IJMPR 2021, 5(6), 165-171

International Journal of Modern Pharmaceutical Research
www.ijmpronline.com

ISSN: 2319-5878
IJMPR
Research Article
SJIF Impact Factor: 5.273

CONTRIBUTION OF REAL-TIME PCR IN THE SCREENING AND EPIDEMIOLOGICAL SURVEILLANCE OF COVID-19 AT THE SITE OF THE NATIONAL REFERENCE UNIVERSITY HOSPITAL OF CHAD

Bessimbaye Nadlaou*[1,2], Saleh Abdelsalam[2], Djimadoum Mbanga[2], Khadidja Gamougame[1], Frank Edgard Zongo Ragomzingba[2], Narbé Djimadengar Maxime[1], Mahamat Abdelaziz[1], Abdelsalam Tidjani[2] and Choua Ouchemi[2]

[1]Laboratories of the National Reference University Hospital (CHU-RN) of N'Djamena. National Reference University Hospital (CHU-RN) of N'Djamena (Chad).
[2]Faculty of Human Health Sciences (FSSH), University of N'Djamena, BP 1117 N'Djamena, Chad.

Received on: 01/11/2021
Revised on: 22/11/2021
Accepted on: 13/12/2021

Dr. Bessimbaye Nadlaou
Specialist in Biochemistry-Microbiology-Biotechnology.

ABSTRACT

The objective of this study is to demonstrate the effectiveness of the PCR-RT test in the screening of travelers and the epidemiological surveillance of Covid-19 at the site of the National Reference University Hospital (CHURN) in Chad. We carried out a prospective study in the virology laboratory of CHURN in N'Djamena over a period of 15 months extending from August 1, 2020 to October 31, 2021. During this period, we included 69,268 virological examinations of nasopharyngeal swabs including 871 positive cases for Covid-19. The overall prevalence of Covid-19 was 1.25%. Significant differences were observed between the proportions of study participants men (59%) and women (41%), between 67% of travelers and 33.3% other cases among patients. At the end of this study, we can say that Covid-19 remains very common among travelers. In this regard, the preliminary findings of this study can be used as a basis for developing strategies and preventive measures emphasizing regular screening followed by adequate treatment, coupled with sanitation, environmental hygiene and compliance with the barrier measures enacted. The results of this control operation are satisfactory. No false positives were noted, which allowed a comparison of results from other laboratories and between reagents used. We recommend that this powerful technique be made available at screening sites in order to carry out effective surveillance for Covid-19 in Chad.

KEYWORDS: PCR-RT, screening, surveillance, Covid-19, CHURN, Chad.

INTRODUCTION

The Covid-19 epidemic appeared in China in December 2019. From February 2020, many cases are observed in northern Italy and then in all countries of Europe. The epidemic has left so many cases with high mortality and great repercussions on everyone's life and work. A first case of Covid-19 was identified in Chad on March 19, 2020 by the mobile laboratory in N'Djamena in a Moroccan traveler from Douala (Cameroon), declared on March 11, 2021 as a pandemic by the WHO.[1] To date, Chad has notified 5,703 cases of patients with Covid-19 and among them 181 have died.[2]

The ever-increasing scale of migratory movements around the world raises specific health questions both in countries of origin and in host countries. Chad, by virtue of its geographical, political and economic situation, is the host country for many migrants from sub-Saharan Africa who may present both pathologies imported from their country of origin as well as acquired pathologies linked to the conditions. socio-economic disadvantage. Among these pathologies, Covid-19 constitutes a serious health problem.[3]

The objective of this study is to demonstrate the effectiveness of the real-time polymerase chain reaction (PCR-RT) test in epidemiological surveillance, screening of travelers, suspected cases and contacts of Covid-19 at the Hospital Center. National Reference University (CHURN) in Chad.

MATERIAL AND METHODS

Framework of the study and progress of the work.

The study took place in N'Djamena from August 1, 2020 to October 31, 2021 at the Virology Unit of the Laboratory of the National Reference University Hospital (CHU-RN) of N'Djamena (Chad)

Choice of samples
The screening strategy for the selection of nasopharyngeal samples is summarized on the qualitative RT-PCR test for the detection of the Covid-19 virus.

Inclusion and exclusion criteria
Was included any traveler, returned from the trip, contact case and suspected case (anyone showing clinically visible signs: hyperthermia at 40 ° C, cough, sore throat, etc.).

Not included, people with no signs.

Collection of samples
Nasopharyngeal swabs were systematically taken from all travelers, contact cases and suspicious or consenting persons with signs suggestive of Covid-19 after filling out the identification forms.

This is a population, aged 0 to 79, of all professions and social categories combined.

We considered the following variables in the people to be sampled such as: sex, age, origin, flu symptoms, marital status, profession, risk behaviors (not wearing a mask, contact with the patient, etc.). The analysis of these parameters provided a better understanding of the risks of transmission of Covid-19.

Data processing
The chi-square test (χ^2) was used for the comparison of qualitative variables with a significance level set at 5%.

MICROBIOLOGICAL ANALYSIS

Presentation of the tests
The biologics we have looked at are nasopharyngeal swabs. These products have been tested in laboratories with RT-PCR screening kits.

Qualitative determination of the COVID19 virus by Polymerization Chain Reaction
Reagent's consumables equipment
- QIAGENTM reagent kit (Buffer Buffer AVL and AVE, Wash1, Wash2, Carrier, Spins columns, Collection tubes)
- DAAN Gene reagent
- Ethanol.
- AB Thermocycler: Applied Biosystems 7300.

The main test necessary for confirmation at the CHURN Chad laboratory for the detection of Covid-19 in an epidemic situation or in the absence of an epidemic was the RT-PCR test.

Standard operating procedure targeting the N and ORF genes of Covid-19
1. Objective: This involves the detection of the N and ORF genes of Covid-19 by the qualitative RT-PCR technique;
2. Principle: Prior to amplification by AB: Applied Biosystems 7300, clinical specimens are inactivated with QiagenTM AVL buffer.

The procedure for extracting and purifying viral RNA consists of 4 main steps: 1: lysis of cells contained in the nasopharyngeal specimen, 2: binding of nucleic acids to magnetic particles, 3: washing and removal of cellular debris and finally 4: elution of nucleic acids.

The RT-PCR test was performed with the DAAN Gene reagent according to the protocol developed by the University of SUN YAT-SEN (China N MPA EUA-CE-IVD) issued by the World Health Organization. The reaction volume: 20 µL (the manufacturer recommends 40 µL)

Internal control in lysis for extraction (as recommended by the manufacturer).

Technical validation
The interpretation of the results is based on the values of Ct (cycle threshold: number of amplification cycles required for the fluorescence emitted by a PCR product to reach the point where it will be greater than the background noises) and the sigmoidal shape of the curve:
- C t≤ 37, positive sample;
- Ct> 37, negative sample.

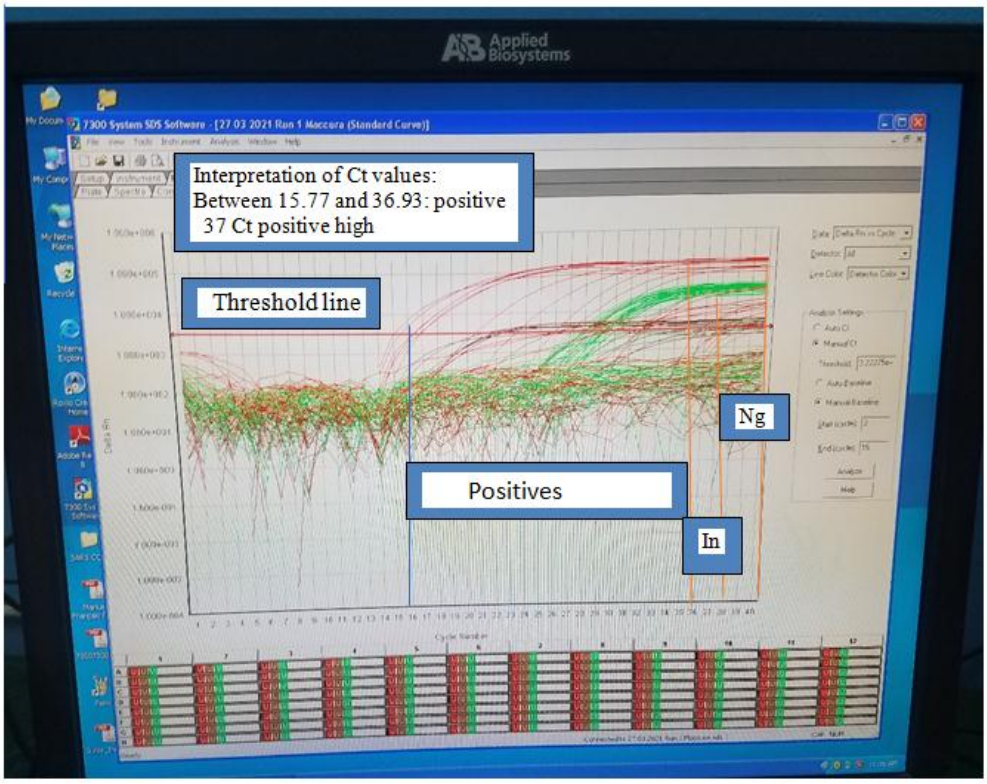
NB: The threshold of Ct varies according to the reagents.

RESULTS

Sociodemographic characteristics of the study population
During 15 months, 69,268 virological examinations of nasopharyngeal swabs were carried out at the CHURN Covid-19 screening site, including 871 positive cases. All genders and different age groups are affected with extremes of 0 to 79 years. Of the 871 cases, 41% (358/871) were female and 59% (513/871) male (x^2 = 8.940> x^2_0> 3.84, p = 0.001, dof = 1), there is a significant difference in favor of men. The male / female sex ratio in our study is 1.4 ((513/358). The most affected age group was 40 to 49 years old with 277 cases (32%). active, the most mobile and certainly the most exposed. Under 10s (Pediatrics) represent 1.26%. Among the patients screened, 67% (581/871) are travelers and 33.3% (290 / 871) of the cases (suspected, contacts, community and hospitalized) (x^2 = 9.196> x^2_0> 3.84, p = 0.001, dof = 1: significant difference in favor of the number of travelers).

Assessment of viremia based on the absence or presence of Covid-19
Figure 1 illustrates the interpretation and validation of RT-PCR assay by Ct values. Due to the limitations of ABI7300, we targeted 2 genes. We repeated the tests for some samples for which interpretation of the results was difficult (example: ORF1ab only positive and N-Gene negative and vice versa).

Figure 1 illustrates the interpretation of Ct values. Between 15.77 and 35: positive values of Ct; between 36.1 and 37.9: undetermined values. Between 38 and 40: negative values. Positive PCR with Ct value> 35: terminal infection with low viremia. Positive PCR with Ct value <35: recent infection with high viraemia. The positive Ct values obtained for a reaction volume of 20 µL ranged from 15.79 to 34.65 for the ORF1ab gene with negative control (0) and positive control (27.89), and from 15.9 to 30.11 for N-gene with the value 0 for the negative control and 27.12 positive control and both with the value 0 for the extraction control.



Ng: negative; In: indeterminate

Figure 1: Validation amplification curves of a result on the ABI 7300 thermal cycler.

Distribution of patients according to the presence or absence of symptoms

Figure 2 illustrates the distribution of patients according to the presence or absence of symptoms. There is a predominance of asymptomatic patients and especially among travelers.

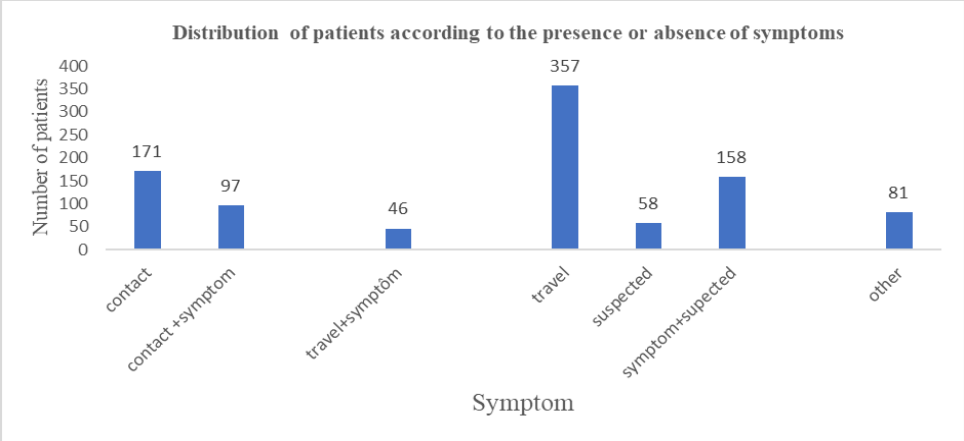


Figure 2: Distribution of patients according to the presence or absence of symptoms.

Distribution of positivity rate by sex

Figure 3 illustrates the distribution of the proportions of patients by sex. Men were more infected than women.

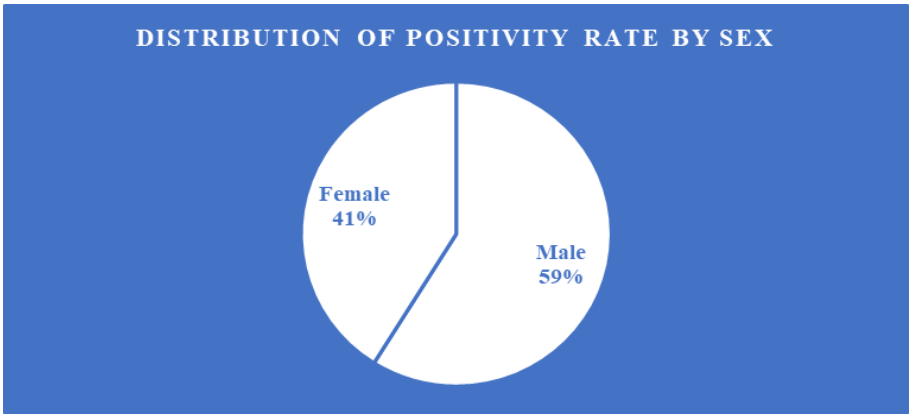


Figure 3: Distribution of patients by sex.

Distribution of patients by month

Figure 4 illustrates the distribution of patients over the 15-month study period. Two peaks were recorded in March and April 2021 (electoral campaign period for the presidential elections of April 2021 in Chad where there is massive displacement of infants) and three other smaller peaks in December 2020, January and February 2021 (cold period).

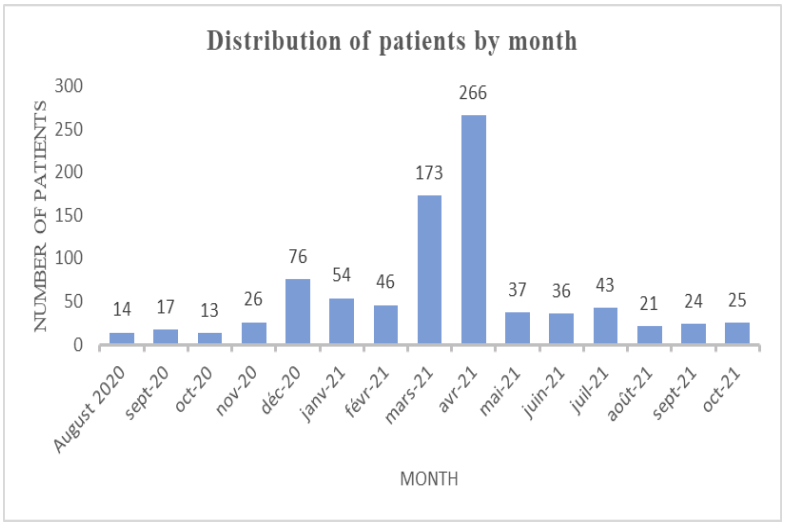


Figure 4: Distribution of patients by month.

Distribution of patients by age group

Figure 5 illustrates the distribution of patients by age group. The most affected age group was 40-49 years old followed by 30-39 years. Overall, adults were the most infected and few children were infected.

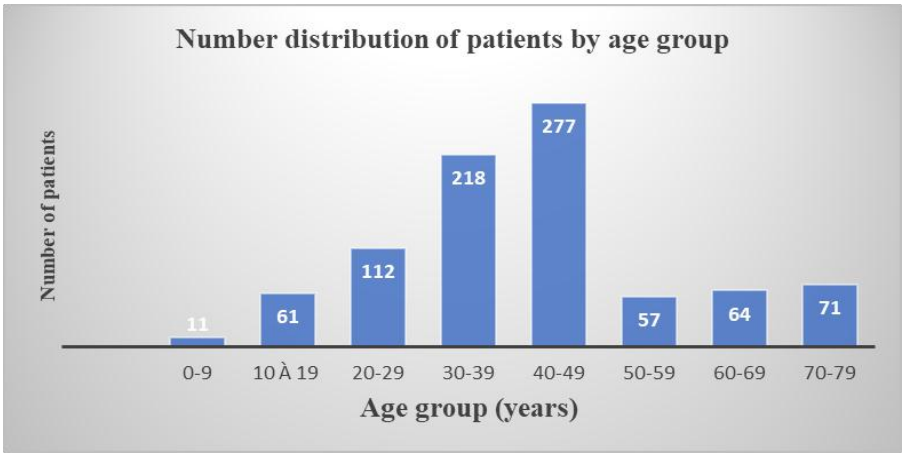


Figure 5: Distribution of patients according to age group.

Distribution of patients by nationality

Figure 6 illustrates the distribution of patients by nationality. Chadians and Chinese were the most infected. The movement of people promotes the transmission of Covid-19. The first case confirmed by the local mobile laboratory was detected on March 19, 2020 in a Moroccan from Douala (Cameroon) and then followed the spread of the disease among the population in Chad.

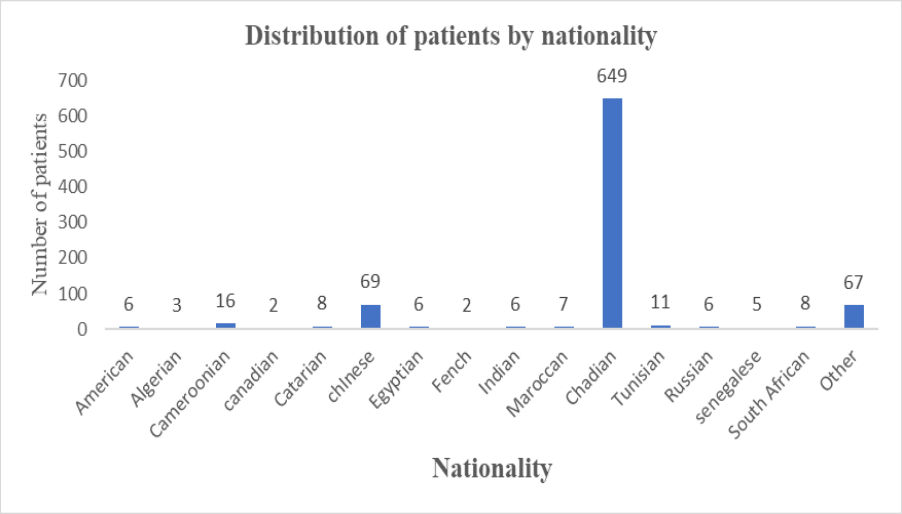


Figure 6: Distribution of patients by nationality.

Distribution of patients by profession

Figure 6 illustrates the distribution of patients by profession. The most infected occupations were manual workers followed by traders and entrepreneurs (Figure 6). The risk of massive transmission within populations is very high for categories of professions such as: traders, drivers, teachers and health workers.

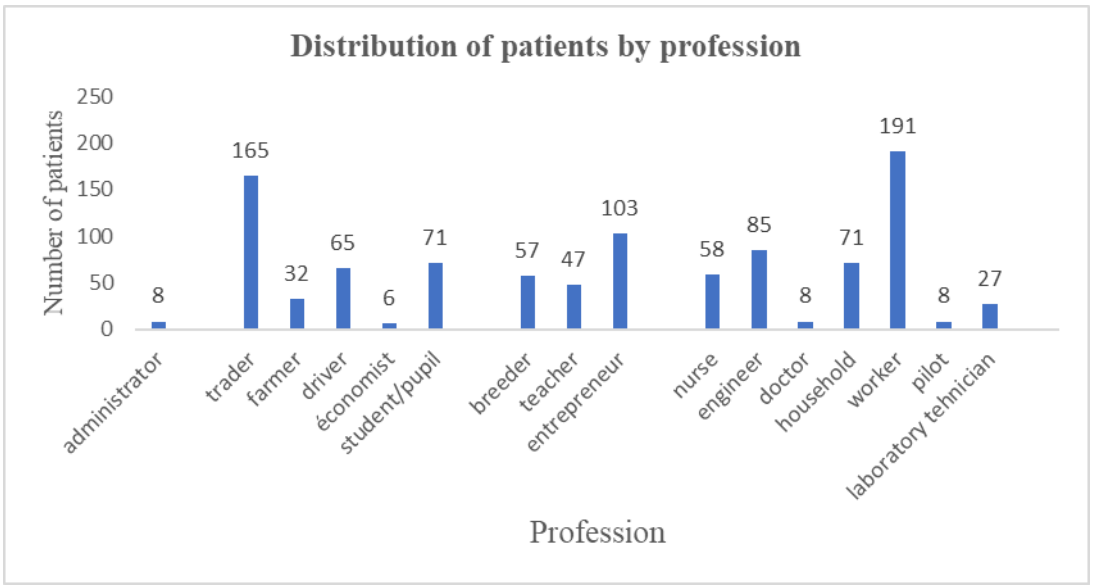


Figure 7: Distribution of patients by profession.

Control and monitoring of patients treated for treatment by the PCR-RT test

Figure 8 illustrates the control and monitoring of patients by the PCR-RT test seven days after treatment. At the first control of the PCR-RT test, that is to say seven days seven days of hospitalization treatment, 844 patients had recovered, 20 patients were cured after the 2nd control, 6 patients were cured in the 3rd and 1 patient had recovered after the 4th control.

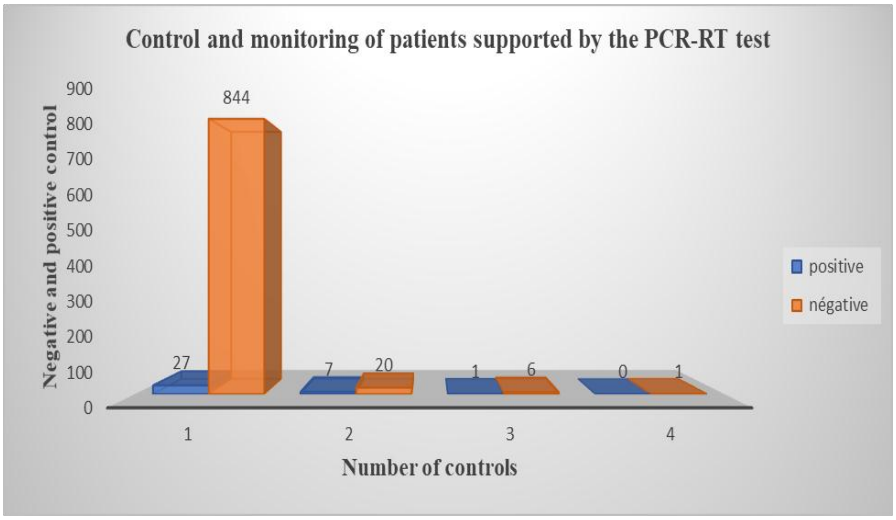


Figure 8: Control and monitoring of patients treated for treatment by the PCR-RT test.

DISCUSSION

Epidemiological surveillance is the way to follow the trends of Covid-19 infections for the estimation of morbidity in a region and the determination of the impact of a new vaccine. However, it must take into account several parameters which have an influence on their frequencies in a given region.[4] Unlike other continents, the progress of the Covid-19 pandemic in Africa has been slower. As of December 1, 2021, while the world had 266 million cases and 5,260,000 deaths, the African continent recorded only 8,786,000 cases and 224,000 deaths.[5] The 1st case of Covid-19 was detected on March 19, 2019 in a traveler in Chad. This would explain that the displacement, the migration of populations from one country to another would have favored the rapid spread of Covid-19 around the world. The results of all the nasopharyngeal samples analyzed show that Covid-19 was detected overall in 1.26% of cases. Among the 871 patients infected with Covid-19, the prevalence of travelers infected with Covid-19 was 67% and it was 33.3% for contact and suspected cases.

The measures put in place by countries, rumors and fear of the virus have had major health, social and economic consequences. Chad unfortunately does not escape this reality, false rumors around Covid-19 as an imaginary disease, the lack of personal protective equipment and the non-observance of barrier measures decreed by governments have contributed to the contamination of 92 health workers in the CHURN screening site including: 8 doctors, 58 nurses and 27 laboratory technicians. The contamination of laboratory technicians would be justified by non-compliance or mistrust in the concept of biosafety and biosecurity but also by a lack of means of protection among this professional category in Chad. The contamination of health professionals in the first place had a huge impact on the observed drop in the use of health services in Chad. Also, the consideration of certain patients from certain social classes (doctors, ministers, diplomats, etc.) who refused to be taken care of in the premises fitted out by the Ministry of Public Health to the detriment of the large hotels in the area contributed much more to the distribution. of Covid-19 among the population. Furthermore, the authors reported similar results to previous studies.[6, 7] As of March 19, 2020 to date, Chad has only 5,703 cases and 181 deaths from Covid-19.[8] The low rates of contamination and death are not real rates because the fear of being stigmatized as having Covid-19 has caused many people with flu-like symptoms to hide and prefer traditional treatments to herbal teas and not to themselves. present at the Covid-19 testing site. The majority of the population remains skeptical. Because for her, the symptoms of Covid-19 are none other than those of the common cold and flu that have always plagued Chad in the past and that she is used to treating with a decoction of lemon leaves and its fruits, lemongrass, tubers and ginger powder combined with antipyretics such as paracetamol. PCR-RT detected 7 post-mortem cases and followed 871 patients until their complete recovery (Figure 8). This would have avoided contamination of the population both at the community level and by grouping people together during mourning ceremonies. The bodies of those who died and were found positive for Covid-19 were handed over to the road service for burial without the parents present. The results of this study showed, as elsewhere [9, 10], the performance of RT-PCR with high specificity (100%) and sensitivity (98.6) during quality control of samples from two laboratories at the national level by targeting the ORF and N genes.

CONCLUSION

Covid-19 infections occur in all age groups, but they are more common in adults, with more cases in cold weather. The majority of cases detected were recorded in March and April 2021 during the presidential election campaigns of April 21, 2021. Thus, surveillance is necessary for epidemiological follow-ups of cases of infections recorded each year. This surveillance must be carried out with efficient means of identification so as not to underestimate or underestimate the number of

people with Covid-19 in Chad. The PCR-RT applied at the CHURN site successfully followed up 871 people who tested positive for Covid-19 until full recovery.

Vaccines against Covid-19, mass screening and compliance with barrier measures could help reduce the prevalence of Covid-19 infections and prevent the emergence of new variants in Chad.

ACKNOWLEDGMENTS

The authors would like to thank the national health response committee (CNRS) for authorizing the Covid-19 screening site at CHURN. We are very grateful to the microbiology laboratory staff at CHURN for their valuable efforts in sample collection and analysis. Thanks, are also extended to the experts from the World Health Organization for their invaluable support in validating the protocol.

CONFLICTS OF INTEREST ETHICAL AND ADMINISTRATIVE CONSIDERATIONS

There are no conflicts of interest. The CHURN Covid-19 screening site has received an agreement from the National Health Response Committee (CNRS). The verbal consent of each person or their beneficiary to whom we have explained the procedure and the importance of screening for Covid-19 and their participation.

REFERENCES

1. OMS. COVID-19- Chronologie de l'action de l'OMS. Consulté le 28/03/2021. https://www.who.int/fr/news/item/27-04-2020-who-timeline---covid-19
2. OMS. COVID-19: https://ourworldindata.org/coronavirus-data
3. OMS. Cartographie des interventions en ressources humaines et en santé, dans le contexte de la Covid-19 : Etude de cas du Tchad. Rapport Draft 1, N'Djamena : 2020, pp.1-93.
4. Moran-Gilad J. Measuring diagnostic performance of COVID-19 tests: lessons for the next pandemic. Euro Surveill, 2021; 26 (45): 1-3.
5. https://ourworldindata.org/coronavirus-data
6. Attoh Touré H, Noufe S, Oussou KR, N'Guessan K, Setchi SM, Ano AMN, Tiembre I, Bénie BVJ. Effets de la pandémie à covid-19 sur les activités vaccinales d'un centre de vaccination de référence de treichville en Côte d'Ivoire. Méd trop et santé int, 2021 ; 5058: 1-5.
7. Sabe SC. Covid-19 : mythe ou réalité ? note sur le cas de la ville de Bossangoa (République Centrafricaine). Méd trop et santé int, 2021; 165: 1-2.
8. http://en.wikipedia.org/wiki/Template:COVID-19_pandemic_data
9. World Health Organization (WHO). Tracking SARSCoV- 2 variants. Geneva: WHO. [Accessed: 1 Sep 2021]. Available from: https://www.who.int/en/activities/tracking-SARS-CoV-2-variants
10. Corman VM, Landt O, Kaiser M, Molenkamp R, Meijer A, Chu DKW, Bleicker T, Brunink S, Julia Schneider1, Marie Luisa Schmidt1, Mulders DGJC, Haagmans BL,Veer BV, Brink S VD, Wijsman L, Goderski G, Romette J-L, Ellis J, Zambon M, Peiris M, Goossens H, Reusken C, Koopmans MPG, Drosten C. Detection of novel Coronavirus (2019-nCov) by reel-time RT-PCR. Euro Surveill, 2020; 25(3): 23-30.