. The main reason for testing was (46.9%) and contact cases (45.1%); symptomatic people accounted for only 3.0% of samples tested (**Table 2**). In order to contain the spread of this pandemic, protocols for testing all symptomatic cases, their contacts, and people wishing to travel was mandatory in the country. At Douala General Hospital, all admissions to hospital wards for hospitalization were validated by a negative COVID PCR test. All these requirements could justify the low rate of symptomatic people tested compared with contact cases and travelers.

Observation of the positive results over the two years showed that 2021 was the year in which the greatest number of tests was carried out, and this year also recorded the highest positivity rate compared with 2022 (**Figure 1**). These data show a decrease in the frequency of detection of COVID-19 at HGD over these two years, from 11.2% in 2021 to 3.3% in 2022, a decrease of 7.9%. In 2022, Fokam *et al.* reported a detection rate of 12.7%, which is very close to our results [18]. These results are in line with national and even international trends over the same periods. WHO reported overall increases in case and death incidence for SARS-CoV-2 infection in the word in 2021, while a general decrease of the cases on 2022 [17] [19]. This epidemiological situation could be the result of good management of the epidemic by the public authorities in our context. In order to limit the spread of SARS-CoV-2, the Cameroonian government had put in place a number of prevention strategies and security measures in line with international recommendations, including containment, awareness campaigns, rapid and systematic testing using rapid antigen diagnostic tests (Ag-TDR), implementation of molecular tests in all regions of Cameroon, and establishment of genomic surveillance inside and outside the country [20].

During our study period, four peaks of COVID-19 epidemics were observed. March and September 2021, then January and August 2022 (**Figure 1**). The highest peak was observed in January 2022 and the lowest in September 2021. During the SARS-CoV-2 epidemic, several variants were identified in the world in general and Cameroon in particular. The different peaks obtained in our study correspond to the different waves of the epidemic in Cameroon and could be related to the appearance of these different variants. Fokam and al. studied the dynamics of the spread of SARS-CoV-2 in Cameroon between 2020 and 2022. They found that the first wave in Cameroon occurred between 2020 and 2022. That first wave in Cameroon occurred between weeks 18 and 33 in 2020. From weeks 2 to 22 of 2021, they obtained co-circulation of the alpha and beta variants, corresponding to wave 2, and the appearance of the delta variant, corresponding to wave 3, in weeks 36 to 46 of 2021. Between week 50 of 2021 and week 5 of 2022, they observed the circulation of the single omicron variant, corresponding to wave 4 [18]. The various peaks observed in our study correspond to waves 2 and 3 for 2021 and waves 3 and 4 for 2022. The high peak observed in January 2022 could reflect the emergence of the delta variant, which is known to have been very aggressive and spreading [21]. It was shown that in January and February 2022, COVID-19 was the leading cause of death for people aged 45 to 84 and was at least among the four leading causes of death for other age groups from 5 years upwards, due to the circulation of the delta variant [22]. It was only in June 2022, more than a year after it first appeared in the world, that the WHO classified it as one of the variants of least concern [21]. In addition, the generally large reduction in these epidemic peaks could be explained by the fact that the collective immunity acquired through natural immunization or the vaccination introduced in Cameroon in 2021 had a better effect on the transmission of SARS-CoV-2 in Cameroon.

With regard to age, cases of SARS-CoV-2 increase with age, and the highest frequency of detection was observed in patients over 65 years of age (**Table 2**), although they are among the least represented in our study population. The co-morbidities and reduced functionality of the immune system generally observed in the elderly would explain the higher frequency of detection in this group of patients, predisposing them to a higher incidence of SARS-CoV-2 infection. A survey conducted in Cameroon on the prevalence of SARS-CoV-2 among adult populations in Yaoundé and Douala showed that comorbidities were associated with seropositivity [23].

Analysis carried out, including the three positive divisions, showed that more than half of the positive samples (50.6%) were low positives, 8.7% could be classified as potential positives, and 40.7% classified as high positives (**Table 3**). Most of the samples with high positivity thresholds came from symptomatic cases (56.7%). In contrast, for the other reasons for testing (contact cases, travel, and sporting events), most Ct values were between 30 and 37 (50.1%, 53.6%, and 47.8%, respectively).

Risk factor analysis showed a risk of 1.7 of having a high viremia in symptomatic patients ($p = 0.001$ [1.3 - 2.3]). The symptoms/signs reported by COVID-19-positive participants were diverse and varied in frequency, indicating multi-organ involvement and suggesting a difference in viral tropism [24]. The presence of symptoms could also be an indicator of the co-circulation of other respiratory viruses at the same time, as demonstrated by Fontanet *et al.* [25]. However, we have not determined whether the sampling conditions (collection, transport, and storage) produced false negatives for SARS-CoV-2 or whether other pathogens were involved. In terms of gender, males were more likely to have high viremia than females. Epidemiological studies in Africa and elsewhere have reported higher levels of COVID-19 in men than in women [26]-[28]. In many studies, male gender has been shown to be an independent risk factor for severe progression of COVID-19 [29] [30]. Although the mechanism underlying this difference has not been elucidated, female sex hormones and X-linked genes have been suggested to be protective in women. This could explain the high viremia observed in men compared with women in our study population. Following the seasonal pattern, the month of December presented the highest risk of high viremia compared with the other months of the year.

5. Study Limitations

One of the limitations of this study is the absence of certain important data on the characteristics of the participants. Not all the socio-demographic and clinical data were systematically recorded on the individual patient identification forms. We did not have information on patients' social and even clinical antecedents (passage through areas of high epidemic, vaccination antecedents). These different parameters could have enabled us to better elucidate the factors associated with high viremia in our study population. In the case of pauci-cellular nasopharyngeal samples,

the viral load is underestimated, as is sampling beyond the first 10 to 15 days of illness. Pre-analytical conditions (type of transport medium, storage time) may influence the result.

6. Conclusion

The DGH Clinical Biology Laboratory has played a major role in the fight against SARS-CoV-2 infection. Two years after the introduction of molecular biology in this laboratory, screening of 45,517 samples was carried out using RT-PCR. This technique made it possible to establish an appropriate biological diagnosis of the infection in a highly turbulent health context, with 4 outbreak peaks over the two years. Symptomatic cases were found to be those with high viremia. There is a need for surveillance of this infection, particularly for high viremia, which seems to be associated with seasonality, mainly in August and July.

Acknowledgements

We express our gratitude to Pasteur Center of Cameroon for its support in setting up our molecular biology platform in the framework of the technology transfer for the COVID-19 response. We thank the staff of all the laboratories concerned for their support and cooperation during COVID-19 response. We would like to thank all the patients for the data provided. All the data presented here were anonymously analyzed and reported.

Availability of Data

All data supporting these findings can be found in the Molecular Biology Unit of the Clinical Biology Laboratory of the DGH.

Authors' Contributions

COE and HNL coordinated the study, ETN-M, ERM, DWW, AT, CN and MNN collected data and participated in its design, ETN-M, COE, J-PNM and CLMO drafted the manuscript, ETN-M and JBTM performed the statistical analysis. All authors read and approved of the final manuscript.

Ethics

This study was carried out within the framework of our mission, such as referential laboratory in the survey in Cameroon of many pathogens, including COVID-19 response. Frequently, Cameroon Ministry of Public Health used our results to update the national response and survey of SARS-CoV-2 infection and other pathogens in the Cameroon context. No biological test was carried out in addition to the original SARS-CoV-2 detection requested by patients and all the results used here were anonymous.

Funding

All the COVID-19 tests were performed in the framework of COVID-19 strategies

riposte in Cameroon conducted by the Ministry of Public Health and the Public Health National Laboratory (PHNL).

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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