EAST AFRICAN COMMUNITY

A two-step molecular and sero-epidemiological cross-sectional study in the East African Community Partner States on the Prevalence of SARS-CoV-2 in Patients with Signs and Symptoms of Severe Acute Respiratory Infection (SARI) between 1st November 2019 and 29th February 2020

Implemented by the East African Community (EAC)
through the East African Health Research Commission (EAHRC)
in collaboration with the EAC Partner States' National Public Health Reference Laboratories (NPHLS) with technical and financial support from the German Government through the Deutsche Gesellschaft für Internationale Zusammenarbeit (GIZ) GmbH and the Robert Koch-Institute

FINAL TECHNICAL REPORT

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Submitted by:

The East African Health Research Commission (EAHRC),
East African Community (EAC),
Quartier Kigobe, P.O. Box 350, Bujumbura, Burundi
Telephone +257 222 799 80; Mobile: +255 784 751 156
Emails: admin-eahrc@eahealth.org; fmashauri@eahealth.org;
ntwungubumwe@eahealth.org;
Website: www.eahealth.org

Study Coordinator: Dr Fabian M. Mashauri

Study Consultant: Dr Stanley Serser Sonoiya

National Coordinators: (1) Dr Joseph Nyandwi, Director General, National Institute of Public Health (INSP), Bujumbura, Burundi; (2) Mr. Abdi Roba, Virologist and Manager, National Virology Reference Laboratory Nairobi, Kenya; Mrs. Alice Kabanda, Senior Medical Laboratory Officer, National Influenza Center (NIC), National Reference Laboratory (NRL), Ministry of Health, Kigali, Rwanda, Mr. Michael Lokore Losuba, Laboratory Supervisor, Public Health Laboratory (NRL), Juba, South Sudan; Dr Julius Julian Lutwama, Deputy Director, Uganda Virus Research Institute (UVRI), Entebbe, Uganda.

Abbreviations

COVID 19: Coronavirus Disease 2019

EAC: East African Community

EAHRC: East African Health Research Commission

EAV: Equine Arteritis Virus

ECHO: European Community Humanitarian Organization

E-Gene: Envelope Protein Gene

GIZ: German Technical Cooperation Agency

HIV/AIDS: Human Immunodeficiency Virus/Acquired Immunodeficiency Syndrome

IDSR: Integrated Diseases Surveillance and Response (IDSR)

MERS-CoV: - Middle East Respiratory Syndrome Corona Virus

MoH: Ministry of Health

N-Gene: Nucleocapsid Protein Gene

-VE: Negative Test Results for COVID-19

NIC: National Influenza Center

+Ve: Positive Test Results for COVID-19

RdRP Gene: RNA-dependent RNA polymerase gene

RKI: Robert Koch Institute

RT-PCR: - Reverse-Transcriptase-Polymerase-Chain-Reaction

SARI: Severe Acute Respiratory Infections

SARS-CoV-2: Severe Acute Respiratory Syndrome Coronavirus 2 (formerly called 2019-nCoV or novel coronavirus 2019)

WHO: World Health Organization?

USAID: United States' Agency for International Development

Summary

The first cases of COVID 19 were reported in the EAC Partner States between 13th March and 5th April 2020. However, between November 2019 and February 2020, the EAC region had seen an increase in patients presenting with Severe Acute Respiratory Infections (SARI)-like signs and symptoms. The East African Health Research Commission (EAHRC) convened the study, described in detail in this report, to answer the question if these cases might already have been caused by COVID-19.

More than 1000 frozen nasal and oropharyngeal swab samples taken at the various Influenza Sentinel Surveillance Sites in Kenya, Rwanda, South Sudan and Uganda from patients who had presented with SARI-like signs and symptoms during that time were re-tested by RT-PCR in the respective National Public Health Reference Laboratories in each country.

All the samples tested negative, which indicates that COVID 19 was not present in the EAC region before the first official cases were reported.

1.0 Introduction

On 31st December 2019, the World Health Organization (WHO)'s Country Office in the Peoples' Republic of China was notified of unusual cases of an acute respiratory syndrome in Wuhan City, Hubei Province of the Peoples' Republic of China. On 7th January 2020, the causative agent was identified to be a novel coronavirus (2019-nCoV), currently referred to as the Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2)[1]. WHO declared the disease a public health emergency of international concern on 30th January 2020 and named it Coronavirus Disease 2019 (COVID19) on 11th February 2020.

As of 29th May 2020, the global number of COVID-19 cases stood at 5,816,706 and 360.437 people had died worldwide of the disease while 2,420,358 had recovered according to the US John-Hopkins-University. For Africa 129,452 cases and 3,792 deaths were confirmed by 28th May while 53,400 people had recovered. In the East African region 3,146 infections and 82 deaths were confirmed by 27th May with 927 recoveries.

Despite the comparatively low numbers of cases, the East African Community (EAC) Partner States suffered considerable from the impact of the pandemic. Trade continued, at least to a certain extent, but the economies suffered severely, and many citizens lost their livelihoods.

[1] WHO. Novel Coronavirus (2019-nCoV) SITUATION REPORT - 1. Who.int. https://www.who.int/docs/default-source/coronaviruse/situation-reports/20200121-sitrep-1-2019-ncov.pdf. Published 2020

Tourism came to a complete standstill and a decrease in agricultural activities raised the fear of food insecurity in the aftermath of the pandemic. As funds and capacities at health facilities were rechannelled towards the COVID-19 response, the treatment of other severe diseases, such as Malaria or non-communicable diseases like Diabetes Mellitus was impaired and resulted in additional deaths. With some essential medication being no longer available and people avoiding seeing doctors there was also growing concern that patients might discontinue their HIV/AIDS treatment and that this might lead to new transmissions and result in increasing numbers of fatalities.

Although an increase in infections and deaths was subsequently reported, the numbers in Africa remained rather low compared to predictions and other parts of the world, even if low testing rates and high rates of underreporting are taken into consideration. Explanations included the low average age of citizens in the countries, the influence of climate and temperatures, the continuous confrontation of the immune system with a variety of pathogens and resulting possible cross-immunisation. Many African countries established strict preventive measures at an early stage and reacted to the pandemic with strict isolation and quarantine measures culminating in total lockdowns of whole cities.

However, anecdotal evidence hinted at another possible explanation: Some African countries seemed to have experienced high numbers of severe respiratory infections with persistent cough, fever and sometimes pneumonia from November 2019 up to early 2020 among citizens. These patients were not tested and/or diagnosed for COVID-19, as public attention to the new virus only started growing in January 2020 and test kits were not yet available. The symptoms might have been related to influenza, but it could not be excluded that these severe acute respiratory infections (SARI) were already caused by SARS-CoV-2.

2.0 Rationale for conducting an indicative COVID-19 antibody study in the EAC region

A French man had suffered from a severe respiratory disease in December 2019 and was admitted to hospital where blood was taken for analysis. The man recovered, but when the pandemic struck France in 2020, his blood sample was re-tested in RT-PCR and clearly diagnosed for COVID-19. Consequently, WHO urged countries to look more closely into past cases of respiratory infections. The reported low numbers of infections and deaths in the EAC region and the **"French case"** shed a new light on the above mentioned severe respiratory infections observed in the EAC region between November 2019 and beginning of 2020. They raised the question, if these infections might already have been related to COVID-19. An answer to this question could have considerable economic and social impact, especially because the course of infections in more severely affected Western countries peaked after

about two months with numbers of new infections starting to decrease.

Against this backdrop, the EAC Secretariat in cooperation with the East African Health Research Commission (EAHRC) decided to conduct a small, indicative COVID-19 antibody study in the Partner States with the objective of collection data to form a hypothesis that could guide political action.

3.0 Conduct of COVID-19 antibody study in the EAC region

The study was initiated with support from the German Government and the German Robert Koch Institute (RKI) within the framework of special measures to mitigate the further spread of COVID 19 and inform the response to the pandemic. It was convened and implemented in line with the EAC regional COVID-19 response plan, which requires the EAC to conduct regional research to guide policy and practice. The study aimed to provide baseline data and information for follow-up investigations to understand the development of SARS-CoV-2 prevalence in the EAC region over time as well as to provide comparative data for further investigations in other study populations and study sites. The study data were also expected to provide indicators for adjustment of local targeted public health measures.

The study was designed as a two-step molecular and sero-epidemiological cross-sectional study. The first step comprised the re-testing in PCR of stored, frozen swab samples from patients with signs and symptoms of Severe Acute Respiratory Infections (SARI) taken at Influenza sentinel sites between November 2019 and February 2020 for COVID 19. In step two patients would be followed up and tested for antibodies in cases of positive test results.

In this study, should all nasal and oropharyngeal swab samples test negative, it would be considered as unlikely that these infections were related to COVID-19 and the study would be discontinued. On the other hand, would a larger number or all swab samples of the test group bring positive results, the study would be indicative for COVID-19 infections in the EAC region as early as 2019.

If felt necessary, such results could subsequently be verified in further population studies that look into the degree of immunity against COVID-19 among EAC citizens *(for details see Study Design and Study sites below as well as the Study Protocol attached as **Annex 1**).*

3.1 Participating Partner States

Five out of six EAC Partner States *(Burundi, Kenya, Rwanda, South Sudan and Uganda)* participated in the study that was coordinated by the EAHRC.

3.2 Study Protocol, Ethics Consideration and Approval, Research Licences

A study protocol was developed and submitted for ethical clearance and approval by all the participating countries and adapted according to the input received. Ethical clearances were obtained from the respective National Ethics and Scientific Review Committees (NERCs). Approvals to conduct the research were obtained from the respective Ministries of Health (**Annex II**). In the Republic of Kenya, a research license for the study was obtained separately from the National Council for Science, Technology and Innovation (NACOSTI) and it is hereto attached as (**Annex III**).

4.0 Overall objective

The overall objective of the study was to verify, if COVID-19 infections had already occurred in the region in 2019 and therefore way before the first cases in Africa were reported by WHO in February 2020.

4.1 Objectives in detail

In detail, the study was designed to

i. Provide an indicative answer to the question if COVID-19-infections occurred already in late 2019. In this case the number of infections might already have peaked. This would explain the comparatively low numbers of current infections;
ii. Provide data to form a sound hypothesis to guide political action;
iii. Provide data for an adapted response.

4.2 Expected outcomes

i) The time of first occurrence of COVID-19 cases in the EAC is more clearly defined, thus providing an indication of an already decreasing or still increasing health event;
ii) Data are available to guide political action including an adapted response that might contribute to limited investment of human, infrastructure and financial resources.

5.0 Testing Methods

Laboratory confirmation of SARS-CoV-2 is key in identifying infected persons to guide appropriate public health interventions of contact tracing and patient isolation to prevent further transmission of infection[2]. The availability of the complete genome of SARS-CoV-2 early in the epidemic facilitated the development of specific primers and standardized laboratory

[2] Wilder-Smith A, Chiew CJ, Lee VJ. Can we contain the COVID-19 outbreak with the same measures as for SARS Lancet Infect Dis 2020; 20: e102–

protocols for COVID-19[3]. The protocol of the first real-time RT-PCR assays targeting the **RNA-dependent RNA polymerase gene (RdRp),** the **envelope protein gene (E)**, and the **nucleocapsid protein gene (N)** of SARS-CoV-2 was published on 23rd January 2020[4]. Reverse-transcriptase-polymerase-chain-reaction (RT-PCR) is the most common method for detection of SARS-CoV-2. Nasal and oropharyngeal swabs are most frequently used samples. However, false negative SARS-CoV-2 and positive RT-PCR test results of SARS-CoV-2 have been reported in patients who recovered from COVID-19[5]. As such uncertainty remains, the nasal and oropharyngeal swabs remain the common methods of sample collection for COVID-19 RT-PCR testing.

6.0 Materials and Methods

6.1. Study design and study sites

This was a laboratory-based retrospective cross-sectional study. Archived swab samples collected from the national influenza virus sentinel surveillance sites for SARIs from the four EAC Partner States, namely **Kenya, Rwanda, South Sudan and Uganda** were analysed by RT-PCR for the presence of SARI-CoV-2.

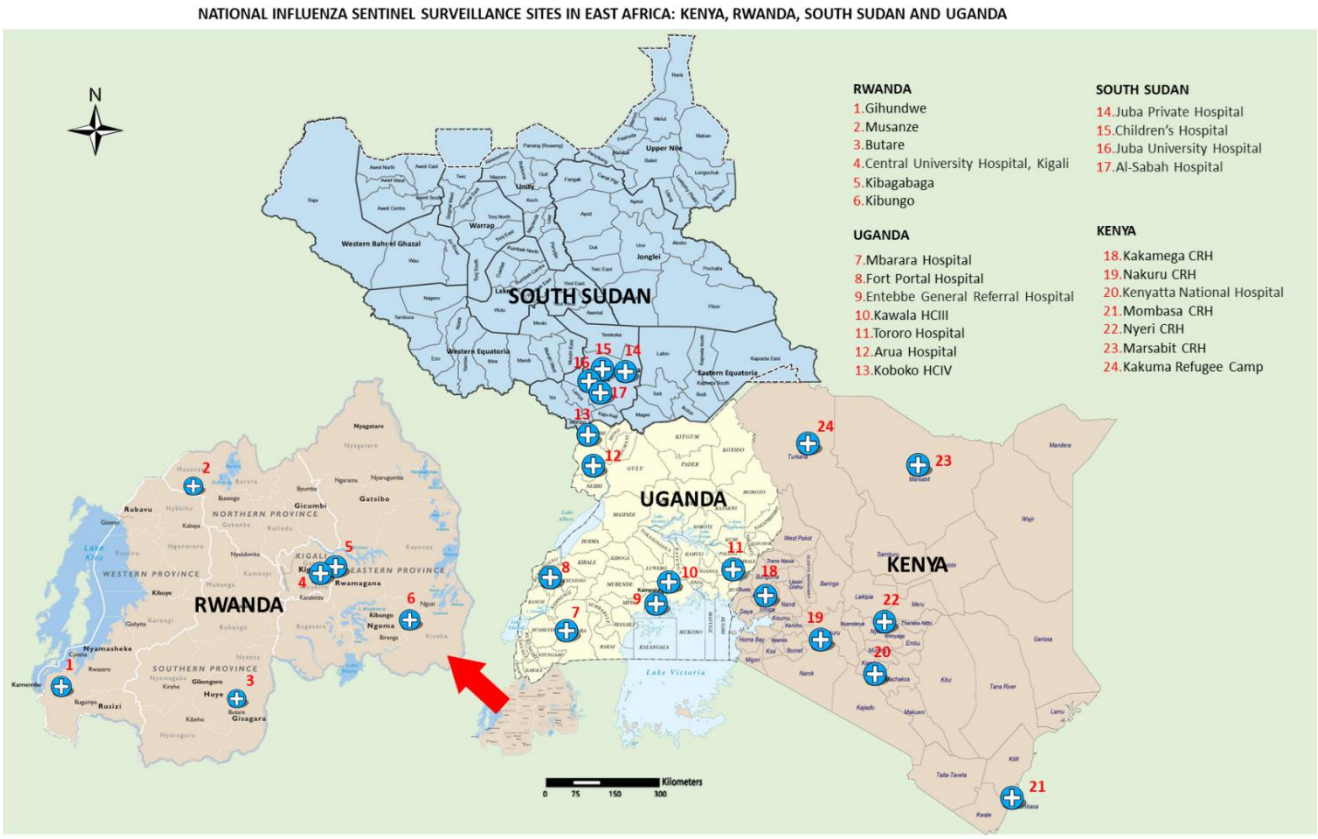
The Federal Government of Germany through the Robert Koch Institute (RKI) in Berlin City, Germany had donated to the EAC Partner States twenty (20) QIAamp Viral RNA Mini Kits (250) for RNA extractions and TIB-Molbiol PCR Detection Kits as follows; 60 Primer Sarbeco E-gen EAV, 25 Primer SARS-CoV-2 (COVID-19) RdRP, 76 Enzymes 1-step RT qPCR 100 rxns, 20 MicroAmp™ Optical Adhesive Film, 20 packs MicroAmp™ Optical 96-Well Reaction Plate, 125 strip MicroAmp™ Fast 8-Tube Strip, 0.1 mL, 300 caps MicroAmp™ Optical 8-Cap Strips, 10 pacts BioRad Hard-Shell® PCR Plates and 20 pacts BioRad Microseal 'B' PCR Plate Sealing Film.

[3] Lu R, Zhao X, Li J, Niu P, Yang B, Wu H, Wang W, Song H, Huang B, Zhu N, Bi Y, Ma X, Zhan F, Wang L, Hu T, Zhou H, Hu Z, Zhou W, Zhao L, Chen J, Meng Y, Wang J, Lin Y, Yuan J, Xie Z, Ma J, Liu WJ, Wang D, Xu W, Holmes EC, Gao GF, Wu G, Chen W, Shi W, Tan W. 2020. Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding. Lancet 395:565–574. https:// doi.org/10.1016/S0140-6736(20)30251-8.

[4] Corman VM, Landt O, Kaiser M, Molenkamp R, Meijer A, Chu DK, Bleicker T, Brünink S, Schneider J, Schmidt ML, Mulders DG, Haagmans BL, van der Veer B, van den Brink S, Wijsman L, Goderski G, Romette JL, Ellis J, Zambon M, Peiris M, Goossens H, Reusken C, Koopmans MP, Drosten C. 2020. Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR. Euro Surveill 25(3):pii_2000045. https://doi.org/10.2807/1560-7917.ES.2020.25.3.2000045

[5] Lan L, Xu D, Ye G, Xia C, Wang S, Li Y, et al. Positive RT-PCR test results in patients recovered from COVID-19. JAMA 2020

Figure 1: Map of EAC showing influenza sentinel surveillance sites



6.2. Study population

The study was carried out on archived nasal and oropharyngeal swab samples which were collected from patients who had presented with SARI-like signs and symptoms between 01st November 2019 and 29th February 2020 and those who had tested negative for Influenza Viruses Infections were then re-tested again for SARS-CoV-2 by RT-PCR in each country.

6.2.1 Eligibility criteria

Patients who presented with SARI-like symptoms between 01st November 2019 and 29th February 2020.

6.2.2. Inclusion criteria

i) Archived swab samples from patients with SARI-like signs and symptoms collected between 01st November2019 and 29th February 2020

ii) Frozen swab samples archived and stored at -80^{0}C.

6.2.3 Exclusion criteria

i) Archived swab samples not stored at -80^{0}C
ii) Archived swab samples which were not collected and not freeze stored between 1st November 2019 and 29th February 2020
iii) Archived swab samples from patients who had tested positive for Influenza Viruses Infections

6.3 Sample size calculations

All available archived swab samples which met the eligibility criteria from the four participating EAC Partner States, were included and tested for SARS-CoV-2 (COVID-19) by RT-PCR. A total of 1,153 archived swab samples met the eligibility criteria and were tested for COVID-19 by RT-PCR in the respective EAC Partner States.

6.4 Sample collection, transportation and storage

The swab samples which had been collected from influenza virus sentinel surveillance sites and transported from the sentinel surveillance sites using the existing cold chain systems to the respective EAC Partner States' National Influenza Center (NIC) for long-term storage and safe keeping in accordance with the established international standards and procedures. At the NIC, the samples were then tested for Flu A and Flu B infections and subsequently stored at -80^{0}C or liquid Nitrogen (for Uganda Virus Research Institute (UVRI).

6.5 Freeze-thawing nasal and oropharyngeal swab samples

The frozen archived swab samples were thawed at room temperature before being tested by RT-PCR for the presence of SARS-COV-2 (COVID-19).

6.6 Validations of the test kits and viability of Viral RNA

Swab samples were randomly selected for testing the viability of RNA. RNA of the randomly selected swab samples was extracted using QIAamp Viral RNA Mini Kit and biosensor extraction kit. The extracted RNA was validated concurrently using TIB-Molbiol PCR kit (WHO kit) and biosensor detection kits. The results of both kits were comparable. Both positive and negative controls were included in the runs and also internal standards. The tests verified that the archived samples were in good condition.

6.7 Viral RNA extractions

Corona Viruses usually affect the lower respiratory system, but the 2019-nCOV is found also in the nose, throat and the intestine. The viral RNA from archived nasal and oropharyngeal swab samples were extracted manually at National Virology Reference Laboratory (NVRL)

using QIAamp Viral RNA Mini Kit for RNA extraction after the samples were thawed at room temperature. The swab samples were extracted within 12 hours after removing them from the -80^0 Centigrade deep freezer. To avoid freeze thawing, only samples to be extracted within the same day were removed from freezer.

The Validity of Viral RNA extraction is verified by running an extraction control RT-PCR. Equine Arteritis Virus (EAV), a positive-sense single-stranded RNA virus, was used as an internal control added to all specimens prior to RNA extraction to ensure quality of the RNA extraction step. The EAV extraction control was added to the samples following the addition of the lysis buffer prior to RNA extraction. RNA extraction was then continued according to the manufacturer's instructions.

6.9 COVID-19 RT-PCR Assay

Real-time RT-PCR assays for SARS-CoV-2 RNA detection were performed using RT-PCR kits (TIB-Molbiol, Berlin, Germany) as previously described[6] including positive control, negative controls and internal standards (**Annex IV**).

7.0 Results

Table 1: Study results from Kenya

	Activity		**Testing method TIB-Molbiol PCR kit**					
			rdrp gene		***e-gene***		***EAV-gene***	
	No. samples tested	Date	-Ve	+ve	-Ve	+Ve	-Ve	+Ve
Week 1	428	01-06/03/ 2021	428	0	428	0	0	428
Week 2	224	08-12/03/ 2021	224	0	224	0	0	224
Week 3	62	15/03/ 2021	62	0	62	0	0	62
Total	**714**		**714**	**0**	**714**	**0**	**0**	**714**

Abbreviations: RdRP Gene: RNA-dependent RNA polymerase gene, **E-Gene:** - Envelope Protein Gene, **EAV:** Equine Arteritis Virus, **-VE:** Negative Test Results, **+Ve:** Positive Test Results

[6] Victor Corman, Tobias Bleicker, Sebastian Brünink, Christian Drosten ; Diagnostic detection of 2019-nCoV by real-time RT-PCR Charité Virology, Berlin, Germany Berlin, Jan 17th, 2020

[7] Long, QX., Tang, XJ., Shi, QL. *et al.* Clinical and immunological assessment of asymptomatic SARS-CoV-2 infections. *Nat Med* **26,** 1200–1204 (2020). https://doi.org/10.1038/s41591-020-0965-6

7.1 Kenya: Study results analysis and interpretations

All seven hundred fourteen (714) eligible swab samples which were archived and analyzed in Kenya tested rdrp-gene and e-gene negative for COVID-19, which indicates that there were no infections with SARS-CoV-2 among patients with SARI like symptoms between 01st November 2019 and 29th February 2020. All swab samples picked Equine Arteritis Virus (EAV) which is an internal control indicating the sample quality was good.

Table 2: Study results from Rwanda

	Activity		**Testing method TIB-Molbiol PCR kit**					
			rdrp gene		***e-gene***		***EAV***	
	No. samples tested	Date	-Ve	+Ve	-Ve	+Ve	-Ve	+Ve
Week 1	20		20	0	20	0	0	20
Week 2	20		20	0	20	0	0	20
Week 3	20		20	0	20	0	0	20
Total	**60**		**60**		**60**			**60**

Abbreviations: RdRP Gene: RNA-dependent RNA polymerase gene, **E-Gene:** - Envelope Protein Gene, **EAV:** Equine Arteritis Virus, **-VE:** Negative Test Results, **+Ve:** Positive Test Results

7.2 Rwanda: Study results analysis and interpretations

i) A total of sixty (60) samples, collected through the influenza sentinel surveillance sites from patients with SARI like signs and symptoms which were previously tested for influenza and stored at 80^0C were later re-tested again for SARS-COV-2. All test results were negative for COVID-19.

Table 3: Study results from Uganda

Sentinel sites	**Activity**	**Testing method- TIB-Molbiol PCR kit**					
		rdrp gene		***e-gene***		***EAV***	
	No. samples tested	-Ve	+ve	-Ve	+Ve	-Ve	+Ve
Entebbe Grade B	102	102	0	102	0	0	102
Nsambya Hospital	28	28	0	28	0	0	28
Fort Portal Hospital	35	35	0	35	0	0	35

Kiseyi Health Centre	19	19	0	19	0	0	19
Kiswa Health Centre	120	120	0	120	0	0	120
Mbarara Hospital	12	12	0	12	0	0	12
Tororo Hospital	15	15	0	15	0	0	15
Total	**331**	**331**	**0**	**331**	**0**	**0**	**331**

Abbreviations: RdRP Gene: RNA-dependent RNA polymerase gene, **E-Gene:** - Envelope Protein Gene, **EAV:** Equine Arteritis Virus, **-VE:** Negative Test Results, **+Ve:** Positive Test Results

7.3 Uganda: Study results analysis and interpretations

All three hundred thirty-one (331) archived swab samples tested rdrp-gene and e-gene negative for SARS-CoV-2. All samples picked EAV which indicates that the sample quality was good.

7.4 South Sudan: Study results analysis and interpretations

All sixty-four (64) archived swab samples tested negative for SARS-CoV-2. The South Sudan swab samples were collected from four (4) sentinel surveillance sites namely Juba private hospital, Children's hospital, Juba University Hospital and Al-Sabah hospital. The South Sudan archived frozen swab samples were previously transported to Uganda through the

World Health Organization (WHO), Country Office in Juba City of South Sudan, and stored at the Uganda Virus Research Institute (UVRI) in Entebbe. Eventually these samples were also tested for SARS-CoV-2 at the Uganda Virus Research Institute (UVRI) together with archived swab samples collected in Uganda itself.

8. Conclusions and Recommendations

The two-step molecular and sero-epidemiological cross-sectional study was planned for May to December 2020, but testing could only start early in 2021 due to long and non-harmonized ethics approval processes, challenges related to the availability of PCR test-kits on the world market, to the import of study material into the EAHRC headquarters and the distribution to the participating Partner States, among others. This provides lessons learned for further regional studies. EAHRC, EAC Secretariat and Partner States should jointly work on easing the framework conditions for the urgently needed research in the East African region.

The sample size and with it the study power were too small to prove with absolute certainty that COVID-19 was not already in the region between 1st November 2019 and 29th February 2020. However, as all one thousand one hundred fifty-three (1,153) archived oropharyngeal and nasopharyngeal swab samples from the four participating EAC Partner States tested negative for COVID-19 in RT-PCR, there is a strong indication that this was not the case. Consequently, the study did not progress to the second phase which would have involved testing for antibodies in those SARS-CoV-2 positive patients and their close family members. Due to the delay in implementation, progression to phase two of the study would even in case of positive results have been difficult due to the time lag between the sample collection and the re-testing. As international research shows that antibodies disappear within few months, the second phase of the study would have had to look into other means of detecting a lasting immune response. A study in Wenzhou District of the Peoples' Republic of China which was published on 18th June 2020 in Nature Medicine[7] showed that people who develop antibodies after becoming infected with SARS-CoV-2 may not keep them for more than a few months, especially if they did not have any signs and symptoms from the beginning.

It is important to note that the existing country-wide infrastructure for the National Influenza Virus Infections Sentinel Surveillance Systems in the East African Community (EAC) Partner States were pivotal in retrieving archived frozen nasal and oropharyngeal swab samples from patients who presented with signs and `symptoms of severe acute respiratory infections (SARIs) at various designated national and sub-national level influenzas viruses infections sentinel surveillance sites in the four participating EAC Partner States. In this regard the national sentinel surveillance sites should be strengthened further and expanded to cover other emerging diseases of national, regional and international epidemic and pandemic potential such as Influenza Viruses, SARS, MERS-CoV, Dengue Fever, Yellow Fever, Rift Valley Fever, Ebola Virus Diseases and other Viral Hemorrhagic Fevers (VHFs) and be expanded to cover more parts of each country including international ports of entry such as in-land borders crossing, sea ports and international airports as well as ports of entry across inland water ways. The Republic of Burundi is recommended to establish and operationalize its own national influenza viruses sentinel surveillance system with its accompanying infrastructure and adequate human resources capacity in line with international guidelines and best practices.

There is also a great need for the Republic of South Sudan to build and strengthen the capacity of its National Public Health Laboratory Service to enable them to carry out various advanced molecular laboratory tests for biosafety level 3 and 4 biological pathogens and reduce reliance on its neighbouring countries or international partners now and in future.

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